1999). However, the only example of a ribozyme that indicates such an evolutionary path is the self-splicing group I intron, which is able to catalyze several closely related reactions (Forconi and Herschlag, 2005). A ribozyme was now found that catalyzes two very different reaction chemistries, both of which are coupling ribose and guanine covalently (Lau and Unrau, 2009). One reaction creates the N-glycosidic bond as seen in today's nucleotides, the other creates a stable connection via Schiff base chemistry and Amadori rearrangement. Therefore, this ribozyme could act as an evolutionary intermediate between two ribozymes, each specific for one reaction. Because this ribozyme probably has the same global fold for both catalytic activities, it is the best available evidence for ribozyme evolution via the third pathway. Future research may elucidate the evolutionary neighbors of this intersection sequence, and in vitro selection experiments could show that this evolutionary pathway actually takes place.

In contrast to RNAs, protein enzymes appear unable or unlikely to walk the first or the second evolutionary pathway because only very few amino acid sequences specify a stable fold. Therefore, the first peptides probably evolved using the third pathway by building onto an RNA scaffold before being able to fold independently (Soding and Lupas, 2003). Later evolutionary steps would have used these independently folding protein scaffolds to develop new catalytic activities (Seelig and Szostak, 2007).

Which of the pathways would have been dominant in an RNA world? Currently, the numbers of identified ribozymes are on the side of "escaping the parent fold" (Curtis and Bartel, 2005). However, the discovery of the promiscuous nucleotide synthase ribozyme (Lau and Unrau, 2009) shows that this picture is still emerging. Further in vitro evolution experiments are needed to determine when a ribozyme follows a specific evolutionary pathway. For example, the evolutionary decision could hinge on the reaction mechanisms or the evolutionary plasticity of an RNA structure. However, only after self-replicating and evolving ribozyme systems are found (Lincoln and Joyce, 2009) that are capable of generating ribozymes with new activities will we be able to pose these burning questions face-to-face with our strange RNA ancestors.

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Putative Fat Fighter Hits the Middle Man

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In this issue, Kamisuki and colleagues characterize fatostatin. This compound inhibits the activity of SREBPs, the master transcription factors of lipid homeostasis. This useful laboratory tool also improved the lipid profile of obese mice; does this have clinical implications?

Obesity is a growth industry, with no prospect of downsizing anytime soon. Associated with cardiovascular disease, hypertension, and diabetes, obesity has become a major health burden in the developed world and is becoming an increasingly prominent issue in developing countries as well. Despite the stigma often attached to lipids in the public consciousness, they are crucial for growth and development. For instance, fatty acids and cholesterol are essential for the synthesis of cell membranes and various signaling molecules.

At the molecular level, lipid levels are tightly regulated by the family of sterol-regulatory element binding proteins (SREBPs) (Goldstein et al., 2006). These transcription factors initially reside in the endoplasmic reticulum (ER), tethered to SREBP cleavage activating protein (SCAP). SCAP escorts SREBP to the Golgi apparatus, where SREBP is processed by site-1-protease (S1P) and site-2-protease (S2P) (Figure 1A). This releases the mature form of SREBP, which migrates into the nucleus to target the genes involved in

lipid homeostasis. There are three SREBP isoforms: SREBP-1c activates genes involved in fatty acid synthesis, SREBP-2 upregulates genes involved in cholesterol synthesis and uptake, and SREBP-1a targets both sets of genes (Goldstein et al., 2006).

These master transcription factors are elegantly controlled by negative feedback; cholesterol and related sterols retain the SREBP-SCAP complex in the ER via the retention protein Insig. The interaction between SCAP and Insig is promoted by cholesterol binding to SCAP and oxygenated sterols binding to Insig (Radhakrishnan et al., 2007). This feedback mechanism is exploited by statins, a class of commonly used cholesterol-lowering drugs. Statins inhibit a rate-limiting step in cholesterol synthesis (catalyzed by HMG-CoA reductase), reducing cholesterol levels and subsequently derepressing SREBP processing. This upregulates SREBP target genes, in particular the gene encoding the low-density lipoprotein (LDL) receptor, which imports LDL ("bad" cholesterol) from the bloodstream, thus reducing blood-cholesterol levels. However, not all patients tolerate statins. Hence, there is a need to examine novel compounds that manipulate lipid homeostasis to, at the very least, complement statins.

In this issue of *Chemistry and Biology*, Kamisuki and colleagues (2009) explore the activity of compound 125B11, which they have bestowed the more catchy name "fatostatin." From a library of 10,000 compounds, fatostatin was one of several compounds that blocked insulin-induced adipogenesis in cell culture (Choi et al., 2003). Here, fatostatin was initially found to reduce the expression of SREBP target genes, directing attention to the SREBP transcription factors. They demonstrated that fatostatin reduced ER-to-Golgi transport of SREBP and thus SREBP processing, but how did this occur?

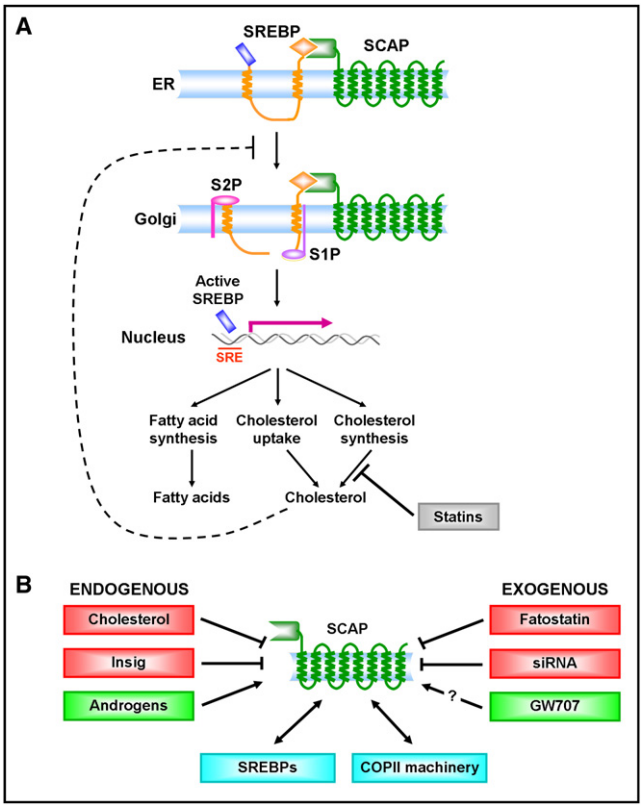

Figure 1. Regulation of Lipid Levels at the Transcriptional Level
(A) The SREBP/SCAP pathway, governed by negative feedback. Details are provided in the text. Statins inhibit cholesterol synthesis, derepressing the SREBP/SCAP pathway.
(B) SCAP acts as the middle man, interacting with SREBPs and the COPII machinery, being negatively regulated by sterols and Insig, and being transcriptionally upregulated by androgens. It is a therapeutic candidate, with siRNA (Soutschek et al., 2009) and fatostatin (Kamisuki et al., 2009) targeting SCAP. Steroid analogs such as GW707 have also been proposed to be SCAP ligands (Grand-Perret et al., 2001), but conflicting evidence has arisen in the literature (Zhang et al., 2004). Green and red boxes indicate agonists and antagonists of SCAP, respectively.

Using modified fatostatin derivatives, microscopy revealed that fatostatin interacted with an ER protein, with a binding assay narrowing down the list of suspects to SCAP. However, fatostatin did not affect SCAP's interaction with SREBP-2 or Insig, and in fact bound to SCAP at a different site to cholesterol. Thus, the authors suggest that fatostatin may interfere with the ability of SCAP to interact with the COPII machinery that is required for ER-to-Golgi transport. Interestingly, fatostatin seems to bind to somewhere within amino acids 449-731 of SCAP—this contains most of the cytosolic loop with the MELADL sequence, which is recognized by the COPII machinery (Sun et al., 2007). Perhaps fatostatin interferes with ER-to-Golgi transport of SREBP by steric hindrance, preventing the interaction between the COPII machinery and the MELADL sequence within SCAP? Once the precise mechanism has been determined, fatostatin may be a useful tool to investigate the SREBP/SCAP pathway, since it inhibits SREBP transport independently of Insig and sterols.

Furthermore, could such a SCAP antagonist be of clinical importance? Kamisuki and coworkers (2009) explored this idea by using *ob/ob* mice, a model for obesity. These mice do not express functional leptin, which induces satiety, and are thus unable to control their food intake. However, these mice are probably not the best model for human obesity because leptin deficiency is rare in obese humans. Nevertheless, treatment with fatostatin was found to reduce weight and improve the lipid profile of these mice, without inducing toxicity or affecting food intake. Genes involved in fatty acid synthesis were downregulated in the liver, reversing fat accumulation (hepatic steatosis, "fatty liver"). This led to an increase in fatty acids (free and triglycerides) and ketone bodies in the bloodstream, suggesting that by inhibiting hepatic SREBP-1c activation and consequently fatty acid synthesis, fatostatin tricks the body into releasing fatty acid stores from the adipose tissue. This raises the question of whether or not fatostatin could be a new fat-reducing drug.

Taking a step back, should we consider SCAP as a candidate for treating obesity? In fact, siRNA was recently developed against SCAP in hamster liver (John et al., 2007), and a patent is in application for using SCAP-targeted RNAi to treat conditions that include hyperlipidemia, obesity, and fatty liver (Soutschek et al., 2009). Importantly, a SCAP antagonist would also reduce SREBP-2 processing,

thus preventing cholesterol synthesis. However, expression of the LDL receptor would also be affected, thus affecting cholesterol uptake from the bloodstream. A high dose of SCAP antagonist would deplete cellular cholesterol levels. So would knocking out SCAP in the *ob/ob* mice model be lethal? While fatostatin reduced LDL and high-density lipoprotein (HDL, ''good cholesterol'') here, the authors caution that mice are not a good model for studying cholesterol homeostasis.

On the other hand, another group considered the use of steroid analogs (e.g. GW707), proposing that these compounds promote SREBP processing by interacting with the cholesterol-binding domain of SCAP (Grand-Perret et al., 2001). However, these ''SCAP ligands'' have been more recently shown to block sterol feedback independently of SCAP by preventing imported (LDL-) cholesterol from reaching the ER (Zhang et al., 2004). Nevertheless, converse to SCAP antagonists, a SCAP agonist would increase LDL-receptor expression and reduce blood-cholesterol levels, but increase fatty acid synthesis, resulting in hepatic steatosis.

Thus, SCAP may be an unsuitable target for adjusting one's lipid profile. Furthermore, taking SCAP out of the picture would negate the effects of statins. Approaches to complement statins could, for instance, involve targeting proprotein convertase subtilisin/kexin type 9 (PCSK9), a SREBP-2 target that degrades the LDL receptor (Chan et al., 2009).

This does not deny the importance of SCAP in lipid homeostasis, however. In a prostate cancer (PCa) setting, androgens (male sex hormones) upregulate SCAP expression, leading to increased SREBP activity and the intracellular accumulation of neutral lipids (reviewed by Heemers et al. (2006)). A high-fat Western diet is associated with PCa risk and dietary cholesterol augmented PCa xenograft growth in vivo (Zhuang et al., 2005), indicating that lipids promote prostate carcinogenesis. Here, Kamisuki and colleagues (2009) found that fatostatin blocks the serum-independent growth of PCa cells, and that knockdown of SREBP-1c inhibits growth of these cells in ''fat-free'' media. From this, they suggested that fatostatin blocks PCa growth by abolishing SREBP processing. While this requires further experiments, these observations suggest that, perhaps, we should start to explore targeting lipid metabolism in PCa therapy.

Overall, Kamisuki and coworkers (2009) have described a compound that may serve as a valuable tool for studying SCAP's role in lipid homeostasis. As a middle man, SCAP interacts with SREBPs, Insig, COPII machinery, and cholesterol, and is upregulated by androgens, so should we consider it to be a therapeutic target (Figure 1B)? Given the divergent regulation of fatty acid and sterol levels downstream of SCAP, altering SCAP alone may yield undesirable broad spectrum effects in treating metabolic disease but could have potential in the treatment of tumors.

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