transcription factors to the promoters of active genes. But, during a prolonged fast, CRTC2 is deacetylated by the NAD-dependent enzyme SIRT1, which allows for ubiquitination of CRTC2 and degradation in the proteosome. The authors provide genetic evidence for this model by demonstrating that in SIRT1-deficient hepatocytes, CRTC2 cannot be degraded even after prolonged exposure to glucagon, which mimics part of the fasting response. The authors suggest that at later stages of the fast, deacetylation of FoxO1 by SIRT1 leads to activation of FoxO1, thereby maintaining glucose homeostasis. This dual regulatory mechanism is schematized in Figure 1.

A few questions remain for future study. First, p300 and CBP acetylate many proteins and, predominantly, histones of genes primed for expression. Thus, it is not clear how much of the effects seen by Liu and colleagues when using p300/CBP inhibitors in mice is due to CRTC2, and how much to the other p300/CBP targets. Second, the effects of CRTC2 RNAi suppression on the expression of PEPCK were relatively mild, and actually larger at 18 hr than at 6 hr. Some of these issues will surely be addressed by gene ablation models for CRTC2 in the future. Third, while Liu and colleagues propose that SIRT1-mediated deacetylation of FoxO1 leads to its activation, prior work had suggested that SIRT1 represses FoxO1 transcription factors (Motta et al., 2004). This discrepancy was not addressed by Liu and colleagues but will need to be resolved in the future. What is clear, however, is that nature has devised multiple redundant systems to ensure that the body's energy balance is maintained even in periods of fasting, and that the liver's central role in this is controlled by a complex system of transcriptional regulators.

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A Cholesterol Toggle Switch

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Cholesterol levels in mammalian cells are controlled by an intricate mechanism in which the transcription factor SREBP plays a key role. Work in this issue (Radhakrishnan et al., 2008) employing direct measurement of endoplasmic reticulum cholesterol levels offers insights into the "switch" that controls this system with surprising precision.

Mammalian cell cholesterol is under constant, mulitfaceted control. A key part of this control is mediated by feedback regulation of SREBP, responsible for the transcription of genes encoding sterol synthetic enzymes, LDL receptor, and other lipid-related proteins. The overarching concept is simple: when cellular cholesterol is low, SREBP is activated, leading to more sterol synthesis and uptake. When cholesterol is high, SREBP becomes inactive, leading to less sterol synthesis and uptake.

The understanding of SREBP regulation sets the stage for the work by Radhakrishnan et al. (2008 [this issue]), showing that mammalian cells have a virtual "toggle switch" for responding to lowering cholesterol. The detailed quantitative picture that begins with these studies will undoubtedly assist in devising new strategies for the clinical manipulation of sterols and provides an example of a biological switching strategy that may be widely used.

Our detailed knowledge of SREBP regulation comes from a remarkable and ongoing odyssey of inquiry by the Brown and Goldstein laboratory leading to an astonishing collection of basic and medical insights (Goldstein et al., 2006). SREBP is an ER membrane protein with an attached cytosolic N-terminal bZIP-HLH

transcription factor. SREBP activation occurs by proteolytic release of the soluble N-terminal transcription factor, allowing access to its nuclear targets.

SREBP cleavage is regulated by cholesterol. SREBP resides in the ER, but the cleaving proteases (S1P and S2P) are in the Golgi. When cholesterol is low, SREBP molecules continuously enter the secretory pathway and travel to the Golgi where they are cleaved and activated, allowing synthesis of sterol synthesis and uptake genes. High cholesterol blocks SREBP trafficking, inhibiting activation by cleavage. Two proteins allow this highly selective traffic control. SREBP is bound to an ER-resident 8-spanning membrane protein called SCAP (SREBP cleavage-activating protein) (Hua et al., 1996). SCAP ferries SREBP to the Golgi by binding to COPII proteins in ER-to-Golgi transport vesicles. SCAP has a bilayer-localized motif called the sterol-sensing domain (SSD) that specifically binds cholesterol with high affinity (Radhakrishnan et al., 2004). Cholesterol binding allows SCAP to bind to Insig, which prohibits SCAP from engaging the transport vesicle, thus preventing SCAP-mediated transport and cleavage of SREBP. This three-protein system is responsible for the entirety of sterol-regulated transcription; that's a lot of regulatory bang for one's protein buck.

This knowledge of SREBP control sets the stage for a quantitative understanding of sterol regulation, which is the focus of the studies by Radhakrishnan et al. The ER membrane has very low levels of sterols compared to the whole cell, making it an appropriate environment for the high-affinity cholesterol sensor SCAP to reside (Steck and Lange, 2002). Furthermore, natural "gain" in the feedback loop exists since ER cholesterol levels are estimated to vary over 10-fold upon ~2 fold changes of total cell cholesterol (Lange and Steck, 1997; Lange et al., 1999). The authors addressed several key questions to connect cellular cholesterol concentrations to SREBP regulation. First, are the predictions about ER and whole-cell cholesterol borne out by direct measurement? Second, what is the relationship between regulated proteolysis of SREPB and ER cholesterol levels? That is, what is the "dose-response" behavior of the system? And finally, can the dose-response behavior of the ER sensing system be modified?

To directly measure ER cholesterol, Radhakrishnan et al. devised a novel three-step centrifugational method to purify ER membranes to a high degree. This step was critical because even slight contamination of the ER membranes with cholesterol-rich fractions from other organelles would cast the analysis into doubt. Mass spectrometry with internal standards was used to measure cholesterol in the highly purified ER membranes and in whole cells. By raising or lowering cellular cholesterol, this direct assay was used to discern the relationship between cellular and ER cholesterol concentration. As predicted, ER cholesterol showed a built-in gain, moving over a 10-fold range in the face of significantly smaller fractional changes in whole-cell content. In the same experiments, the degree of cleavage of SREBP was also measured, so that the ER "dose" of cholesterol and the SREBP cleavage "response" were discernable over a range of sterol levels. When SREBP cleavage was plotted against the ER cholesterol concentration, the response of the SCAP sensing system was strikingly sigmoidal, showing a sudden transition from little to nearly maximal SREBP cleavage when ER cholesterol falls over a surprisingly small range, from about 6% to 4%. That is, the dose-response curve is highly cooperative, resulting in a toggle switch for SREBP activation. The switch occurs at the same position along the ER mole % axis whether the curve is generated by depletion of cholesterol-rich cells or feeding cholesterol-poor ones and was observed with various ways of adding or removing cholesterol from the cells. Importantly, a sterol analog (25-OH cholesterol) that promotes SCAP-Insig interaction caused the same switch without changing ER levels of cholesterol (Radhakrishnan et al., 2007), indicating the amenability of the switch as a drug target.

The switching point can be shifted by physiological conditions. SCAP-mediated cleavage depends on the presence of Insig proteins (Yang et al., 2002). When cells with elevated Insig1 expression were tested, the same steep sigmoid was shifted 2-fold to the left, meaning that the SREBP switch occurred at significantly lower levels of cholesterol. This is interesting for at least two reasons. Since Insigs are under elaborate regulation (Yabe et al., 2003), this may be part of how the "switch point" is determined in a given cell or physiological circumstance. Also, the fact that the cholesterol transition is movable indicates that it might be altered by other means, such as small molecules or dietary protocols, possibly providing novel approaches to clinical management of cholesterol. For example, a drug that shifted the curve to regions of no SREBP activation might be able to lower cholesterol independently of or even synergistically with statin inhibition of sterol synthesis.

Traditional biochemistry shows that highly cooperative behavior requires interacting multiple subunits of an enzyme. The SCAP protein appears to be a tetramer (Radhakrishnan et al., 2004), and the measured Hill coefficient of near 4 for the cholesterol transition would be consistent with a highly cooperative tetrameric system. Before deciding SCAP is a membrane-embedded version of hemoglobin, it is humbling to consider a few other features. The Insigs also appear to be multimeric (Radhakrishnan et al., 2007), and the response to sterols requires the interaction between SCAP and Insig. Furthermore, the SREBP cleavage measured as an endpoint in these studies is the result of numerous cell processes that occur after the game of cholesterol-triggered musical chairs between SCAP and Insig. It will be interesting to evaluate the dose-response curve for more upstream events, such as the structural transition that cholesterol causes in SCAP. Ultimately the structures of these key proteins will have to be discerned in all their cholesterol binding and multimultimer interacting glory. Until that time, this cholesterol toggle switch will activate a new level of quantitative thinking about lipid regulation and cholesterol homeostasis. This type of analysis is a key step in the creation of a predictive model for use in both the basic and clinical understanding of mammalian sterol homeostasis.

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