the nature of the NPC2-NgBR interaction will come from the metabolic labeling studies, coupled with examination of the mode of NPC2 degradation. Such studies will establish whether, in the absence of NgBR, NPC2 is degraded within the ER or is targeted for proteosomal degradation from the lysosome. Because NPC2-mediated sterol transfer in vitro does not require NgBR (Cheruku et al., 2006; Infante et al., 2008), NgBR may principally exert its effect by retaining sufficient levels of NPC2 protein, which one could hypothesize may be rate limiting, to facilitate rapid intralysosomal transfer of lipoprotein-derived cholesterol from internal membranes to NPC1 at the limiting lysosomal membrane.

The NPC1-NPC2 pathway acts as a critical checkpoint for sensing the flux of lipoprotein-derived cholesterol through endocytic pathway. This is achieved through delivery of free cholesterol to the ER sterol-sensing machinery and by promoting synthesis of side-chain oxysterols, which act cooperatively with ER membrane cholesterol to downregulate cholesterol synthesis and uptake (Frolov et al., 2003). Regulation of NPC2 protein levels by NgBR would potentially offer an additional point of regulation to the diverse transcriptional and posttranslational regulatory pathways that contribute to maintenance of cellular cholesterol balance. In this event, it would be expected that NgBR protein levels might be subject to sterol regulation, perhaps at the level of protein expression or even subcellular localization.

The study by Harrison et al. underscores the power of unbiased genetic screens. In future studies, investigation of the specificity and nature of the NPC2-NgBR binding will shed light onto the physiological relevance of this unanticipated protein interaction. It will be instructive to define the regions of NPC2 necessary to support NgBR binding and whether glycosylation-deficient forms of NPC2 (i.e., Asn-39 and Asn-116 mutants) permit binding to NgBR. A critical question is whether the stabilizing effect of NgBR is specific for NPC2 or more broadly extends to other prenlyated or N-glycosylated proteins. If NgBR ultimately is shown to regulate NPC2 protein levels in a specific and possibly sterol-regulated fashion, then the NgBR-NPC2 interaction may serve as a molecular rheostat that responds to the magnitude of lipoprotein cholesterol flux through the endocytic pathway. Thus, regulation of NPC2 protein stability would appear to provide yet another layer of control to limit free cholesterol-mediated cytotoxicity.

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Bile Acids Have the Gall to Function as Hormones

Thomas Quad de Aguiar Vallim[1] and Peter A. Edwards[1,2,*]
[1]Department of Biological Chemistry
[2]Department of Medicine
David Geffen School of Medicine, University of California, Los Angeles, BSRB Room 310, Los Angeles, CA, 90095, USA
*Correspondence: pedwards@mednet.ucla.edu
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In this issue of *Cell Metabolism*, Thomas et al. (2009) show that specific activation of the bile-acid-activated G protein-coupled receptor TGR5 improves pancreatic and hepatic function and impairs the development of obesity following administration of a high-fat diet.

Despite the obvious advantage of increased exercise and restricted calorie intake and the availability of treatments that target obesity and associated disorders, the worldwide fat epidemic continues unabated. Recent studies suggest that the development of bile acid mimetics may prove useful in the treatment of obesity-related disorders. In a tour de force published in this issue of *Cell Metabolism*, Thomas et al. (2009) demonstrate that activation of TGR5 provides significant protection from many physiological changes that normally arise from ingestion of a high-fat diet.

This is the latest in a series of unexpected findings that have expanded the role of bile acids from simple detergents

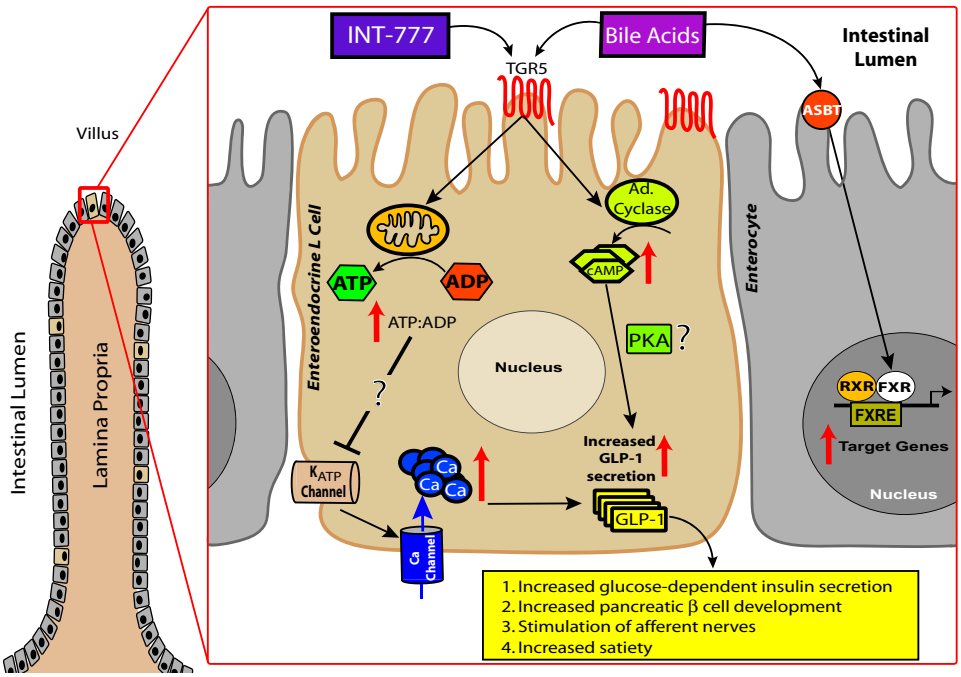

Figure 1. Overview of the Effects of Bile Acid Activation of TGR5 and FXR in the Gut

Bile acids present in the intestine activate both TGR5 on the surface of enteroendocrine L cells and the nuclear receptor FXR in enterocytes. Ninety-five percent of the bile acids entering the intestinal lumen from the gall bladder are subsequently resorbed via the apical sodium-dependent bile acid transporter (ASBT), leading to activation of the FXR:RXR heterodimer bound to the FXR response element (FXRE) and induction of target genes. Bile acids in the intestinal lumen also activate TGR5 on the surface of enteroendocrine L cells, leading to an increase in oxidative phosphorylation and the ratio of ATP:ADP. The increased levels of ATP are thought to inhibit the ATP-sensitive potassium channel, leading to a depolarization of the cell membrane and opening of calcium channels. TGR5 activation also activates adenylate cyclase (Ad. Cyclase), thus increasing cellular cAMP levels. Increases in intracellular calcium and cAMP both lead to increased secretion of GLP-1 from L cells. Secreted GLP-1 has a number of effects. It augments glucose-dependent insulin release from β cells, it promotes β cell development, and it stimulates afferent nerves. GLP-1 can also induce satiety, and it has neuro- and cardioprotective roles.

necessary for lipid absorption to that of potent hormones. In 1999, three groups made the remarkable observation that specific bile acids bound to and activated the nuclear receptor FXR, leading to increased transcription of specific target genes (Figure 1). These genes control a number of diverse pathways, including bile acid, cholesterol, and glucose metabolism (reviewed in Zhang and Edwards, 2008; Lefebvre et al., 2009). Subsequent studies demonstrated that bile acids also activated three other nuclear receptors (VDR, PXR, and CAR), consistent with pleiotropic effects in vivo (Schmidt and Mangelsdorf, 2008; Zhang and Edwards, 2008).

In yet another surprise, specific bile acids were shown in 2002–2003 to activate a cell surface G protein-coupled receptor, now named TGR5, and to increase cellular cAMP levels (Maruyama et al., 2006; Kawamata et al., 2003). TGR5 expression levels vary greatly from tissue to tissue. For example, high expression is observed in the intestine and gall bladder, which are normally exposed to high levels of bile acids circulating in the enterohepatic circulation, whereas low levels of TGR5 are present in muscle and BAT, tissues that are exposed to lower levels of bile acids present in peripheral blood. TGR5 may, therefore, have different effects depending on the cell type. Indeed, bile acid activation of TGR5 in either BAT or muscle cells was previously shown to result in increased cAMP levels and activation of type 2 thyroid hormone deiodinase (D2) that converts inactive T4 to the active thyroid hormone T3. Consistent with this finding, a diet enriched in bile acids increased both D2 in BAT and energy expenditure and resulted in resistance to fat-induced obesity (Watanabe et al., 2006). In contrast, activation of TGR5 in enteroendocrine L cells that are scattered throughout the jejunum, ileum, and colon stimulates the secretion of the incretin glucagon-like peptide-1 (GLP-1) (reviewed in Thomas et al., 2008).

Thomas et al. (2009) now report on additional studies aimed at delineating the physiological function of TGR5 (Figure 1). The synthesis of INT-777, a novel bile acid mimetic that activates TGR5, but not FXR (Pellicciari et al., 2007), and the generation of both gain-of-function (*Tgr5*-Tg) and *Tgr5*$^{-/-}$ mice (Thomas et al., 2009) enabled complementary and extensive in vitro and in vivo studies. Overexpression/activation of TGR5 was shown to improve liver function, protect pancreatic islets from high-fat-induced hypertrophy and insulin reduction, improve glucose tolerance and insulin sensitivity in obese mice, and reduce hepatic steatosis and obesity in fat-fed mice.

One important player in these effects produced by activation of TGR5 in vivo appears to be the incretin GLP-1. Importantly, administration of drugs that either impair GLP-1 degradation in the blood or activate the GLP-1 receptor are currently used clinically to improve insulin sensitivity in type 2 diabetics (Reimann et al., 2008). Thus, understanding the mechanisms controlling GLP-1 release is

important and might prove efficacious in the development of novel clinical treatments.

The combined approaches by Thomas et al. (2009) show that activation of TGR5 results in increases in intracellular levels of cAMP, oxygen consumption, cytochrome c oxidase (thus linking TGR5 with mitochondrial function), and the ATP:ADP ratio (Figure 1). The authors propose that the increase in ATP:ADP could account for the inactivation of the K_{ATP} channel, membrane depolarization, and subsequent opening of a calcium-gated voltage channel on enteroendocrine L cells (Figure 1). Importantly, the increase in cellular calcium levels in enteroendocrine L cells stimulates GLP-1 secretion. The authors also show that oral administration of either glucose or a high-fat meal to *Tgr5*-Tg mice improved glucose tolerance and increased GLP-1 secretion. One possibility is that the high-fat meal caused the gall bladder to contract, thus increasing bile acid levels in the intestinal lumen and activating TGR5 on L cells. Surprisingly, although fat-fed *Tgr5*-Tg mice exhibited higher energy expenditure, they did not lose body weight relative to control mice. The authors suggest that this may be a result of the decreased locomotion of *Tgr5*-Tg mice, a finding consistent with the known hyperactivity observed in GLP-1 knockout mice.

To directly address the effects of pharmacological activation of TGR5, Thomas et al. (2009) fed wild-type mice a high-fat diet to promote obesity and generate symptoms normally associated with diabetes. Some of the fat-fed mice were also treated with the TGR5 agonist INT-777. The results were striking, as compared to fat-fed controls, the INT-777-treated mice exhibited a 17% reduction in body weight and had reduced adiposity, improved liver function, less steatosis (fat in the liver), decreased plasma levels of triglycerides and free fatty acids, and an improved glu-cose tolerance test. Importantly, food intake, which can be repressed by high levels of GLP-1, was unaffected by INT-777 treatment. Indeed, calorimetric studies indicate that the changes in body weight are likely a result of increased energy expenditure.

The *Tgr5*-floxed mice generated in the current study provide a new resource, as they can now be used to generate mice lacking TGR5 in specific cell types. In this regard, it will be particularly interesting to assess whether the physiological effects of INT-777 are attenuated following deletion of TGR5 from enteroendocrine L cells or from pancreatic β cells if TGR5 is expressed in the latter cells.

In conclusion, studies during the last decade have identified bile acids as potent hormones that activate nuclear receptors and a G protein-coupled receptor. Hopefully, these studies will lead to the development of specific agonists for FXR or TGR5 that will prove efficacious in the treatment of various metabolic disorders.

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Metabolism by Remote Control

Marc Tatar[1,*]
[1]Brown University, Providence, RI 02912, USA
*Correspondence: marc_tatar@brown.edu
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Drosophila melanogaster **produce insulin-like peptides in specialized neuroendocrine cells to regulate growth, metabolism, aging, and reproduction. In this issue of *Cell Metabolism*, Géminard et al. (2009) describe how secretion of insulin-like peptides is remotely controlled by the fat body (an adipose, liver-like tissue) in response to dietary amino acids.**

Diseases of metabolism are appearing in alarming proportions in many societies. These complex pathologies reflect a loss of homeostasis between our environment, endocrine physiology, and molecular mechanisms within cells. Scientists have struggled to understand these interactions, even in mammalian model systems. Enter, then, the fruit fly *Drosophila*