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Does This Schlank Make Me Look Fat?

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Abstract

Chaurasia and colleagues discuss the provocative new finding that certain enzymes in the de novo sphingolipid synthesis pathway have dual roles as transcriptional regulators.

As fatty acids enter adipocytes (mammals) or fat bodies (*Drosophila*), the majority are shunted into a glycerolipid biosynthesis pathway that generates triglycerides—the major fuel store in the body—and other membrane lipids. A smaller percentage of the fatty acids spillover into an alternative biosynthetic pathway that produces sphingolipids such as ceramides. Under conditions of nutrient overload, ceramides accumulate and impair metabolic processes involved in lipid and nutrient storage (e.g. insulin-stimulated glucose uptake, *de novo* lipogenesis, oxidative phosphorylation, etc.), thus contributing to the sequelae of pathogenic events that underlie diabetes and cardiovascular disease [1]. New studies by Sociale and colleagues [2] suggest a novel mechanism by which enzymes in this sphingolipid biosynthesis pathway, independent of their effects on ceramide production, also translate changes in fatty acid concentrations into an altered transcriptional program that further modifies lipid metabolism. Indeed, certain enzymes were shown to have DNA-binding properties and the capacity to alter expression of lipolytic genes. These provocative studies lend credence to the idea that enzymes in the sphingolipid synthesis pathway serve as gauges of fuel status and reveal new mechanisms by which the body alters biological responses in relation to nutritional overload.

Ceramides consist of a sphingoid base, generated by the condensation of palmitoyl-CoA and serine, coupled to a fatty acid via an amide bond. In mammals, six different ceramide synthases (CERS 1–6), differing in tissue distribution and substrate specificity [3, 4], catalyze the acylation reaction. Unlike mammals, *Drosophila melanogaster* express only one ceramide synthase enzyme, termed *schlank*, produces the entire spectrum of organismal (dihydro)ceramides. Sociale and colleagues were intrigued by the fact that *schlank*, like most mammalian CERS enzymes (i.e. CERS1,2,4,5 and 6), contains a nuclear localization sequence and a homeodomain, suggesting that they may regulate gene expression. Using knock-in constructs to dissect the functional *schlank* domains, they determined that the enzyme responds to fatty acids flowing through the pathway by redistributing to the nucleus and repressing expression of several lipases (e.g. *lipase 3*, *magro*)(Figure 1)[2]. Mutation of

the nuclear localization sequence recapitulated the phenotype of *schlank* knockout flies, producing a slimness phenotype associated with a persistent upregulation of lipase genes [2]. Indeed, the authors demonstrated that lipid availability (cells) or feeding (organism) led to nuclear enrichment of the *schlank* protein, revealing a mechanism by which fatty acid availability represses lipolysis by altering the intracellular location of *schlank* [2]. Moreover, they found that the nuclear localization sequence was required for the feeding responses that included repression of *lipase 3* and *magro* gene expression [2]. Lastly, they showed that these effects were independent of ceramide synthase activity [2]. Thus, the study revealed an alternative definition of *schlank*, as the enzyme was shown to have dual functions as both a (i) ceramide synthase and a (ii) fatty acid-inducible transcriptional repressor (Figure 2).

The idea that mammalian CERS enzymes could have transcriptional activity has been considered previously, owing to the presence of the aforementioned homeobox-like domain (HOX) that is present in five of the six *Cers* isoforms. However, the HOX domains show many sequence discrepancies with other HOX-domain containing proteins, lacking key residues that were thought to be required for DNA binding [3, 4]. Since the domain juxtaposes a region critical for catalytic activity, many investigators thus suggested that this domain acts as a modulator of synthase activity, rather than as a direct modulator of transcription. Indeed, a highly conserved region of 12 amino acids near the C-terminal portion of the Hox-like domain was found to be essential for activity of some CERS (i.e. CERS5 and 6)[3, 4]. Nonetheless, Sociale and colleagues demonstrated that the mammalian CERS2 enzyme, like *schlank*, likely bound DNA and/or modulated transcription from the *lip3* promoter [2]. These findings support the relevance of this additional role for mammalian CERS enzymes as direct modulators of gene expression.

The transmembrane domain that binds lipid bilayers was required for the repression of the *lip3* gene by *schlank*, as overexpression of the enzyme's pure homeodomain, which is entirely nuclear, was without effect. This finding raises obvious questions about mechanism, particularly regarding the mechanisms of nuclear translocation and the identity of the lipid moieties that might influence enzyme activity in the nucleus. Sociale and colleagues note that a few other metabolic enzymes, such as glyceraldehyde-3-phosphate and inosine monophosphate dehydrogenase, have been found to have dual roles as transcriptional regulators [2]. Their work could nonetheless *reveal a paradigm-shifting mechanism that underlies lipid sensing*.

The work sheds new light on prior studies using *Cers* knockout mice to elucidate their functions in lipid metabolism. For example, studies in mice lacking one or both alleles of the *Cers2* gene revealed numerous effects on liver lipid metabolism. Complete knockout of the gene greatly reduced liver fat, inducing several hepatic abnormalities (e.g. hepatopathy, hepatocyte death, and recurrent regeneration)[5–8]. By comparison, haploinsufficiency for the gene produced no ill-effects in mice maintained on a normal chow diet, but predisposed them to diet-induced steatohepatitis [9]. In both the heterozygous and homozygous null animals, *Cers2* depletion induced hepatic insulin resistance [5–9]. The studies described Sociale and colleagues suggest that some of these effects could result from an altered transcriptional repression of lipolysis. Nonetheless, at least some of the effects of *Cers2* gene ablation were likely attributable to a compensatory increase in *Cers6* and C16-ceramide

biosynthesis, as treatment with inhibitors of earlier enzymes in the pathway (i.e. myriocin) negated the effects of *Cers2* heterozygosity [9]. Moreover, ablation of the *Cers6* gene had effects that opposed those of *Cers2* haploinsufficiency, protecting mice from diet-induced steatohepatitis and hepatic insulin resistance [10]. Far more work is needed on the differences in gene expression programs that might be driven by different CERS transcriptional modifiers.

The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC), which evaluated GWAS data from 152,635 individuals of European, East Asian, and African ancestry, identified a particularly strong association between a SNP in the coding region of *Cers2* (i.e. rs267738) and measures of metabolic dysfunction (i.e. HbA1C)[1]. Other publications also revealed that the SNP correlates with markers of diabetic kidney disease (e.g. albuminuria)[1]. The glutamate to alanine substitution caused by this polymorphism, which is present in this HOX domain, would be predicted to impair lipase gene expression. However, polyphen-2 analysis reveals that it also likely inhibits enzyme function, and further work is needed to probe its relevance as a causative agent in diseases associated with abnormal lipid homeostasis. Nonetheless, the work unveils the provocative possibility that the point mutation introduced alters transcriptional activity without modifying enzyme activity, a finding that could have important clinical ramifications.

Collectively, the preponderance of data on enzymes in the ceramide-synthesizing pathway indicate that the pathway serves as an important sensor of lipid overload. Indeed, we previously surmised that the spillover of lipids into this pathway gave rise to lipid modulators that antagonize de novo lipid synthesis (i.e. glucose uptake and activation of lipid-synthesizing genes) and oxidation (i.e. oxidative phosphorylation)[11]. The new findings by Sociale and colleagues reveals an additional role for the CERS enzymes as modulators of the transcriptional response to lipid overload. These findings introduce a new level of complexity to our understanding of nutrient sensing, uncovering a new paradigm in our understanding of the role of this pathway in diseases associated with lipid accumulation.

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Figure 1. Schematic summarizing the studies suggesting that nuclear translocation of schlank (*Drosophila*) or ceramide synthases (CerS enzymes, Mammals) represses transcription of lipolytic genes.

Schlank

slaNGk/

verb

verb: **schlank**; 3rd person present: **schlanks**; past tense: **schlunk**; past participle: **Schlunk**; gerund or present participle: **schlanking**

1.

To catalyze production of dihydroceramides from 3-ketosphinganine

2.

To modulate transcription of genes involved in lipid metabolism

Figure 2.
Schlank takes on a new meaning.