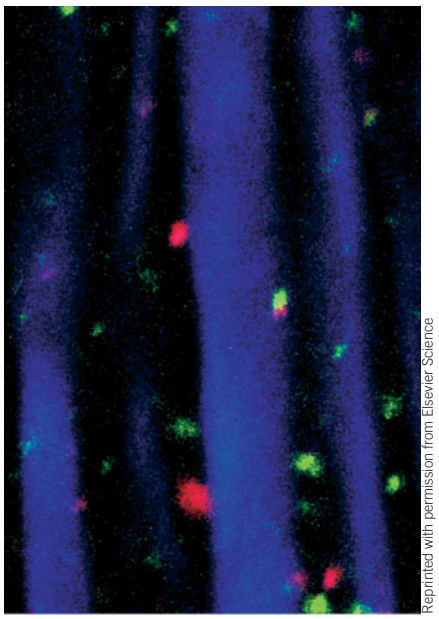

Figure 2 Channel meets receptor. Triple-labeled sections show L-type calcium channels (green), ryanodine receptor (red) and axons, visualized with staining for neurofilaments (blue). Clusters of calcium channels and ryanodine receptors colocalize near the outer membrane of the axon. Clusters also occur elsewhere, probably within glial structures (*from ref. 2*).

damage. Either this sort of axonal injury was not mediated by calcium, or it had to come from intra-axonal stores.

The authors found that removing intra-axonal calcium with a chelating agent protected the axons, suggesting that the calcium comes from within. But what about sodium? Tetrodotoxin also reduced the injury, but not when sodium in the medium was replaced by lithium, a monovalent cation that allows injured axons to depolarize even in the presence of the channel blocker. The authors postulate that axonal depolarization must trigger the release of calcium stored within the axon.

They then took a cue from the excitation-contraction coupling mechanism in skeletal muscle. Depolarization at the neuromuscular junction causes L-type calcium channels at the surface of the muscle cell to activate intracellular ryanodine receptors (RyR). These receptors release calcium from stores in the sarcoplasmic reticulum. A similar sequence seems to occur during ischemic axonal injury. The authors found that blockade of either L-type calcium channels or RyR reduced functional loss after oxygen-glucose deprivation.

The effect of the drugs, however, was not additive, suggesting a common pathway. Both immunoprecipitation and immunolocalization experiments showed a link between L-type calcium channels and RyR in white matter (**Fig. 2**), and immunoelectron microscopy revealed elements of smooth endoplasmic reticulum near the axonal membrane at the myelin internodes. Finally, calcium imaging in a live dorsal column white matter preparation *in vitro* during oxygen-glucose deprivation revealed a robust release of intra-axonal calcium within individual axon segments.

Previous studies have provided evidence for the involvement of L-type calcium channels in axonal injury[8] and RyR in white matter compression injury[9]. However, Ouardouz *et al.* are the first to provide both morphological and physiological insight into how the calcium channel and the RyR work together to produce axonal injury (**Fig. 1**). The physiological and pharmacological studies, along with the immunoprecipitation, immunolocalization and calcium imaging data, provide compelling evidence that RyR and L-type calcium channels interact in at least some axons, in a manner highly similar to their functional interaction in skeletal muscle.

Of course, there are many remaining questions about axonal injury. The extent to which extracellular and intracellular sources of calcium contribute to loss of axon function seems to vary in the different *in vitro* models. We do not know how much each calcium source contributes to different types of injury *in vivo*, and to secondary axonal damage at different times after injury. However, drugs that target specific channels and receptors involved in axonal injury now include those developed from studies of muscle and cardiac arrhythmia.

With greater understanding of the contribution of specific mechanisms to different types and stages of axonal injury, it may be possible to customize therapeutic approaches to optimize recovery from white matter injury.

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Setting fat on fire

Robert Walczak & Peter Tontonoz

One approach to developing antiobesity drugs is to shift the energy balance in the body in favor of burning fat. A transcriptional coactivator is now assigned this task.

Obesity and its associated ills, such as diabetes and high blood pressure, are increasing at an alarming rate worldwide. Between 1995 and 2001, according to the World Health Organization, the number of obese individuals increased from 200 million to 300 million.

The quest for antiobesity drugs has been bolstered by recent work suggesting that orphan nuclear receptors and their coactivators may represent tractable targets. In a recent issue of *Proceedings of the National Academy of Sciences*, Kamei *et al.* examine one such coactivator, peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1β (PGC-1β)[1]. They report that overexpression of PGC-1β increased energy expenditure and blocked the development of obesity in mice. These observations suggest that pharmacologic agents aimed at increasing the activity of PGC-1β or its interacting transcription factors might alter the body's energy balance in favor of burning fat rather than storing it.

PGC-1β belongs to a larger family of transcriptional coactivators[2–4]. PGC-1α, for instance, is induced in brown adipose tissue skeletal muscle, heart and liver in response to increased energy demands such as cold exposure, short-term exercise or fasting. PGC-1α controls thermogenesis in brown fat[2], affects gluconeogenesis in the liver[5] and drives the formation of slow-twitch fibers in

Robert Walczak and Peter Tontonoz are at the Howard Hughes Medical Institute, Department of Pathology and Laboratory Medicine, University of California, Los Angeles, California 90095-1662, USA.
e-mail: ptontonoz@mednet.ucla.edu

skeletal muscle[6]. PGC-1β is expressed in similar tissues as PGC-1α, but its *in vivo* function is still unclear. Also like PGC-1α, PGC-1β is a coactivator for nuclear respiratory factor-1, a key component in the expression of mitochondrial genes. Ectopic expression of PGC-1α or PGC-1β in cells triggers mitochondrial biogenesis and increases respiration[7].

Kamei *et al.* found that PGC-1β-transgenic mice were lean and resistant to obesity. Even on a high-fat diet, the transgenic mice developed less adipose tissue and had lower blood glucose and insulin levels than their wild-type littermates, despite consuming more food. The transgene expressing PGC-1β also antagonized the development of obesity in a genetically obese background. How does PGC-1β do this?

The authors found that PGC-1β increased the rate of fatty acid oxidation in skeletal muscle by as much as 50%, and significantly elevated whole-body energy expenditure. Thus, increased expression of PGC-1β appears to burn up fat.

Kamei *et al.* propose that the interaction of PGC-1β with the estrogen-related receptor (ERR) group of nuclear receptors is responsible for these substantial effects on energy balance. They found that PGC-1β interacted preferentially with ERRs when tested for its ability to coactivate a panel of nuclear receptors in the absence of ligand. Previous work established that ERRs regulate the expression of genes involved in mitochondrial fatty acid oxidation, such as medium-chain acyl-CoA dehydrogenase (MCAD)[8]. MCAD mediates the initial step in medium-chain fatty acid β-oxidation, and regulation of MCAD expression is an important mechanism of metabolic control. Indeed, Kamei *et al.* found increased expression of MCAD in the skeletal muscle of PGC-1β-transgenic mice. Interestingly, the related coactivator PGC-1α also interacts with ERRs to drive the expression of MCAD in cultured cells[9,10].

The interaction with ERRs provides a plausible explanation for the fat-burning tendencies of PGC-1β-transgenic mice. But it is premature to conclude that ERRs are the only transcription factors interacting with PGC-1β to promote energy expenditure. Other groups have shown that PGC-1β interacts with glucocorticoid, thyroid hormone and retinoic acid receptors, as well as with nuclear respiratory factor-1 (ref. 4). In addition, studies in cultured cells have established that PGC-1β, like PGC-1α, is a powerful regulator of mitochondrial biogenesis and function[7]. Additional studies, including an analysis of the effects of PGC-1β in mice lacking ERRs, will be required to definitively address this issue.

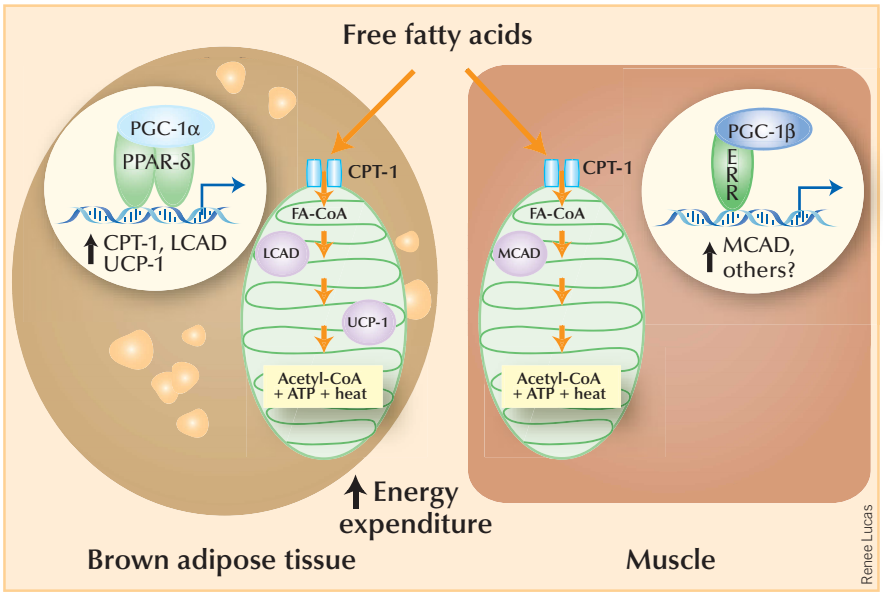

Figure 1 Control of fatty acid oxidation and thermogenesis by nuclear receptors and their coactivators. Transcription of the gene encoding MCAD is induced in skeletal muscle in response to the interaction between the orphan nuclear receptor, ERR, and the coactivator, PGC-1β. MCAD catalyzes the limiting step in the pathway of mitochondrial fatty acid (FA) β-oxidation. Kamei *et al.* propose that in PGC-1β-transgenic mice, activation of MCAD in skeletal muscle increases fatty acid oxidation and energy expenditure. Similarly, genes encoding mitochondrial fatty oxidation enzymes, such as carnitine palmitoyl transferase-1 (CPT-1) and long-chain acyl-CoA dehydrogenase (LCAD) are induced in brown adipose tissue by the action of PPAR-δ; its coactivator, PGC-1α, increases the expression of uncoupling protein-1 (UCP-1) in adipose tissue. Transgenic mice expressing PGC-1β or constitutively active PPAR-δ are resistant to diet-induced and genetic obesity.

Another limitation of the study is that the authors did not investigate the expression of genes involved in fatty acid oxidation in brown adipose tissue or heart, and did not examine the expression of a broader panel of genes involved in mitochondrial function. Such studies will be needed in the future to fully understand the phenotype of the PGC-1β-transgenic mice.

The findings of Kamei *et al.* bear a striking resemblance to recent studies on a different nuclear receptor, PPAR-δ. Earlier this year, Wang *et al.* reported that mice expressing a dominant active form of PPAR-δ in adipose tissue were also resistant to genetic and diet-induced obesity[11]. The authors of that study showed that PPAR-δ interacts with PGC-1α and drives the expression of genes involved in fatty acid oxidation and thermogenesis in adipose tissue *in vivo*. Taken together, these two studies point to PGC-1 coactivators and their orphan receptor partners as potential targets for treating human obesity. Agents that specifically increase the activity of the PPAR-δ–PGC-1 or ERR–PGC-1β axes might increase fatty acid oxidation and energy expenditure and limit the development of excess adipose tissue (**Fig. 1**).

In theory, this might be accomplished by increasing the expression of the coactivator in specific cells, either by increasing the affinity of the coactivator for the nuclear receptor, or by activating the receptor with a specific small-molecule ligand. In fact, synthetic ligands for PPAR-δ have already been shown to inhibit the development of obesity in mice[11]. Of course, given the numerous cellular processes PGC-1 proteins participate in, one must be prepared for the possibility that chronic activation of these pathways may have undesirable effects.

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