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# PGC-1α: Looking behind the Sweet Treat for Porphyria

**Porphyrias are a group of genetic disorders caused by mutations in enzymes of the heme biosynthesis pathway. Acute attacks of porphyria, reputedly the disease that incapacitated the British sovereign King George III (see this *Cell* cover), are precipitated by fasting and are treated by infusing heme or glucose, although the underlying molecular mechanisms remain unclear. In this issue of *Cell*, Handschin et al. (2005) reveal that a transcriptional coactivator called PGC-1α is a key player in both induction of porphyria by fasting and amelioration of the symptoms by glucose treatment.**

The porphyrin heme is the crucial iron bound prosthetic group of hemoglobins that enables red blood cells to carry oxygen. All nucleated cells require heme for production of functional respiratory cytochrome proteins. About 85% of heme biosynthesis takes place in erythroid cells, and this heme is destined for incorporation into hemoglobins (reviewed in Kauppinen, 2005). Most of the heme that is not produced in red blood cells is made by the liver and then incorporated into various heme proteins such as members of the cytochrome P450 family.

De novo heme biosynthesis is carried out by the sequential actions of eight distinct enzymes (see Figure 1A). The first and rate-limiting step of the pathway is catalyzed by the enzyme δ-aminolevulinic acid (ALA) synthase (ALAS), which promotes condensation of glycine and succinyl CoA to form ALA. Mammals have two *ALAS* genes: the housekeeping gene *ALAS-1* is ubiquitously expressed, whereas *ALAS-2* is specific to erythroid cells. Given that excess heme is toxic to cells, it is not surprising that the heme biosynthetic pathway is tightly regulated. For example, both the expression and activity of ALAS-1 are inhibited by heme via feedback mechanisms. In addition, heme biosynthesis can be upregulated in response to an increase in demand, perhaps best illustrated by the drug-induced increase in the production of cytochrome P450 family members in the liver, which metabolize drugs. Reflecting the requirement of cytochrome P450 proteins for heme, many drugs concomitantly induce expression of the *ALAS-1* gene (Podvinec et al., 2004). Ironically, such drug-mediated induction of ALAS-1 expression in turn can precipitate a porphyria attack in susceptible individuals.

Porphyrias are caused by loss-of-function mutations in any of the seven enzymes of the heme biosynthetic pathway apart from ALAS-1 (Kauppinen, 2005). These mutations lead to overproduction and accumulation of different heme biosynthetic intermediates depending on which enzyme is affected. Mutations in four of the enzymes cause inducible hepatic porphyria (see Figure 1A), which is characterized by the occurrence of acute attacks with symptoms including abdominal pain and neurological disturbances, accompanied by a dramatic increase in production and excretion of porphyrin precursors such as ALA and porphobilinogen (Kauppinen, 2005). Acute attacks are often precipitated by drugs, hormones, or fasting. They are treated by removal of the precipitating agent, together with infusion of heme and/or glucose (Anderson et al., 2005; Kauppinen, 2005). Although heme is known to inhibit its own synthesis through negative feedback loops, the molecular mechanisms that underlie the beneficial effects of glucose and the precipitating effects of fasting are not known.

In this issue of *Cell*, Spiegelman and colleagues report that a transcriptional coactivator called PGC-1α (PPAR γ coactivator 1α) holds the key to unraveling the mystery of acute porphyria attacks, the disease that reputedly incapacitated the British sovereign King George III and induced his episodes of "madness" (Handschin et al., 2005; see the *Cell* cover). PGC-1α was originally identified by the Spiegelman group from a cDNA library derived from brown fat tissue. These investigators showed that PGC-1α is a cofactor that interacts with the nuclear receptor family member PPAR γ (reviewed in Puigserver and Spiegelman, 2003). In response to cold temperature, its expression is induced in key thermogenic tissues such as brown fat and skeletal muscle. In these locations, PGC-1α plays an important role in adaptive thermogenesis by enhancing the activities of specific transcription factors (including PPAR γ) that regulate the expression of UCP-1, a key molecule that uncouples ATP synthesis from mitochondrial respiration, resulting in heat production (Puigserver and Spiegelman, 2003).

At first glance, there does not seem to be much of a link between the ability of PGC-1α to regulate energy metabolism and its connection with the genetic disease porphyria. Subsequent studies by the Spiegelman group and others revealed that PGC-1α has another important function in energy homeostasis: the regulation of gluconeogeneis in the liver (reviewed in Puigserver and Spiegelman, 2003). The term gluconeogenesis refers to the process of de novo synthesis of glucose from noncarbohydrate precursors (such as pyruvate and alanine) upon prolonged fasting. It takes place principally in the liver and is essential for providing the sugar needed for organs such as the brain. PGC-1α expression in the liver is rapidly induced by fasting (see Figure 1B); and this increase in PGC-1α in turn activates the gluconeogenic program by coactivating key transcription factors that turn on genes encoding gluconeogenic enzymes (Puigserver and Spiegelman, 2003; Puigserver et al., 2003).

Given that fasting is a powerful inducer of acute attacks of porphyria and that PGC-1α is dramatically induced in the liver upon fasting, Handschin et al. were intrigued by the possibility that PGC-1α might mediate *ALAS-1* expression induced by fasting. Indeed, an initial time course analysis showed that liver ALAS-1 and PGC-1α exhibited almost identical temporal patterns of upregulation in response to fasting. The authors then

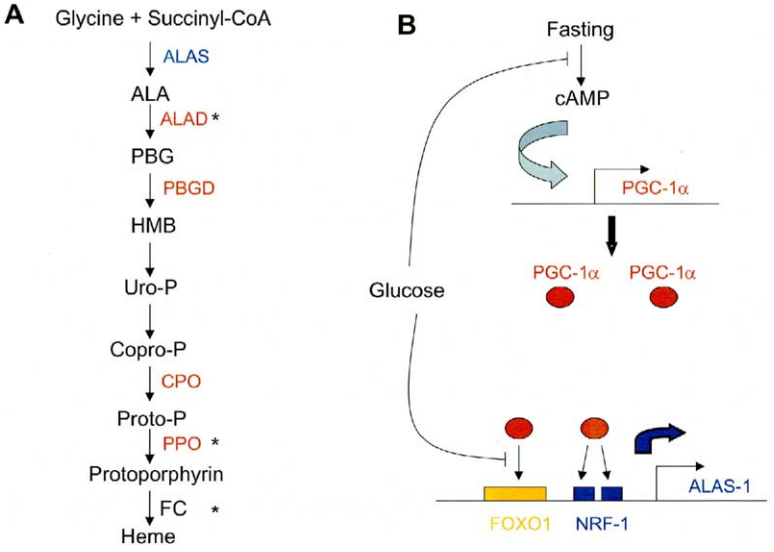


Figure 1. Insights into the Cause and Treatment of Porphyria

(A) The heme biosynthetic pathway. Abbreviations for intermediates: ALA, δ-aminolevulinic acid; PBG, porphobilinogen; HMB, hydroxymethylbilane; Uro-P, Uroporphyrinogen; Copro-P, Coproporphyrinogen; Proto-P, Protoporphyrinogen. Abbreviations for enzymes: ALAS, ALA synthase; ALAD, ALA dehydratase; PBGD, PBG deaminase; CPO, Copro-P oxidase; PPO, Proto-P oxidase; and FC, Ferrochelatase. The rate-limiting enzyme ALAS is depicted in blue. The four enzymes affected in inducible hepatic porphyrias are depicted in red. The asterisks indicate enzymes that are inhibited by chemicals used for inducing chemical porphyria. Two other enzymes of the pathway are Uro-P synthase and Uro-P decarboxylase (not shown).

(B) PGC-1α regulates expression of the *ALAS-1* gene in response to fasting. Fasting activates PGC-1α expression in the liver through the cAMP/CREB pathway (reviewed in Puigserver and Spiegelman, 2003). PGC-1α (red ovals) then binds to and coactivates the transcription factors NRF-1 and FOXO1, greatly enhancing *ALAS-1* expression (thick blue arrow). Glucose administration and the subsequent increase in insulin reverses this effect (1) by antagonizing the induction of PGC-1α expression and (2) by disrupting the interaction of PGC-1α with FOXO1 (through promoting phosphorylation of FOXO1 by the Akt kinase). The promoter of the *ALAS-1* gene also contains cAMP response elements (Giono et al., 2001) and thus could be subjected to direct regulation by the cAMP/CREB pathway upon fasting, an effect that would also be antagonized by glucose (not shown).

used an adenovirus-based system to ectopically express PGC-1α in either cultured cells in vitro or in rat liver in vivo. They then showed that in all cases ectopic expression of PGC-1α markedly increased the production of *ALAS-1* mRNA. Interestingly, none of the other genes of the heme biosynthetic pathway were induced by PGC-1α. To further probe the role of endogenous PGC-1α in ALAS-1 expression, Handschin et al. isolated primary hepatocytes from either wild-type mice or those lacking PGC-1α and treated them with a cocktail of hormones to mimic fasting. They found that whereas the hormone cocktail markedly induced expression of *ALAS-1* in wild-type cells, induction was blunted in PGC-1α-deficient cells. Notably, lack of PGC-1α also blunted the induction of gluconeogenic genes, which are known to be regulated by PGC-1α.

These data clearly suggest an important role for PGC-1α in ALAS-1 expression. Thus, the authors turned to the promoter of *ALAS-1* to further dissect how PGC-1α exerts its effects. Inspection of known elements in the ALAS-1 promoter revealed several informative features: a region comprising two binding sites for the transcription factor NRF-1 (Braidotti et al., 1993) and a region containing the insulin-response element (see Figure 1B). The insulin-response element is important for insulin-mediated inhibition of *ALAS-1*, but the transcription factor that binds to this region has remained elusive (Scassa et al., 2004). Interestingly, previous studies from the Spiegelman group have shown that PGC-1α can act as a coactivator of NRF-1 during, for example, mitochondrial biogenesis. Furthermore, yet another transcription factor called FOXO1 interacts with PGC-1α to activate gluconeogenic genes, and this interaction is disrupted by insulin, which promotes phosphorylation of FOXO1 by the Akt kinase (Puigserver and Spiegelman, 2003; Puigserver et al., 2003). Thus, it is conceivable that PGC-1α could regulate the ALAS-1 promoter through two nonexclusive mechanisms. In the first, PGC-1α interacts with and coactivates NRF-1 bound at the NRF-1 sites, an action that is presumably insulin insensitive. In the second, PGC-1α interacts with and potentiates the activity of FOXO1, which binds to the insulin-response element; this interaction can be disrupted by insulin signaling. Using a variety of molecular approaches—chromatin immunoprecipitation, gel-shift and reporter assays, as well as mutagenesis analyses of regulatory elements—the authors convincingly document that this is indeed the case (see Figure 1B).

To prove the crucial part played by PGC-1α in the metabolic regulation of ALAS-1 in vivo, Handschin et al. generated mice with a liver-specific deficiency in PGC-1α. As expected, fasting induced expression of ALAS-1 as well as PGC-1α in the liver of wild-type animals. In animals with liver-specific PGC-1α deficiency, however, PGC-1α expression was ablated and, importantly, the induction of ALAS-1 expression in the liver was blunted. A similar effect was observed for gluconeogenic genes that depend on PGC-1α for induction of their expression. The authors also examined the induction of ALAS-1 expression by the drug phenobarbital and found that the drug increased the production of liver *ALAS-1* mRNA to a similar extent in both wild-type and PGC-1α-deficient animals. As the effect of phenobarbital is mediated by the nuclear receptors PXR and CAR (Podvinec et al., 2004), this result suggests that these receptors do not require PGC-1α for activation of *ALAS-1*.

Having demonstrated a critical role of PGC-1α in fasting-induced expression of ALAS-1, the authors

probed its functional relevance in porphyria using a chemical model of this disease. Mice were treated with chemicals such as lead that inhibit specific enzymes of the heme biosynthetic pathway (see Figure 1A) resulting in "priming," a condition that mimics the partial loss of function in heme biosynthesis seen in genetic porphyria. Chemical treatment was combined with fasting to precipitate a "porphyria attack" in mice. In a gain-of-function experiment, overexpression of PGC-1α by virus-mediated transduction, in combination with fasting and chemical treatment, led to a dramatic increase in expression of ALAS-1. Plasma levels of the porphyrin precursors ALA and PBG were dramatically increased in these animals, to levels comparable to those seen in genetic mouse models of porphyria (Lindberg et al., 1996). Thus, ectopic expression of PGC-1α in conjunction with fasting and chemical treatment created a "porphyria attack" in otherwise normal animals. In contrast, wild-type animals subjected to the same fasting and chemical regimen but in the absence of ectopic PGC-1α exhibited a moderate increase in *ALAS-1* mRNA and in porphyrin precursors such as ALA. Remarkably, this moderate increase in ALAS-1 and in heme precursors was completely ablated in animals with liver-specific deletion of PGC-1α, suggesting that these animals are protected from the milder "porphyria-like" symptoms induced by the combined fasting and chemical treatment. Taken together, the gain- and loss-of-function experiments strongly indicate that PGC-1α is a key factor that mediates the expression of ALAS-1 during porphyria attacks.

Short of directly examining the effects of PGC-1α loss in a genetic mouse model of porphyria, the study by Handschin et al. nevertheless clearly indicates that PGC-1α is the culprit behind the precipitation of porphyria attacks by fasting. Thus, fasting induces expression in the liver of PGC-1α, which then acts as a powerful coactivator for the transcription factors NRF-1 and FOXO1, resulting in enhanced *ALAS-1* expression and an increased risk of a porphyria attack (see Figure 1B). Glucose administration, together with the resulting secondary increase in insulin, ameliorates this risk through two independent mechanisms: first, by directly antagonizing the induction of PGC-1α expression and second, by reducing the association between PGC-1α and FOXO1 (see Figure 1B).

Given that the power of the sweet treat for porphyria lies in the ability of glucose to tame PGC-1α, one could envisage alternative (less calorific) treatments that could target PGC-1α in the liver directly by, for example, modulating its expression, activity, or its interaction with relevant transcription factors. Although the development of such therapeutics lies in the future, the new study by Handschin et al. represents a vivid example of an initial endeavor in a seemingly unrelated research area (adipocyte metabolism) that has yielded therapeutic insights into a human disease (porphyria). No doubt, future studies will use mouse genetic models of porphyria (Lindberg et al., 1996) to directly examine the function of PGC-1α. It will also be intriguing to test whether loss of PGC-1α would sufficiently dampen the overall activity of the ALAS-1 promoter such that this loss might offer protection from attacks induced by drugs such as phenobarbital despite the fact that these drugs activate *ALAS-1* in a PGC-1α-independent manner.

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