# Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis[1–4]

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**ABSTRACT**

Mechanisms responsible for energy management in the cell and in the whole organism require a complex network of transcription factors and cofactors. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) has emerged as a master regulator of mitochondrial biogenesis and function, thus becoming a crucial metabolic node. We present an overview of the mechanisms by which PGC-1α is regulated, including the transcriptional regulation of PGC-1α expression and the fine-tuning of its final activity via posttranslational modifications. *Am J Clin Nutr* 2011;93 (suppl):884S–90S.

## INTRODUCTION

For optimal performance, all living systems must maintain a tight equilibrium between energy intake, storage, and expense. This equilibrium must be both robust and flexible to allow for adaptation to very different situations, such as exercise or rest and famine or feast. Organisms rely on finely tuned and complex signaling networks to confront all of these possibilities. In turn, dysfunction and perturbation of these networks can lead to metabolic imbalances, which if uncorrected induce diseases such as obesity or diabetes.

Metabolic equilibrium is maintained in the cell by an intricate regulatory circuitry, which is controlled to a large extent by transcriptional mechanisms (1, 2). These pathways imply many transcription factors that directly contact DNA and execute major changes in gene expression and transcriptional coregulators, which are responsible for the fine-tuning of the transcriptional response. Transcriptional coregulators are hence proposed to act as metabolic sensors, which translate changes in metabolism into alterations in gene expression, and it is suggested that aberrant signaling by these cofactors could contribute to the pathogenesis of common metabolic disorders (3). The role of coregulators in metabolic control is perhaps nowhere better shown than with peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (4), the master regulator of mitochondrial biogenesis and energy expenditure. Several metabolic functions have been attributed to PGC-1α. In brown adipose tissue (BAT), PGC-1α acts as a cold-inducible protein that controls adaptive thermogenesis. Fasting induces hepatic PGC-1α expression, thereby increasing gluconeogenesis, whereas in skeletal and cardiac muscle exercise increases PGC-1α–mediated mitochondrial biogenesis and respiration. Thus, PGC-1α expression seems finely tuned to reflect cellular energy needs, with conditions of increased energy demands inducing its expression. PGC-1α performs all these tasks by regulating the activity of a large number of transcription factors, including, among others, peroxisome proliferator-activated receptor (PPAR) γ (4), PPARα (5), estrogen receptor–related α (ERRα) (6), FoxO1 (7), hepatocyte nuclear factor 4α (HNF4α) (8), and nuclear respiratory factor 1 (NRF1) (9). By regulating the transcriptional activities of these proteins, PGC-1α modulates a number of genes involved in metabolic pathways as gluconeogenesis and fatty acid synthesis and oxidation or glycolysis.

An extensive discussion of the biological effects of PGC-1α is beyond the scope of this article, and we refer the interested reader to several excellent recent reviews on this subject (3, 10, 11). We focus here only on the recent developments concerning the regulation of both the expression and posttranslational modifications of this fascinating metabolic coregulator.

## REGULATION OF PGC-1α GENE EXPRESSION

PGC-1α expression levels are regulated in response to a plethora of stimuli, exemplifying the spectrum of situations to which mitochondrial biogenesis and activity must respond (**Figure 1**). The mechanisms governing the transcription of PGC-1α have been extensively studied and vary between distinct tissues and different situations, even though there is a common pattern of regulation emerging.

### *PGC-1α* expression in muscle after exercise: $Ca^{2+}$, p38 mitogen-activated protein kinase, AMP-actived protein kinase, and insulin

In muscle, PGC-1α expression is induced after exercise (Figure 1). There are several pathways involved in this process. First, it has been shown that PGC-1α expression responds to nerve stimulation–mediated calcium signaling through the activation of $Ca^{2+}$/calmodulin-dependent protein kinase IV

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[2] Presented at the symposium "Transcriptional Controls of Energy Sensing," held at Experimental Biology 2010, 25 April 2010.

[3] PJF-M is supported by a long-term fellowship of the Federation of the European Biochemical Societies. The work in the laboratory of the authors is supported by the Ecole Polytechnique Fédérale de Lausanne, the NIH (DK59820), the EU Ideas program (Sirtuins; ERC-2008-AdG-23118), the Swiss National Science Foundation (SNF 31003A-124713), and the Danone International Nutrition Award 2009.

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First published online February 2, 2011: doi: 10.3945/ajcn.110.001917.



Am J Clin Nutr 2011;93(suppl):884S–90S. Printed in USA. 

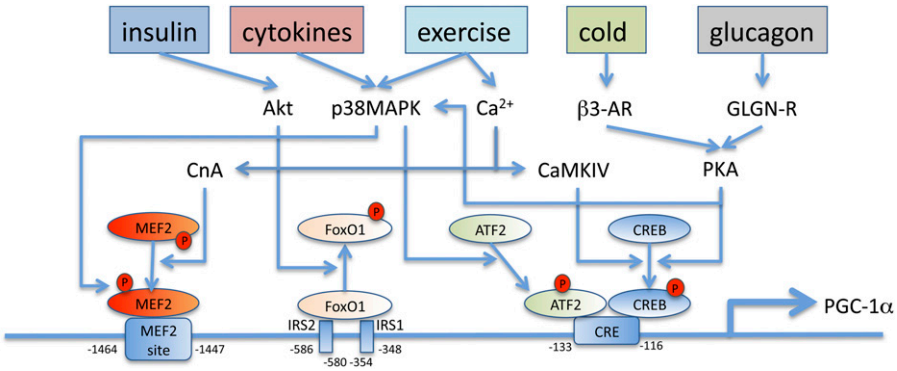


**FIGURE 1.** Regulation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) transcription. At the PGC-1α promoter, there are binding sites for transcription factors myocyte enhancer factor 2 (MEF2), forkhead box class-O (FoxO1), activating transcription factor 2 (ATF2), and cAMP response element–binding protein (CREB), all of which enhance PGC-1α transcription. These factors, in turn, are modulated by different signaling pathways: insulin activates Akt, which leads to cytoplasmic sequestration and inhibition of FoxO1; cytokines and exercise activate p38 mitogen-activated protein kinase (p38MAPK), which phosporylates and activates MEF2 and ATF2; exercise also stimulates $Ca^{2+}$ signaling, which, through calmodulin-dependent protein kinase IV (CaMKIV) and calcineurin A (CnA), will induce CREB and MEF2-mediated PGC-1α transcription; and cold activates $\beta_3$-adrenergic receptors ($\beta$3-AR) in muscle and brown fat, leading to protein kinase A (PKA)–mediated activation of CREB. IRS, insulin response sequence; GLGN-R, glucagon receptor; P, phosphate; CRE, cAMP response element.

(CaMKIV) and calcineurin A (CnA; *see* Figure 1). CnA has been shown to interact and activate the myogenic transcription factors myocyte enhancer factor 2C (MEF2C) and MEF2D, which subsequently drive PGC-1α transcription (12). On the other hand, one of the targets of CaMKIV is the transcription factor cAMP response element (CRE)-binding protein (CREB). The *PGC-1α* gene possesses a binding site for CREB, which is well conserved between humans (spanning the region −133 to −116 in PGC-1α promoter) and mice (region −146 to −129) and renders PGC-1α transcription highly reactive to CREB activation (12, 13). $Ca^{2+}$-induced CaMKIV phosporylation and activation of CREB enhances PGC-1α expression in a CREB-dependent manner (12, 14). In confirmation of these observations, in vivo imaging experiments show a nerve stimulation–mediated induction of PGC-1α mRNA expression that is CREB and MEF2 dependent (15). Interestingly, MEF2C and MEF2D are also well-known targets of PGC-1α (16, 17), thereby creating an autoregulatory feed forward loop by which PGC-1α increases its own expression.

Another mechanism regulating PGC-1α expression in muscle after exercise involves the activation of the p38 mitogen-activated protein kinase (p38 MAPK). p38 MAPK can activate both MEF2 (18) and activating transcription factor 2 (ATF2). In muscle, p38 MAPK activation during exercise or by overexpression increases the expression of PGC-1α, and this induction is dependent on the transcription factor ATF2 (20).

AMP-activated protein kinase (AMPK) is a crucial sensor of the energy status of the cell, becoming activated when the AMP/ATP ratio is high and triggering a wide range of catabolic pathways directed to increase cellular levels of ATP. It is logical that AMPK is activated in muscle during exercise (21). One of the catabolic pathways enhanced by activated AMPK is mitochondrial biogenesis and function, and PGC-1α activity has been shown to be necessary for AMPK-mediated mitochondrial activation (22). AMPK can be specifically activated by using the drug 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR). Treating muscle cells with AICAR leads to an increase in PGC-1α transcription (21, 23). However, the mechanisms by which AMPK activation increases the transcription of PGC-1α have not yet been clarified.

Finally, PGC-1α amounts in muscle seem to be also regulated by insulin signaling, although the mechanisms taking place are complex and have not yet been fully elucidated. Ling et al (24) concluded that insulin increases PGC-1α transcription. However, insulin signaling mediates energy storage and diminishes catabolic pathways, suggesting that insulin could also decrease PGC-1α activity. Supporting this view, forkhead box class-O (FoxO1) has been shown to bind and stimulate the PGC-1α promoter in muscle. In this scenario, insulin inhibits PGC-1α transcription by activating Akt, which phosphorylates and inhibits FoxO1 (25).

### *PGC-1α* expression regulation by temperature: PKA-CREB and p38 MAPK-ATF2

PGC-1α was originally found to be activated in brown fat and muscle cells on cold exposure (*see* Figure 1). Cold temperatures are sensed by the sympathetic nervous system through the β3-adrenergic receptor (β3-AR) (4, 26, 27), leading to enhanced cAMP signaling and the activation of protein kinase A (PKA). One of the best-characterized PKA targets is CREB. As expected, the cAMP-stimulating compound forskolin enhances PGC-1α expression, and a dominant negative mutant of CREB blocks this activation (12). The activation of β3-AR and cAMP/PKA in brown fat also stimulates p38 MAPK, subsequently inducing PGC-1α transcription through ATF2 in a fashion reminiscent of muscle contraction (19).

### *PGC-1α* expression in liver

In liver, PGC-1α expression is increased during fasting in response to glucagon, a pancreatic hormone that induces cAMP and CREB (13). PGC-1α then leads to an induction of the expression of several gluconeogenic enzymes via its association with several transcription factors, such as HNF4-α (28) or FoxO1 (7). As in muscle and brown fat, p38 MAPK activation in

liver by the fasting-glucagon-cAMP-PKA axis was also shown to increase PGC-1α transcription and induce gluconeogenesis (29). In addition, p38 MAPK has also been shown to be necessary for free fatty acid–mediated activation of PGC-1α expression in liver, which in turn enhances gluconeogenic genes (30).

### Other mechanisms regulating *PGC-1α* expression

PGC-1α expression has also been shown to be regulated by nitric oxide in brown fat and endothelial cells via the generation of cyclic GMP (cGMP) by guanylate cyclase (31–33). However, the precise mechanisms for this increase in PGC-1α expression have not yet been defined.

Recently, a link has been established between elevated fatty acids in muscle and DNA methyltransferase 3B (DNMT3B)–mediated methylation of PGC-1α promoter, leading to its repression with a subsequent reduction in mitochondrial biogenesis (34). Interestingly, PGC-1α promoter methylation in skeletal muscle was found to be more prevalent in patients with diabetes compared with healthy subjects. This novel way of regulation of the *PGC-1α* gene leads to longer-lasting changes of PGC-1α transcription with a potential relevance for the pathophysiology of diabetes.

From all of the described mechanisms, a clear common pattern of PGC-1α regulation emerges, linking increases of PGC-1α expression to situations of energy stress, such as exercise, fasting, or cold exposure. Molecularly, this response is transduced by a discrete list of transcription factors, including FoxO1, ATF2, MEF2, and CREB, which respond to physiologic stimuli in a tissue-specific manner; every factor will have a different relative importance depending on the specific situation and cell type (Figure 1), providing fine adjustments to meet the needs of each particular tissue over a wide range of conditions.

### Regulation of *PGC-1α* activity by posttranslational modifications

Posttranslational mechanisms, as equally important as the transcriptional mechanisms, also extensively regulate PGC-1α. To date, phosphorylation, ubiquitination, methylation, acetylation, and GlcNAcylation or PGC-1α have all been described (**Figure 2**).

### Regulation of *PGC-1α* by phosphorylation

PGC-1α activity has long been known to be regulated by phosphorylation. AMPK, p38 MAPK, and Akt are the best-characterized protein kinases known to target PGC-1α. AMPK enhances mitochondrial biogenesis not only by inducing PGC-1α transcription (*see* previous text) but also through activation of PGC-1α by phosphorylating threonine-177 and serine-538, thereby enhancing the co-transcriptional activity of PGC-1α (22). Insulin reduces *PGC-1α* gene expression (25); it also induces phosphorylation of PGC-1α at serine-570 through the action of Akt and thereby inhibits its activity (35). Recently, another mechanism for PGC-1α regulation through the insulin/Akt axis was reported, by which the feeding/insulin/Akt pathway resulted in phosphorylation and stabilization of the protein

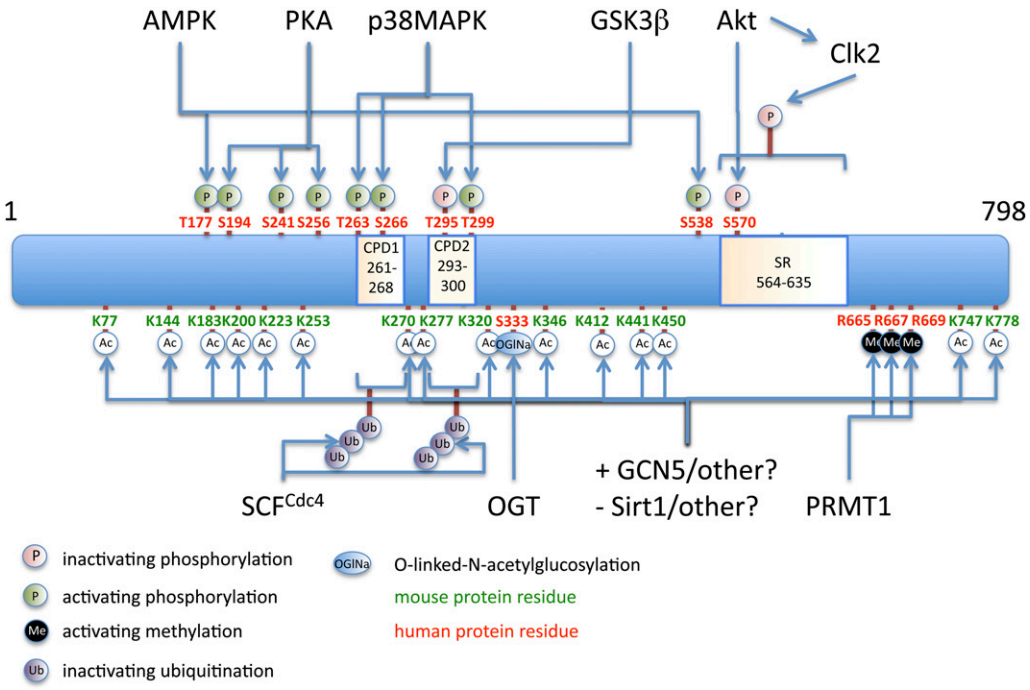


**FIGURE 2.** Posttranslational modifications of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α). Numerous modifications have been described to affect PGC-1α, modulating its levels and activity through phosphorylation, acetylation, methylation, ubiquitination, and *O*-linked *N*-acetylglucosylation. The respective residues or regions where these modifications occur are indicated. Certain modification sites are mapped in the mouse or the human PGC-1α protein (as indicated in the key). AMPK, AMP-activated protein kinase; PKA, protein kinase A; p38MAPK, p38 mitogen-activated protein kinase; GSK3*β*, glycogen synthase kinase 3*β*; $SCF^{Cdc4}$, Skp1/Cullin/F-box cell division control 4; OGT, *O*-linked *N*-acetylglucosamine transferase; Clk2, Cdc2-like kinase 2; Ac, acetylation; CPD, Cdc4 phosphodegron; SR, serine-arginine domain; GCN5, general control of amino acid synthesis 5; Sirt1, silence information regulator 2-like 1; PRMT1, protein arginine methyltransferase 1.

kinase Clk2. In turn, Clk2 phosphorylates PGC-1α in its serine- and arginine-rich region (SR; *see* Figure 2), reducing its co-transcriptional activity (36) specifically toward FoxO1, thereby blunting gluconeogenesis. In effect, Clk2 acts as a "long-acting" insulin-induced gluconeogenic repressor that sustains the inhibition of glucose production during refeeding once Akt is already down-regulated.

p38 MAPK, another kinase that phosphorylates and activates PGC-1α (37), phosphorylates threonine-262, serine-265, and threonine-298 in response to cytokine stimulation in muscle cells (Figure 2). This pathway is thought to mediate some of the effects of cachexia. p38 MAPK phosphorylation enhances PGC-1α activity by at least 2 mechanims. First, p38 MAPK phosphorylation of PGC-1α enhances its stability (37); because PGC-1α's half life is relatively short (2 or 3 h), modulation of its stability constitutes an important mechanism of control of PGC-1α activity (38). p38 MAPK also disrupts the inactivating interaction between PGC-1α and the co-repressor p160MBP in myoblasts, resulting in enhanced PGC-1α transactivation capacity (39). It is interesting to note that p38 MAPK becomes activated in muscle after exercise (20), but, to our knowledge, no reports have been published on the eventual phosphorylation of PGC-1α by p38 MAPK during exercise in this tissue, which would be a fruitful subject for future research. In liver, p38 MAPK can also be activated by FFAs, as discussed above. Besides increasing PGC-1α transcription, activated p38 MAPK also phosphorylates and enhances PGC-1α activity (30).

PGC-1α is also phosphorylated by glycogen synthase kinase 3$\beta$ (GSK3$\beta$) (40), which inhibits PGC-1α by enhancing its proteasomal degradation in the nucleus during oxidative stress. The physiologic function of this inhibitory phosphorylation is not yet clear. Because silence information regulator 2-like 1 (Sirt1) and GSK3$\beta$ are both activated by oxidative stress, GSK3$\beta$ inhibitory phosphorylation is claimed to limit Sirt1-mediated activation of PGC-1α after acute stress. A sustained stress signal would, however, replenish PGC-1α nuclear amounts through increased PGC-1α transcription and cytoplasmic translation, thereby overcoming GSK3$\beta$-mediated degradation of nuclear PGC-1α.

Very recently, a splicing isoform of PGC-1α [called NT-PGC-1α because it lacks the C-terminus of the protein and the nuclear localization signal (NLS) residing in this region that keeps the full-length PGC-1α in the nucleus], has also been reported to be phosphorylated by PKA, a kinase also involved in the regulation of PGC-1α transcription (41). This splicing variant is more stable than the classical isoform, and its activity is thought to rely more on nuclear import and export than on protein stability. PKA phosphoryates NT-PGC-1α at serine-194, serine-241, and threonine-256 (Figure 2); and this phosphorylation blocks the nuclear export of PGC-1α by inhibiting its binding to the nuclear exporter CRM1 and enhances its nuclear accumulation, thereby increasing NT-PGC-1α–mediated transcription.

### Regulation of *PGC-1* by acetylation

PGC-1α is heavily acetylated by the acetyltransferase GCN5, whereas it is deacetylated by the deacetylase Sirt1. Both proteins are exquisite sensors of the energy status of the cell (**Figure 3**). Acetylated residues on PGC-1α span the whole sequence of the protein (42), which makes it difficult to ascribe this posttranslational modification to one specific structural or functional pattern.

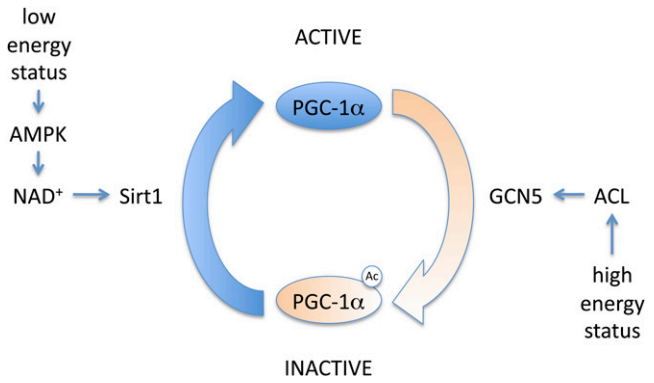


**FIGURE 3.** Cellular sensing of energy status through acetylation of peroxisome proliferator-activated receptor $\gamma$ coactivator 1α (PGC-1α). In situations of low energy status, AMP-activated protein kinase (AMPK)–increased $NAD^+$ amounts enhance Sirt1 activity and lead to the activating deacetylation of PGC-1α and increased mitochondrial biogenesis and function. When energy is abundant in the cell, GCN5 (general control of amino acid synthesis) acetylates and inhibits PGC-1α; the acetyl-CoA necessary for this reaction is provided by ATP-citrate lyase (ACL), which acts as a rate-liming factor for GCN5-mediated acetylation of PGC-1α. Ac, acetylation.

Sirt1 is one of the mammalian homolog of yeast silent information regulator 2 (Sir2), a protein responsible for transcription silencing (43) and linked to lifespan extension in yeast (44), worms (45), and flies (46). In mice, Sirt1 activation or moderate overexpression leads to healthier aging and protects against cancer induction (47–49), which makes Sirt1 an attractive drug target. Because Sir2 and its mammalian homologs Sirt1–7 require the coenzyme nicotinamide adenine dinucleotide ($NAD^+$) as a substrate, it is generally understood that $NAD^+$, NADH, or the ratio $NAD^+$/NADH modulates Sirt1 activity, linking it to the energy status of the cell (47). In line with this, Sirt1 activity generally increases on fasting, exercise, or redox stress, as a response aimed at increasing the energy loads of the cell to overcome these situations. PGC-1α deacetylation by Sirt1 increases the co-activation of its target transcription factors. Because Sirt1 is most active in times of energy demand, when $NAD^+$ amounts or the $NAD^+$/NADH ratio are at their highest levels, Sirt1-mediated deacetylation and activation of PGC-1α becomes an important response of the cell to increase mitochondrial metabolism when energy is needed (Figure 3). In skeletal muscle, Sirt1-mediated deacetylation of PGC-1α leads to its activation and an increase in the transcription of its targets (48, 49). In mouse liver, PGC-1α–mediated gluconeogenic genes are down-regulated after Sirt1 knockdown and up-regulated when Sirt1 is overexpressed (42, 50), which also reflects a Sirt1-mediated regulation of PGC-1α activity in this tissue.

Whereas AMPK induces PGC-1α expression and directly enhances its activity though phosphorylation (*see* previously in text), there is yet another, complementary, and converging mechanism by which AMPK regulates PGC-1α activity, albeit indirectly. AMPK increases $NAD^+$ amounts by increasing fatty acid oxidation (48) or possibly by enhancing its biogenesis through Nampt (51), thereby enhancing Sirt1 activity and inducing PGC-1α deacetylation and activation (48). It is interesting to note that the phosphorylation of PGC-1α by AMPK

is required for the subsequent Sirt1-mediated deacetylation, perhaps suggesting that these 2 converging posttranslational modifications increase specificity (48). These multiple mechanisms by which AMPK regulates PGC-1α reinforce the notion of the vital role of PGC-1α for AMPK function and the response to energy stress situations (Figure 3).

PGC-1α interacts with several acetyl transferases, including p300, SRC-1, SRC-3, and GCN5 (52–54). However, only the histone acetyltransferase GCN5 has been shown to acetylate and inhibit PGC-1α (and PGC-1β) activity in vitro and in vivo (53, 55) (it cannot be excluded that these or other acetyltransferases can acetylate PGC-1α in certain conditions). Interestingly, GCN5 activity is enhanced by SRC-3, another cofactor with acetyltransferase activity. Like GCN5 and Sirt1, SRC-3 amounts are sensitive to the energy status of the cell. The expression of both GCN5 and SRC-3 are induced by caloric excess, whereas Sirt1 amounts are reduced, leading to a decrease in PGC-1α activity by enhancing its GCN5-mediated acetylation (Figure 3). The opposite occurs with calorie restriction, when GCN5 and SRC3 expression are reduced and that of Sirt1 is induced, thereby favoring PGC-1α deacetylation and activity (52).

Another potential mechanism of regulation of PGC-1α acetylation status by GCN5 could be the control of the amounts of nuclear acetyl-CoA in the cell. A recent report suggests that, in mammals, the nuclear amounts of acetyl-CoA are controlled by the enzyme ATP-citrate lyase (ACL), which generates acetyl-CoA from tricarboxylic acid–derived citrate both in the cytoplasm and in the nucleus (56). Accordingly, knockdown of ACL blocks histone acetylation in a wide set of cell types in vitro, a mechanism highly dependent on nuclear acetyl-CoA. Interestingly, the effects on histone acetylation after silencing of ACL were very similar to those observed after silencing GCN5, suggesting that GCN5 is the main acetyltransferase responsible for ACL-induced histone acetylation (56). Therefore, it is tempting to speculate (although it has not yet been proven) that not only histone acetylation but also PGC-1α acetylation could be gated by nuclear acetyl-CoA amounts (Figure 3).

All of these data suggest that the proteins responsible for the reversible acetylation/deacetylation of PGC-1α—ie, Sirt1 and GCN5—act as robust and flexible sensors of the cellular energy status and direct an appropriate mitochondrial adaptation via the regulation of PGC-1α activity.

### Regulation by ubiquitination

*PGC-1α* amounts can also be regulated by proteasomal degradation. p38 MAPK phosphorylation of PGC-1α increases the stability of PGC-1α (37), although the exact mechanism for the stabilization of PGC-1α was not investigated at the time. GSK-3β phosphorylation of PGC-1α leads to its proteasomal degradation in the nucleus (40). Recently, Skp1/Cullin/F-box-cell division control 4 ($SCF^{Cdc4}$) was identified as an E3 ubiquitin ligase that regulates PGC-1α through ubiquitin-mediated proteolysis (57). Curiously, phosphorylation by p38 MAPK and GSK-3β was found to enhance $SCF^{Cdc4}$ binding to PGC-1α, targeting it for proteasomal degradation. Whereas this mechanism fits with that described for GSK-3β regulation of PGC-1α, it contrasts at first sight with the previously shown p38 MAPK–mediated enhancement of PGC-1α transcriptional expression and activity. This apparent contradiction awaits further research.

### Other posttranslational modifications on *PGC-1α*

PGC-1α is methylated by the protein arginine methyltransferase 1 (PRMT1) at arginine 665, 667, and 669, which strongly enhances PGC-1α–mediated transcription (58). PGC-1α is also a target for *O*-linked *N*-acetylglucosamine (O-GlcNAc) transferase (OGT), which transfers the O-GlcNAc group to serine-333 of PGC-1α (59); interestingly, this residue lies within the regulatory domain where p160MBP binds (39). However, the physiologic relevance of these 2 posttranslational modifications is yet unclear.

## DISCUSSION AND PERSPECTIVES

The understanding of the transcriptional regulation of diverse metabolic pathways during the last decades has contributed to the elucidation of mechanisms of metabolic control and to a better knowledge of the pathogenesis of metabolic diseases. Clearly, transcriptional regulation not only involves direct regulation through transcription factors that bind DNA but also a more discrete fine-tuning process executed by numerous transcriptional coregulators. The discovery of PGC-1α and the pleiotropic and robust effects it has on metabolic homeostasis unveiled how the PGC-1α cofactor network is central to the regulation of mitochondrial biogenesis and function, thereby having an effect on whole-body energy expenditure.

Dysfunction of these pathways through abnormal PGC-1α activity has a profound effect on general metabolism and, if uncorrected, could predispose and contribute to metabolic diseases such as obesity, the metabolic syndrome, and type 2 diabetes. PGC-1α activity is modulated both through the regulation of its expression and through the regulation of its activity by a panoply of posttranslational modifications, such as phosphorylation, acetylation, and ubiquitination, which enable it to fine-tune the activity of several transcription factors and the downstream pathways that they control.

From this analysis, a surprising redundancy in the signaling pathways regulating PGC-1α has become evident. In fact, most signaling pathways regulating PGC-1α, such as AMPK, PKA, Akt, or p38 MAPK, act both through modulation of PGC-1α transcription and by posttranslational modification of the PGC-1α protein. This poses the interesting question of the physiologic sense of these redundant regulatory pathways. It is quite tempting to infer that posttranslational modifications constitute the first, fast response to an acute stimulus, whereas the modulation of PGC-1α amounts would represent a slower adaptive response to more prolonged signals. Such a double regulation at the level of expression and protein activity will furthermore entail a more robust control of overall PGC-1α action.

Posttranslational modifications of PGC-1α affect different regions of the protein, allowing for a quick, multifaceted, and flexible regulation of PGC-1α activity (Figure 2). Some of these modifications are clearly associated with a precise structural or functional mechanism; for example, phosphorylation at residues threonine-262, serine-265, and threonine-298 target precisely the binding site of the inhibitor p160MBP (39). Another example is the inhibition of the binding of PGC-1α to CRM1 and the subsequent nuclear export through phosphorylation by PKA at residues serine-194, serine-241, and threonine-256 of the splicing isoform NT-PGC-1a. However, other posttranslational

modifications on PGC-1α cannot yet be clearly linked to a precise structural mechanism: an example would be PGC-1α acetylation, which affects lysine residues spread throughout the protein sequence. In this case, however, the functional consequences of the modification have been clearly defined.

Several of the pathways that regulate PGC-1α interact functionally. This can be the case for pathways that induce posttranslational modifications of PGC-1α. The deacetylation of PGC-1α by Sirt1 on AMPK activation, which also requires PGC-1α phosphorylation by AMPK, provides evidence that these posttranslational modifications act convergently (48). It is also interesting to note that all of these mechanisms of regulation of PGC-1α activity will also affect its own expression through an autoregulatory feed forward loop (12), thereby leading to a prolongation of the acute inducing signal through a long-term increase in PGC-1α expression.

Combined with the regulation of expression, the elevated number of posttranslational modifications on PGC-1α allow for a powerful and flexible system of regulation. These modifications can occur in concert or in a mutually exclusive manner. Some modifications will influence the ability of others to take place or to affect PGC-1α function. The presence of a specific pattern of posttranslational modifications on PGC-1α protein can direct PGC-1α toward a precise set of transcriptional targets as a reaction to energy needs and tissue-specific conditions that drive these posttranscriptional modifications. This incredibly complicated network of transcriptional and posttranslational modifications that determine PGC-1α activity has only started to emerge and will be a rich field for further study in the years to come. Note that a large number of other transcriptional cofactors have context-specific activities that converge on PGC-1α as a nodal point. This is notoriously the case for the ligand-dependent nuclear receptor corepressor RIP140 in white adipose tissue (60), CRTC2 (61) in the control of hepatic gluconeogenesis, and PRDM16 (62) and the retinoblastoma protein during muscle/BAT development (63). Hence, all of these signaling pathways and transcription cofactors constitute an intricate cofactor network that enables PGC-1α to fine-tune mitochondrial metabolism and guarantee metabolic flexibility.

We thank Richardus Houtkooper for valuable suggestions that improved the manuscript. We apologize to those authors whose original work could not be cited due to space limitations.

The authors' responsibilities were as follows—JA and PJF-M: mined the published literature to provide the structure for the original thesis in the manuscript and wrote, read, and edited the text including references, figures, and tables. The authors declared no conflicts of interest.

## REFERENCES

1. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. Science 2001;294:1866–70.
2. Francis GA, Fayard E, Picard F, Auwerx J. Nuclear receptors and the control of metabolism. Annu Rev Physiol 2003;65:261–311.
3. Spiegelman BM, Heinrich R. Biological control through regulated transcriptional coactivators. Cell 2004;119:157–67.
4. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998;92:829–39.
5. Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 2000;20:1868–76.
6. Huss JM, Kopp RP, Kelly DP. Peroxisome proliferator-activated receptor coactivator-1alpha (PGC-1alpha) coactivates the cardiac-enriched nuclear receptors estrogen-related receptor-alpha and –gamma: identification of novel leucine-rich interaction motif within PGC-1alpha. J Biol Chem 2002;277:40265–74.
7. Puigserver P, Rhee J, Donovan J, et al. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. Nature 2003;423:550–5.
8. Yoon JC, Puigserver P, Chen G, et al. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 2001;413:131–8.
9. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98:115–24.
10. Puigserver P. Tissue-specific regulation of metabolic pathways through the transcriptional coactivator PGC1-alpha. Int J Obes (Lond) 2005;29 (suppl 1):S5–9.
11. Canto C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 2009;20:98–105.
12. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proc Natl Acad Sci USA 2003;100:7111–6.
13. Herzig S, Long F, Jhala US, et al. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 2001;413:179–83.
14. Wu H, Kanatous SB, Thurmond FA, et al. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. Science 2002;296:349–52.
15. Akimoto T, Sorg BS, Yan Z. Real-time imaging of peroxisome proliferator-activated receptor-gamma coactivator-1alpha promoter activity in skeletal muscles of living mice. Am J Physiol Cell Physiol 2004;287:C790–6.
16. Michael LF, Wu Z, Cheatham RB, et al. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proc Natl Acad Sci USA 2001;98:3820–5.
17. Lin J, Wu H, Tarr PT, et al. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 2002;418:797–801.
18. Zhao M, New L, Kravchenko VV, et al. Regulation of the MEF2 family of transcription factors by p38. Mol Cell Biol 1999;19:21–30.
19. Cao W, Daniel KW, Robidoux J, et al. p38 Mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. Mol Cell Biol 2004;24:3057–67.
20. Akimoto T, Pohnert SC, Li P, et al. Exercise stimulates PGC-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J Biol Chem 2005;280:19587–93.
21. Jorgensen SB, Wojtaszewski JF, Viollet B, et al. Effects of alpha-AMPK knockout on exercise-induced gene activation in mouse skeletal muscle. FASEB J 2005;19:1146–8.
22. Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci USA 2007;104:12017–22.
23. Suwa M, Nakano H, Kumagai S. Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. J Appl Physiol 2003;95:960–8.
24. Ling C, Poulsen P, Carlsson E, et al. Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins. J Clin Invest 2004;114:1518–26.
25. Southgate RJ, Bruce CR, Carey AL, et al. PGC-1alpha gene expression is down-regulated by Akt- mediated phosphorylation and nuclear exclusion of FoxO1 in insulin-stimulated skeletal muscle. FASEB J 2005;19:2072–4.
26. Boss O, Bachman E, Vidal-Puig A, Zhang CY, Peroni O, Lowell BB. Role of the beta(3)-adrenergic receptor and/or a putative beta(4)-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor-gamma coactivator-1. Biochem Biophys Res Commun 1999;261:870–6.
27. Gomez-Ambrosi J, Fruhbeck G, Martinez JA. Rapid in vivo PGC-1 mRNA upregulation in brown adipose tissue of Wistar rats by a beta (3)-adrenergic agonist and lack of effect of leptin. Mol Cell Endocrinol 2001;176:85–90.

28. Rhee J, Inoue Y, Yoon JC, et al. Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. Proc Natl Acad Sci USA 2003;100:4012–7.
29. Cao W, Collins QF, Becker TC, et al. p38 Mitogen-activated protein kinase plays a stimulatory role in hepatic gluconeogenesis. J Biol Chem 2005;280:42731–7.
30. Collins QF, Xiong Y, Lupo EG Jr, Liu HY, Cao W. p38 Mitogen-activated protein kinase mediates free fatty acid-induced gluconeogenesis in hepatocytes. J Biol Chem 2006;281:24336–44.
31. Nisoli E, Falcone S, Tonello C, et al. Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. Proc Natl Acad Sci USA 2004;101:16507–12.
32. Nisoli E, Clementi E, Paolucci C, et al. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science 2003;299: 896–9.
33. Borniquel S, Valle I, Cadenas S, Lamas S, Monsalve M. Nitric oxide regulates mitochondrial oxidative stress protection via the transcriptional coactivator PGC-1alpha. FASEB J 2006;20:1889–91.
34. Barres R, Osler ME, Yan J, et al. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab 2009;10:189–98.
35. Li X, Monks B, Ge Q, Birnbaum MJ. Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature 2007;447:1012–6.
36. Rodgers JT, Haas W, Gygi SP, Puigserver P. Cdc2-like kinase 2 is an insulin-regulated suppressor of hepatic gluconeogenesis. Cell Metab 2010;11:23–34.
37. Puigserver P, Rhee J, Lin J, et al. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. Mol Cell 2001;8:971–82.
38. Sano M, Tokudome S, Shimizu N, et al. Intramolecular control of protein stability, subnuclear compartmentalization, and coactivator function of peroxisome proliferator-activated receptor gamma coactivator 1alpha. J Biol Chem 2007;282:25970–80.
39. Fan M, Rhee J, St-Pierre J, et al. Suppression of mitochondrial respiration through recruitment of p160 myb binding protein to PGC-1alpha: modulation by p38 MAPK. Genes Dev 2004;18:278–89.
40. Anderson RM, Barger JL, Edwards MG, et al. Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell 2008;7: 101–11.
41. Chang JS, Huypens P, Zhang Y, Black C, Kralli A, Gettys TW. Regulation of NT-PGC-1α subcellular localization and function by PKA-dependent modulation of nuclear export by CRM1. J Biol Chem 2010;285:18039–50.
42. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 2005;434:113–8.
43. Shore D, Squire M, Nasmyth KA. Characterization of two genes required for the position-effect control of yeast mating-type genes. EMBO J 1984;3:2817–23.
44. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 1999;13:2570–80.
45. Tissenbaum HA, Guarente L. Increased dosage of a Sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 2001;410:227–30.
46. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci USA 2004; 101:15998–6003.
47. Houtkooper RH, Canto C, Wanders RJ, Auwerx J. The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. Endocr Rev 2010;31:194–223.
48. Canto C, Gerhart-Hines Z, Feige JN, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 2009;458:1056–60.
49. Gerhart-Hines Z, Rodgers JT, Bare O, et al. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J 2007;26:1913–23.
50. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. Proc Natl Acad Sci USA 2007;104:12861–6.
51. Fulco M, Cen Y, Zhao P, et al. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell 2008;14:661–73.
52. Coste A, Louet JF, Lagouge M, et al. The genetic ablation of SRC-3 protects against obesity and improves insulin sensitivity by reducing the acetylation of PGC-1α. Proc Natl Acad Sci USA 2008;105: 17187–92.
53. Lerin C, Rodgers JT, Kalume DE, Kim SH, Pandey A, Puigserver P. GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1alpha. Cell Metab 2006; 3:429–38.
54. Puigserver P, Adelmant G, Wu Z, et al. Activation of PPARgamma coactivator-1 through transcription factor docking. Science 1999;286: 1368–71.
55. Kelly TJ, Lerin C, Haas W, Gygi SP, Puigserver P. GCN5-mediated transcriptional control of the metabolic coactivator PGC-1beta through lysine acetylation. J Biol Chem 2009;284:19945–52.
56. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 2009;324:1076–80.
57. Olson BL, Hock MB, Ekholm-Reed S, et al. SCFCdc4 acts antagonistically to the PGC-1alpha transcriptional coactivator by targeting it for ubiquitin-mediated proteolysis. Genes Dev 2008;22:252–64.
58. Teyssier C, Ma H, Emter R, Kralli A, Stallcup MR. Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation. Genes Dev 2005;19:1466–73.
59. Housley MP, Udeshi ND, Rodgers JT, et al. A PGC-1alpha-O-GlcNAc transferase complex regulates FoxO transcription factor activity in response to glucose. J Biol Chem 2009;284:5148–57.
60. Hallberg M, Morganstein DL, Kiskinis E, et al. A functional interaction between RIP140 and PGC-1alpha regulates the expression of the lipid droplet protein CIDEA. Mol Cell Biol 2008;28:6785–95.
61. Wu Z, Huang X, Feng Y, et al. Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1alpha transcription and mitochondrial biogenesis in muscle cells. Proc Natl Acad Sci USA 2006;103: 14379–84.
62. Kajimura S, Seale P, Tomaru T, et al. Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. Genes Dev 2008;22:1397–409.
63. Scime A, Grenier G, Huh MS, et al. Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha. Cell Metab 2005;2:283–95.