Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis

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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 post translational modifications. Am J Clin Nutr doi: 10.3945/ajcn.110.001917.

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 changes in gene expression and transcriptional coregulators, transcription factors that directly contact DNA and execute major transcriptional mechanisms (1, 2). These pathways imply many regulatory circuitry, which is controlled to a large extent by transcriptional mechanisms (1, 2).

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: Ca2+, p38 mitogen-activated protein kinase, AMP-activated 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 Ca2+/calmodulin-dependent protein kinase IV

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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, 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. Molecules, 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; GSK3β, glycogen synthase kinase 3β; SCFCdc4, 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 aminoacid synthesis 5; Sirt1, silence information regulator 2-like 1; PRMT1, protein arginine methyltransferase 1.](image-url)
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 mechanisms. 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 3β (GSK3β) (40), which inhibits PGC-1α by enhancing its proteosomal 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 (Sir2) and GSK3β are both activated by oxidative stress, GSK3β inhibitory phosphorylation is claimed to limit Sir2-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 GSK3β-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 phosphorylates 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 Sir1. 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. Sir1 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, Sir1 activation or moderate overexpression leads to healthier aging and protects against cancer induction (47–49), which makes Sir1 an attractive drug target. Because Sir2 and its mammalian homologs Sir1–7 require the coenzyme nicotinamide adenine dinucleotide (NAD+), as a substrate, it is generally understood that NAD+, NADH, or the ratio NAD+/NADH modulates Sir1 activity, linking it to the energy status of the cell (47). In line with this, Sir1 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 Sir1 increases the co-activation of its target transcription factors. Because Sir1 is most active in times of energy demand, when NAD+ amounts or the NAD+/NADH ratio are at their highest levels, Sir1-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, Sir1-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 Sir1 knockdown and up-regulated when Sir1 is overexpressed (42, 50), which also reflects a Sir1-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 Sir1 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 Sir1-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 acetyltransferases, 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 is 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—in, 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<sup>Calc</sup>) 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<sup>Calc</sup> 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-1α. 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 post-translational 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 post-translational 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), CRCT2 (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.

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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.

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