The hitchhiker's guide to PGC-1 α isoform structure and biological functions

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Abstract Proteins of the peroxisome proliferator-activated receptor γ (PPAR γ) coactivator 1 (PGC-1) family of transcriptional coactivators coordinate physiological adaptations in many tissues, usually in response to demands for higher nutrient and energy supply. Of the founding members of the family, *PGC-1 α* (also known as *PPARGC1A*) is the most highly regulated gene, using multiple promoters and alternative splicing to produce a growing number of coactivator variants. *PGC-1 α* promoters are selectively active in distinct tissues in response to specific stimuli. To date, more than ten novel PGC-1 α isoforms have been reported to be expressed from a novel promoter (PGC-1 α -b, PGC-1 α -c), to undergo alternative splicing (NT-PGC-1 α) or both (PGC-1 α 2, PGC-1 α 3, PGC-1 α 4). The resulting proteins display differential regulation and tissue distribution and, most importantly, exert specific biological functions. In this review we discuss the structural and functional characteristics of the novel PGC-1 α isoforms, aiming to provide an integrative view of this constantly expanding system of transcriptional coactivators.

Keywords Alternative promoter · Alternative splicing · Brown adipose tissue · Hypertrophy · Isoforms · NT-PGC-1 α · PGC-1 α · PGC-1 α -b · PGC-1 α 4 · Review · Skeletal muscle

Abbreviations

Aa	Amino acid
AMPK	5' AMP-activated protein kinase
BAT	Brown adipose tissue
ERR	Oestrogen-related receptor
NLS	Nuclear localisation signal
PGC-1 α	PPAR γ coactivator 1 α
PPAR γ	Peroxisome proliferator-activated receptor γ
PRC	PGC-1-related coactivator
RRM	RNA recognition motif
RS	Arginine–serine-rich
SIRT	Sirtuin
TSS	Transcription start site

Introduction

It is now more than 15 years since the cloning of the transcriptional coactivator known as peroxisome proliferator-activated receptor γ (PPAR γ) coactivator-1 α (PGC-1 α) [1]. Since then, PGC-1 α and its homologues, PGC-1 β and PGC-1-related coactivator (PRC), have been the subject of intense research in a constantly growing number of fields, such as energy metabolism, cardiovascular disease, skeletal muscle physiology, neurodegenerative diseases and even mood disorders. Numerous studies have investigated the roles of PGC-1 coactivators in different physiological and disease settings, and brought to light several mechanisms by which these transcriptional regulators contribute to cellular adaptation to diverse challenges. At the same time, this research has uncovered new levels of complexity in transcriptional regulation mediated by PGC-1 coactivators. Of the three founding members of the family, PGC-1 α has received the most attention, partly because of the many and highly

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genes express appreciable levels of at least two populations of distinct mRNAs [5]. The lack of consensus in nomenclature has populated scientific literature with a growing constellation of PGC-1 α variants. For clarity, we use *PGC-1 α /Pgc-1 α* to refer to the human/rodent gene, PGC-1 α when discussing the collective of α proteins, and PGC-1 α 1 to specify the original protein ([1]; Fig. 1). Here we discuss current advances in our understanding of PGC-1 α -related biology, with particular focus on its variants, their structure and biological functions, to give an integrated view of the PGC-1 α system of transcriptional coactivators.

*Highly conserved in mammals;
transcripts only detected in
human tissues

PGC-1s: meet the parents

PGC-1 α was first identified in a yeast two-hybrid screen for factors that interact with the nuclear receptor PPAR γ in brown adipose tissue (BAT) in the context of cold-induced thermogenesis [1]. Soon after, two homologous genes, *PGC-1 β* [6] and *PRC* [7] were cloned by cDNA and protein sequence homology, respectively. Although these proteins are encoded by different genes, their modular structure is remarkably similar [8], which explains the partial overlap in their physiological roles [3]. The three PGC-1 homologues share an amino-terminal activation domain with LXXLL signature motifs involved in nuclear receptor docking [6] and interaction with other transcriptional coactivators such as the steroid receptor coactivator-1 (SRC-1)/p300 complex [9], a nuclear localisation signal (NLS), and a C-terminal RNA recognition motif (RRM) [1]. PGC-1 α and PRC share an additional carboxy-terminal sequence, the arginine–serine-rich (RS) domain [1], that is characteristic of proteins involved in mRNA processing and splicing [10]. The C-terminal region of PGC-1 α 1 has been shown to be essential for interaction with the Mediator complex, and thus the transcriptional machinery, via Mediator of RNA polymerase II transcription subunit 1 (MED1) [9]. Additionally, PGC-1 α mutants lacking the RS and RRM domains cannot associate with splicing factors and fail to modulate specific splicing events in an in vitro system. This suggests that PGC-1s play a role not only in quantitative regulation of gene expression, but also collaborate with the co-transcriptional processing of nascent mRNAs [11].

Most gene programs regulated by PGC-1 α are bioenergetically demanding and result in coordinated cellular actions to adapt to higher energy demands. For this reason, cells keep tight control over PGC-1 α activity through diverse transcriptional and post-translational mechanisms. Some of the signalling pathways controlling *PGC-1 α* gene expression involve calcium signalling [12], calcineurin A [13], cyclic AMP [14] and 5' AMP-activated protein kinase (AMPK) [15]. Activation of these pathways results in the mobilisation of different transcription factors to *PGC-1 α* gene regulatory regions, in a context- and tissue-specific manner. These include several members of the nuclear hormone receptor family, as well as other tissue-specific transcription factors [3]. Access of transcriptional regulators to the appropriate genomic regions can be limited through epigenetic modifications such as DNA methylation [16]. Accordingly, *PGC-1 α* promoter methylation is reduced by physical exercise, a classical inducer of PGC-1 α expression in skeletal muscle [17]. Conversely, methylation of the same regions has been found to increase in pathological situations associated with reduced *PGC-1 α* gene expression [18, 19]. In addition, PGC-1 α activity can be modulated by a plethora of post-translational modifications, including phosphorylation, acetylation and ubiquitylation. Kinases that target PGC-1 α 1 include p38

mitogen-activated protein kinase [20], protein kinase B [21], S6 kinase [22], AMPK [23] and glycogen synthase kinase 3 β (GSK3 β) [24]. These different phosphorylation events constitute a code that changes PGC-1 α 1 biological activity by, for example, dictating association with specific transcription factors [3]. The acetylation state of PGC-1 α 1 can be modified by acetyltransferases such as GCN5 [25] or deacetylases like sirtuin 1 (SIRT1) [26, 27]. Additionally, ubiquitylation by specific E3 ligases directly regulates PGC-1 α protein stability [24, 28, 29]. Together, these multiple levels of regulation ensure that under any given condition the cellular PGC-1 α activity is appropriate to regulate the gene programs necessary to adapt to the new biological environment.

Where do we start? Multiple origins for PGC-1 α transcripts

Most of the studies on PGC-1 α protein structure and function have focused on the 797 amino acid (aa)-long murine full-length protein (94.7% sequence identity with the 798 aa-long human PGC-1 α). However, early on in the PGC-1 α history there were reports of other transcripts induced by challenging energy situations, such as cold exposure in BAT [30] and exercise in skeletal muscle [31]. Only recently have various laboratories made efforts to further characterise these PGC-1 α variants. By analysing expressed sequence tag (EST) libraries, two independent studies identified two novel *Pgc-1 α* transcripts highly induced in mouse skeletal muscle by exercise and β -adrenergic stimulation [32, 33]. Genomic mapping of the newly identified 5' sequences revealed that the novel transcripts are expressed from an evolutionarily conserved alternative promoter of the *Pgc-1 α* gene, located approximately 14 kb upstream of the previously characterised transcription start site (TSS) [33]. Transcripts originating from this distal promoter contain a novel exon 1 sequence (exon 1b) that can follow one of two splicing options and thus encode two different amino termini (12 and 3 aa long, respectively; Fig. 1) slightly shorter than the proximal exon 1a (16 aa long; Fig. 1) [32, 33]. Miura et al cloned *Pgc-1 α -b* and *Pgc-1 α -c* (for the long and short exon 1b, respectively) from a muscle cDNA library, thus renaming the original protein as PGC-1 α -a [32] (Fig. 1).

PGC-1 α -b and PGC-1 α -c differ only in their N-termini while the rest of the protein is identical to the canonical PGC-1 α 1. Concurrently, two other transcripts were identified that contained the long and short exon 1b, those for *Pgc-1 α 2* and *Pgc-1 α 3*, but their primary structure was not investigated in this study [33]. Consistent with their nomenclature, the canonical isoform was renamed *Pgc-1 α 1*. The alternative gene promoter is highly conserved between species and has been shown to be active in human skeletal muscle [34–36].

Accordingly, the novel PGC-1 α isoforms are detected in skeletal muscle after exercise [35–37].

These first studies showed that the proximal *Pgc-1 α* gene promoter, from where *Pgc-1 α 1* is expressed, seems to have higher basal expression whereas the alternative promoter is more responsive to stimulation. Indeed, physical exercise or cold exposure induces a promoter activity shift towards the upstream TSS in skeletal muscle and BAT (respectively), favouring the expression of the novel PGC-1 α isoforms [32, 33]. This alternative *Pgc-1 α* gene promoter is highly responsive to β -adrenergic stimulation, both in BAT and skeletal muscle. In line with this, mice treated with clenbuterol (a β_2 -adrenergic agonist) show levels of expression of the novel PGC-1 α isoforms comparable to those induced by exercise training. Conversely, the β_2 -adrenergic antagonist ICI-118,551 blunts *Pgc-1 α -b* and *Pgc-1 α -c* induction by physical exercise [32], whereas the non-selective β -blocker propranolol significantly reduces the magnitude of muscle *Pgc-1 α* induction by voluntary running [33].

PGC-1 α -b and PGC-1 α -c are not significantly divergent from PGC-1 α 1 (i.e. PGC-1 α -a) in terms of structure or function. For example, like PGC-1 α 1 they coactivate PPAR nuclear receptors [32] and all these proteins regulate transcriptional programs that elicit some of the effects of endurance exercise training in skeletal muscle by promoting mitochondrial biogenesis, fatty acid oxidation [32] and angiogenesis [38] (Fig. 2). Chinsomboon and collaborators observed that exercise-mediated induction of *Pgc-1 α 2* and *Pgc-1 α 3* is associated with vascular endothelial growth factor (VEGF) expression and promotion of angiogenesis in an oestrogen-

related receptor (ERR) α -dependent manner [33]. Further characterisation of a *Pgc-1 α -b* skeletal muscle-specific transgenic mouse model revealed that the improved oxidative capacity and exercise performance were directly correlated to mitochondrial volume rather than increased mitochondrial function [38]. *Pgc-1 α -b* has also been detected in rat cardiomyocytes after fasting, an event that is dependent on cAMP signalling [39].

PGC-1 α isoforms: ABC, L/B, NT and 2, 3, 4

L(iver)- and B(rain)-PGC-1 α In addition to the novel upstream alternative *PGC-1 α* gene promoter [32, 33], other TSSs have been described for the human *PGC-1 α* gene [40, 41]. One site is located within intron 2 of the *PGC-1 α* gene and, despite being in a highly conserved region, it seems to be used only in human fasted liver [40]. The resulting transcript, *L-PGC-1 α* , encodes the novel exon 1L that is spliced to exon 3 and then follows the canonical *PGC-1 α* transcript sequence (Fig. 1). Because the start codon for L-PGC-1 α is now located in exon 3, this protein lacks the first 127 aa of PGC-1 α 1 (encoded by exons 1, 2 and part of 3). Consequently, the first LXXLL motif of the activation domain that facilitates recruitment of SRC-1/p300, and the GCN5 interaction region (aa 1–97 of PGC-1 α 1) are missing from L-PGC-1 α . Since L-PGC-1 α maintains the C-terminal NLS, the protein localises in the nucleus, where it coactivates PPAR α , PPAR γ and hepatocyte nuclear factor 4 α (HNF4 α) but not liver X receptor α (LXR α) [40]. Like PGC-1 α 1, L-PGC-1 α is induced in response to glucocorticoids and cAMP. It is interesting to note that the lack of aa 1–127 could impact both post-translational modifications, e.g. acetylation, as well as interactions with other transcription factors.

Subsequent work from the same group identified another TSS, located 587 kb upstream of *PGC-1 α* exon 2, which can produce several brain-specific isoforms [41]. In human brain, these novel transcripts seem to be more abundant than the canonical *PGC-1 α 1* and encode several isoforms of varying length that have been confirmed at the transcript level in human brain samples and at the protein level in a SH-SY5Y neuroblastoma cell line [41]. These transcripts contain novel exons B1–5 located upstream of the canonical exon 2. Most of these transcripts include exons B1 followed by B4 and/or B5, and then exons 2–13 [41]. Whether the brain-specific isoforms have functional activation domains remains to be determined. Interestingly, the newly identified promoter that mediates the transcription of these brain *PGC-1 α* isoforms is located in a genomic region associated with age of onset of Huntington's disease [41], a disease that has been linked to altered PGC-1 α function [42–44].

Fig. 2 PGC-1 α isoforms have overlapping and specific biological activities

NT(erminal)-PGC-1 α Using a BAT cDNA library, Zhang and collaborators cloned a *Pgc-1 α* variant expressed under the control of the proximal gene promoter that contains an alternative splicing event between exons 6 and 7 [45], which introduces a premature stop codon (Fig. 1). This particular intronic sequence of the *Pgc-1 α* gene, which is highly conserved in mammals, contains two distinct splicing acceptor sites. When the upstream acceptor site is favoured, it yields a 270 aa protein named NT-PGC-1 α that corresponds to the activation domain of PGC-1 α 1 (aa 11–80) and part of the repression domain (aa 180–403 in PGC-1 α 1; 180–267 in NT-PGC-1 α). NT-PGC-1 α lacks all the central and C-terminal PGC-1 α 1 protein modules, including the RS/RRM domains and the NLS [1]. The absence of these sequences unmasks a nuclear export signal (NES) that under basal conditions localises NT-PGC-1 α mainly to the cytosol (90%) [45]. Cold exposure or β -adrenergic stimulation of BAT triggers a double mechanism that regulates NT-PGC-1 α transcriptional activity. On the one hand, in vitro stimulation of brown adipocytes with a cAMP analogue induces rapid shuttling of cytoplasmic NT-PGC-1 α into the nucleus [45]. Additionally, cold exposure induces BAT expression of equivalent levels of full-length and NT-PGC-1 α isoforms. The same group investigated which *Pgc-1 α* gene promoter regulates the expression of these inducible isoforms. Two studies showed that stimulation of BAT [46] or skeletal muscle [47] shifts promoter activation, favouring the expression of either full-length or NT-PGC-1 α from the novel upstream gene promoter [33]. In this way, the repertoire of *Pgc-1 α* isoforms is increased by the addition of two *NT-Pgc-1 α* variants that contain both versions of the novel exon 1b, namely *NT-Pgc-1 α -b* (which is identical to *Pgc-1 α 4*, described by Ruas et al in 2012 [36]) and *NT-Pgc-1 α -c*. Consequently, NT-PGC-1 α was renamed NT-PGC-1 α -a.

Although NT-PGC-1 α proteins lack the C-terminal domain that in PGC-1 α 1 is required for interaction with the Mediator complex [9], they still coactivate PPAR α and PPAR γ transcriptional activity [45]. However, this association is strictly ligand-dependent, probably because NT-PGC-1 α lacks the amino acid sequences that in PGC-1 α 1 mediate ligand-independent coactivation of PPARs (aa 338–403 in PGC-1 α 1) [48]. The lack of C-terminal domains make NT-PGC-1 α proteins resistant to Twist-1-mediated inhibition in brown adipocytes [49]. This suggests that, unlike PGC-1 α 1, NT-PGC-1 α cannot be targeted by the negative feedback loop mediated by Twist-1 [50].

The biological activity of NT-PGC-1 α -a overlaps considerably with that of PGC-1 α 1. Indeed, expression of NT-PGC-1 α -a or PGC-1 α 1 in differentiated brown adipocytes drives similar gene programs, with enhanced mitochondrial biogenesis (Fig. 2) and increased carnitine palmitoyltransferase-1b (CPT-1b) and uncoupling protein 1 (UCP1) expression [45]. *NT-Pgc-1 α -a* expression is induced by exercise training, does

not depend on β -adrenergic stimulation, and promotes a classical oxidative phenotype in C2C12 cells [47]. Accordingly, the expression profile of *NT-Pgc-1 α -a* is closer to that of *Pgc-1 α 1* and differs from the isoforms transcribed from the alternative gene promoter. *NT-Pgc-1 α* can be found in several tissues and is particularly abundant in mouse brain [45]. *NT-Pgc-1 α -a* and *Pgc-1 α 1* (together with L-PGC-1 α in humans) are the only isoforms to date that have been reported to be induced in fasted liver (Fig. 2).

PGC-1 α 2, - α 3 and - α 4 Activation of the alternative *Pgc-1 α* promoter located 14 kb upstream of the proximal TSS (Fig. 1) results in the transcription of three additional PGC-1 α variants with unique structural features [36]. Two of these variants, *Pgc-1 α 2* and *Pgc-1 α 3*, share with *Pgc-1 α -b* and *Pgc-1 α -c* (respectively) the splice variants of exon 1b, but differ vastly in structure from any of the other isoforms (Fig. 1). This is due to a series of splicing events common to both *Pgc-1 α 2* and *Pgc-1 α 3*, which eliminate exons 4–6 and 9–13. The splicing of exon 8 to the 3' untranslated region (UTR) of the *Pgc-1 α* gene then creates a new stop codon common to both transcripts. Consequently, the two resulting proteins (379 and 370 aa long, respectively) retain part of the activation and repression domains and completely lack all the C-terminal motifs of PGC-1 α 1. The third *Pgc-1 α* isoform described in the same study [36] is 266 aa long and is named PGC-1 α 4 (Fig. 1). *Pgc-1 α 2*, *Pgc-1 α -b* and *Pgc-1 α 4* share the same transcriptional origin (and hence the same N-terminal aa sequence) but *Pgc-1 α 4* undergoes the downstream alternative splicing options seen in the *NT-Pgc-1 α* isoforms (i.e. the inclusion of the new exon 6 and the premature stop codon at 266 aa). *Pgc-1 α 2*, - α 3 and - α 4 mRNA and protein levels are induced by hypertrophic stimuli in skeletal muscle [36, 37] and by cold exposure in BAT, but do not drive the well-known oxidative phenotype induced by other variants [36]. Notably, PGC-1 α 4 has been shown to exert physiological effects previously unreported for a member of the PGC-1 family. When expressed in skeletal muscle, PGC-1 α 4 promotes robust muscle hypertrophy by inducing IGF-1 expression and reducing the levels of myostatin, a negative regulator of muscle growth (Fig. 2). Through this mechanism, differential activation of one single *Pgc-1 α* gene in skeletal muscle can result in some of the most-well established benefits of endurance and resistance exercise training (i.e. fatigue resistance and increased muscle mass and strength, respectively). Moreover, high PGC-1 α 4 levels in mouse skeletal muscle confers resistance to cancer cachexia and disuse-induced atrophy. Muscle hypertrophy mediated by the PGC-1 α system is independent of ERR α / γ [36] since PGC-1 α 4 expression in ERR α or - γ genetically defective myotubes still promotes a hypertrophic phenotype. On the other hand, hypoxia promotes skeletal muscle PGC-1 α 4 and NT-PGC-1 α expression that, together with ERR α , increase *VEGF* gene

transcription and angiogenesis [51]. The biological roles PGC-1 α 2 and PGC-1 α 3 remain unknown.

Changes in the PGC-1 α system of coactivators in skeletal muscle can crosstalk to other tissues and have systemic effects. Several recent reports demonstrate that elevating specific PGC-1 α isoforms in skeletal muscle can impact other tissues by secreting circulating factors (myokines). Irisin and β -aminoisobutyric acid (BAIBA) [52, 53], both under the control of PGC-1 α 1, or PGC-1 α 4-regulated myostatin and meteorin-like protein [54, 55], all convey skeletal muscle signals to adipose tissue. Thus, skeletal muscle overexpression of either PGC-1 α 1 or PGC-1 α 4 results in browning of adipose tissue, although this is achieved through distinct pathways and mechanisms specific to each isoform.

We have recently uncovered a mechanism by which skeletal muscle PGC-1 α 1 activation can protect against stress-induced depression. This crosstalk between muscle and the brain is not mediated by secretion of myokines, but results from skeletal muscle detoxification of the tryptophan metabolite kynurenine that upon accumulation in the brain has been linked to neuroinflammation and depression [56].

Challenges and perspectives

Which promoter to promote? One of the pressing questions about PGC-1 α isoform expression is how do cells specifically regulate the different *Pgc-1 α* gene promoters? How do specific stimuli dictate which promoter will be activated? Upon cold exposure, BAT clearly shifts the preference from the proximal to the alternative *Pgc-1 α* promoter and every induced isoform, either full-length or spliced, contains the novel exon 1b [32, 45, 46]. The biological meaning of this specific regulation remains elusive, since the expression of the diverse PGC-1 α isoforms in BAT does not seem to trigger a differentiated biological function, and independently of promoter origin or splicing choice, all seem to conduct the mitochondrial thermogenesis program.

Promoter choice and PGC-1 α isoform expression is also regulated in skeletal muscle. The 14 kb upstream alternative *Pgc-1 α* gene promoter is highly sensitive to stimulation, and most exercise interventions, from high- to low-intensity endurance training [57] to resistance training [36] seem to activate it to some extent. The classical proximal promoter seems to be less inducible but consistently responsive to high-intensity endurance exercise [57] or freewheel running [33, 58]. Pre-treatment of exercised animals with a β -adrenergic antagonist impairs *Pgc-1 α -b* and *Pgc-1 α -c* induction but does not affect the induction of *Pgc-1 α -1* [32]. In effect, in vivo administration of the β ₂-adrenergic agonist clenbuterol induces the expression of all isoforms derived from the novel promoter while repressing *Pgc-1 α -1* expression (V. Martínez-Redondo, J. L. Ruas, unpublished observation).

This shift adds PGC-1 α 4-mediated muscle hypertrophy to the bioenergetic effects induced by other alternative isoforms. Understanding how these mechanisms are regulated will allow each of the specific pathways to be targeted therapeutically.

Technical considerations for the detection of PGC-1 α isoforms Since the *Pgc-1 α* gene can generate a variety of mRNAs under different biological conditions and in different tissues, it is important to accurately design the detection reagents when studying specific *Pgc-1 α* isoforms. When performing gene expression analysis by real-time PCR (the most widely used method), it is possible to use isoform-specific combinations of primers that target unique sequences in the different transcripts (Table 1). Moreover, detecting total *Pgc-1 α* levels (which can be done by targeting exon 2, shared by all known isoforms except *L-PGC-1 α*) will, in many cases, mask important changes in the levels of specific isoforms. For example, targeting exon 1a to analyse the effects of clenbuterol administration on mouse muscle PGC-1 α expression will detect the overall change in isoforms expressed from the canonical promoter, but fail to measure the induction of the alternative promoter [59]. However, since most transcripts share part of their sequence, real-time PCR is not always a viable approach for reliably measuring PGC-1 α isoform expression levels. In many cases, the primer pair combinations that would be truly isoform-specific generate amplicons too large for this technique (i.e. generate non-linear signals). One way of refining PGC-1 α isoform analysis is by combining information from two separate primer sets targeting different parts of the transcript and by determining protein molecular sizes by immunoblotting (Table 1). Finally, a third layer of complexity comes from the fact that different PGC-1 α isoforms seem to have distinct mRNA or protein stability. For example, equivalent mRNA expression of *Pgc-1 α -1*, *Pgc-1 α -2*, *Pgc-1 α -3* and *Pgc-1 α -4* in primary myotubes results in different protein levels [36]. Regardless of the transcript proportions in the different *Pgc-1 α* mRNA populations, many of the novel isoforms are more easily detected by immunoblotting than the predominant transcript isoform, PGC-1 α 1.

Despite the high degree of sequence conservation observed throughout the *Pgc-1 α* gene region, not all the isoforms are expressed in all species. This is the case for the L-PGC-1 α isoform, which has only been detected in human tissues [40, 41]. To our knowledge, no available database contains comprehensive information about all the described PGC-1 α isoforms. For the human *PGC-1 α* isoforms, Ensembl (www.ensembl.org) (ENSG00000109819) contains entries for *PGC-1 α -1* (001), *NT-7a* (homologous to mouse *NT-Pgc-1 α -a*) (007), brain-specific isoform *8a* (008) and *L-PGC-1 α* (202), which link to the Uniprot (www.uniprot.org) protein database (entry Q9UBK2) as isoforms 1, NT-7a, 8a and 9, respectively. In the same entry are also documented

Table 1 Strategies to detect PGC-1 α isoforms by quantitative real-time PCR and immunoblotting using specific antibodies

Isoform	Strategy 1: Primer pair 1	Strategy 2: Primer pair 2	Predicted size (kDa)	Apparent size (kDa)	Reference
PGC-1 α 1	ex1a Fw, ex2 Rv	ex3 Fw, ex5 Rv	90	115	[36]
PGC-1 α 2	ex1b Fw, ex2 Rv	ex3 ^a Fw, ex8 ^a Rv	42	50	[36]
PGC-1 α 3	ex1b' Fw, ex2 Rv	ex3 ^a Fw, ex8 ^a Rv	41	50	[36]
PGC-1 α 4	ex1b Fw, ex2 Rv	ex7b Fw, ex8 Rv	29	37	[36]
PGC-1 α -b	ex1b Fw, ex2 Rv	ex3 Fw, ex5 Rv	90	115	[32]
PGC-1 α -c	ex1b Fw, ex2 Rv	ex3 Fw, ex5 Rv	90	115	[32]
NT-PGC-1 α -a	ex1a Fw, ex2 Rv	ex7b Fw, ex8 Rv	30	37	[45]
NT-PGC-1 α -b	ex1b Fw, ex2 Rv	ex7b Fw, ex8 Rv	29	37	[46]
NT-PGC-1 α -c	ex1b' Fw, ex2 Rv	ex7b Fw, ex8 Rv	30	37	[46]
L-PGC-1 α	ex1L Fw, ex3 Rv	–	77	77	[40]
B4	exB1 Fw, exB4 Rv	–	90	~115	[41]
B5	exB5 Fw, ex2 Rv	–	90	~115	[41]
B4-8a	exB1 Fw, exB4 Rv	–	32	~40	[41]
B5-NT-7a	exB5 Fw, ex2 Rv	–	32	~35	[41]
B4-3ext	exB1 Fw, exB4 Rv	–	15	–	[41]
Ex1-8a	ex1a Fw, ex2 Rv	–	33	~40	[41]

^aPrimer pair' columns indicate exons that should be targeted for PGC-1 α isoform-specific primer design

^a Primers must target sequences in exon 3 and 8 retained in these isoforms. This generates an amplicon suitable for real-time PCR due to exclusion of exons 4–6 in isoforms PGC-1 α 2 and - α 3

Fw, forward; Rv, reverse

other human brain isoforms B5, B4, B4-8a, B5-NT and B4-3ext (as isoforms 3 to 7). For the mouse variants, only isoforms *Pgc-1 α 1* (001), *NT-Pgc-1 α -a* (002) and *Pgc-1 α 4* (003) can be found in Ensembl (ENSMUSG00000029167), while in Uniprot (entry O70343), isoforms PGC-1 α 1, - α 2, - α 3 and - α 4 are all documented. The protein sequence of NT-PGC-1 α -a can be found in entry Q3LIG2.

As the number of variants described for PGC-1 α grows, it would be of great value to agree on a unified, systematic and non-descriptive nomenclature.

A personalised workout for PGC-1s Another subject of debate is whether different types of exercise training specifically regulate *Pgc-1 α* promoter activation and isoform expression. Alternative promoter-derived *Pgc-1 α 4*, which drives muscle hypertrophy, is induced only in exercise programmes that include a resistance component, whereas its expression is unaffected by 8 weeks of aerobic cycling [36]. In addition, a 12-week progressive weight lifting programme specifically induces isoforms *Pgc-1 α 2*, *Pgc-1 α 3* and *Pgc-1 α 4* from the novel promoter [37]. However, other research shows that both promoters can be activated independent of the exercise intervention [60, 61]. PGC-1 α isoform expression patterns display distinct temporal dynamics [55] and until we understand the mechanisms behind these different expression profiles it is important to unify experimental

procedures such as exercise intervention, sampling and gene expression analysis techniques. One common outcome of the exercise training studies with human volunteers is that concurrent training programmes, with both endurance and resistance components, have a synergistic effect on the expression of all PGC-1 α isoforms, although the effect is more pronounced on the alternative promoter.

Resistance exercise training protocols are difficult to translate to rodents, so different experimental protocols are used to induce skeletal muscle hypertrophy. Using the hindlimb suspension–reloading model [62] we have seen specific induction of *Pgc-1 α 4* and concomitant repression of *Pgc-1 α 1* during the reloading/hypertrophic phase [36]. In the same study, we demonstrated that PGC-1 α 4 is sufficient to induce skeletal muscle hypertrophy. A different group has reported that mice lacking expression of all (known) PGC-1 α proteins in skeletal muscle still undergo hypertrophy induced by the synergistic ablation model [63]. We cannot rule out either the existence of additional isoforms that might account for those particular effects or compensatory mechanisms due to the disruption of the *Pgc-1 α* gene since the embryonic state. Until we have the appropriate mouse genetic models of PGC-1 α isoform-specific gain- and loss-of-function, it will be difficult to establish the in vivo contribution of each protein to the overall effects of PGC-1 α activation.

In conclusion, future studies will need to address the mechanisms of regulation and interplay between PGC-1 α variants in different tissues and physiological contexts. Finally, we would like to highlight the importance of a more systematic nomenclature for the constantly expanding PGC-1 α system of transcriptional coactivators.

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