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Yubin Zhang
Pennington Biomedical Research Center

Peter Huypens
Pennington Biomedical Research Center

Aaron W. Adamson
Pennington Biomedical Research Center

Ji Suk Chang
Pennington Biomedical Research Center

Tara M. Henagan
Pennington Biomedical Research Center

See next page for additional authors

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Alternative mRNA Splicing Produces a Novel Biologically Active Short Isoform of PGC-1α

The transcriptional co-activator PGC-1α regulates functional plasticity in adipose tissue by linking sympathetic input to the transcriptional program of adaptive thermogenesis. We report here a novel truncated form of PGC-1α (NT-PGC-1α) produced by alternative 3′ splicing that introduces an in-frame stop codon into PGC-1α mRNA. The expressed protein includes the first 267 amino acids of PGC-1α and 3 additional amino acids from the splicing insert. NT-PGC-1α contains the transactivation and nuclear receptor interaction domains but is missing key domains involved in nuclear localization, interaction with other transcription factors, and protein degradation. Expression and subcellular localization of NT-PGC-1α are dynamically regulated in the context of physiological signals that regulate full-length PGC-1α, but the truncated domain structure conveys unique properties with respect to protein-protein interactions, protein stability, and recruitment to target gene promoters. Therefore, NT-PGC-1α is a co-expressed, previously unrecognized form of PGC-1α with functions that are both unique from and complementary to PGC-1α.

PGC-1α was identified as a brown adipocyte-enriched, cold-inducible, and transcriptional co-activator of the nuclear receptor PPARγ (1). PGC-1α function is central to transcriptional programs that underlie not only brown adipocyte differentiation (1, 2) but also fiber-type switching in muscle (3), gluconeogenesis in liver (4–7), fatty acid oxidation in heart (8, 9), and protection from oxidative stress in the central nervous system (10). Although the physiological responses affected by PGC-1α differ among tissues, mitochondrial biogenesis and increased cellular respiratory capacity are common elements of the transcriptional program in all sites. In some tissues, PGC-1α functions as a nutrient sensor, and in others its activity is regulated by endocrine, environmental, or circadian signals (6, 11–14). In (brown) adipose tissue, PGC-1α is a key factor in sympathetic nervous system-induced functional plasticity, responding to increased cAMP and translating sympathetic nervous system input into transcriptional programs that remodel the mitochondrial proteome, increase oxidative capacity, and enable thermogenesis (1, 2, 15, 16).

PGC-1α has a complex structure with multiple domains that act in concert to define the biological and physiological properties of the full-length protein (1, 9, 17–20). Distinct surfaces on the PGC-1α protein enable the co-activation of a diverse array of nuclear receptors (NRs) and transcription factors. N-terminal leucine-rich domains (L2 and L3) mediate interactions with the ligand binding domain of NRs, whereas central and C-terminal domains mediate interactions with PPARγ, NR1F1, host cell factor, MEF2C, and FOXO1. Interaction with transcription factors directs PGC-1α to the regulatory region of target genes and facilitates recruitment of other co-activators, such as SRC-1 and CREB-binding protein (via an N-terminal transactivation domain) and the Mediator complex (via C-terminal domains) (17, 21, 22). In any given cellular environment, productive interactions between PGC-1α and downstream effectors are subject to signaling inputs that regulate PGC-1α expression and activity through extensive post-translational modification of the protein. Nutrient-sensing systems regulate hepatic gluconeogenesis in part by regulating acetylation of PGC-1α (11), which increases activation of gluconeogenic genes via HNF4 (5) and FOXO1 (6). Phosphorylation of specific sites by p38 MAPK (13), AMP kinase (12), AKT (23), and GSK-3β (14) regulate the transcriptional activity and/or protein levels of PGC-1α. Transcriptional activity of PGC-1α is also regulated by methylation of arginine residues by PRMT1 (24), and C-terminal domains regulate PGC-1α protein stability (25). Thus, the versatility of PGC-1α as a transcriptional co-activator in multiple contexts derives from a complex domain structure that enables the following: first, interactions...
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with multiple transcriptional factors via several distinct protein–protein interaction domains, and second, post-translational modifications (affecting activity and protein levels) by diverse regulators and signaling inputs.

Alternative splicing of multiexon genes is common and particularly relevant to multidomain proteins where splicing events produce protein isoforms with unique properties (26). The splice variant of PGC-1α identified herein (N-truncated PGC-1α) retains the N-terminal transcriptional activation and NR-interacting domains but is missing all domains within aa 268–797 of the full-length protein. Thus, the distinct properties of NT-PGC-1α are defined not only by the subset of domains retained from the parent protein but also by the missing domains that are downstream of the splice site. Using a combination of approaches, we show that NT-PGC-1α expression, subcellular localization, nuclear receptor interaction, and target gene activation are dynamically regulated within the context of the physiological signals that regulate full-length PGC-1α. Collectively, these data support the concept that NT-PGC-1α is an important, previously unrecognized component of the transcriptional network that links signaling input to PGC-1α-dependent transcriptional responses.

EXPERIMENTAL PROCEDURES

Animals and Tissues—Six-week-old male C57BL/6J mice or 3-month-old Fisher F344 rats were individually housed and exposed to cold (4 °C) or room temperature (22–23 °C) for 5 h prior to sacrifice using CO2 and cervical dislocation. Tissues from PGC-1α null mice (15) were a kind gift from Bruce Spiegelman. Human tissue (heart) harvested at autopsy was obtained from National Disease Research Interchange (Philadelphia).

RNA and Protein Analysis—Total RNA was isolated from tissues or cultured brown adipocytes using TRIzol reagent (Invitrogen) and reverse-transcribed using oligo(dT) primers and Moloney murine leukemia virus reverse transcriptase (Promega). Twenty ng of cDNA was used as template for quantitative reverse transcription-PCR on a Smart Cycler (Cepheid) using cyclophilin as internal control. The primers for mouse and rat Pgc-1α and NT-Pgc-1α mRNA (S1) used a common forward primer and a reverse primer that spanned the junction of exons 6 and 7 for Pgc-1α or a reverse primer that spanned the beginning of exon 7 and the intronic sequence retained in NT-Pgc-1α mRNA. The specificity of the respective primer sets for Pgc-1α and NT-Pgc-1α was confirmed by sequencing cDNA fragments amplified by each primer set from a BAT cDNA library. The respective cDNA fragments were carefully quantitated and used to prepare standards by serial 10-fold dilution of each purified fragment. The slopes of the resulting standard curves relating log mass of Pgc-1α and NT-Pgc-1α cDNA to cycle threshold were −3.3 (S2). In addition, serial dilution of BAT cDNA samples produced changes in cycle threshold that paralleled the standard curves. The following primers were used: mouse Ucp1 F, 5' GAT CCA AGG TGA AGG CCA GG 3', and Ucp1 R, 5' GTT GAC AAG CTT TCT GTG GTG G 3'; cyclophilin F, 5'CCA TCG TGT CAT CAA GGA CTT CAT-3' and cyclophilin R, 5'-CTT GCC ATC CAG CCA GGA GTT CTT-3'.

For measurement of protein expression, tissues were homogenized under liquid nitrogen and lysed in RIPA buffer containing 50 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM EDTA, and protease inhibitor mixture. Brown adipocytes were washed with phosphate-buffered saline prior to lysing in the same buffer. UCP1 was detected using our previously described polyclonal antibody (27). For detection of PGC-1α or NT-PGC-1α, lysates (100 μg) were analyzed by immunoblotting using a rabbit polyclonal antibody raised against a GST fusion of PGC-1α (1–200 aa) or a monoclonal antibody raised against a GST fusion of PGC-1α (1–120 aa). Both antibodies were epitope-mapped using a series of overlapping 15-aa-long peptides corresponding to aa 1–200 of mouse PGC-1α (JPT Peptide Technologies). The 15-mer peptides were spotted onto glass slides and hybridized with each antibody to identify the specific sequences recognized by each antibody. Immuneblots were hybridized with antibodies raised against β-actin as a loading control (Santa Cruz Biotechnology). Finally, the immunoreactive proteins in each blot were visualized using species-specific second antibodies and ECL reagent (Amersham Biosciences).

Generation and Culture of Brown Adipocyte Cell Lines—Interscapular brown adipose tissue was isolated from newborn FVB mice by collagenase digestion and immobilized by transfection with the retroviral pBabe vector expressing SV40T antigen as described previously (28, 29). After a 14-day selection using 1 μg/ml puromycin, the immortalized preadipocytes were grown in Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum and antibiotics. Subsequently, the cells were stably transformed with empty lentiviral vector or lentiviral constructs expressing NT-PGC-1α-HA or PGC-1α-V5. Following a 12-day selection with blastidicin (7.5 μg/ml), 4–5 lines from each group were selected, and expression of NT-PGC-1α-HA or PGC-1α-V5 was confirmed by Western blotting. The respective cell lines were maintained in growth medium containing Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum and antibiotics. At 80% confluence, culture medium was supplemented with 20 nM insulin, 1 nM triiodothyronine, 0.5 mM isobutylmethylxanthine, 2 μg/ml dexamethasone, and 250 μM indomethacin for 48 h to induce differentiation. Thereafter, cells were maintained in differentiation medium (Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum, antibiotics, 20 nM insulin, and 1 nM triiodothyronine) until full differentiation was achieved on day 7.

To examine induction of NT-PGC-1α by cAMP, differentiated brown adipocytes were treated with 100 μM 8-CPT-cAMP for 14 h. Induction of the thermogenic gene program by NT-PGC-1α was examined by treating differentiated brown adipocyte cell lines expressing NT-PGC-1α-β-actin, PGC-1α-V5, or empty vector with a ligand mixture containing 100 μM 8-CPT-cAMP, 10 μM WY14693, or BRL49653, and 5 μM 9-cis-RA.

Immunocytochemistry and Cell Volume Measurements—CHO-K1 cells, brown preadipocytes stably transformed with NT-PGC-1α-β-actin, or fully differentiated, stably transformed brown adipocytes were seeded at a density of 30,000 cells per well and grown overnight on polylysine-coated coverslips. CHO-K1 cells were transiently transfection with NT-PGC-1α.
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1α-HA in pcDNA3.1 using FuGENE (Roche Applied Science). After 12 h, serum-deprived CHO-K1 cells or the stably transformed preadipocytes and adipocytes were treated as described in each figure legend, washed with prewarmed phosphate-buffered saline, and fixed using 4% paraformaldehyde. The fixed cells were permeabilized with 0.5% Triton X-100 and sequentially incubated with rabbit anti-HA antibody (Abcam), goat anti-rabbit second antibody coupled to fluorescein isothiocyanate (Invitrogen), and DAPI. Following stringent washes with 0.5% Triton X-100 and 0.1% Tween 20, coverslips were mounted with Vectashield (Vector Laboratories). Subcellular localization of NT-PGC-1α and DAPI was determined by confocal microscopy using a Zeiss LSM 510 Meta, and the image stacks were analyzed using ImageJ software. Treatment-dependent changes in nuclear NT-PGC-1α content were assessed by expressing NT-PGC-1α signal intensity co-localized with DAPI as a percentage of NT-PGC-1α signal intensity measured in the whole cell. In addition, the relative concentrations of NT-PGC-1α in the nucleus and cytosol were determined independently by measuring nuclear and cytosolic volume in cells stained with DAPI and CellTracker Green CMFDA (Invitrogen). The cells were imaged using confocal microscopy and analyzed using Imaris software (Bitplane). Nuclear and cytoplasmic NT-PGC-1α concentrations were calculated as the ratio of NT-PGC-1α signal intensity in the nucleus to nuclear area and NT-PGC-1α signal intensity in the cytosol to cytosolic area. Treatment-dependent changes in subcellular localization of NT-PGC-1α were assessed using the ratio of nuclear to cytosolic NT-PGC-1α concentration, with ratios approaching 1 indicative of equilibration of NT-PGC-1α across the nuclear membrane and ratios <1 or >1 indicative of cytosolic or nuclear sequestration of NT-PGC-1α, respectively.

Interaction of NT-PGC-1α with Nuclear Receptors—To examine interaction of NT-PGC-1α with PPARs, CHO-K1 cells were transiently transfected with GAL4-responsive luciferase reporter gene (pGK), plasmids expressing the GAL4 DNA binding domain fused to either full-length PPARα or PPARγ, pcDNA3.1 plasmids expressing either mouse PGC-1α or NT-PGC-1α, and a pRL-SV40 control plasmid expressing Renilla luciferase for normalization. After 24 h, the cells were treated with vehicle (DMSO), 10 μM WY14693 (Sigma), or 10 μM BRL49653 (Cayman Chemicals) for 24 h. Cells were harvested in lysis buffer (Promega) for measurement of luciferase activity. For in vitro binding assays, GST-PPARα or GST-NT-PGC-1α were expressed in bacteria (BL21) by β-1-thio-d-galactopyranoside induction for 3 h at 30 °C and purified on Sepharose beads containing glutathione. 35S-Labeled PPARα and PPARγ were produced in the TnT reticulocyte lysate system (Promega) and incubated for 1 h with equal amounts of the in vitro translated proteins in binding buffer (20 mM Hepes, 75 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl2, 0.05% Nonidet P-40, 2 mM dithiothreitol, and 10% glycerol (pH 7.7)). After pull-down and washing of each fusion protein bound to glutathione-agarose beads, bound proteins were separated by SDS-PAGE, and autoradiographs were analyzed by scanning densitometry (Bio-Rad).

Co-immunoprecipitation of NT-PGC-1α with PPARα—Whole cell extracts from COS cells expressing PPARα-FLAG and NT-PGC-1α-HA were pre-cleared with protein G and incubated with either anti-HA, rabbit preimmune IgG (Santa Cruz Biotechnology), and either anti-FLAG (Sigma; FLAG-M2) or mouse preimmune IgG (Santa Cruz Biotechnology) at 4 °C. After incubation with protein G, the supernatants were used to evaluate immunoprecipitation efficiency, although the remaining pellets were probed with anti-FLAG or anti-HA antibody to measure protein-protein interaction, followed by re-probing with either anti-HA or anti-FLAG to evaluate the immunoprecipitation efficiency.

Recruitment of NT-PGC-1α to Target Gene Promoters—Chromatin immunoprecipitation assays were used to examine protein-DNA interaction of NT-PGC-1α with the Ucp1 or Cpt-1β promoters in brown adipocytes stably transformed with NT-PGC-1α-HA (30, 31). In brief, 4 ODs of the cross-linked, pre-cleared chromatin was immunoprecipitated with rabbit anti-HA antibody or anti-rabbit IgG (Santa Cruz Biotechnology). Real-time PCR was used to quantify protein-DNA interaction of NT-PGC-1α with the Ucp1 or Cpt-1β promoters, and specificity of the PCRs was confirmed by agarose gel electrophoresis. The respective primers were as follows: Ucp1 promoter F, 5′-GAG AAG AAC ACG GAC ACT AGG, and Ucp1 promoter primer R, 5′-GGG ACT AGG GAG ATG TGG AG; Cpt-1β promoter F, 5′-ACC TTG AGC CCT GGA ATT AGG-3′, and Cpt-1β promoter R, 5′-ACC AGA ACA CCC TCC TTT TGG-3′. Intragenic regions 3′ of the respective Cpt-1β and Ucp1 promoters were amplified as negative controls with the following primers: Cpt-1β gene F, 5′-GTC CTT GAC TCT TTT GTG GAC ACC, and Cpt-1β gene R, 5′-GGT CAT AGG GTA TGC TTT GGA CAC T; Ucp1 gene F, 5′-CTT GCA TTA AGG CAG TTT CCC ACT G and Ucp1 gene R, 5′-GGA TGT CTA TTT AAT AGC AGC TTT GTC.

Assessment of Mitochondrial Biogenesis—Changes in mitochondrial DNA copy number were assessed by measuring the ratio of mitochondria to nuclear DNA (32) in differentiated brown adipocytes stably transformed with empty vector, NT-PGC-1α-HA or PGC-1α-V5. In companion experiments, mitochondrial mass was measured using fluorescence microscopy with TRITC filters (excitation 484/15 and emission 555/25) in differentiated brown adipocytes pretransformed with Mitofluor Red 589 (Invitrogen).

RESULTS

Alternative 3′-Splicing of Pgc-1α Gene Produces Truncated Form of PGC-1α—In the process of cloning full-length Pgc-1α from a mouse BAT cDNA library, we identified cDNA clones that contained an extra 31 nucleotides between nucleotides 891 and 892 of the published sequence. The insert corresponded to 31 bp at the 3′ end of the sixth Pgc-1α intron, which is normally spliced out during gene transcription (Fig. 1A). Examination of the intronic sequence revealed two consensus 3′ splice acceptor sites in intron 6 as follows: an upstream site that leads to the 31-bp inclusion we identified, and a downstream site that leads to the known, reported Pgc-1α mRNA isoform. Splicing at the upstream acceptor site is noteworthy because it introduces an in-frame stop codon (Fig. 1A). Translation of the alternatively spliced mRNA with the 31-bp insert is predicted to produce a truncated form of PGC-1α containing the first 267 aa of
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PGC-1α and 3 additional aa from the first three codons of the insert before the stop codon. We denote the truncated form of PGC-1α as N-truncated PGC-1α (NT-PGC-1α). Examination of the NCBI nucleotide data base shows a variant form of human Pgc-1α mRNA (accession number AB061325) that uses the same alternative splicing site we have detected for mouse Pgc-1α mRNA (accession number AB061324). In addition, alignment of the genomic DNA sequences from the end of exon 6 to the beginning of exon 7 for mouse, rat, human, chimp, cow, dog, horse, and cat Pgc-1α reveals that the sequence spanning the 3’ splice sites for NT-Pgc-1α and Pgc-1α identified in the mouse sequence are completely conserved across all of these species (Fig. 1B).

Examination of the predicted NT-PGC-1α protein indicates retention of two essential PGC-1α domains as follows: the N-terminal domain (Fig. 1C) that recruits SRC-1 and CREB-binding protein and carries the transcriptional activation function, and the two LXXLL-like (L2–L3) motifs that mediate interactions with NRs. NT-PGC-1α also retains two of the three described p38 MAPK phosphorylation sites (13). NT-PGC-1α lacks the nuclear localization sequences of the parent protein, the ligand-independent PPARγ binding region (aa 338–403), the SR-rich and RRM domains, the FOXO1, MEFC2, and TRAP220 interaction domains (6, 33), and multiple sites of post-translation modification and regulation (e.g. the GSK-3β, AMP kinase, and AKT phosphorylation sites (12–14, 23), the arginine methylation sites (24), multiple lysine acetylation sites (11, 34), and the C-terminal domains involved in regulating protein stability (25)). Thus, the truncated domain structure of NT-PGC-1α infers that it would possess unique properties encompassing functions that are both complementary to and unique from full-length PGC-1α (Fig. 1C).
In Vivo and in Vitro Regulation of NT-PGC-1α and PGC-1α Expression—A biological role for NT-PGC-1α is predicated on it being expressed. To evaluate NT-Pgc-1α expression in a relevant physiological context, we determined the levels of NT-Pgc-1α and Pgc-1α mRNA (using isofrom-specific primers, see supplemental Fig. S1 and Fig. S2 for details) and protein in the BAT of C57BL/6J mice kept at room temperature or exposed to cold (4 °C) for 5 h. At the mRNA level, NT-Pgc-1α and Pgc-1α were expressed at a ratio of 1 to 1.75 in BAT from control mice retained at room temperature (23 °C) (Fig. 2A). Cold exposure induced both mRNA species dramatically, by 16.3-fold (NT-Pgc-1α) and 14.7-fold (Pgc-1α), resulting in comparable expression of the two isoforms (Fig. 2A). At the protein level, NT-Pgc-1α was weakly detected at ~35 kDa in BAT whole cell extracts from mice at room temperature, whereas Pgc-1α was essentially undetectable under these conditions (Fig. 2A). Cold exposure produced a significant increase in the expression of both isoforms (Fig. 2A). Thus, in BAT, NT-Pgc-1α mRNA is an abundant message that is present at levels comparable with those of Pgc-1α mRNA, is increased by exposure to cold, and leads to expression of a truncated PGC-1α protein of the expected size.

Expression to cold leads to β-adrenergic stimulation and increased cAMP in BAT. To examine whether NT-PGC-1α expression was regulated by cAMP in an in vitro model of adaptive thermogenesis, differentiated brown adipocytes were treated with vehicle or the cell-permeable cAMP analog 8-CPT-cAMP for 12 h. As observed in BAT from mice at room temperature, basal expression of both proteins was low to undetectable (Fig. 2B). The cAMP analog mimicked the effect of cold exposure in BAT and produced a significant increase in expression of both proteins in the cells (Fig. 2B). These results complement our in vivo findings and illustrate that NT-PGC-1α expression is similarly regulated in both models by the physiological signals that regulate full-length PGC-1α.

In liver, PGC-1α is known to be induced upon fasting (4, 7). Hepatic PGC-1α and NT-PGC-1α mRNA levels were similar in fed mice and induced by fasting by >10-fold (Fig. 2C). As in BAT, transcriptional activation of the hepatic PGC-1α gene by fasting did not alter the splicing ratio of the respective isoforms. NT-PGC-1α protein was faintly detected as a doublet around 35 kDa in hepatic nuclear extracts from fed mice, whereas full-length PGC-1α was essentially undetectable under these conditions (Fig. 2C). Fasting induced a significant increase in expression of both protein isoforms, proportional to the increase in mRNA of each isoform under these conditions (Fig. 2C).

To assess whether NT-PGC-1α is expressed in other tissues where PGC-1α function is important, NT-Pgc-1α and Pgc-1α mRNA levels were also examined in brain and kidney (Fig. 2D), as well as skeletal muscle, white adipose tissue, spleen, and heart. Brain was unique among the tissues studied in having the highest ratio of NT-Pgc-1α to Pgc-1α mRNA, whereas NT-Pgc-1α and Pgc-1α mRNA levels were comparable in skeletal muscle, white adipose tissue, spleen, and heart (data not shown). Thus, alternative splicing of Pgc-1α does not appear to favor formation of either transcript, except in brain where NT-Pgc-1α mRNA appears to be slightly favored. To confirm that the 35-kDa protein we detect with our PGC-1α antibody is indeed derived from the Pgc-1α-encoding gene, we probed tissue lysates from control and PGC-1α null mice (15). NT-PGC-1α was specifically detected at ~35–38 kDa in BAT, brain, and kidney in control but not PGC-1α null mice (Fig. 2E). NT-PGC-1α protein expression was highest in brain and lowest in liver, whereas expression in BAT and kidney was comparable and intermediate to liver and brain (Fig. 2E). It is currently unclear why NT-PGC-1α migrates at slightly different sizes among the tissues. Given the potential for post-translational modification and the existence of alternative promoters and first exons (35), it seems likely that tissue-specific modifications and/or promoter usage contribute to differential mobility (Fig. 2E). Finally, the rank order of mRNA (Fig. 2D) and protein expression (Fig. 2E) was roughly comparable among tissues.

As noted in Fig. 1B, the highly conserved sequence around the alternative 3′ splice sites of PGC-1α suggests that NT-PGC-1α should be readily detected in tissues from these species. To test this hypothesis, total RNA and whole cell lysates from BAT of rats exposed to cold (4 °C) or room temperature for 5 h were examined, along with rat brain and human brain and heart. In rat BAT, neither PGC-1α isoform was detectable at room temperature by Western blot (Fig. 2F), whereas cold exposure produced a robust increase in expression of both isoforms. The fold increase is difficult to estimate, even when 3-fold more BAT whole cell extract is loaded from room temperature versus cold-exposed rats (Fig. 2F). However, the relative expression of both isoforms appears comparable at the 5-h time point (Fig. 2F), and as noted previously, the NT-PGC-1α isoform is expressed as a doublet in this tissue. In contrast, NT-PGC-1α is expressed as a single, more slowly migrating protein in whole cell lysates from rat brain (Fig. 2G). In addition, full-length PGC-1α is faintly detected in these extracts (Fig. 2G). NT-PGC-1α protein is also readily detected in whole cell extracts of human heart collected 3–4 h post-mortem (Fig. 2H). Full-length PGC-1α is not evident in this Western blot (Fig. 2H), although isolation of nuclei and probing of nuclear extracts revealed expression of PGC-1α (data not shown). Messenger RNA for both protein isoforms was readily detected in our human heart sample and in both tissues from rat (data not shown). Together, these findings strongly support the conclusion that NT-PGC-1α is co-expressed with PGC-1α in human, rat, and probably many other species.

We noted that although NT-Pgc-1α and Pgc-1α mRNA levels were similar in most tissues, NT-Pgc-1α protein was detected at higher levels than full-length PGC-1α (e.g. Fig. 2, A–C). Although we cannot exclude that differences in the physical properties of the two isoforms (e.g. higher solubility in the...
extraction buffer or more efficient transfer of NT-PGC-1α from the gel to the membrane) contribute to the differences in detected levels, we considered that the two isoforms could have different turnover rates. We thus assessed the relative targeting of PGC-1α and NT-PGC-1α to the proteosome. Following transient transfection of CHO-K1 cells with similar PGC-1α and NT-PGC-1α expression vectors, the respective protein levels were determined after treating the cells with vehicle or the proteosome inhibitor MG132 for 5 h. In vehicle-treated conditions, NT-PGC-1α expression was ~5-fold higher than PGC-1α (Fig. 2I). However, the proteins were expressed at comparable levels after inhibition of the proteosome, the result of a 10.6-fold increase in PGC-1α expression but only a 1.8-fold increase in NT-PGC-1α (Fig. 2I). The results support the view that NT-PGC-1α is degraded less quickly by the proteosome than the relatively short lived PGC-1α.

Post-translational Regulation of Subcellular Localization of NT-PGC-1α—Although NT-PGC-1α lacks the nuclear localization sequences of full-length PGC-1α, the size of the translated protein should not impede it moving through nuclear pores and equilibrating across the nuclear membrane (36). To confirm the presence of NT-PGC-1α in the nucleus and to assess the effects of signaling inputs on its cellular distribution, we measured nuclear and cytoplasmic content of HA-tagged NT-PGC-1α in Chinese hamster ovary cells using confocal microscopy. Analysis of confocal image stacks from cells under basal conditions indicated that ~10% of the total cell NT-PGC-1α-HA signal co-localized with DAPI (Fig. 3A). Stated another way, ~90% of the cellular content of NT-PGC-1α-HA was found in the cytosol under these conditions (Fig. 3A). To examine whether signaling inputs known to regulate PGC-1α under physiological conditions affected subcellular distribution of NT-PGC-1α, cells were treated with 8-CPT-cAMP for
1 h in the absence and presence of PKA (H89) and p38 MAPK (SB203580) inhibitors. Activation of PKA increased nuclear content of NT-PGC-1α from 11.1 ± 0.4% (n = 58) to 19.2 ± 0.6% (n = 58) (Fig. 3, A, B, and E). Forskolin, which increases cAMP by directly activating adenyl cyclase, produced a similar enrichment in nuclear content of NT-PGC-1α (data not shown). Inhibition of PKA with H89 blocked the 8-CPT-cAMP response (Fig. 3, C and E) and even reduced nuclear content of NT-PGC-1α to below basal levels (11.1 ± 0.4%) to 8.6 ± 0.3% (Fig. 3, C and E), suggesting that basal PKA activity provides input to subcellular distribution of NT-PGC-1α. The PKA-dependent increase in nuclear content of NT-PGC-1α was also decreased by ~38% when p38 MAPK was inhibited by SB203580 (Fig. 3, D and E). Collectively, these results show that PKA regulates nuclear content of NT-PGC-1α through both p38 MAPK-dependent and -independent mechanisms.

To assess whether the observed fraction of NT-PGC-1α in the nucleus could be the result of NT-PGC-1α equilibrating across the nuclear membrane, we next used a cell-specific probe in conjunction with DAPI to measure cytoplasmic and nuclear volumes in confocal image stacks of Chinese hamster ovary cells under basal and PKA-stimulated conditions. Nuclear volume included 14.7 ± 0.9% of total cell volume under basal conditions and 18.6 ± 0.7% of total cell volume in 8-CPT-cAMP-treated cells. These measures, used in conjunction with measures of total NT-PGC-1α signal intensity in the cytosol and in the nucleus, allowed us to calculate the ratio of nuclear to cytoplasmic NT-PGC-1α concentration. Under basal conditions, the ratio of nuclear to cytoplasmic NT-PGC-1α was 0.680 ± 0.003, indicating a higher concentration of NT-PGC-1α in the cytosol versus the nucleus. Upon activation of PKA with 8-CPT-cAMP, the ratio increased to 1.200 ± 0.09, indicating accumulation of NT-PGC-1α in the nucleus slightly beyond equilibration across the nuclear membrane. Collectively, these findings suggest a system of regulated access of NT-PGC-1α to the nucleus under signaling conditions that favor its participation in transcriptional activation. It will be important in future studies to establish how NT-PGC-1α is being excluded from the nucleus under basal conditions and how signaling inputs from PKA and p38 MAPK regulate this process.

To examine subcellular distribution of NT-PGC-1α in a biologically relevant cell context, studies were conducted in immortalized brown adipocytes stably transformed with HA-tagged NT-PGC-1α. Although NT-PGC-1α is an endogenous protein in our brown adipocyte cell line, the induction of its expression by PKA (Fig. 2B) has the potential to confound measurement of changes in subcellular localization under these conditions. The strategy of measuring subcellular localization of epitope-tagged NT-PGC-1α in this cell line avoids these caveats and provides the added benefit of examining the issue in cells before and after differentiation. In undifferentiated preadipocytes (Fig. 4A), 10.0 ± 0.3% of total cellular NT-PGC-1α was found in the nucleus under basal conditions (Fig. 4, A and E), and treatment with 8-CPT-cAMP increased nuclear content to 27.7 ± 0.7% (Fig. 4, B and E). In differentiated brown adipocytes, a similar 10.7 ± 0.3% of cellular NT-PGC-1α was found in the nucleus under basal conditions (Fig. 4C), and activation of PKA with 8-CPT-cAMP increased nuclear content to 18.7 ± 0.6% (Fig. 4, D and E). Together, these data indicate that NT-PGC-1α is primarily cytosolic under basal conditions, but in both preadipocytes and adipocytes PKA activation significantly increases the nuclear content of the protein.

**Transactivation and Physical Interaction of NT-PGC-1α with PPARs**—NT-PGC-1α retains important functional domains, including the leucine-rich motifs that mediate interactions with nuclear receptors (9, 17, 20). It is of particular interest that the domain at aa 338–403 of PGC-1α reported to confer ligand-independent interaction with PPARγ (1) would be absent from NT-PGC-1α. We used three distinct but complementary approaches to assess the ligand dependence of the interaction of NT-PGC-1α with PPARγ and PPARα. In the first approach, interaction of NT-PGC-1α or PGCG1α with PPARγ or PPARα fused to Gal4 was measured using the Gal4-responsive luciferase reporter pGK1. Expression relative to pGK1 activation by PPARγ-Gal4 or PPARα-Gal4 alone, transactivation of PPARα-Gal4 by NT-PGC-1α (40 ± 4-fold) was 102 ± 2-fold in the absence of ligand (Fig. 5A). Addition of ligand increased PPARα-Gal4 activity to the same maximal level with NT-PGC-1α and PGC-1α (Fig. 5A), although the fold increase with NT-PGC-1α (8.4-fold) was significantly larger than PGC-1α (3-fold). In the absence of ligand, transactivation of PPARγ-Gal4 by PGC-1α (308 ± 38-fold) was also significantly greater than by NT-PGC-1α (40 ± 4). Addition of ligand produced only a modest 1.9-fold increase in PPARγ-Gal4 activity in the presence of PGC-1α, consistent with a significant ligand-independent activation of PPARγ by PGC-1α (1). In contrast, transactivation of PPARγ-Gal4 by NT-PGC-1α, which was low in the absence of ligand, was increased 8.4-fold by addition of ligand.
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To examine transcriptional activation of PGC-1α target genes by NT-PGC-1α, immortalized brown adipocytes were stably transformed with lentiviral constructs expressing HA-tagged NT-PGC-1α, V5-epitope tagged PGC-1α, or empty vector. Selection pressure (blasticidin) was applied for 16 days, and 4–5 clones from each line were screened to select lines expressing NT-PGC-1α-HA and PGC-1α-V5 (Fig. 6A) for further study. Endogenous NT-PGC-1α was detected at 35–37 kDa and comparably expressed in the empty vector (EV) and PGC-1α-V5 cell lines, whereas NT-PGC-1α was detected as a doublet expressed at significantly higher levels in the NT-PGC-1α-expressing lines (Fig. 6A). In the PGC-1α-V5 transformed lines, full-length PGC-1α was detected at 113 kDa and at this exposure the protein was not visible in the EV or NT-PGC-1α-HA cell lines (Fig. 6A). In contrast, expression of aP2 was similar among the lines, indicating that the cell lines were comparably differentiated (Fig. 6A).

To examine the ability of NT-PGC-1α to increase transcription of PGC-1α target genes, differentiated adipocytes derived from the respective cell lines were treated with vehicle or a mixture containing PPARα + retinoid X receptor α ligands or PPARγ + retinoid X receptor α ligands for 12 h, followed by treatment with 8-CPT-cAMP for 6 h. *P<0.001 vs Control

FIGURE 3. Regulation of subcellular distribution of NT-PGC-1α in CHO-K1 cells. A, representative confocal images of subcellular distribution of NT-PGC-1α-HA in CHO-K1 cells transfected with expression construct for NT-PGC-1α-HA for 24 h and treated with vehicle for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. B, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in CHO-K1 cells transfected with expression construct for NT-PGC-1α-HA for 24 h and treated with 100 μM 8-CPT-cAMP for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. C, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in CHO-K1 cells transfected with expression construct for NT-PGC-1α-HA for 24 h and treated with PKA inhibitor (H89) and 100 μM 8-CPT-cAMP for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. D, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in CHO-K1 cells transfected with expression construct for NT-PGC-1α-HA for 24 h and treated with p38 MAPK inhibitor (SB203580) and 100 μM 8-CPT-cAMP for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. E, image stacks from confocal imaging of cells represented in A–D were analyzed using ImageJ software, and the signal intensity from NT-PGC-1α co-localized with DAPI was expressed relative to signal intensity in the whole cell. Means ± S.E. are representative of 80–100 cells per treatment from three to four separate experiments.
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lines, and treatment with either mixture induced Ucp1 mRNA and protein expression in all three lines (Fig. 6B). However, the increase in Ucp1 mRNA in the NT-PGC-1α cell lines was 10-fold greater than the response in the empty vector line, reaching 5.2 ± 0.5 fmol of Ucp1 mRNA/μg of RNA. These levels are similar to levels seen in intact BAT (37) and are 2–4-fold higher than the induction observed in the PGC-1α-V5-transformed cell line. This may be related in part to higher expression of NT-PGC-1α versus PGC-1α in the respective cell lines (Fig. 6A), even though one should keep in mind that only 10–20% of NT-PGC-1α protein is nuclear. UCP1 protein expression paralleled Ucp1 mRNA in that it was nearly undetectable in all three vehicle-treated lines. The ligand mixtures increased UCP1 expression in all three lines, although the increase with NT-PGC-1α was ∼3-fold higher than the comparable increase observed between the empty vector and PGC-1α-V5 lines (Fig. 6B).

In addition to the thermogenic target UCP1, we determined the effect of PGC-1α and NT-PGC-1α on Cpt-1β, which regulates the entry of fatty acids into the mitochondria for oxidation. In the absence of the ligand mixture, basal expression of Cpt1β mRNA was 18-fold higher in the NT-PGC-1α-HA line versus the EV line (Fig. 6C). In contrast, basal expression of Cpt1β mRNA was only modestly higher (2-fold) in PGC-1α-V5 compared with the EV lines (Fig. 6C). The similar expression of aP2 among the lines indicates that Cpt-1β mRNA expression was compared at similar states of differentiation (Fig. 6C). The induction of Cpt-1β by the ligand mixtures was similarly enhanced by PGC-1α (3.9-fold) and NT-PGC-1α (4.1-fold) relative to EV, which differs from the significantly greater induction of Ucp1 by NT-PGC-1α relative to PGC-1α (see Fig. 6B).

Together, these data illustrate first that PGC-1α target genes can be transcriptionally activated by NT-PGC-1α and second that PGC-1α and PGC-1α are likely to display differential activity at different targets and/or contexts, with PGC-1α preferentially enhancing expression of a subset of PGC-1α targets (e.g. basal expression of CPT1β).

Cyclic AMP-dependent Recruitment of NT-PGC-1α to Transcriptional Complexes Bound to Target Gene Promoters—The ability of NT-PGC-1α to enhance expression of known PPAR/PGC-1α target genes suggests that NT-PGC-1α is recruited to the Cpt1β and Ucp1 promoters in a cAMP-dependent manner. To assess NT-PGC-1α recruitment at these targets, we performed chromatin immunoprecipitations from differentiated brown adipocytes treated with vehicle or a ligand mixture containing 8-CPT-cAMP, WY14693, and 9-cis-RA. The amount of

![FIGURE 4. Regulation of subcellular distribution of NT-PGC-1α in undifferentiated and differentiated brown adipocytes. A, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in undifferentiated brown adipocytes stably transformed with NT-PGC-1α-HA and treated with vehicle for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. B, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in undifferentiated brown adipocytes stably transformed with NT-PGC-1α-HA and treated with 100 μM 8-CPT-cAMP for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. C, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in fully differentiated brown adipocytes stably transformed with NT-PGC-1α-HA and treated with vehicle for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. D, representative confocal images showing subcellular distribution of NT-PGC-1α-HA in fully differentiated brown adipocytes stably transformed with NT-PGC-1α-HA and treated with 100 μM 8-CPT-cAMP for 1 h prior to fixation and imaging. The images on the left and right are presented with and without DAPI staining. E, image stacks from confocal imaging of all cells represented in A–D were analyzed using ImageJ software, and the signal intensity from NT-PGC-1α co-localized with DAPI was expressed relative to signal intensity in the whole cell. Means ± S.E. are representative of 50–60 cells per treatment from three to four separate experiments.](image)
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FIGURE 5. Physical and functional interaction of NT-PGC-1α-HA with PPARα and PPARγ. A, ligand-dependent transactivation of Gal4-PPARα and Gal4-PPARγ by PGC-1α and NT-PGC-1α in Chinese hamster ovary cells transiently transfected with GAL4-responsive luciferase reporter gene (pGK) and a pRL-SV40 plasmid expressing Renilla luciferase for normalization. Luciferase activity was measured 24 h after treatment with vehicle (DMSO), 10 μM WY14693, or 10 μM BRL49653. Fold changes in luciferase activity were expressed relative to cells transfected with pGK alone. Data represent means ± S.E. from three experiments. B, ligand-dependent interaction of 35S-PPARγ or 35S-PPARα by GST-tagged PGC-1α and NT-PGC-1α. The 35S-labeled nuclear receptors were produced by Tnt T7 in vitro translation system and incubated with GST-tagged PGC-1α or NT-PGC-1α for 1 h in the absence and presence of ligands for the respective receptors. Autoradiograms are representative of three experiments. C, co-immunoprecipitation analysis of protein-protein interaction of HA-tagged NT-PGC-1α with FLAG-tagged PPARα in COS cells. 24 h after treatment with vehicle (DMSO) or 10 μM WY14693, whole cell extracts were immunoprecipitated with anti-FLAG (top panel), anti-HA (bottom panel), mouse preimmune IgG (top panel), or rabbit IgG (bottom panel). Using Western blots (WB), the respective immunoprecipitates were sequentially probed with anti-HA and anti-FLAG (top panel) or anti-FLAG and anti-HA (bottom panel) to test for co-IP of NT-PGC-1α and PPARα and IP efficiency. Blots are representative of four experiments.

DISCUSSION

Alternative splicing expands the translational repertoire of numerous genes by producing multiple transcripts that encode protein-protein interaction of HA-tagged NT-PGC-1α with FLAG-tagged PPARα in COS cells. 24 h after treatment with vehicle (DMSO) or 10 μM WY14693, whole cell extracts were immunoprecipitated with anti-FLAG (top panel), anti-HA (bottom panel), mouse preimmune IgG (top panel), or rabbit IgG (bottom panel). Using Western blots (WB), the respective immunoprecipitates were sequentially probed with anti-HA and anti-FLAG (top panel) or anti-FLAG and anti-HA (bottom panel) to test for co-IP of NT-PGC-1α and PPARα and IP efficiency. Blots are representative of four experiments.
different protein sequences, and most transcribed genes contain sequences that can be alternatively spliced (26). Tile-based microarrays and massive parallel sequencing have been used to interrogate the transcriptional complexity among tissues (39) and in cells during different growth phases and conditions (40). The studies show reproducible patterns of alternative splicing that are tissue- and cell type-specific, regulated during proliferation and differentiation, and responsive to post-translational inputs. Thus, regulated splicing of the transcriptome may be deterministic with respect to cell identity and function. The degree to which transcriptional complexity is actually translated is uncertain, given that 30% of alternative mRNA isoforms contain premature termination codons (41, 42). Premature termination codons are predicted to target these transcripts for rapid decay by nonsense-mediated RNA decay (43, 44), a surveillance system that preemptively rids the cell of truncated versions of proteins with potentially deleterious dominant negative or gain of function activity (43, 44). Accordingly, to pro-
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The expression of endogenous PGC-1α protein is often difficult to detect due to its relatively short half-life (13) and also due to numerous post-translational modifications that may modify antibody recognition of epitopes in affected sequences (11–13, 20, 24, 34, 45). Measurement of PGC-1α and NT-PGC-1α expression in this study was accomplished using two antibodies recognizing epitopes spanning the N-terminal transactivation domain of PGC-1α, a region common to the two isoforms where post-translational modification has not been reported. The predicted molecular mass of NT-PGC-1α is ~30 kDa, but both in vitro translated and overexpressed NT-PGC-1α are detected at ~35–38 kDa. This is similar to the difference between predicted (92 kDa) and observed (113 kDa) molecular mass of PGC-1α (1). Expression of endogenous PGC-1α and NT-PGC-1α was increased in BAT by short term fasting and cold exposure, and in immortalized brown adipocytes after treatment with a cell-permeable cAMP analog. Induction of PGC-1α and NT-PGC-1α was also detected after an overnight fast in liver nuclear extracts. Brain was the site of highest NT-Pgc-1α expression among the tissues surveyed, and it was also the site where splicing appeared to favor formation of NT-Pgc-1α mRNA over PGC-1α. We found no evidence that cold exposure or fasting altered NT-Pgc-1α expression in the whole brain, and the signals or physiological states that regulate its expression in this site remain unclear. In addition to differences in relative expression levels of NT-Pgc-1α among tissues, we observed subtle differences in apparent molecular mass. The basis for this is unclear, although tissue-specific differences in alternative promoter/first exon usage and the type or extent of post-translational modifications could be involved.

In tissues studied to date, basal expression of NT-PGC-1α protein is consistently higher than full-length PGC-1α despite similar or lower levels of mRNAs. We hypothesized that the splice variant may be more stable based on the absence of the second Cdc4 phosphodegron (aa 293–300) (14) and C-terminal arginine/serine-rich domains involved in targeting PGC-1α for ubiquitination and degradation (25). Our findings that short term proteosome inhibition produced only modest increases in NT-PGC-1α (~2-fold) compared with dramatic increases in PGC-1α (10–11-fold) are consistent with this prediction.

Based on its size, it is surprising that NT-PGC-1α is primarily cytosolic in untreated cells and equilibrated across the nuclear membrane only after activation of PKA. One possibility is that NT-PGC-1α is bound to cytosolic proteins that affect its ability to diffuse into the nucleus. Although activation of PKA allows NT-PGC-1α to equilibrate across the nuclear membrane, it is unclear whether NT-PGC-1α is actually a substrate of PKA. At least part of the response can be attributed to PKA-dependent activation of p38 MAPK, which phosphorylates PGC-1α (13, 46); however, the major proportion of the response is retained after inhibition of p38 MAPK (Fig. 3, D and E). In contrast, PKA inhibition completely blocked cAMP-dependent redistribution of NT-PGC-1α to the nucleus and actually lowered nuclear content to a level below that seen in vehicle-treated cells. These findings indicate that PKA provides input to regulating NT-PGC-1α distribution within the cell over the full range of its activation state. To understand this response, it will be important to determine whether PKA is regulating the interaction between NT-PGC-1α and a protein(s) that alters its ability to equilibrate across the nuclear membrane.

Despite the fact that only 10–20% of exogenously expressed NT-PGC-1α protein is nuclear, we provide unambiguous data that NT-PGC-1α physically interacts with and transactivates nuclear receptors in a ligand-dependent manner, suggesting that nuclear NT-PGC-1α is active. The conditions that increase nuclear content of NT-PGC-1α (i.e., PKA activation) also increase transcriptional activity of PPARγ, PPARα, and recruitment of NT-PGC-1α to associated transcriptional complexes. However, it should be noted that under these conditions, a significant amount of total cellular NT-PGC-1α is retained in the cytoplasm. The implications of this unique property of NT-PGC-1α could be related to recent work showing that C-terminal domains in PGC-1α that are not present in NT-PGC-1α
target nuclear PGC-1α for polyubiquitination and proteosomal degradation (25). One interpretation is that regulation of PGC-1α activity is accomplished by somewhat different means for the two PGC-1α isoforms. For example, full-length PGC-1α activity may be predominantly regulated by ubiquitination and degradation and NT-PGC-1α activity by nuclear/cytoplasmic relocalization. Distinct regulatory mechanisms may increase adaptability in responses to specific signals (e.g. to signals that activate a specific protein isoform) and strengthen or extend the duration of responses (e.g. to signals that activate both proteins). Clearly, it will be important to understand how the responses of the PGC-1α isoforms are integrated in tissues where both are expressed.

Despite the fact that NT-PGC-1α lacks C-terminal domains proposed to be important for transcriptional activation of endogenous genes (21, 22), we show that NT-PGC-1α leads to a profound enhancement of Ucp1 and Cpt-1β expression in stably transformed brown adipocytes (Fig. 6). In the same cells, NT-PGC-1α, as well as PGC-1α, also led to increased expression of other known PGC-1α target genes (e.g. Tfam, Mctad, Cyt c1, and CoxII) and produced a comparable 2-fold increase in mitochondrial density. A limitation of these studies is that endogenous NT-PGC-1α and PGC-1α are present in the control (empty vector), NT-PGC-1α, and PGC-1α transformed lines so we cannot rule out some contribution of the endogenous proteins to the phenotype of the cells. However, our comparison of protein expression indicates that endogenous PGC-1α is quite low (Fig. 6A). The enhancement of Ucp1 and Cpt-1β expression by NT-PGC-1α was paralleled by clear data showing that NT-PGC-1α coordinates the assembly of transcriptional complexes whose binding to the Ucp1 and Cpt-1β promoters is enhanced by cAMP (Fig. 6, D and E). Although expression of both genes and binding of NT-PGC-1α to the respective promoters was enhanced by cAMP and ligands, a clear distinction in the role of ligands in transactivating each gene was evident among the cell lines. In particular, the basal expression of Ucp1 mRNA and protein was comparable between the NT-PGC-1α and empty vector cell lines, whereas Cpt-1β mRNA was ~15–20-fold higher in the NT-PGC-1α line compared with the empty vector or PGC-1α lines. In contrast, the induction of Ucp1 mRNA by the cAMP ligand mixture was 10-fold higher in the NT-PGC-1α line, and the induction of Cpt-1β mRNA by ligands was comparable between the lines. Although the underlying reasons are unclear, these findings support the idea that NT-PGC-1α and PGC-1α are differentially active in different contexts, with Cpt-1β in the absence of cAMP and PPAR ligands representing a target that is uniquely sensitive to NT-PGC-1α.

Strategies for evaluating the in vivo functions of NT-PGC-1α in the absence of PGC-1α are technically challenging due to the difficulty of selectively disrupting expression of the full-length PGC-1α transcript. However, comparison of PGC-1α null mice produced by different gene targeting strategies (15, 16) reveals some differences that may relate to the function of NT-PGC-1α. PGC-1α null mice produced by Lin et al. (15) lack exons 3–5 (which encode the NR interaction domains) and lead to a frameshift in the remaining exons, consistent with a complete lack of PGC-1α activity. PGC-1α null mice produced by Leone et al. c retain exons 1–5, which, as noted by the authors, may permit the expression of a truncated form (aa 1–254) of PGC-1α. This protein would be only 16 aa shorter than NT-PGC-1α and would include all major motifs and domains of NT-PGC-1α except for the p38 phosphorylation sites (aa 262 and 268), which could of course be important for regulation of a truncated protein (see Fig. 1C). The mice of Lin et al. (15) display a more severe phenotype with early postnatal mortality (50%) and profound hyperactivity, and the mice of Leone et al. (16) showed no increase in mortality and reduced locomotor activity. Moreover, the induction of Ucp1 mRNA in BAT after 5 h of cold exposure was severely compromised in the Lin et al. (15) mice but uncompromised in the Leone et al. (16) mice. Based on the biological activity of NT-PGC-1α described here, it seems likely that a 1–254-aa form of PGC-1α could be responsible for the phenotypic differences between the two mouse lines. It will be important in future experiments to test for expression and abundance of this form of PGC-1α in the mouse line described by Leone et al. (16).

The data presented here provide a compelling case that NT-PGC-1α is an abundant, previously unrecognized form of PGC-1α that is produced by alternative splicing between exons 6 and 7. Expression of NT-PGC-1α in BAT and liver is dynamically regulated in the context of the physiological signals that regulate full-length PGC-1α. NT-PGC-1α is also expressed in other tissues where PGC-1α plays important roles in regulation of cell function and energy metabolism and is particularly abundant in the central nervous system, where PGC-1α has a neuroprotective function (10, 15). The truncated domain structure of NT-PGC-1α conveys unique properties with respect to subcellular localization, protein stability, transcription factor interaction, and target gene activation. We propose that the unique properties of NT-PGC-1α enable it to produce a cell context-specific subset of responses that complement, overlap, or prolong the actions of PGC-1α. As such, it represents a novel component and an additional level of complexity within the signaling network that mediates adaptive responses in metabolically active tissues.

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