The Expression of CNS-Specific PPARGC1A Transcripts Is Regulated by Hypoxia and a Variable GT Repeat Polymorphism

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Abstract
PPARGC1A encodes a transcriptional co-activator also termed peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha (PGC-1α) which orchestrates multiple transcriptional programs. We have recently identified CNS-specific transcripts that are initiated far upstream of the reference gene (RG) promoter. The regulation of these isoforms may be relevant, as experimental and genetic studies implicated the PPARGC1A locus in neurodegenerative diseases. We therefore studied cis- and trans-regulatory elements activating the CNS promoter in comparison to the RG promoter in human neuronal cell lines. A naturally occurring variable guanidine thymidine (GT) repeat polymorphism within a microsatellite region in the proximal CNS promoter increases promoter activity in neuronal cell lines. Both the RG and the CNS promoters are activated by ESRRα, and the PGC-1α isoforms co-activate ESRRα on their own promoters suggesting an autoregulatory feedback loop. The proximal CNS, but not the RG, promoter is induced by FOXA2 and co-activated by PGC-1α resulting in robust activation. Furthermore, the CNS, but not the RG, promoter is targeted by the canonical hypoxia response involving HIF1α. Importantly, the transactivation by HIF1α is modulated by the size of the GT polymorphism. Increased expression of CNS-specific transcripts in response to hypoxia was observed in an established rat model, while RG transcripts encoding the full-length reference protein were not increased. These results suggest a role of the CNS region of the PPARGC1A locus in ischemia and warrant further studies in humans as the activity of the CNS promoter as well as its induction by hypoxia is subject to inter-individual variability due to the GT polymorphism.

Keywords PPARGC1A · PGC-1α · CNS-specific isoforms · FOXA2 · ESRRα · Hypoxia · Microsatellite

Introduction
Peroxisome proliferator–activated receptor gamma co-activator 1A (PGC-1α) encoded by PPARGC1A, is a versatile transcriptional co-activator involved in multiple transcriptional programs [1–3]. A key issue to understand the diverse functions of PGC-1α is to gain insight into the mechanisms that confer specificity to its interactions with

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numerous transcription factors. The regulation of PGC-1α function occurs at several levels. Expression of PGC-1α and the transcription factors co-activated by it are critical for some pathways [4]. Furthermore, PGC-1α is targeted by several signaling pathways at the posttranslational level, as site-specific phosphorylation [5, 6], acetylation [7], ubiquitination [8, 9], and methylation [10] have been shown to alter the stability and/or activity of PGC-1α. Alternative splicing and/or transcriptional initiation represent another level of control that results in gain or loss of domains interacting with signaling targets. The truncated NT-PGC-1α resulting from alternative splicing contains the N-terminal activation and nuclear interaction domains and displays functional differences in comparison to the full-length protein [11, 12]. In skeletal muscle, two novel promoters have been described 14 kbp upstream of the reference promoter that are differentially regulated in response to metabolic cues such as fasting and exercise [13, 14]. In the human liver, an alternative promoter in intron 2 of the reference gene gives rise to a 75-kDa protein that showed subtle differences in co-activation selectivity compared to the reference protein [15]. We recently described several distinct CNS-specific PPARγ1A mRNAs that are transcribed from a novel promoter located ~586 kbp upstream of the reference promoter. These transcripts are more abundant than reference gene (RG) mRNAs in human post-mortem brain samples and are partially conserved in rodents [16].

PGC-1α activates transcriptional programs that are relevant for neurodegenerative diseases such as mitochondrial biogenesis and function, the defense against reactive oxygen species, and autophagy [17–21]. Indeed, several functional studies in animal or cell culture models suggest that reduced PGC-1α function plays a role in neurodegenerative diseases [22–27]. In addition, PGC-1α has been implicated in multiple sclerosis [28, 29]. Genetic studies in humans have suggested associations of the PPARγ1A locus with Alzheimer’s, Huntington’s, and Parkinson’s disease and amyotrophic lateral sclerosis [16, 30–36]. As some of these associations involved the CNS-specific region of the PPARγ1A gene, it is reasonable to suspect that the CNS-specific isoforms and/or their regulation play a role in these disorders. While the signaling pathways that converge at the RG promoter have been identified in mouse muscle and liver [reviewed in 37] as well as in human tissues and cell lines [15, 38], virtually nothing is known about the regulation of the CNS-specific promoter with the exception of its activation by USF1 [16]. We therefore have compared the regulation of the CNS-specific and RG PPARγ1A promoters in human neuronal cell lines and a rat model of ischemia. We identified not only similarities but also substantial differences in the transcriptional regulation of the two promoters.

Materials and Methods

Plasmids

Expression plasmids pPGC-1α [39], B4-PGC-1α, B5-PGC-1α, B4-7a-PGC-1α, B5-7a PGC-1α [16], and FOXA2 [39] were described previously. The human ESRRA (NM_001282450) ORF Clone in pcDNA 3.1/C-(K)-DYK was purchased from GenScript (Piscataway, NJ, USA). HA-HIF-1α-pcDNA3 and HA-HIF-2α-pcDNA3 were gifts from William Kaelin (Addgene plasmids #18949 and #18950 respectively [40]). Long and short promoters of both the RG [15] and CNS-specific PPARγ1A [16] have been described. Short CNS-specific promoters with increasing guanidine thymidine (GT) sizes (602 bp, −539 to +63 relative to the transcription start site in promoters containing 11 GT) were cloned into pGL4.11[luc2P] (Promega, Madison, WI, USA) from DNA of subjects that harbored identical CNS promoter sequences but differed in their GT repeat size. A very short promoter luciferase construct (−84 to +63 bp relative to the transcription start site) was produced by cloning amplification products of the proximal promoter into pGL4.11[luc2P] (Promega). Primers used are shown in Supplementary Material, Table S1.

Cell Culture and Transfection Experiments

SH-SY5Y and NTERA-2cl.D1 (NT2/D1) cells were obtained from ATCC and cultured in DME (D046)/F12 1:1 (Sigma-Aldrich, St. Louis, MO, USA) and DME (D0547)/F12 (Sigma-Aldrich) supplemented with 0.21% NaHCO3, respectively. The murine hippocampal cell line HT22 was a kind gift from D. Schubert, Salk Institute, La Jolla, CA, USA and cultured in DME (D0547)/F12 1:1 supplemented with 0.21% NaHCO3. Media for all cells were supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). All cells were cultured in a humidified 5% CO2 atmosphere at 37 °C. Hypoxia culture experiments were performed in an O2-regulatable incubator (Binder GmbH, Tuttingen, Germany) or in a H135 HEPA hypoxystation. Ciclopirox olamine and deferoxamine were obtained from Sigma-Aldrich. Cells were plated in 24-well plates 1 day before transient transfection with plasmid constructs for 24–48 h using Lipofectamine 3000 (Invitrogen) or DNAfectinTM Plus (ABM Inc., Vancouver, Canada). The pRL-TK plasmid (Promega) was used as a transfection control. Luciferase activities were measured using the Dual Luciferase Reporter Assay System (Promega) as described [15]. Results are repre-
sentative of two or more experiments, each performed in quadruplicate, and are shown as means ± SD.

**RNA and Protein Extraction**

Total RNA from SH-SY5Y, NT2/D1 cells, and rat brain regions was extracted using the QIAzol Lysis Reagent (Qiagen, Hilden, Germany) and the RNeasy Lipid Tissue Mini kit (Qiagen). Concentrations of RNA were measured using the Nanovue Plus spectrophotometer (GE Healthcare, Chicago, IL, USA). In addition, the QIAxcel Advanced Instrument (Qiagen) was used to ascertain RNA quality. RNA integrity scores were between 8 and 9 for all samples used. For total protein preparation of whole cultured cells, cell pellets were solubilized in RIPA buffer containing protease inhibitors (HaltTM Protease Inhibitor Cocktail, Thermo Fisher Scientific, Waltham, MA, USA). Clear supernatants were obtained after centrifugation (10,000×g for 20 min at 4 °C) of suspensions that were intermittently incubated on ice and heated at 95 °C and sonicated (Sonoplus HB 27, Bandelin, Berlin, Germany). Nuclear and cytoplasmic extracts were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. Protein concentrations were determined by the RCDC Protein Quantification Assay (Bio-Rad Laboratories, Inc. Hercules, CA, USA).

**Western Blot Analysis**

Immunoblotting was performed as described [16]. In brief, equal amounts of NT2/D1 cell extracts (20 or 40 μg) were subjected to SDS electrophoresis in 7–10% polyacrylamide gels. Separated proteins were transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories, Inc.) that were blocked with Tris-buffered saline (TBS), pH 7.6, containing 10% Blotting Grade Blocker (Bio-Rad Laboratories, Inc.) for 2 h at room temperature. Membranes were incubated with the primary antibodies directed against HIF1A (HIF-1α, NB100-105, Novus Biologicals, Centennial, CO, USA), diluted 1:1000 in 0.1% Tween 20 in 1× TBS overnight at 4 °C. Membranes were then incubated for 1 h at room temperature with the respective secondary antibody (IRDye® 800CW Goat anti-Rabbit IgG(H + L) or IRDye® 800CW Goat anti-Mouse IgG(H + L) from LI-COR, Lincoln, NE, USA) and analyzed using the ODYSSEY infrared imager (LI-COR). β-Actin (#4967, Cell Signaling technology, Danvers, MA, USA) or Histone H3 (NB500-171, Novus Biologicals) antibodies were used as loading controls. For densitometric analysis of Western blots, Image Studio™ software (LI-COR) was used.

**Real Time PCR**

DNase I-treated total RNA from cell cultures (1 μg/reaction) was reverse transcribed with the QuantiTect Reverse Transcription kit (Qiagen), using a mix of random hexamers and oligo-dT primers. cDNAs were amplified in duplicate by real-time PCR using Maxima SYBR Green (Thermo Scientific) or GoTaq qPCR Master Mix (Promega) and primers targeting exons B1 and B4 or B5 and exon 2 to quantify the two main CNS-specific PPARGC1A transcripts. Primers targeting exon 1 and exon 2 were used to target RG transcripts. The transcripts encoding the class of NT-PGC-1α isoforms were quantified by targeting exon 5 and exon 6A (Supplementary Material, Table S1 for primer sequences). To directly compare the amounts of PPARGC1A transcripts, gene segments containing the sequences targeted by the respective transcript-specific assays were cloned and used for the construction of standard curves to normalize for the efficiency of assays. Primers to quantify VEGF transcripts are also shown in Table S1. Total RNA from rat brain regions was reverse transcribed as described above. As the B5 encoded isoform is not present in rats, we measured B1b4, exon 1, exon 2, exon 5, and exon 6A containing transcripts as well as transcripts encoding VEGF and GLUT1. The respective primers are shown in Supplementary Table S1. For transcript amplification, the Rotor-Gene TM Q (Qiagen) instrument or the LightCycler TM 480 Instrument (Roche, Basel, Switzerland) was used. Relative mRNA levels were calculated using the comparative threshold cycle method (ΔΔCt). Constitutively expressed RPLP0 (Ribosomal Protein, large, P0) mRNA was used for normalization of mRNA abundance.

**ChIP Assays**

The chromatin immunoprecipitation (ChIP)-IT Express Enzymatic kit (Active Motif Europe, La Hulpe, Belgium) was used. Cross-linking of 10⁶ NT2/D1 cells, enzymatic shearing and preparation of input DNA were performed as described [41]. After an overnight incubation with 4 μg of HIF1A-specific monoclonal antibodies at 4 °C, chromatin/ DNA complexes were immunoprecipitated using protein G magnetic beads. After DNA purification, primers described in Supplementary Material, Table S1 were used to amplify fragments of the CNS-specific promoter. As positive control, we designed the human equivalent primers of those described [42] spanning an HRE in the mouse Ntrk2 (TrkB) promoter known to be activated by HIF1A. As a negative control, primers spanning Exon B5 of CNS-PPARGC1A that did not contain a predicted HRE were used. IgG instead of the HIF1A
antibody was used to confirm the absence of non-specific bands.

**Animal Experiments**

The experiments were carried out in accordance with the protocol for animal care approved by European Communities Council Directive (2010/63/EU) with permission of The State Veterinary and Food Administration of the Slovak Republic (4451/14-221 and 4247/15-221) under the supervision of Ethical Council of the Institute of Neurobiology, Bratislava Slovak Academy of Sciences. Every effort was made to minimize animal suffering and reduce the number of animals used. Adult male albino Wistar rats weighing 300–500 g obtained from Velaz (Czech Republic) were housed in the certified vivarium of the Institute of Neurobiology, Biomedical Research Center of the Slovak Academy of Sciences, maintained on a 12-h light/dark cycle and given food and water ad libitum. Food was withdrawn 1 day before surgery.

**Experimental design** Four groups of animals were used: two ischemic groups of 3 rats each with different post-ischemic reperfusion intervals (ischemia 5 min followed by 1 h or 3 h of reperfusion) and two sham control animal groups (n = 3 each) sacrificed at the time points of the experimental ischemic animals.

**Model of ischemia** Transient forebrain global ischemia was induced [43] with modifications [44]. The rats were anesthetized with 4% halothane in anesthetic cages and maintained with 1.5% halothane during surgery. On the first day, both vertebral arteries were irreversibly occluded by coagulation through the alar foramina. The next day, rats were reanesthetized and the common carotid arteries were dissected free and clamped just before awakening by small atraumatic clips to induce forebrain ischemia. After 5 min of ischemia, blood flow was restored by releasing the clips and verified visually. Normothermic conditions (approximately 37 °C) were maintained by a feedback-controlled heating lamp and pad during all surgical procedures. Animals that became unresponsive within 30 s after clip tightening, lost the righting reflex during bilateral carotid artery occlusion, and showed no seizures during and after ischemia were used for the experiments. Sham-operated controls were surgically treated the same as the ischemic group, but the common carotid arteries were not clamped on the second day.

**Tissue sample collection** Animals were sacrificed by decapitation under halothane anesthesia; the brains were quickly removed and maintained at 0 °C. The fronto-parietal and occipital cortex, striatum (dorsolateral part and rest of the stratum, respectively), hippocampus, thalamus, and cerebellum were separated. To compare the selectively vulnerable CA1 versus the relatively resistant dentate gyrus and CA3 regions, the hippocampus was divided into the CA1 region and the rest of the hippocampus (DG) under a dissecting microscope. Collected tissue was weighed, divided into halves, and frozen at −20 °C in 5 volumes of RNAlater (Sigma-Aldrich) until RNA analysis.

**Statistical Analysis**

In transient transfection studies, ANOVA was used for comparisons of means and the Tukey honest significance test for post-hoc comparisons. Two-way ANOVA was used to ascertain effects of transcriptional activators, co-activators, and their interactions. ANOVA was used to compare effects of reduced oxygen tension and CPX. Three-way ANOVA was used to analyze effects on transcript levels in the in vivo model of transient ischemia. Treatment (sham/transient ischemia), post-ischemic reperfusion time (1 h/3 h) and brain regions sampled (1–7) were used as independent variables. Reported p values are 2-tailed.

**Results**

We previously identified two microsatellite regions located at −164 and −24 bp relative to the transcription start site in the CNS-specific promoter region. The more proximal (GT)4GC(GT)2GC(GT)4G(TG)3(GC)3 microsatellite showed no variation in DNA from 50 subjects, but the more distal (GT)GC(GT)GC(GT)5T(GT)5(GC)5 microsatellite revealed considerable variation in the number of GT repeats (N). In DNA from 2000 subjects, the GT insertion polymorphism (N) ranged from 9 to 28 repeats with 11 GTs as the most common allele [16]. Both SH-SY5Y and NT2/D1 cells contained 17 GTs. Hence, the numbers indicating the relation to the transcription start site in various clones are given for the 11 GT size, unless otherwise indicated. The instable microsatellite region includes interspersed GC repeats. This region has a high potential for Z-DNA formation [45] known to frequently influence promoter activation [46–48]. To determine possible effects of the variable microsatellite on promoter activity, we amplified proximal CNS promoters (−539 to +63 bp relative to the transcription start site) differing in GT sizes, but otherwise containing identical sequences, from human subjects, cloned the fragments into reporter vectors, and transiently transfected the constructs into SH-SY5Y cells. Constructs with increasing GT size displayed increased promoter activity up to 19–21 GTs without further increases by the longest GT inserts (>21 GTs). Similar, but even more pronounced effects of the 21 GT constructs relative to the 11 GT constructs were observed in NT2/D1 cells and HT22 cells (Fig. 1a; Supplementary Material Fig. S1A, B).
Robust expression of PGC-1α isoforms may be maintained by positive feedback loops. As demonstrated in mouse skeletal muscle, Mef2c binds and activates the reference promoter and co-activation of Mef2c by PGC-1α further increases promoter activity resulting in sustained PGC-1α expression [49].

To determine whether the CNS-specific promoter is subject to autoregulation in a human neuronal cell model, SH-SY5Y cells were transiently co-transfected with reporter constructs harboring the sequences of the reference (2.6 kbp) or CNS-specific (2 kbp) promoters cloned upstream of the luciferase gene. All promoter constructs were comparably activated by FL reference and the FL-B4 and FL-B5-PGC-1α proteins, even though minor differences in the extent of co-activation were apparent (Fig. 1b). Thus, reference and CNS-specific promoters are co- and cross-regulated by their encoded proteins via co-activation of transcription factors not further identified in these experiments.

Fig. 1 Distinct and common regulations of the reference and the CNS-PPARGC1A promoters. A common GT repeat polymorphism in the CNS promoter increases its activity up to a repeat size of 21 GT \( (p < 0.001) \); proximal CNS promoters from human subjects differing in GT size, but otherwise of identical sequence \((-539 \text{ to } +63 \text{ bp relative to the transcription start site in the common allele harboring 11 GTs})\) were cloned in reporter vectors and transiently transfected into SH-SY5Y cells (a). Both the reference and the CNS promoters are activated by the reference protein and the brain-specific isoforms B4- and B5-PGC-1α via co-activation of transcription factors not further identified. Equimolar amounts of reporter constructs encoding the reference promoter (2.6 kbp) and the CNS promoter (2 kbp) were transfected into SH-SY5Y cells along with expression plasmids encoding the reference protein or CNS-specific isoforms (b). ESRRA activates and PGC-1α co-activates ESRRA on both the reference and the CNS promoter. The reference or CNS promoter reporter constructs were co-transfected with empty expression plasmid or plasmids encoding ESRRA, PGC-1α, or both; \(p < 0.001\) for interactions of ESRRA and PGC-1α on both the reference and the CNS promoters (c). FOXA2 induces the 2.0-kbp CNS, but not the 2.6-kbp reference, promoter and PGC1α co-activates FOXA2 at the CNS promoter. Activation by FOXA2 and co-activation of FOXA2 are maintained on a truncated CNS promoter \((-0.539 \text{ to } +63 \text{ bp})\); \(p < 0.001\) for interactions of FOXA2 and PGC1α on both the 2.0 kbp and truncated CNS promoters. Similar effects in C and D were observed when reference PGC-1α was substituted by B4-PGC-1α. *\(p < 0.01\); **\(p < 0.001\).
FOXA2, a member of the forkhead family of winged-helix transcription factors, plays an essential role in the development and maintenance of mid-brain neuronal function [50, 51] and has been shown to influence Ppargc1α expression in mice [52]. We therefore compared the transactivation of the reference and CNS promoter by FOXA2 in SH-SY5Y cells in transient co-transfection assays. The CNS-specific promoter, but not the reference promoter, was induced by FOXA2, and synergistic activation of the CNS-specific promoter by FOXA2 and FL-PGC-1α was noted (p < 0.001). As we identified putative FOXA2 binding sites in the distal (−1175 bp) and proximal (−218 bp) CNS promoter using the PROMO 3.0 transcription factor binding site tool/ALGGEN Research Software (http://alggen.lsi.upc.edu, [53, 54]), we repeated the experiment with a truncated promoter construct comprising −539 to +63 bp relative to the transcription start site. As the activation pattern was comparable between the full and truncated CNS-specific promoters, the putative FOXA2 binding site located at (−218 bp) was likely the cis-functional site (Fig. 1d).

Since neuronal cells are particularly sensitive to ischemia, we studied potential effects of reduced oxygen tension on the expression levels of CNS and RG transcripts in NT2/D1 cells cultured in 20% and 1% O2, respectively. After 6 h in culture, levels of the CNS-specific transcripts B1B4 and B5E2 as well as VEGF transcripts were increased in cultures exposed to 1% oxygen in comparison to cells cultured in 20% O2. A similar pattern was noted at 24 h of culture, but average levels of E1E2 and E5E6A transcripts tended also to be higher under oxygen deprived culture conditions (Fig. 2a, b). To determine whether non-hypoxic stimuli, known to stabilize HIF1A via inhibition of proline hydroxylase domain (PHD) isoforms [55, 56], also influenced the expression of PPARG1A transcripts, we incubated cells with the iron chelators ciclopirox olamine (CPX) and deferoxamine (DFO). In NT2/D1 cells, cellular levels of VEGF transcripts and CNS-specific transcripts B1B4 and B5E2 were increased by CPX, but levels of RG transcripts E1E2 and E5E6A transcripts, encoding NT-PGC-1α, were similar to levels in control cells (Fig. 3c). CPX treatment resulted in clearly detectable levels of HIF1A in cell nuclei as expected (Fig. 3d). In SH-SY5Y cells incubated with DFO, we observed upregulation of B1B4, but not E1E2 transcripts (Supplementary Material Fig. S2). As the latter results were consistent with stabilization of HIF1A and a role of the canonical hypoxia response in the upregulation CNS-specific transcripts, we performed transient transfection studies of SH-SY5Y cells using reporter constructs harboring the CNS-specific and the reference promoter and HIF1A expression plasmids. HIF1A activated the CNS promoter nearly 2-fold, and activation was further increased in the presence of the HIF stabilizer CPX. In contrast, no activation of the reference promoter was observed (Fig. 3a). Plasmids encoding FL reference and/or CNS-PGC-1α activated the CNS promoter as described above, but co-transfection of plasmids encoding HIF1A and FL-PGCs had an additive, but not a synergistic effect on promoter activity, strongly suggesting that HIF1A is not co-activated by PGC-1α (Supplementary Material Fig. S3). To interrogate a possible role of the GT repeat size in the activation by HIF1A, we performed co-transfections of SH-SY5Y cells with truncated promoters (−539 to +63) harboring various GT repeat sizes and HIF1A expression plasmids (Fig. 4d). Activation of CNS-specific promoters by HIF1A increased up to 21 GTs and a significant interaction was observed between HIF1A and GT size (p < 0.0001). The truncated promoter constructs were activated by HIF1A to a similar extent as the 2.0 kbp promoter (matched for GT size). Based on the consensus sequence ((A/G)CGTG) [57], the truncated promoters contained eight putative HREs. Truncation of the CNS promoter to 147 bp (−84 to +63) resulted in loss of activation by HIF1A, suggesting that the deleted sequence harbored functional HREs (Fig. 3c). To substantiate this result, we determined the region of the CNS promoter that interacts with HIF1A. We performed ChIP assays using NT2/D1 cells treated with 15 μM CPX or vehicle for 24 h and a monoclonal antibody directed against HIF1A. The region encompassing HREs 5 to 8 and containing 17 GT repeats was readily amplified from NT2/D1 cells treated with CPX, while the same DNA region from NT2/D1 cells without prior CPX treatment was barely amplified. Targeting the proximal promoter region harboring putative HREs 1 to 4 did not produce amplification products in control or CPX treated cells (Fig. 4a-d). A bona fide HRE site [42] of the human NTRK2 (TRKB) promoter used as a positive control was also amplified in CPX treated cells, while no amplification products were noted with the antibody from the exon B5 region serving as negative control. Hence, these results are consistent with the transactivation assays and support promoter activation by HIF1A via HREs located between −218 and −84 of the CNS promoter.

Even though human cell lines were used for the hypoxia experiments, the results may not necessarily emulate the in vivo situation in mammals. Hence, we performed an experiment in rats, whose CNS promoter is conserved and also contains a conserved HRE. Furthermore, we previously described the presence of B1b4 containing Ppargc1α transcripts in mice and rats [16, 31], but B5e2 transcripts were not detected and such transcripts would have a very short open reading frame of 11 amino acids. To determine whether hypoxia and/ or nutrient deprivation influences the expression of CNS-specific PPARG1A transcripts in vivo, we used an established rat model of transient forebrain global ischemia. This model involves the occlusion of both vertebral and carotid arteries and affects all brain regions to a variable degree. To keep the number of animals as low as possible, we used a study design that included transient ischemia/sham, 7 different brain regions, and post-ischemic reperfusion time as
independent variables. Transient ischemia resulted in an increase of Vegf and Glut1 transcripts as expected. Furthermore, transcript levels of B1b4 and E5e6a, but not E1e2, were also increased (Fig. 5a, b). Thus, the response of CNS-specific transcripts to ischemia occurred in both the rat ischemia model and the human cell lines studied. For all the transcripts measured, effects of brain regions were highly significant, while reperfusion time only affected Glut1 transcript abundance (Supplementary Material Figs. S4 and S5).

### Discussion

Alternative promoter usage and/or splicing occur in the great majority of mammalian genes and are key mechanisms in transcriptional regulation and generation of protein diversity [58–60]. Several tissue-selective promoters have been described for PPARGC1A, but the CNS-specific promoter is unique in that it is localized far upstream of the reference gene promoter and is responsible for the majority of PPARGC1A transcripts in human brain, especially in neuronal cells. To this end, we compared some relevant regulations of the CNS-specific and the RG promoter and report not only similar but also distinct factors controlling their activities.

Transcriptional activity of the CNS promoter was modulated by a variable GT tandem repeat region in that a larger GT size enhanced promoter activity. As such, a result was obtained in three neuronal cell types; it is likely to occur also in vivo. Hence, the GT size is a likely source of inter-individual variability in human CNS transcript expression. This conclusion is in keeping with a recent study that highlighted the importance of short tandem repeats in the genetic architecture of quantitative human traits. In a survey, more than 2000 tandem repeats were identified that influenced transcript expression. Such repeats were enriched in conserved regions, co-localized with regulatory elements and modulated certain
histone marks [61]. In addition, the instable GT microsatellite is part of a highly predicted Z-DNA region. Sequences transiently forming Z-DNAs are non-randomly distributed, occur more frequently proximal to transcription start sites, and influence promoter activity [46–48].

Self- and cross-co-activation of both the RG and CNS promoters by the reference protein and CNS-specific isoforms were consistent findings in our transient transfection studies of SH-SY5Y cells. Even though we did not further identify the transcription factors co-activated in the initial experiments, a synergistic co-activation of ESRRA on both the RG and CNS promoters was clearly demonstrated. Expression profiles of ESRRA and PPARGC1A transcripts are similar in various tissues and both genes are induced by cold-exposure [62, 63]. An autoregulatory feedback loop consisting of ESRRA response elements in the ESRR and the reference PPARGC1A promoters has been discovered previously. The evolutionary conserved ESRR binding site in the human RG promoter is located 1978-bp upstream of the transcription start site [64], while a 23-bp sequence that contains a functional ERR response element and is present in 1 to 4 copies has been mapped within −700 bp of the transcription start site of the ESRRA promoter [65]. In embryonic fibroblasts of Esrra null mice, the transcriptional activity of Pgc-1α was reduced in comparison to cells from wild-type mice but could be restored by the ectopic expression of Esrra. [66]. We also identified two putative ESRRA binding sites in the CNS promoter, suggesting that a similar feedback loop augments the expression of CNS transcripts. This regulatory loop may be of clinical relevance, as loss of ESRRA function in brain regions of mice or loss-of-function mutations in humans have been associated with eating disorders [67, 68].

Unlike ESRRA, FOXA2 selectively activated the CNS-specific promoter in a positive feedback loop with FL-PGC1α. This result is supported by data showing that adenoviral overexpression of murine Foxa2 in Neuro-2a cells resulted in a dramatic increase in CNS-specific transcripts. In addition, murine Foxa1, another member of the forkhead family of
Fig. 4 Chip assays demonstrate binding of HIF1A to a region between −77 and −218 relative to the transcription start site and harboring the putative HREs 5 to 8 as well as the GT repeat tract. Schematic describing the location of putative HREs, the GT repeat tract, and primers used to amplify Chip regions 1 and 2 (a). Sequence of Chip region 1 and 2 with primers (in italics) and putative HREs (underlined); numbers indicate position relative to transcription start site (b, c). Representative Chip assays showing interaction of HIF1A with region 2 of the CNS promoter. NT2/D1 cells were cultured for 24 h without or with 15 μmol CPX prior to harvesting of cells. Note that amplification products reflecting the binding of HIF1A were obtained only in NT2/D1 cells treated with CPX. The TRKB promoter shown to have a bona fide HIF1A binding site was used as positive control and the CNS-specific exon B5 was used as negative control. Input, 1% of input DNA; NTC no template control.

Fig. 5 Effect of transient ischemia on the expression of selected transcripts in brains of rats in whom transient forebrain global ischemia of 5 min followed by 1 or 3 h of reperfusion was induced. Samples were collected from 7 brain regions. Sham-operated animals served as controls. Log-transformed transcript levels were used as dependent variables using a 3-way ANOVA with transient ischemia/sham operated, post-perfusion time (1/3 h), and brain regions as independent variables. Note significant effects of transient ischemia for all transcripts analyzed except for E1e2 which reflects the reference gene transcript; MS mean square; df degrees of freedom (a). Average values for all transcripts except for E1e2 increased in the experimental animals (b). *p < 0.01; **p < 0.001.
winged-helix transcription factors, induced the transcriptional activity of the CNS-specific promoter in HEK293T cells [52]. FOXA1 and FOXA2 share over 95% homology between mice and humans and near perfect sequence homology in their DNA binding and transactivation domains [69, 70]. The two transcription factors play important, but redundant roles in the development and maintenance of dopaminergic midbrain neurons [51, 71], arguing for the involvement of CNS-specific PP ARGC1A isoforms. Interestingly, genetic studies implicated the CNS-specific PPARG1C region in the pathogenesis of Parkinson’s disease [31].

PGC-1α, specifically the truncated NT-isoform, has been shown to be upregulated in myotubes by hypoxia, but the canonical hypoxia response pathway characterized by HIF-1α stabilization was not involved [72, 73]. The increase in NT-PGC-1α strongly induced Vegf expression by co-activation of Esrra via conserved binding sites in the Vegf promoter and in the first intron. The increased levels of NT-PGC-1α likely result from enhanced alternative splicing and perhaps increased transcription, but the exact mechanisms have not been elucidated. Our results are consistent with these findings as hypoxia, but not HIF1A stabilization, increased the levels of E5E6A transcripts. Furthermore, the E5E6A level was also increased in the transient ischemia rat model. As demonstrated in the current and previous studies [72, 73], HIF1A does not activate transcription from the reference promoter, but the autoregulatory loop with ESSRA may play a role, as treatment of cardiomyocytes with an ESRRRA inverse agonist caused knockdown of Esrra protein expression and blocked the increase in Ppareg1a mRNA levels in response to hypoxia [66]. Importantly, the activation of CNS-specific transcripts in hypoxia does occur via the canonical HIF-1α pathway. This conclusion is based on effects of both hypoxia and non-hypoxic HIF1A stabilizing stimuli on CNS-specific PPARG1C transcripts, the activation of CNS-specific promoter reporter constructs by HIF1A expression plasmids, and the data from ChIP assays. In addition, the in vivo results in an established transient ischemia rat model also are consistent with this conclusion. Our data also showed that the average values of Vegf and CNS-specific, but not RG transcripts increased after ischemia in all brain regions sampled, except in the cerebellum, where the levels did not change (data not shown). Due to the sample number, the data on individual brain regions must be viewed as preliminary. Clearly, the effects of ischemia require further studies on the time course of response and effects in specific brain regions, as their vulnerability and response may differ. A link between hypoxia and CNS-specific PPARG1C expression is supported by studies in the subterranean rodent Namnospalax galli. In this blind mole that survives at 3% O₂ for up to 11 h as compared to 2–4 h for rats [74], CNS-specific transcripts are upregulated [75].

HIFs interact with HREs with the core sequence 5′-RCGTG-3′ [57], but only a small proportion of the potential binding sites are bound by HIFs across the genome [76]. Several sequence preferences outside the core binding motif have been described but are not absolute. In addition, two or more HREs and/or binding sites for other transcription factors are often found near functional HIF sites and interactions of HIF1A with USF1 and FOXA2 (both located near HREs in the CNS-specific promoter) have been reported [77, 78]. Furthermore, various epigenetic marks are likely playing an important role in HIF binding [76, 79]. An interesting finding of our studies is the interaction of GT repeat size with HIF1A on the transcriptional activity of the CNS promoter. A similar interaction has been described at the NRAMP1 (SLCA11A1) promoter, as an ACGTG site next to a GT microsatellite with high Z-DNA potential affected transcriptional activity and susceptibility to inflammatory and infectious diseases [83]. HIF2A also has been implicated in the hypoxic response, has been shown to transactivate HRE containing target genes [81], and may therefore influence the expression of the CNS-specific promoter. Indeed, our preliminary studies suggest that HIF2A is expressed in nuclei of CPX-treated cultures and activates the CNS promoter in transient transfection studies (Soyal SM, Patsch W, unpublished results), but additional studies will be required to gain more insight into the regulation of the CNS-specific promoter by HIF2A.

Upregulation of CNS-specific PGC-1α could have major implications for the pathology of cerebral ischemia as well as neuronal physiology. Our results on the interaction of HIF1A and the GT repeat size would imply inter-individual differences in the response to hypoxia. CNS-specific transcripts encode for proteins that differ from the reference protein only at the N-terminus. The N-terminal activation and repression domains appeared to be responsible for gene programs and splicing events that are specific for some PGC-1α isoforms [82]. Our preliminary data in SH-SY5Y in which the CNS-specific or the RG promoters were selectively upregulated using the CRISPR-dCAS9 approach show overlapping as well as distinct targets for the CNS isoforms and the reference protein. Importantly, CNS-specific forms appear to participate in pathways supporting neural and axonal growth, while the reference protein was mainly related to mitochondrial biogenesis and function (Kwik M, Patsch W, Soyal SM, unpublished results). The generation of new mitochondria, specifically at the peripheral axonal tree, has been shown to be induced by reference PGC-1α and is essential for the axonal growth [83]. Thus, the functions of the CNS isoforms and the reference protein may be intertwined by cross-talk between the CNS and reference proteins as demonstrated by the co-activation of each other’s promoters as shown in the current study.

In conclusion, the CNS-specific, but not the RG, promoter is activated by FOXA2 via an autoregulatory loop, suggesting a role of CNS-specific transcripts in the development and maintenance of dopaminergic mid-brain neurons. The activation of the CNS-specific promoter by HIF1A supports a role of CNS isoforms in ischemia. The hypoxic/ischemic activation may differ between individuals, as an interaction is likely to occur between a variable GT repeat and HIF1A. Finally,
cross-talk between the CNS and RG promoters via transcriptional co- and cross-activation may support a balanced expression of the reference protein and the CNS-specific isoforms.

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**Conflict of Interest** The authors declare that they have no conflict of interest.

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**References**

1. Lin J, Handschin C, Spiegelman BM (2005) Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1(6):361–370
2. Vega RB, Kelly DP (2017) Cardiac nuclear receptors: architects of mitochondrial structure and function. J Clin Invest 127(4):1155–1164
3. Soyal S, Krempfer F, Oberkofer H, Patsch W (2006) PGC-1alpha: a potent transcriptional coactivator involved in the pathogenesis of type 2 diabetes. Diabetologia 49(7):1477–1488
4. Spiegelman BM, Heinrich R (2004) Biological control through regulated transcriptional activators. Cell 119(2):157–167
5. Lustig Y, Ruas JL, Estall JL, Lo JC, Devarakonda S, Lazenik D, Choi JH, Ono H et al (2011) Separation of the gluconeogenic and mitochondrial functions of PGC-1alpha through S6 kinase. Genes Dev 25(12):1232–1244
6. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK et al (2001) Cytochrome stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. Mol Cell 8(5):971–982
7. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434(7029):113–118
8. Sano M, Tokudome S, Shimizu N, Yoshikawa N, Ogawa C, Shirakawa K, Endo J, Katayama T et al (2007) Intramolecular control of protein stability, subnuclear compartmentalization, and co-activator function of peroxisome proliferator-activated receptor gamma coactivator 1alpha. J Biol Chem 282(35):25970–25980
9. Olson BL, Hock MB, Ekholm-Reed S, Wohlschlegel JA, Dev KK, Kralli A, Reed SI (2008) SCF(Cdc4) acts antagonistically to the PGC-1alpha transcriptional coactivator by targeting it for ubiquitin-mediated proteolysis. Genes Dev 22(2):252–264
10. Teyssier C, Ma H, Emter R, Kralli A, Stallcup MR (2005) Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation. Genes Dev 19(12):1466–1473
11. Zhang Y, Huypens P, Adamson AW, Chang JS, Henagan TM, Boudreau A, Lenard NR, Burk D et al (2009) Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1alpha. J Biol Chem 284(7):32813–32826
12. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, Greene NF, Wu J et al (2012) A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell 151(6):1319–1331
13. Miura S, Kai Y, Kamei Y, Ezaki O (2008) Isoform-specific increases in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1alpha) mRNA in response to beta2-adrenergic receptor activation and exercise. Endocrinology 149(9):4527–4533
14. Tadaishi M, Miura S, Kai Y, Kano Y, Oishi Y, Ezaki O (2011) Skeletal muscle-specific expression of PGC-1alpha-b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake. PLoS One 6(12):e28290
15. Felder TK, Soyal SM, Oberkofer H, Hahne P, Auer S, Weiss R, Gadermaier G, Miller K et al (2011) Characterization of novel peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha) isoform in human liver. J Biol Chem 286(50):42923–42936
16. Soyal SM, Felder TK, Auer S, Hahne P, Oberkofer H, Witting A, Paulmichl M, Landwehrmeyer GB et al (2012) A greatly extended PPARGC1A genomic locus encodes several new brain-specific isoforms and influences Huntington disease age of onset. Hum Mol Genet 21(15):3461–3473
17. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jager S et al (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 119(1):121–1235
18. Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443(7113):787–795
19. Handschin C, Spiegelman BM (2006) Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 27(7):728–735
20. Tsunemi T, Ashe TD, Morrison BE, Soriano KR, Au J, Roque RA, Lazaro-Azarowski ER, Damian VA et al (2012) PGC-1alpha rescues Huntington’s disease proteotoxicity by preventing oxidative stress and promoting TFEB function. Sci Transl Med 4(142):142ra97
21. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K et al (2006) Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 127(2):397–408
22. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D et al (2006) Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 127(1):59–69
23. Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER, Gilbert ML, Morton GJ et al (2006) Thermoregulatory and metabolic defects in Huntington’s disease transgenic mice implicate PGC-1alpha in Huntington’s disease neurodegeneration. Cell Metab 4(5):349–362
24. Zheng B, Liao Z, Locascio JJ, Lesniak KA, Roderick SS, Watt ML, Eklund AC, Zhang-James Y et al (2010) PGC-1alpha, a potential therapeutic target for early intervention in Parkinson’s disease. Sci Transl Med 2(52):52ra73
25. Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, Troconso JC, Dawson VL et al (2011) PARIS (ZNF746) repression of PGC-1alpha transcriptional coactivator by targeting it for ubiquitin-mediated proteolysis. Genes Dev 22(2):252–264
26. Skardal A, Pelleatisfied H, Hagerman RM, Skutnik K, Hagerman RJ, Baehner RL, Siegel D, Goodfriend AL et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
27. Mizuno Y, Yamakawa T, Sasazuki T, Irie K, Aoki T, Shibasaki K, Tsuchiya Y, Nakamura H et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
28. Miyazaki T, Tanaka H, Shiozaki H, Kurimoto K, Itoh H, Koyama H, Sata M, Kanda M et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
29. Miyazaki T, Tanaka H, Shiozaki H, Kurimoto K, Itoh H, Koyama H, Sata M, Kanda M et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
30. Miyazaki T, Tanaka H, Shiozaki H, Kurimoto K, Itoh H, Koyama H, Sata M, Kanda M et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
31. Miyazaki T, Tanaka H, Shiozaki H, Kurimoto K, Itoh H, Koyama H, Sata M, Kanda M et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
32. Miyazaki T, Tanaka H, Shiozaki H, Kurimoto K, Itoh H, Koyama H, Sata M, Kanda M et al (2011) A PGC-1alpha isoform regulated by TFE3 promotes trophoblast cell proliferation and angiogenesis. J Biol Chem 286(50):42923–42936
1alpha contributes to neurodegeneration in Parkinson’s disease. Cell 144(5):689–702
26. Katsouri L, Lim YM, Blondrath K, Eleftheriadou I, Lombardero L, Birch AM, Mirzaei N, Irvine EE et al (2016) PPARgamma-coactivator-1alpha gene transfer reduces neuronal loss and amyloid-beta generation by reducing beta-secretase in an Alzheimer’s disease model. Proc Natl Acad Sci U S A 113(43): 12292–12297
27. Zhao W, Varghese M, Yemul S, Pan Y, Cheng A, Marano P, Hassan S, Vempati P et al (2011) Peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1alpha) improves motor performance and survival in a mouse model of amyotrophic lateral sclerosis. Mol Neurodegener 6(1):51
28. Nijland PG, Witte ME, van het Hof B, van der Pol S, Bauer J, Lassmann H, van der Valk P, de Vries HE et al (2014) Astroglial PGC-1alpha increases mitochondrial antioxidant capacity and suppresses inflammation: implications for multiple sclerosis. Acta Neuropathol Commun 2:170
29. Witte ME, Nijland PG, Drexhage JAR, Gerritsen W, van der Valk P, de Vries HE et al (2013) Reduced expression of PGC-1α partly underlies mitochondrial changes and correlates with neuronal loss in multiple sclerosis cortex. Acta Neuropathol 125(2):231–243
30. Eschbach J, Schwaltenstocker B, Soyal SM, Bayer H, Wiesner D, Akimoto C, Nilsson AC, Birve A et al (2013) PGC-1alpha is a male-specific disease modifier of human and experimental amyotrophic lateral sclerosis. Hum Mol Genet 22(17):3477–3484
31. Soyal SM, Zara G, Ferger B, Felder TK, Kwik M, Nofziger C, Dossena S, Schwabenbacher C et al (2018) The PPARGC1A locus and CNS-specific PGC-1alpha isoforms are associated with Parkinson’s disease. Neurobiol Dis 121:34–46
32. Weydt P, Soyal SM, Gellera C, Didonato S, Weidinger C, Oberkofler H, Landwehrmeyer GB, Patsch W (2009) The gene coding for PGC-1alpha modifies age at onset in Huntington’s disease. Mol Neurodegener 4(3):3
33. Weydt P, Soyal SM, Landwehrmeyer GB, Patsch W, European Huntington Disease N (2014) A single nucleotide polymorphism in the coding region of PGC-1alpha is a male-specific modifier of Huntington disease age-at-onset in a large European cohort. BMC Neuro 14:1
34. Che HV, Metzger S, Portal E, Deyle C, Riess O, Nguyen HP (2011) Localization of sequence variations in PGC-1alpha influence their thermodynamic approach for predicting the formation of Z-DNA in naturally occurring sequences. EMBO J 5(10):2737–2744
35. Schroth GP, Chou PJ, Ho PS (1992) Mapping Z-DNA in the human genome. Computer-aided mapping reveals a nonrandom distribution of potential Z-DNA-forming sequences in human genes. J Biol Chem 267(17):11846–11855
36. Oh DB, Kim YG, Rich A (2002) Z-DNA-binding proteins can act as potent effectors of gene expression in vivo. Proc Natl Acad Sci U S A 99(26):16666–16671
37. Lin W, Metzakopian E, Mavromatakis Y, Gao N, Balasaks N, Sasaki H, Briscoe J, Whitsett JA et al (2009) Foxa1 and Foxa2 function both upstream of and cooperatively with Lmx1a and Lmx1b in a feedforward loop promoting mesodiencephalic dopaminergic neuron development. Dev Biol 333(2):386–396
38. Pristera A, Lin W, Kaufmann AK, Brimblecombe KR, Threlfell S, Dodson PD, Magill PJ, Fernandes C et al (2015) Transcription factors FOXA1 and FOXA2 maintain dopaminergic neuronal properties and control feeding behavior in adult mice. Proc Natl Acad Sci U S A 100(12):7111–7116
39. Lin W, Metzakopian E, Mavromatakis Y, Gao N, Balasaks N, Sasaki H, Briscoe J, Whitsett JA et al (2009) Foxa1 and Foxa2 function both upstream of and cooperatively with Lmx1a and Lmx1b in a feedforward loop promoting mesodiencephalic dopaminergic neuron development. Dev Biol 333(2):386–396
40. Pristera A, Lin W, Kaufmann AK, Brimblecombe KR, Threlfell S, Dodson PD, Magill PJ, Fernandes C et al (2015) Transcription factors FOXA1 and FOXA2 maintain dopaminergic neuronal properties and control feeding behavior in adult mice. Proc Natl Acad Sci U S A 100(12):7111–7116
41. Auer S, Hahne P, Soyal SM, Felder T, Miller K, Paulmichl M, Krempler F, Oberkofler H et al (2012) Potential role of upstream stimulatory factor 1 gene variant in familial hyperlipidemia and related disorders. Arterioscler Thromb Vasc Biol 32(6): 1535–1544
42. Martens LK, Kirschchner KM, Warnecke C, Scholz H (2007) Hypoxia-inducible factor-1 (HIF-1) is a transcriptional activator of the TrkB neurotrophin receptor gene. J Biol Chem 282(19): 14379–14388
43. Pulselinna WA, Brierley JB (1979) A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10(3):267–272
44. Schmidt-Kastner R, Frischin W, Oppo BH, Hossmann KA (1989) A modified four-vessel occlusion model for inducing incomplete forebrain ischemia in rats. Stroke 20(7):938–946
45. Ho PS, Ellison MJ, Quigley GJ, Rich A (1986) A computer aided thermodynamic approach for predicting the formation of Z-DNA in naturally occurring sequences. EMBO J 5(10):2737–2744
46. Schroth GP, Chou PJ, Ho PS (1992) Mapping Z-DNA in the human genome. Computer-aided mapping reveals a nonrandom distribution of potential Z-DNA-forming sequences in human genes. J Biol Chem 267(17):11846–11855
47. Oh DB, Kim YG, Rich A (2002) Z-DNA-binding proteins can act as potent effectors of gene expression in vivo. Proc Natl Acad Sci U S A 99(26):16666–16671
48. Shin SI, Ham S, Park J, Seo SH, Lim CH, Jeon H, Huh J, Roh TY (2016) Z-DNA-forming sites identified by ChIP-Seq are associated with actively transcribed regions in the human genome. DNA Res 23(5):477–486
49. Handschin C, Rhe J, Lin J, Tarr PT, Spiegelman BM (2003) An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proc Natl Acad Sci U S A 100(12):7111–7116
50. Lin W, Metzakopian E, Mavromatakis Y, Gao N, Balasaks N, Sasaki H, Briscoe J, Whitsett JA et al (2009) Foxa1 and Foxa2 function both upstream of and cooperatively with Lmx1a and Lmx1b in a feedforward loop promoting mesodiencephalic dopaminergic neuron development. Dev Biol 333(2):386–396
51. Pristera A, Lin W, Kaufmann AK, Brimblecombe KR, Threlfell S, Dodson PD, Magill PJ, Fernandes C et al (2015) Transcription factors FOXA1 and FOXA2 maintain dopaminergic neuronal properties and control feeding behavior in adult mice. Proc Natl Acad Sci U S A 100(12):7111–7116
52. Gasser E, Johannsen HC, Rulicke T, Zeilhofer HU, Stoffel M (2016) Foxa1 is essential for development and functional integrity of the subthalamic nucleus. Sci Rep 6:38611
53. Messegueur X, Escudero R, Farré D, Nuñez O, Martínez J, Albá MM (2002) PROMO: detection of known transcription regulatory elements using species-tailored searches. Bioinformatics 18(2): 333–334
54. Farré D, Roset R, Huerta M, Adsuara JE, Roselló L, Albá MM, Messegueur X (2003) Identification of patterns in biological sequences at the ALGEN server: PROMO and MALGEN. Nucleic Acids Res 31(13):3651–3653
55. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Sálic A, Asara JM et al (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292(5516):464–468
56. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF et al (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292(5516):464–468
57. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Messeguer X (2003) Identification of patterns in biological sequences at the ALGEN server: PROMO and MALGEN. Nucleic Acids Res 31(13):3651–3653
58. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Sálic A, Asara JM et al (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292(5516):464–468
59. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF et al (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292(5516):464–468
60. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Messeguer X (2003) Identification of patterns in biological sequences at the ALGEN server: PROMO and MALGEN. Nucleic Acids Res 31(13):3651–3653
58. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP et al (2008) Alternative isoform regulation in human tissue transcriptomes. Nature 456(7221):470–476
59. Nilsen TW, Graveley BR (2010) Expansion of the eukaryotic proteome by alternative splicing. Nature 463(7280):457–463
60. Carminci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T et al (2005) The transcriptional landscape of the mammalian genome. Science 309(5740):1559–1563
61. Gymrek M, Willem T, Guilmot A, Zeng H, Markus B, Georgiev S, Daly MJ, Price AL et al (2016) Abundant contribution of short tandem repeats to gene expression variation in humans. Nat Genet 48(1):22–29
62. Ichida M, Nemoto S, Finkel T (2002) Identification of a specific molecular repressor of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1alpha). J Biol Chem 277(52):50991–50995
63. Schreiber SN, Knutti D, Brogli K, Uhlmann T, Kralli A (2003) The transcriptional activator of the orphan nuclear receptor estrogen-related receptor alpha (ERRalpha) promoter dictates peroxisome proliferator-activated receptor gamma coactivator-1alpha control of ERRalpha expression. J Biol Chem 278(11):9013–9018
64. Ramjiawan A, Bagchi RA, Albak L, Czubryt MP (2013) Mechanism of cardiomyocyte PGC-1alpha gene regulation by ERRalpha. Biochem Cell Biol 91(3):148–154
65. Laganiere J, Tremblay GB, Dufour CR, Giroux S, Rousseau F, Giguere V (2004) A polymorphic autoregulatory hormone response element in the human estrogen-related receptor alpha (ERRalpha) promoter directs peroxisome proliferator-activated receptor gamma coactivator-1 alpha control of ERRalpha expression. J Biol Chem 279(18):18504–18510
66. CunninghamKF, Beeson GC, Beecon CC, Baicu CF, Zile MR, McDermott PJ (2015) Estrogen-related receptor alpha (ERRalpha) is required for adaptive increases in PGC-1 isoform expression during electrically stimulated contraction of adult cardiomyocytes in sustained hypoxic conditions. Int J Cardiol 187:393–400
67. Cui H, Moore J, Ashimi SS, Mason BL, Drewbridge JN, Han S, Hing B, Matthews A et al (2013) Eating disorder predisposition is associated with ESRRα and HDAC4 mutations. J Clin Invest 123(11):4706–4713
68. Cui H, Khan MZ, Anderson RM, McDaniel L, Wilson HE, Yin TC, Radley JJ, Pieper AA et al (2015) Behavioral disturbance in estrogen-related receptor alpha-null mice. Cell Rep 11:1–7
69. Jackson BC, Carpenter C, Nebert DW, Vasiliou V (2010) Update of human and mouse forkhead box (FOX) gene families. Hum Genomics 4(5):345–352
70. Friedman JR, Kaestner KH (2006) The Foxa family of transcriptional coactivators. J Biol Chem 281(13):8810–8817
71. Ariela, R, Nevo E (1991) Hypoxic survival differs between two mole rat species (Spalax ehrenbergi) of humid and arid habitats. Comp Biochem Physiol A Comp Physiol 11:543–545
72. Malik A, Korol A, Hübner S, Hernandez AG, Thimmappuram J, Ali S, Glaser F, P A et al (2011) Transcriptome sequencing of the blind subterranean mole rat, Spalax galili: utility and potential for the discovery of novel evolutionary patterns. PLoS One 6:e21227
73. Thom R, Rowe GC, Jang C, Safdar A, Arany Z (2014) Hypoxic induction of vascular endothelial growth factor (VEGF) and angiogenesis in muscle by truncated peroxisome proliferator-activated receptor gamma coactivator (PGC)-1alpha. J Biol Chem 289(13):8810–8817
74. Ariel R, Nevo E (1991) Hypoxic survival differs between two mole rat species (Spalax ehrenbergi) of humid and arid habitats. Comp Biochem Physiol A Comp Physiol 11:543–545
75. Malick A, Koral A, Hübner S, Hernandez AG, Thimmappuram J, Ali S, Glaser F, P A et al (2011) Transcriptome sequencing of the blind subterranean mole rat, Spalax galili: utility and potential for the discovery of novel evolutionary patterns. PLoS One 6:e21227
76. Qi J, Nakayama K, Cardiff RD, Borowsky AD, Kaul K, Williams R, Krajewski S, Mercola D et al (2010) Siah2-dependent concerted activity of HIF and FoxA2 regulates formation of neuroendocrine phenotype and neuroendocrine prostate tumors. Cancer Cell 18(1):23–38
77. Hu J, Stiehl DP, Selzer C, Wichmann D, Shinde DA, Rehrauer H, Hradecky P, Gassmann M et al (2011) Interaction of HIF and USF signaling pathways in human genes flanked by hypoxia-response elements and E-box palindromes. Mol Cancer Res 9(11):1520–1535
78. Xia X, Kung AL (2009) Preferential binding of HIF-1 to transcriptionally active loci determines cell-type specific response to hypoxia. Genome Biol 10(10):R113
79. Bayele HK, Peyssonnaux C, Giartromanolaki A, Arrais-Silva WW, Mohamed HS, Collins H, Giorgio S, Koukourakis M et al (2007) HIF-1 regulates heritable variation and allele expression phenotypes of the macrophage immune response gene SLC11A1 from a Z-DNA forming microsatellite. Blood 110(8):3039–3048
80. Carroll VA, Ashcroft M (2006) Role of hypoxia factor (HIF)-1α versus HIF-2α in the regulation of HIF target genes in response to hypoxia, insulin-like growth factor –I, or loss of von Hippel-Lindau function: implications for targeting the HIF pathway. Cancer Res 66(12):6264–6270
81. Martinez-Redondo V, Jannig PR, Correia JC, Ferreira DM, Cervenka I, Lindvall JM, Sinha J, Izadi M et al (2016) Peroxisome proliferator-activated receptor gamma coactivator-1 alpha isoforms selectively regulate multiple splicing events on target genes. J Biol Chem 291(29):15169–15184
82. Vaarmann A, Mandel M, Zeb A, Wareski P, Liiv J, Kuum M, Antsov E, Liiv M et al (2016) Mitochondrial biogenesis is required for axonal growth. Development 143(11):1981–1992
83. Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.