Journal of Steroid Biochemistry & Molecular Biology 133 (2013) 58–65

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Distinct ligand-dependent and independent modes of thyroid hormone receptor (TR)/PGC-1α interaction

Chaoshen Yuan[b,1], Phuong Nguyen[b,2], John D. Baxter[a,b,**,3], Paul Webb[a,*]

[a] The Methodist Hospital Research Institute, Genomic Medicine Program, Houston, TX 77030, United States
[b] University of California Medical Center, Diabetes Center, San Francisco, CA 94122, United States

ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form 31 August 2012
Accepted 2 September 2012

Keywords:
Thyroid hormone receptors
Thyroid hormone
Peroxisome proliferator activated receptor coactivators
Gene expression
Coactivator
Activation function

ABSTRACT

Thyroid hormone receptor (TR)/peroxisome proliferator activated receptor coactivator (PGC-1α) interactions are required for T_3-dependent transcriptional responses involved in adaptive thermogenesis and liver. Thus, it is important to define TR/PGC-1α contact modes and to understand their significance in gene expression. Previous studies have shown that TRβ1 recruits PGC-1α to target promoters *via* contacts between the hormone-dependent TRβ1 activation function 2 (AF-2) in the C-terminal ligand binding domain (LBD) and a major PGC-1α nuclear receptor (NR) interaction box (consensus LxxLL) at amino acids 142–146. While our studies verify the existence and importance of this interaction, we present evidence that TRβ1 also binds PGC-1α in a second ligand and LxxLL motif independent mode and show that this interaction requires the TRβ1 N-terminal domain (NTD) and the PGC-1α N-terminal activation domain (AD) at amino acids 1–130. Transfection assays suggest that optimal PGC-1α coactivation requires the TRβ1 NTD and that these contacts are needed for utilization of the PGC-1α C-terminal AD, which does not bind TR and is implicated in basal transcription machinery contacts. We propose that TR AF-1/PGC-1α contacts are needed for transition between activities of PGC-1α N-and C-terminal ADs in gene expression. Our findings provide insights into possible roles for TR and NR AF-1 in gene expression.
© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Thyroid hormone receptors (TRs) are members of the nuclear hormone receptor (NR) family of transcription factors [1,2]. TRs bind thyroid hormone response elements (TREs) located near thyroid hormone responsive target genes, usually as heterodimers with retinoid X receptors (RXRs). Hormone (triiodothyronine, T_3) triggers changes in the conformation of the TR C-terminal ligand binding domain (LBD) that result in changes in gene expression [3]. Specifically, T_3 binding repositions C-terminal helix (H) 12, occluding a hydrophobic surface that binds NR corepressors and completing a coactivator binding surface (activation function 2, AF-2), which is comprised of residues from H3, H5 and H12 and acts as a docking site for short α-helical coactivator peptides (consensus Leu-X-X-Leu-Leu, LxxLL) [4–6].

NRs harbor other cofactor binding sites in addition to AF-2 [5,7,8]. Many of these surfaces map to the N-terminal domain (NTD) and comprise a second activation function (AF-1), although the central DNA binding domain (DBD), the region that bridges the DBD and LBD, often called the hinge, and other LBD surfaces have also been implicated in NR/cofactor contact. While TR AF-1 activities have been documented [9,10], its mechanism of action is not clear. TR AF-2 recognizes several families of general NR coregulators [2], including steroid receptor coactivators (SRCs), and one report suggests that the AF-1 function of the pituitary-specific TRβ2 subtype makes auxiliary contacts with the SRC C-terminal domain [11]; a pro/glu rich region that binds NTDs of other NRs and lacks LxxLL motifs [12,13]. Presently, however, the partners of TRβ1 AF-1 and the range of cofactor binding sites within the TR are not well understood.

Peroxisome proliferator activated receptor (PPAR) coactivators (PGCs) are TR interacting proteins [14–16]. PGC-1α was discovered as a cold-inducible NR coactivator required for PPARγ and TR induction of uncoupling protein 1 (UCP1) in brown fat [17]. Subsequently, PGCs have been linked to a stimuli that signal requirements for increased mitochondriol activity and altered metabolic responses [14,15]. For example, PGC-1α is induced by fasting in liver and coordinates induction of genes involved in gluconeogenesis and

* Corresponding author at: The Methodist Hospital Research Institute, Genomic Medicine Program, Department of Medicine, 6565 Fannin Street F8-045, Houston TX 77030, United States. Tel.: +1 713 441 2516; fax: +1 713 793 7162.
** Corresponding author.
E-mail address: pwebb@tmhs.org (P. Webb).
[1] Current address: Department of Nutritional Science and Toxicology, University of California, Berkeley, United States.
[2] Current address: Department of Biochemistry and Biophysics, University of California Medical Center, San Francisco, United States.
[3] Deceased.

0960-0760/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2012.09.001

fatty acid β-oxidation [18] and TRβ/PGC-1α contacts are important in T_3-induction of several liver genes, including carnitine palmitoyl transferase 1α (CPT-1α) and pyruvate dehydrogenase kinase (PDK4) [19–21].

NRs utilize a mix of conventional LxxLL-dependent and unusual LxxLL-independent contact modes in PGC-1α recruitment [14,22]. PPARγ, TRs and other NRs bind to a variant LxxLL at PGC-1α amino acids 142–146 [14,23]; here, the LxxLL hydrophobic triad is conserved but there are differences in flanking amino acids that result in subtle differences in requirements for specific AF-2 surface amino acids relative to those needed for binding to SRC LxxLL motifs [24–26]. There is also evidence for a second non consensus NR box at amino acids 210–214 (LLxxL) in NR/PGC-1α contacts [27–30]. In addition, there are NR/PGC-1α contact modes that appear completely independent of the LxxLL motif. The PPARγ hinge binds an auto-inhibitory region (AIR) of approximately 200 amino acids, C-terminal of the major LxxLL motif [28,31]. This enhances activity of the PGC-1α N-terminal activation domain (AD) at amino acids 1–130, which binds coregulators with histone modification activities, SRC1 and the associated histone acetyl transferases CBP and p300 [31]. Estrogen receptors (ERs) and hepatocyte nuclear factor 4α (HNF-4α) also participate in non-LxxLL dependent PGC-1α contact modes [29,32,33].

TRs interactions with the major PGC-1α LxxLL motif initiate dynamic three-way interactions between TR, PGC-1α and the mediator complex, which is part of the basal transcription machinery and binds to a PGC-1α C-terminal AD that is implicated in RNA polymerase II recruitment [34]. The C-terminal AD is not required for PGC-1α recruitment to TR regulated genes [35] and analysis of TR interactions with PGC-1α in cultured cells and *in vitro* suggests that TR recruits PGC-1α to target promoters such as UCP1 *via* LxxLL contacts, mediator complex then binds PGC-1α and the mediator TRAP220 subunit LxxLL motif displaces the PGC-1α LxxLL motif from TR AF-2, leading to altered cofactor presentation and a transition between PGC-1α associated chromatin remodeling and transcriptional initiation activities [34]. The existence and roles of alternate non-LxxLL dependent modes of TR/PGC-1α remain open questions.

In this study, we reinvestigated the basis of TRβ/PGC-1α interactions and show that TRβ utilizes LxxLL dependent and independent modes to bind PGC-1α. We suggest that the LxxLL independent mode is related to requirements for the C-terminal AD in optimal PGC-1α coactivation.

2. Materials and methods

2.1. Plasmids

TRβ mutations were generated in the context of a human TRβ cDNA cloned into the *p*CMX expression vector [2]. TRβ LBD cDNA was generated by PCR amplification of corresponding sequences in full length TRβ. GAL-TR LBD (amino acid residues 202–460) and GST-TR expression vectors were generated by cloning PCR fragments into pM (Clontech) and pGEX-5X vector (Amersham Pharmacia) [4]. PGC-1α expression vector was a gift of Dr. Bruce Spiegelmann (Harvard Medical School, Boston, MA) [17]. *PGC-1mut* (LxxAA) was generated by PCR-based mutagenesis using QuickChange^{TM} Site-Directed Mutagenesis kit (Stratagene). Full length PGC-1α was cloned into PM vector to create Gal4-PGC-1α. GST-PGC-1α LxxLL peptide and mutant peptide expression vectors were created by cloning synthetic oligonucleotides corresponding to amino acids 130–160 into pGEX-5X vector. PGC-1α N- and C-terminal deletions were generated by PCR-amplification and cloned into pCMV-Tag 2B vector (Stratagene). For western blot and immunoprecipitation, PGC-1α cDNA was cloned into pCMV-Myc-tag vector (Clontech) and TRβ cDNA was cloned into pCMV-Tag 2B to generate flag tagged TRβ.

2.2. Cell culture

U2-OS, HeLa and CV-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Hyclone Laboratories) at 37 °C and 5% CO_2. For transfection, cells were seeded in 12-well plates at density of 1×10^5 cells/well in 10% FBS-DMEM overnight. Transfections used TransFectin^{TM} Lipid Reagent (Bio-Rad, Hercules, CA) using manufacturer's suggested protocol. T_3 or other ligands were added to the culture media 4 h after the plasmid–TransFectin mixture.

2.3. Luciferase assays

Transfected cells were washed with phosphate-buffered saline (PBS) and harvested with cell lysis buffer containing 0.1 M Tris (pH 7.8) and 1% Triton100. Luciferase Activity was measured in a TopCount NXT luminescent counter (PerkinElmer). In some experiments, electroporation was used as previously described [4,36]. The plasmid pJ3 LacZ containing the β-galactosidase (β-Gal) gene was cotransfected in all assays as an internal control and β-Gal activity was measured by standard methods.

2.4. Co-immunoprecipitation (co-IP) and western blot

U2OS cells were seeded in 10 mm dishes at density of 3×10^6 (cells) and cotransfected with plasmid DNA including Flag-TRβ, Myc-PGC-1α and Myc-PGC-1α mutant. After incubation with plasmid DNA–Transfectin (1:3 ratio) mixture overnight, cells were treated with T_3 (10^{-7} M) for 4 h. The monolayer was washed with PBS and lysed with buffer containing 1% Triton-100, 1 M Tris, PMF and proteinase inhibitor cocktail. Cell lysates were sonicated for 10 s three times on ice and then centrifuged at maximum speed for 15 min. The protein concentration of the supernatants was measured and 500 μg of cell lysate was taken for immunoprecipitation. Cell lysates were pre-cleaned with normal rabbit IgG (approximately 4 μg) at 4 °C for at least 1 h. Non-specific protein-IgG complexes were precipitated with protein G-sepharose (50 μl) followed by centrifugation. Pre-cleaned cell lysates were then incubated with 4 μg rabbit anti-Flag antibody (Rockland) and shaken at 4 °C overnight. To precipitate IgG-TR-PGC-1α complex, 50 μl of pre-washed protein G-sepharose beads (GE Health Care) was added to the cell lysates and continuously shaken at 4 °C for another 2–4 h followed by centrifugation (eppendorf centrifuge) at max speed for 30 s. After removing supernatant, the precipitate was washed with lysis buffer for at least 5 times. After the last wash, all supernatant was removed and bound IgG-TRβ-PGC-1α complex was eluted from the beads by boiling in SDS loading buffer. The sample was heated and spun down at max speed for 1 min. Approximately 15–20 μl of supernatant was subjected to SDS-PAGE (7.5% polyacrylamide). For western, 15–20 μl of starting material was used. After electrophoresis, the proteins were transferred to a PVDF membrane. The membrane was then soaked in block buffer containing 5% milk in TTBS for 45 min and probed by mouse anti-Myc antibody and HRP conjugated goat anti mouse-IgG. The proteins were visualized with ECL system.

2.5. Pull-downs

Fusions of glutathione-S-transferase (GST) to TRβ and TRβ domains or PGC-1α and GRIP1 fragments were prepared by bacterial expression and linked to solid supports using standard methods [37]. Bait proteins were synthesized and labeled with

Download English Version:

https://daneshyari.com/en/article/8339514

Download Persian Version:

https://daneshyari.com/article/8339514

Daneshyari.com