Jab1 is a $T_2$-dependent coactivator or a $T_3$-dependent corepressor of TRB1-mediated gene regulation

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Abstract

Thyroid hormones (THs) induce pleiotropic effects in vertebrates, mainly through the activation or repression of gene expression. These mechanisms involve thyroid hormone binding to thyroid hormone receptors, an event that is followed by the sequential recruitment of coactivator or corepressor proteins, which in turn modify the rate of transcription. In the present study, we looked for specific coregulators recruited by the long isoform of the teleostean thyroid hormone receptor beta 1 (L-Trb1) when bound to the bioactive TH, $3,5$-$T_2$ ($T_2$). We found that jun activation domain-binding protein1 (Jab1) interacts with L-Trb1 + $T_2$ complex. Using both the teleostean and human TRB1 isoforms, we characterized the Jab1–TRB1 by yeast two-hybrid, pull-down and transactivation assays. Our results showed that the TRB1–Jab1 interaction was ligand dependent and involved the single Jab1 nuclear receptor box, as well as the ligand-binding and N-terminal domains of TRB1. We also provide evidence of ligand-dependent, dual coregulatory properties of Jab1. Indeed, when $T_2$ is bound to L-Trb1 or hTRB1, Jab1 acts as a coactivator of transcription, whereas it has corepressor activity when interacting with the $T_3$-bound S-Trb1 or hTRB1. These mechanisms could explain some of the pleiotropic actions exerted by THs to regulate diverse biological processes.

Introduction

Thyroid hormone (TH) signaling is essential for multiple biological processes including growth, development, reproduction and energy balance. The genomic effects of THs respond to intracellular $T_3$ levels and are transduced by the thyroid hormone receptors (TRs), which promote the activation or repression of a wide collection of genes (Grøntved et al. 2015). Two isoforms for the thrb gene have been identified in teleosts: long or L-Trb1 and short or S-Trb1, which differ by the presence of a nine amino-acid insert located in the ligand-binding domain (LBD) of L-Trb1. Previous work from our laboratory has shown that 3,5-diiodothyronine ($T_2$) is a specific ligand for L-Trb1, whereas S-Trb1 activates transcription only in the presence of 3,3’-5-triiodothyronine ($T_3$) (Mendoza et al. 2013). In a current consensus model, ligand binding
to the TR induces conformational changes facilitated by the packing of the C-terminal helix-12 against the LBD into an active position (Brélivet et al. 2012, Billas & Moras 2013). This molecular event results in the dismissal of corepressors and the exposure of the coactivator-binding surface, which in turn facilitates the interaction of the primary coactivators (Figueira et al. 2011). Primary coactivators interact directly with active TRs through the nuclear receptor (NR) recognition motif (NR box) ‘LxxLL’ (Savkur & Burris 2004). This allows the recruitment of secondary coactivators to ultimately form a transcriptional complex that harbors a vast enzymatic tool chest, which modulates chromatin structure and transcription-associated proteins through post-translational modifications (PTM) (Lonard & O’Malley 2007).

Previous findings from our laboratory using in vivo teleost models (García-G et al. 2007) as well as structure-function studies with teleost and human TRB1 isoforms (Mendoza et al. 2013) suggest that the binding of T2 or T3 differentially affects TRB1 conformation to promote the recruitment of specific sets of coregulators. To follow-up on this hypothesis, in the present study, we aimed to identify possible ligand-specific recruitment of coregulators to TRB1 isoforms. Here, we identified Jab1 as a TRB1 partner and showed that this protein acts as a coactivator or corepressor of TRB1 and that this Jab1-mediated activation or repression of gene transcription is ligand specific, suggesting that T2 and T3 could elicit opposite effects on a specific promoter when bound to the TRB1–Jab1 complex.

Materials and methods

Plasmids

Native and mutated TRB1 clones used in the present work were previously described (Mendoza et al. 2013). Full-length tilapia (Oreochromis niloticus) Jab1 was cloned as follows: total RNA from tilapia liver was reverse transcribed (oligo dT). Based on the available tilapia sequence (Accession No. XM_003443454.2; RefSeq Genome), the entire tilapia Jab1 ORF (1005 bp, 334 aa) was amplified using specific oligonucleotides for the 5′- and 3′-flanking regions. The sequence obtained presented 95.2% amino acid identity with the human counterpart. A Jab1 chimera (Jab1A) was generated by PCR-based site-directed mutagenesis using internal hybrid primers that introduced a substitution of the last two leucines of the NR box ‘RKLLELL’ by alanines (RKLLEAA). All constructs were ligated into pcDNA 3.3-TOPO-TA (Invitrogen) for transient transactivation assays; TRB1 clones were inserted into pGEX4-T (Amersham Pharmacia) for pull-down assays; L-Trb1 and L-Trb1-LBD were subcloned into pGBKT7 for yeast two-hybrid assays (see below). All clones are shown schematically in Fig. 1.

Library construction and yeast two-hybrid assay

The MATCHMAKER Library Construction and Yeast two-hybrid assay (Clontech) was used to construct a cDNA library using tilapia liver mRNA. The cDNA library was cotransformed with pGADT7-Rec vector into the haploid Mat-a yeast strain (Y187), whereas the L-Trb1-pGBKT7 expression vector was transformed into the haploid Mat-α yeast strain (Y2H Gold). The yeast two-hybrid assay (Y2H) was performed by mating the two transformed haploid yeast strains, according to the manufacturer’s protocol. Diploids expressing the interacting proteins were identified by nutritional selection using four reporters (ADE2, TRP, LEU and MEL1) in the presence of 10−6 M T2. After selection, positive clones were rescued in Escherichia coli and sequenced to identify their molecular identity by using the Basic Local Alignment Search Tool (BLAST).
program (National Center for Biotechnology Information; NCBI). The L-Trb1-Jab1 interaction was confirmed by yeast mating. Briefly, the Jab1-pGADT7-Rec vector was transformed into the Y187 yeast strain, and the L-Trb1-pGBK7 vector was transformed into the Y2H Gold yeast strain. Both strains were seeded on selective medium, in the absence or presence of 10⁻⁶ M T₃, interaction was confirmed by reporter activation.

**GST pull-down assays**

GST fusion proteins were expressed in *BL21 Escherichia coli* induced by 0.4 × 10⁻³ M IPTG at 37°C for 3.5 h and incubated with glutathione–agarose beads to purify the GST fusion protein. Jab1 and Jab1A proteins were also expressed as GST fusion proteins as described previously, but were further digested with 0.1U/µg thrombin (Amersham Pharmacia). The presence of the digested protein was evaluated by Coomassie staining (Supplementary Figure 1, see section on supplementary data given at the end of this article). Jab1 or Jab1A proteins were incubated with GST, GST-L-Trb1, GST-S-Trb1, GST-L-Trb1-LBD and GST-S-Trb1-LBD fusion proteins bound to glutathione–agarose columns in binding buffer (2 × 10⁻² M Hepes pH 8, 1 × 10⁻³ M NaCl, 1 × 10⁻³ M EDTA, 4 × 10⁻³ M MgCl₂, 0.01 (v/v) NP-40, 10 (v/v) glycerol, 1 × 10⁻³ M DTT, 0.1 (v/v) saturated PMSF and 10 mg/mL BSA) and incubated for 4 h at 4°C with or without 10⁻² M T₃ or T₄. After incubation, the beads were washed, and the Jab1 or Jab1A agarose-bound proteins were analyzed by immunoblot using a primary monoclonal Jab1 antibody from Abcam (ab124720) and probed with a secondary anti-mouse IgG antibody coupled to HRP (Genetex, GTX213111-01).

**Transactivation assays**

GH3 cells (1 × 10⁴ cells/well) were seeded onto 96-well, white-wall plates and maintained in F12-K media, which was supplemented with 5 (v/v) of dialyzed fetal calf serum during the 24 h prior to transfection. All transfections were performed using Lipofectamine 2000 (Invitrogen), as previously described (Mendoza et al. 2013, Hernández-Puga et al. 2016). Cells were transfected with 125 ng pGL3-empty plasmid (Promega) as control reporter gene or SERCA-pGL3 plasmid (Promega) as TH reporter gene and 62 ng of one of the following plasmids: L-Trb1, S-Trb1, hTRB1 or delta N-terminal domain of L-Trb1 (ΔNTD-L-Trb1). Jab1 or Jab1A was cotransfected in increasing amounts ranging from 4 to 64 ng. Renilla pRL-CMV (12.5 ng) (Promega) was used as a reference reporter gene. Cells were treated with vehicle (NaOH, 0.05 N) or 10⁻² M T₃ or T₂ added in the culture media for 24 h, after which a dual-luciferase assay (Promega) was carried out using DLR-ready Varioskan (Thermo Scientific). For every experiment, renilla luciferase activity was used to control the number of cells transfected per well, whereas the empty pGL3 vector was used for subtracting the background activity from the SERCA-pGL3 reporter gene. Then, data were normalized as fold induction using the vehicle-treated cells as control. For clarity, the charts only show the results for TH-treated cells to depict the effect of increasing amounts of Jab1. All experiments were carried out independently at least three times.

**Western blotting**

Western blot analysis of cell lysates was performed to evaluate protein expression levels of L-Trb1, S-Trb1 and hTRB1 in GH3-transfected cells (see above). Total proteins were extracted with the ProteoJET Cytoplasmic and Nuclear Protein Extraction Kit (Fermentas) following the instructions of the manufacturer. Protein concentrations were determined by the Bradford Protein Assay. Subsequently, 50 µg of total protein were separated by 12% SDS-PAGE and transferred onto a nitrocellulose membrane using a standard protocol. The membrane was incubated with a primary monoclonal TR antibody from Abcam (ab2743) and probed with a secondary anti-IgG antibody coupled to HRP (Genetex, GTX213111-01). The membrane was exposed to an Amersham Hyperfilm ECL (GE Health Care Lifesciences), and the images obtained were digitalized. Actin was used as loading control using a polyclonal antibody from Santa Cruz Biotechnology (sc-1616).

**Supershift assays**

Supershift assays were carried out using the Dig Gel Shift Kit 2nd Generation (Roche). Nuclear proteins from tilapia liver (10 µg) were obtained as previously described (García-G et al. 2007) and incubated on ice for 30 min with 2 µg of Jab1 antibody (Abcam, ab124720) and/or 8 µg of TR antibody (Abcam, ab2743) in binding buffer (1 × 10⁻⁵ M Tris–HCl pH 8.0, 3 × 10⁻⁴ M KCl, 10 (v/v) glycerol, 0.2 × 10⁻³ M MgCl₂, 0.5 µg/µL BSA and 1 µg/µL poly dI-dC). A DIG-labeled Direct Repeat 4 response element (DR4, 5′-AGC TTC AGT CAC AGG AGG TCA GAG AG-3′)
was added, and the binding reactions were incubated for 15 min on ice followed by a 15-min incubation at room temperature. The reaction was loaded onto a 6.5% native polyacrylamide gel and resolved at 120 V over the course of 2 h. The DNA–protein complexes were visualized by following the instructions of the manufacturer. Excess cold DR4 or chicken β-actin oligonucleotides (5′-CTG GGA TGA TAT GGA GAA GAT CTG GCA CC-3′) were added to the binding reaction to evaluate specific and non-specific binding, respectively.

Quantitative PCR

Total RNA was extracted from tilapia tissues and cDNA was reverse transcribed from 2 µg of total RNA using oligo (dT) primer. Quantitative PCR was carried out in duplicate using tilapia β-actin and ubiquitin-conjugating enzyme E2Z (UBCE) as reference genes. The following oligonucleotides were used: β-actin, 5′-ACT TCG AGC AGG AGA TGG-3′ and 5′-GTT GTT TTC GTG GAT TCC-3′; UBCE, 5′-CTC TCA AAT CAA TGC CAC TTC C-3′ and 5′-CCC TGG TGG AGG AGG AGG-3′; Jab1, 5′-GAT CCC ACT CCG ACT ATT TCT G-3′ and 5′-GGG GGA GCT TTC TAT C-3′; Jab1A, 5′-GAT CCC ACT CCG ACT ATT TCT G-3′ and 5′-GGG GGA GCT TTC TAT C-3′. All products were amplified at 10′ at 95°C, 10′ at 95°C, 10′ at 61°C and 10′ at 62°C for 40 cycles and were cloned into pGEM-T vector (Promega). A plasmid standard curve that ranged from 10^2 to 10^9 molecules/µL was constructed. In all cases, reactions contained 1 µL of the reverse transcribed reaction, 6 µL Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Waltham, MA, USA), and 500 nM forward and reverse oligonucleotides in a final volume of 12 µL. A Step One instrument was used for detection and data analysis according to the manufacturer’s instructions (Applied Biosystems). The absolute mRNA concentration was expressed as molecules per microgram of total mRNA used in the RT reaction (2 µg) and obtained by interpolation with the standard curve and normalized to the concentration of β-actin or UBCE in each experimental sample. The data obtained with both reference genes were similar (Fig. 5).

Statistical analysis

Results were analyzed using ANOVA coupled to a Tukey post hoc test (control vs treatments) with the software GraphPad Prism 6. Differences were considered statistically significant at P values ≤ 0.05.

Results

Jab1 interacts with teleost L- and S-Trb1 isoforms

The screening of the tilapia liver cDNA library using L-Trb1 as bait in the presence of T_2 identified Jab1 as an interacting partner. This interaction was confirmed by yeast mating, where an activation of the reporter genes is observed in the absence of T_2, but enhanced when this hormone is present (Supplementary Figure 2). To characterize TRB1–Jab1 interaction, pull-down assays were performed using Jab1 and two full-length TRB1 isoforms, L-Trb1 and S-Trb1, in the presence or absence of T_2 or T_3. The results showed L-Trb1–Jab1 interaction in the absence of THs; however, the presence of T_2 or T_3 significantly enhanced the interaction between Jab1 and L-Trb1 (Fig. 2A).

We then analyzed the TRB1 and Jab1 domains that participate in the interaction. Most primary coregulators interact directly with the active coactivator-binding surface located at the NR LBD (Feng et al. 1998, Moras & Gronemeyer 1998). To elucidate if Jab1 interacted directly with this TR domain, we performed pull-down
Jab1 is a ligand-specific dual coregulator of TRB1

To explore the possible coregulatory effect of Jab1 on the transactivation activity of the two isoforms of TRB1, we transiently transfected the thyroid-responsive GH3 cells with increasing concentrations of Jab1 and treated them with a single dose of TH (10^{-7}M/48h). The results showed that Jab1 enhanced L-Trb1 transactivation in the presence of T_3 in a concentration-dependent manner, revealing Jab1 to be a coactivator; this response seems to be T_3 specific as Jab1 did not affect the expression of the TH-reporter gene in the presence of T_2 (Fig. 3). On the other hand, when Jab1 was cotransfected with S-Trb1, a receptor that binds only to T_3, a surprisingly opposite response was observed: repression of the transactivating activity was evident from the lowest Jab1 concentration used (Fig. 3).

We then explored this apparent coactivator/corepressor capacity of Jab1 by employing the human TRB1 (hTRB1), a receptor activated by both T_2 and T_3 (Mendoza et al. 2013). Our results revealed that Jab1 increased hTRB1 transactivation activity in the presence of T_3 (Fig. 2A). Furthermore, pull-down assays showed reduced signal intensities when Jab1 interacted with the LBD of S-Trb1 (S-Trb1-LBD), and no signal was obtained when full-length S-Trb1-Jab1A interaction was tested (Fig. 2B and C), suggesting that the S-Trb1-Jab1 interaction requires binding surfaces similar to those of L-Trb1.

Jab1 is a dual-coregulator of TRB1
of \(T_2\), whereas it induced a significant suppressing effect upon hTRB1 activity when the cells were stimulated with \(T_3\) (Fig. 3). The L-, S- and h-TRB1 protein levels from transfected GH3 cells were comparable in all experimental situations, as showed by Western blot (Fig. 3).

**Jab1 NR box and L-Trb1 N-terminal domain are determinant for a functional TRB1–Jab1 interaction**

As pull-down assays showed that the \(LxxLL\) motif was essential for TRB1–Jab1 interaction, we evaluated the functional relevance of this coregulator NR box. Transactivation assays were performed by co-transfecting Jab1A with L-Trb1, S-Trb1 or hTRB1 in GH3 cells, in the presence of \(T_2\) or \(T_3\). In contrast to our observations with Jab1 (Fig. 3), Jab1A did not modify the transactivating capacity of either the L-Trb1 + \(T_2\) and hTRB1 + \(T_2\) or the S-Trb1 + \(T_3\) and hTRB1 + \(T_3\) complexes (Supplementary Figure 3), demonstrating that a functional TRB1–Jab1 transactivating complex requires the interaction through the Jab1 NR box.

Pull-down assays suggested that the LBD was not the only TR domain involved in TRB1–Jab1 interaction (Fig. 2B); thus, we evaluated the participation of the TRB1 NTD, a domain known to function as a coregulator-binding surface (Tian et al. 2006). Transactivation assays were performed using NTD-truncated L-Trb1 (ΔNTD L-Trb1) co-transfected with either Jab1 or Jab1A in the presence of \(T_2\) or \(T_3\). The lack of the NTD practically abolished the transactivating capacity of the \(T_2\)-bound L-Trb1, and Jab1 did not induce the previously observed upregulatory effect on the transactivating capacity of this TRB1 isoform (Supplementary Figure 4); neither Jab1 nor Jab1A had an effect upon its transactivating capacity in the presence of \(T_3\). Together, these results suggest that both the NR box and NTD are necessary for the \(T_2\)-liganded L-Trb1 to adopt the holoreceptor conformation. As NTD is identical in L- and S-Trb1, it could be predicted that in the absence of NTD, Jab1 repressor effect over S-Trb1 transactivation capacity would be abolished; however, further experiments are required to evaluate this idea.

**Jab1 is present in TR-transcriptional complexes**

As an initial approach to gain insights on Jab1 relevance in the context of teleost TR signaling pathways, we looked for the presence of Jab1 and TR in transcriptional complexes. Supershift assays were performed using nuclear proteins from tilapia liver incubated with Jab1 and/or TR antibody and a canonical DR4-response element. The results showed that TR and Jab1 are indeed present in the same transcriptional complexes (Fig. 4). TR-complex specificity was probed with specific and non-specific competition assays (Fig. 4 and Supplementary Figure 5).

**Jab1 is expressed in vivo**

Jab1 expression was detected in juvenile tilapia spleen, gill, heart, liver, gut, muscle, skin, kidney and brain. In all
In the present study, we show that Jab1 acts as a dual coregulator when interacting with different TRB1 isoforms. Furthermore, this activation or repression of gene transcription is ligand dependent, suggesting that T₂ and T₃ have differential roles in gene modulation, adding further evidence that T₂ is a physiologically relevant ligand.

Our previous results showed that L-Trb1 and hTRB1 are active in the presence of T₂, and suggested that TRB1 could adopt different conformations in the presence of T₂ or T₃, exposing binding surfaces that favor the interaction with specific sets of coactivators (Mendoza et al. 2013). To test this last idea, we took advantage of the high affinity of L-Trb1 for both T₃ and T₂ to search for possible TRB1 ligand-specific coregulators. A yeast two-hybrid assay approach revealed Jab1 as a binding partner of L-Trb1 + T₂. Jab1 (AKA COPSS) has been primarily described as part of the COP9 signalosome complex (Cope et al. 2002) and is known to be a major modulator of E3 ubiquitin ligase involved in protein degradation through the proteosome pathway (Wei et al. 2008, Kato & Yoneda-Kato 2009). However, some subunits of the COP9 signalosome have also been shown to have independent activity as monomers; e.g., COPS2 (AKA Alien) has been characterized as a corepressor of TR (Papaioannou et al. 2007) and Jab1 was previously identified as a coactivator of several liganded NRs, including TRα1 (Chauchereau et al. 2000).

Our study shows that Jab1 exhibits opposite roles upon gene regulation. As we found Jab1 to be bound to L-Trb1 in the presence of T₂ (Supplementary Figure 2), we expected it to be a coactivator of the complex. Indeed, Jab1 enhanced T₂-dependent transactivation when interacting with L-Trb1 and also with hTRB1, and the two isoforms that bind and are activated by T₂. However, Jab1 had no effect upon gene transactivation when interacting with L-Trb1 bound to T₃ (Fig. 3), whereas it repressed S-Trb1 and hTRB1 transactivation activity below T₃-induced levels when bound to this TH (Fig. 3). Thus, in teleosts that express two TRB1 isoforms, one of which is preferentially activated by T₂ (L-Trb1) and the other exclusively by T₃ (S-Trb1), Jab1 exerts a ligand- and isoform-specific function. Interestingly, in the case of hTRB1, which is activated by both T₃ and T₂, Jab1 induced ligand-dependent dual effects. Other studies have shown ligand-specific coregulatory effects; for example, tesmin acts as a coactivator of mineralocorticoid receptor when bound to aldosterone but not to cortisol (Rogerson et al. 2014); likewise, repressor of tamoxifen transscriptional activity protein, RTA, acts as a corepressor of estrogen receptor-a when bound to selective hormone response modulators like tamoxifen, but not when bound to pure agonists (Norris et al. 2002). As far as we know, a dual coactivator and corepressor effect has only been reported for the receptor-interacting protein 140 (RIP140), which repressed gene activation mediated by glucocorticoid receptor and peroxisome proliferator-activated receptor gamma but enhanced androgen receptor activity. Although the mechanisms were not described, the authors suggested that PTM of RIP140 might be involved in the coregulator duality (Subramaniam et al. 1999). Because Jab1 participates in the proteosome pathway, the observed repression of gene activation when interacting with the T₃ bound to S-Trb1 or hTRB1 could be interpreted as TR degradation. However, this possibility is precluded by the results obtained after the overexpression of Jab1 and the different TR isoforms in GH3 cells (Fig. 3). Thus, to our knowledge, this is the first description of a dual coregulator that activates or represses, in a ligand-dependent manner, the activity of isoforms of the same NR. These findings raise the question of how Jab1 induces such opposite transcriptional effects. One possibility is that T₂ and T₃ induce specific conformational changes in the LBD of the TR that would expose particular coactivator-binding surfaces influencing the functional outcome, as seen with other NR bound to different ligands (Moore et al. 2004, Nettles & Greene 2005, Jeyakumar et al. 2008). This hypothesis is consistent with the findings of Chauchereau and coworkers (Chauchereau et al. 2000) who showed that Jab1 can bind to both TR and steroid receptor coactivator-1 (SRC-1), possibly participating as a functional bridge between the TR and other coregulators. In contrast, the repressor effects of Jab1 could be secondary to its interaction with corepressor proteins, such as NCoR, which has been detected as a binding protein in MCF7 cells (Lu et al. 2016). However, further studies must be performed to unravel the dual effect mechanism of Jab1.

We then characterized the Jab1–TRB1 interaction by using Jab1A, a protein with a mutated NR box (Fig. 1), in transactivation assays. Interestingly, both Jab1-mediated activation (T₂) and repression (T₃) activities were lost when Jab1A was co-expressed with TRB1 isoforms (Supplementary Figure 3), showing that Jab1 requires its unique NR box to interact as a primary
coregulator with the receptors. On the other hand, the TR coactivator-binding surface located in the LBD of the receptor is known to provide a primary coactivator-binding surface (Feng et al. 1998, Moras & Gronemeyer 1998). This protein domain was not sufficient for TRB1–Jab1 binding as only a weak interaction was observed between Jab1 and the LBDs of both L-Trb1 and S-Trb1 in the presence of T2 and T3 (Fig. 2), suggesting that other protein domains are essential for TRB1–Jab1 binding. In support of this idea, our functional assays showed that when a NTD-truncated L-Trb1 was tested, the L-Trb1–Jab1 complex lost all activity when bound to T2 (Supplementary Figure 4), confirming the importance of the full conformation of the receptor to provide a functional coactivator-binding surface, as reported previously by others (Hollenberg et al. 1995, Tomura et al. 1995, Blessing et al. 2015). Overall, the present results suggest that ligand binding induces a specific, three-dimensional conformation of the TRB1, as shown for other NR agonists and selective nuclear receptor modulators (SNUrMs) (Margeat et al. 2003, Kremoser et al. 2007), promoting a cooperative interaction between the NTD and LBD to provide a stable coregulator interaction surface. Thus, ligand-specific TRB1 ‘shaping’ could determine the orientation of the bound primary coregulator (Jab1), which would in turn influence the recruitment of secondary coregulators that could play a central role in modulating gene expression. Whether the ligand- or TR isoform-specific interaction with Jab1 triggers the recruitment of specific secondary coregulators remains an open question.

Finally, in the context of a functional correlation of the L-Trb1–Jab1 interaction, we found that Jab1 was recruited to TH-transcriptional complexes (Fig. 4), strongly suggesting that Jab1 could be a relevant coregulator in tilapia TR signaling and T2 a potent coregulator interaction. In support of this idea, Jab1 expression was detected in all analyzed juvenile tilapia tissues (Fig. 5) and is also ubiquitously expressed in human tissues (http://www.genecards.org/cgi-bin/cardisp.pl?gene=COPS5). Although the physiological role of Jab–TRB1 remains to be elucidated, both transcription factors are co-expressed in the same tissues (Fig. 5 and Ref. Cheng et al. 2010). This could allow their joint participation in the regulation of positively or negatively TH-regulated genes. In this context, ongoing transcriptomic analysis in tilapia liver and cerebellum show Jab1 to be a TH-sensitive gene as both T2 and T3 regulate its expression in a tissue-specific manner, suggesting a role of Jab1 in TH-dependent signaling pathways. Further experiments are required to shed light on the physiological implication of this novel mechanism.

Overall, the present work supports the idea that the pleiotropic effects of thyroid hormones could be explained in part by a mechanism for the activation or repression of gene transcription by specific ligands such as T2, which would in turn recruit a specific set of primary and/or secondary coregulators to differentially modulate chromatin structure and gene transcription.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-16-0485.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
G Hernández-Puga and A Mendoza performed the experiments; G Hernández-Puga, A Mendoza, A León-del Río and A Orozco designed the research and wrote the paper.

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