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A method for efficient production of recombinant thyroid hormone receptors reveals that receptor homodimer–DNA binding is enhanced by the coactivator TIF2

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Abstract

Thyroid hormone receptors (TRs) are ligand-activated transcription factors that mediate the biological effects of thyroid hormone (T3) by binding to thyroid hormone response elements (TREs), typically located in the promoter regions of T3-responsive genes. It is generally held that T3-induced gene activation is mediated by retinoid X receptor (RXR)–TR heterodimers. Although TR homodimers can bind to some TREs, T3 destabilizes this interaction, calling into question the ability of TR to activate transcription in the absence of RXR. TR–DNA binding has been difficult to study in vitro because mammalian TRs are notoriously difficult to produce in *Escherichia coli*. We considered that this may be due to codon bias. Therefore, we produced TRβ1 in *E. coli* Rosetta 2(DE3) which contains a plasmid that overexpresses the tRNAs corresponding to the seven rarest *E. coli* codons. This resulted in an improved yield of full-length TRβ1, which we then used in electrophoretic mobility shift assays. We found the coactivator TIF2 greatly enhances binding of T3-occupied TRs to a subset of TREs, suggesting TRs may activate transcription from these TREs in an RXR-independent manner.

Keywords: Thyroid hormone receptor; Retinoid X receptor; Triiodothyronine; Thyroid hormone response element; Homodimer; Coactivator

Thyroid hormone receptors (TRs) and retinoid X receptors (RXRs) are members of the nuclear hormone receptor superfamily. TRs are ligand-activated transcription factors that mediate the biological effects of thyroid hormone (3,5,3′-triiodothyronine, T3). TRs exert their effects on gene expression by binding to thyroid hormone response elements (TREs), which typically are located in the promoter regions of T3-responsive genes. TREs usually are composed of two receptor-binding "half sites" with the consensus sequence AGGTCA arranged as a direct repeat separated by 4 bp [1,2]. It is generally held that RXR–TR heterodimers mediate gene induction by T3, although the evidence for this is largely circumstantial. By electrophoretic mobility shift assay (EMSA), TRs prefer to bind to most TREs as RXR–TR heterodimers [3–5], with RXR occupying the 5′ half site and TR the 3′ half site [6,7]. However, TRs bind with highest affinity to an 8 bp sequence, $T^{A}/_{G}AGGTCA$ [8]. When the TRE 5′ half site contains this sequence, TRs can bind even in the absence of RXR. Interestingly, these TR homodimer–TRE complexes are unstable in the presence of T3 [9], calling into question the ability of TRs to mediate T3 responsiveness independent of RXR. The role of RXR in T3-mediated gene regulation cannot be tested directly due to the absence of RXR-null mammalian cells. However, studies in *Saccharomyces cerevisiae* (which lack endogenous nuclear receptors) suggest that TRs can regulate gene expression in the absence of RXR if the TRE has the extended 8 bp 5′ half site [10].

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Thyroid hormone receptors do not themselves activate transcription, but rather, function by recruiting a series of coregulatory proteins to the target promoter. Unliganded TRs interact with a corepressor complex that possesses histone deacetylase activity, thereby repressing transcription. The binding of ligand triggers a conformational change in the TR that results in release of the corepressor and recruitment of a coactivator complex. One very important group of coactivators is the p160 family, which is typified by the protein transcription intermediary factor 2 (TIF2)/glucocorticoid receptor interacting protein 1 (GRIP1) [11–13]. The p160 proteins possess histone acetyltransferase activity, which loosens chromatin structure and hence leads to gene activation.

In vitro studies of TRs are hampered by the lack of a simple method to produce substantial quantities of purified recombinant protein. In contrast to RXRs, mammalian TRs are notoriously difficult to produce in *Escherichia coli*. Various approaches have been used to overcome this [such as coexpression of thioredoxin and expression as a glutathione *S*-transferase (GST) fusion protein], but these only help marginally. We considered that codon bias might be an important factor limiting the ability to produce TRs in *E. coli*. Certain codons that are commonly used in mammalian mRNAs are rarely used in *E. coli*. The tRNAs that recognize these rare codons are present at very low levels in *E. coli*, which can impede the production of full-length mammalian proteins [14]. To this end, we compared *E. coli* BL21(DE3) with Rosetta 2(DE3) as hosts for producing recombinant TRβ1. Rosetta 2(DE3) cells are *E. coli* BL21 derivatives that carry a plasmid expressing tRNAs that recognize the seven codons rarely used in *E. coli* but commonly found in mammalian mRNAs: AGA, AGG, AUA, CUA, GGA, CCC, and CGG. Purification of TRβ1 from Rosetta 2(DE3) competent cells substantially improved the yield of full-length protein, which was functional in DNA binding, ligand binding, and interaction with coactivators. Using this recombinant TR in EMSAs, we found that the coactivator TIF2 greatly enhances the binding of T3-occupied TRs to a subset TREs, suggesting that TRs may activate transcription from these TREs in an RXR-independent manner.

Materials and methods

Protein expression and purification

Rat TRβ1, mouse RXRα, and the human TIF2 nuclear receptor interaction domain (amino acids 624–869 [15]) were expressed in *E. coli* as GST fusion proteins from the vector pGEX-KG [16]. This vector places a thrombin cleavage site immediately after GST, allowing the recombinant protein of interest to be purified by thrombin digestion of the fusion protein while it is adsorbed to glutathione–agarose beads. The polymerase chain reaction was used to add a 5′ *Eco*RI site and a 3′ *Sal*I site to the TRβ1 and RXRα cDNAs, allowing ligation into pGEX-KG. The 3′ PCR primers also added six histidines to the carboxyl termini of both receptors, resulting in tags at both ends of the proteins to facilitate purification of full-length receptors. PCR was used to add a 5′ *Nco*I site and a 3′ *Xho*I site to the TIF2 nuclear receptor interaction domain cDNA, allowing ligation into pGEX-KG. All constructs were verified by sequencing. The protein expression plasmids were transformed into either *E. coli* BL21(DE3) or Rosetta 2(DE3) (Novagen). The BL21(DE3) cells also contain a plasmid that overexpresses thioredoxin, as this has been shown to marginally enhance the yield of TRs [17,18]. An overnight culture in SB (32 g tryptone, 20 g yeast extract, 5 g NaCl, 5 ml of 1 N NaOH, and water to 1 L total volume) was diluted 1:50 into 1 L SB. The cultures were shaken at 300 rpm at 30 °C until an $A_{600\,nm}$ of 0.7–0.8 was obtained (~4 h). Recombinant protein expression was induced with the addition of isopropyl-β-D-thiogalactopyranoside to 0.1 mM, followed by further incubation at 30 °C with shaking at 300 rpm for 3 h. The bacteria were centrifuged at 4000*g*, 30 min, 4 °C, and the pellets were quick-frozen in liquid N_2 and stored at −70 °C overnight. The following day the bacterial pellets were thawed rapidly and suspended in 25 ml of ice-cold lysis buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 5 mM dithiothreitol (DTT), and 0.05% Triton X-100) supplemented with EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals) followed by lysis in a French press. The lysate was centrifuged at 24,000*g* for 30 min at 4 °C. The supernatant was added to 1 ml glutathione–agarose beads (Pierce) that had been washed with 10 ml ice-cold lysis buffer. After 30 min of gentle rocking at 4 °C, the agarose bead–protein mixture was centrifuged at 1000*g* for 5 min at 4 °C. The pellet was washed three times with 10 ml of ice-cold lysis buffer. To liberate the recombinant protein of interest, the beads were washed once in 0.5 ml of ice-cold thrombin buffer (50 mM Tris, pH 8, 150 mM NaCl, 2.5 mM $CaCl_2$, and 5 mM DTT), then resuspended in 0.5 ml thrombin buffer with 3 U thrombin (Sigma), and incubated at room temperature with gentle rocking for 20 min. The resin was microfuged at 10,000 rpm for 2 min at 4 °C, the supernatant was stored on ice, and a second thrombin digestion was performed as before. Each of the supernatants from the thrombin cleavage step was kept in separate tubes on ice. Protein concentrations were determined using BioRad Reagent (Cat. # 500-0006) according to the manufacturer's directions. Glycerol was added to each sample to attain 10%, and the protein samples were aliquoted, quick-frozen in liquid N_2, and stored at −70 °C.