Thyroid Hormone Dependent Transcriptional Programming by TR β Requires SWI/SNF Chromatin Remodelers

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ABSTRACT

Transcriptional regulation in response to thyroid hormone (T_3) is a dynamic and cell-type specific process that maintains cellular homeostasis and identity in all tissues. However, our understanding of the mechanisms of thyroid hormone receptor (TR) actions at the molecular level are actively being refined. We used an integrated genomics approach to profile and characterize the cistrome of TR β , map changes in chromatin accessibility, and capture the transcriptomic changes in response to T_3 in normal thyroid cells. There are significant shifts in TR β genomic occupancy in response to T_3 , which are associated with differential chromatin accessibility, and differential recruitment of SWI/SNF chromatin remodelers. We further demonstrate selective recruitment of BAF and PBAF SWI/SNF complexes to TR β binding sites, revealing novel differential functions in regulating chromatin accessibility and gene expression. Our findings highlight three distinct modes of TR β interaction with chromatin and coordination of coregulator activity.

INTRODUCTION

Thyroid hormones play key roles in maintaining cell identity, metabolism, and homeostasis in all tissues. Thyroid hormone receptors (TRs) function primarily as transcription factors that regulate broad networks of target genes in response to fluctuations in thyroid hormone (T_3) levels. Two distinct genes encoding TR α (*THRA*) and TR β (*THRB*) are expressed in a tissue-specific patterns. TR β is the predominant TR expressed in the liver, kidney, and thyroid, while TR α is expressed the heart, bone, and brain¹. The classic bimodal switch model (reviewed in^{2,3}) where TR is constitutively bound to chromatin and T_3 binding promotes dissociation of corepressors and recruitment of coactivators, was the predominant model used to describe transcriptional regulation by TRs for many years. However, a number of studies that point to more nuanced mechanisms have recently been put forth. For example, multiple genome-wide studies of TR binding have shown significant T_3 -dependent recruitment of TR β to chromatin and *de novo* chromatin remodeling, in opposition to the bimodal switch model⁴⁻⁶. Additionally, it has been demonstrated that a large proportion of TR β binding occurs in distal regulatory elements, and that TR β coordinates histone acetylation of enhancer regions and higher order chromatin structure⁷. Recruitment of cofactors by TR β has been recently described as a T_3 -dependent coregulator shift, rather than a complete loss of corepressors and gain of co-activators upon ligand binding⁸. Our data supports a multi-modal regulation model for TR β interaction with chromatin that integrates each of these concepts.

ATP-dependent chromatin remodeling complexes are known to act as coregulators for nuclear receptor transcriptional regulation and have been implicated in chromatin remodeling specifically by TR β . SWI/SNF components were found to be associated with NCOR, a well-known co-repressor for TRs and other non-steroidal hormone receptors⁹. This provided indirect evidence that SWI/SNF chromatin remodeling might be required for target gene repression by TRs. We have described an interaction between the SWI/SNF core subunit BRG1 and TR β for repression the oncogene RUNX2¹⁰. Recruitment of the SWI/SNF complex to T_3 -activated promoters has been suggested to be dependent upon interactions between SRC and p300, rather than a direct interaction between BRG1 and TR¹¹. Direct interaction between BAF57, a key SWI/SNF subunit, and TR β have been observed at T_3 -responsive promoters¹². Given the limited studies of SWI/SNF participation in T_3 -regulated gene expression, a specific mechanism for TR interactions with SWI/SNF complexes has yet to be defined.

In this study, we mapped the binding sites of endogenous TR β in normal thyroid cells and integrated this data with changes in chromatin accessibility to classify three distinct modes of TR β chromatin interaction and remodeling. However, a majority of TR β binding sites were found

in distal regulatory elements. In order to identify the protein-protein interactions involved in T_3 -dependent transcriptional regulation, we performed a proximity labeling assay followed by mass spectrometry. Some interactions between TR β and its binding partners are gained or lost with T_3 , but notably the majority do not exhibit a T_3 -dependent switch. Differential enrichment of interactions between TR β and members of BAF and PBAF SWI/SNF complex subspecies were identified. We further demonstrated that TR β differentially recruits SWI/SNF complexes to its binding sites. Based on our comprehensive genomic and proteomic analyses, we propose a new model whereby selective recruitment of BAF and PBAF SWI/SNF complexes to TR β binding sites regulates chromatin accessibility and gene expression.

RESULTS

TR β binds to and remodels chromatin in three distinct modes.

We profiled the genomic binding patterns of TR β in the human normal thyroid epithelial cell line Nthy-ORI using CUT&RUN¹³. This approach allows high-resolution detection of genomic binding sites of low abundance transcription factors compared with ChIP-seq. Replicate CUT&RUN peaks were used to generate high confidence peak sets for TR β with and without 10nM T_3 for 6 hours (**Supplemental Figure 1A**). We identified a total of 8,200 TR β binding sites that we classified into three distinct groups based on response to T_3 treatment (**Figure 1A**). The largest group is the liganded TR β binding sites ($n = 6,768$), which have significantly increased TR β enrichment when T_3 is present. Unliganded TR β binding sites ($n = 959$) are enriched with TR β only in the absence of T_3 , and ligand-independent binding sites ($n = 473$) are detected both in the presence and absence of T_3 . The enrichment profile of TR β binding at each of the three groups of sites was visualized in a heat map (**Figure 1B**), and in representative genome browser shots (**Figure 1C**). Accordingly, we observed a nearly complete loss of TR β enrichment at unliganded binding sites with T_3 treatment, while the enrichment remained stable at ligand-independent binding sites with T_3 treatment. Liganded binding sites exhibited a significant gain in TR β enrichment upon T_3 treatment. The gain in signal at liganded binding sites is consistent with the dynamic assisted loading model^{14,15}, the concept whereby nuclear receptors have sparse transient interactions with chromatin and ligand binding increases the residency time to stabilize interactions with response elements.

Annotation of TR β binding sites revealed differences in the distribution of TR β binding relative to transcriptional start sites (TSS) (**Figure 1D**). 30% of ligand-independent sites mapped to proximal promoter regions (within 500bp of TSS), while only 18% of liganded and 9% of unliganded TR β binding sites mapped to a proximal promoter. Unliganded binding sites were more likely to map to distal regulatory elements (28%), compared to liganded binding sites (19%). Calculating the distance to the nearest TSS of each of these three types of binding sites revealed that ligand-independent binding sites tend to be closer to transcriptional start sites than others (**Figure 1E**). Transcription factor motif analysis revealed that thyroid hormone response elements (TRE) were strongly overrepresented in each of the three groups of TR β binding sites (**Supplemental Figure 2**). Ligand-independent binding sites have the highest frequency of the full-length direct-repeat palindromic TRE (DR4), while liganded sites have the highest frequency of TRE half-sites.

To map changes in chromatin accessibility in response to T_3 in Nthy-ORI cells, we performed ATAC-seq after 6 hours (early) and 24 hours (late) hours after treatment with T_3 (**Supplemental Figure 3**). Differential accessibility analysis revealed a significant increase in the chromatin accessibility with early and late T_3 treatments (7,754 and 21,678 opened regions, respectively; FDR < 0.05). In contrast, we found that only 107 regions were closed by T_3 treatment, all of which were closed with early T_3 treatment (**Figure 2A**). We next examined the chromatin

accessibility associated with TR β binding. Unliganded TR β binding sites, which are lost upon T₃ treatment, had relatively little T₃-induced chromatin accessibility with a modest decrease in accessibility with T₃ treatment (**Figure 2B**). However, chromatin accessibility increased at liganded binding sites with early T₃ treatment and further increased with late T₃ treatment (**Figure 2C**). Ligand-independent binding sites also had significant increases in chromatin accessibility after 6 and 24 hours of T₃ treatment (**Figure 2D**). To estimate the size of differentially accessible regions of chromatin following T₃ treatment, we compared the average peak width of differentially accessible ATAC-seq peaks within 5kb of each of the groups of TR β binding sites (**Figure 2E**). Unliganded TR β binding sites had the smallest average peak width (190 bp), consistent with the modest changes in accessibility near those sites. Differentially accessible peak width was greatest for ligand-independent TR β binding sites (355 bp) followed by liganded sites (253 bp). This suggests that remodeling around liganded binding TR β binding sites is more focused while remodeling around ligand-independent TR β binding sites stretches across broader regions.

T₃ induces changes in the TR β interactome.

To detect protein-protein interactions between TR β and potential coregulators, including transient cofactors that are exchanged in a T₃-dependent fashion, we used a live cell proximity-dependent biotin labeling assay (**Figure 1A**). Nthy-ORI cells were transfected with vectors that express a TR β -miniTurboID fusion construct that can rapidly biotinylate proximal proteins, which were isolated via biotin-affinity purification and subsequently identified by mass spectrometry (**Figure 3A, Supplemental Figure 4**). We identified a total of 1,328 high-confidence proteins that interact with TR β either in the presence and absence of T₃. Differential enrichment analysis revealed that 75 of these were gained in the presence of T₃, and 70 were lost in the presence of T₃ (**Figure 3B, Supplemental Table 1**). The largest group are unchanged interactions (1,183 proteins). Pathway analysis showed an enrichment of distinctive biological processes associated with the different groups of TR β interaction partners that change with T₃ treatment (**Figure 3D**). Interacting proteins that were lost with T₃ treatment were enriched with transcription repressor activity, demethylase activity, and translation factor activity, while gained proteins were enriched with transcriptional coactivator and RNA polymerase binding activity. Interacting proteins that remained stable were classified as nucleosome remodeling, ATPase activity, and histone binding proteins.

To examine the impact of ligand-dependent interactions and DNA-binding dependent interactions, we compared the interaction profiles of a ligand-binding domain mutant (TR β^{PV})¹⁶ and a DNA-binding domain mutant (TR β^{GS125})¹⁷ with that of wildtype TR β . The subset of 143 T₃-induced differential interactions with wildtype TR β were altered by TR β^{PV} and TR β^{GS125} mutants (**Figure 3C**). TR β^{PV} mutant retained many of the wildtype interactions, but did not exhibit differential binding upon T₃ treatment, while TR β^{GS125} mutant lost many interactions entirely. Our analysis of the TR β interactome is consistent with a co-regulatory shift model⁸, where, rather than an all-or-nothing switch, T₃ alters the ratio of corepressor to coactivator binding partners.

We focused our analysis on interacting proteins that were likely to participate in chromatin remodeling. Several multi-subunit chromatin remodeling complexes were identified in our proximity labeling assay such as SWI/SNF, Mi-2/NURD, the NCOR and SIN3 co-repressors, and the CBP/p300 and TRAP/DRIP/Mediator co-activators (**Figure 3E**). Each of these has been previously linked to TR β gene regulation¹⁸⁻²². We identified several members of each of these complexes as in our proteomic dataset. An interaction score was calculated for TR β -associated chromatin remodeling complexes by dividing the sum of the signal intensity of each subunit in the complex by the total subunits identified (**Figure 3F**). The Mi-

2/NURD, SIN3, and TRAP/DRIP/Mediator complexes did not have significant changes in their interaction scores. There was an increase in the CBP coactivator complex interaction score and decrease in the NCOR corepressor complex interaction score with T₃, consistent with previous studies of their interaction with TRβ¹⁸. The SWI/SNF complex also significantly increased with T₃. TRβ^{PV} and TRβ^{GS125} mutations both inhibited these T₃-dependent interactions.

TRβ differentially recruits SWI/SNF complexes.

Upon closer examination of the SWI/SNF complex subunits that were identified as TRβ interacting proteins, it became apparent that TRβ interacts with two different subspecies of SWI/SNF complexes: canonical BAF and polybromo-associated BAF (PBAF). Each of these contains one of the mutually exclusive ATPase catalytic subunits, (BRG1 or BRM) which are necessary for nucleosome displacement, array of accessory components, and a few subspecies-specific subunits²³ (**Figure 4A**). Our proteomic analysis identified the BRG1 core subunit, many of the accessory components, and three subspecies-specific subunits. Enrichment of the BAF-specific subunit ARID1A (BAF250) was decreased with T₃ treatment, while the PBAF-specific subunits ARID2 (BAF200) and PBRM1 (BAF180) were increased in the presence of T₃ (**Figure 4B**). BAF57 enrichment was also increased in the presence of T₃. BRG1 was not differentially enriched.

Based on differential enrichment observed in our proximity ligation data, we performed CUT&RUN to determine T₃-induced changes in genomic binding of ARID1A as a representative of BAF complexes, PBRM1 as a representative of PBAF complexes, as well as BRG1 (**Supplemental Figure 1B-D**). We then examined the binding of each of these factors specifically at TRβ binding sites. BRG1 CUT&RUN tag density was unchanged with T₃ at unliganded sites, while ARID1A tag density was low with a slight increase with T₃, and PBRM1 tag density was low (**Figure 4C**). This indicates that unliganded TRβ may recruit BRG1 to prime its binding sites in a manner similar to a mechanism described for the glucocorticoid receptor²⁴. Ligand-independent sites had an increase in all three SWI/SNF components with T₃, indicating that both BAF and PBAF complexes are recruited (**Figure 4D**). Liganded binding sites had an increase BRG1 tag density, while ARID1A and PBRM1 remained stable (**Figure 4E**).

To further examine differential recruitment of BAF and PBAF complexes, TRβ peaks were annotated based on whether they occurred near a promoter (< 5kb from nearest TSS) or in a distal regulatory region (> 5kb from nearest TSS). The CUT&RUN tag density of SWI/SNF subunits were quantified near two subsets of peaks (**Figure 5A**). As expected, BRG1 was recruited to both sites in the presence and absence of T₃. ARID1A was recruited in a T₃-dependent manner to both TRβ-bound promoters and distal binding sites. PBRM1, however, was recruited preferentially to TRβ-bound promoters and not to distal binding sites. Visualization of representative binding sites in genome browser shots (**Figure 5D**) further demonstrated differential recruitment. There is a much greater degree of change in chromatin accessibility at 6 and 24 hours near TRβ-bound promoters (**Figure 5B**) than at distal binding sites (**Figure 5C**). Given that both BAF and PBAF complexes are recruited to promoters, both complexes may be required to facilitate the large changes in chromatin accessibility observed (**Figure 5B**).

TRβ chromatin interactions are correlated with target gene expression.

To determine the effect of T₃ treatment on gene expression, Nthy-ORI cells were treated with 10nM T₃ or vehicle control for 6 and 24 hours and global transcriptomic analysis by RNA-seq was performed. Differential gene expression analysis was performed comparing each treatment to the corresponding control. We determined that 366 and 368 genes were up and downregulated, respectively, by T₃ at 6 hours (**Figure 6A, Supplemental Table 2**). The effect was increased at the 24-hour period where 480 and 667 up and downregulated genes,

respectively. Enriched biological function pathways within early and late DEGs were compared using Ingenuity Pathway Analysis software (**Figure 6C**). Highly enriched upregulated pathways included cellular homeostasis, survival, viability, and cell cycle progression. Transcription and protein synthesis switched from down- to upregulated between early and late T₃ treatment, while carbohydrate metabolism switched from up- to downregulated. Apoptosis, cell transformation, and ER stress response were downregulated. BETA²⁵ was used to predict whether T₃-regulated genes are likely to be direct transcriptional targets of TR β based on proximity of a TR β peak to the TSS (**Figure 6B**). 63% (230/366) of early upregulated genes and 51% (245/480) of late upregulated genes were predicted to be direct targets of TR β . This suggests that increases in chromatin accessibility that occur in the time between early and late T₃ timepoints (**Figure 2A**) may allow TR β to access additional binding sites for induction of a subsequent set of target genes. Conversely, 48% (177/368) of early and 18% (120/667) of late downregulated genes were predicted to be direct targets of TR β , which indicates that direct TR β -induced gene repression occurs more immediately and the additional downregulation we observed may be secondary effects downstream of the early transcriptomic effects of T₃-treatment.

We examined SWI/SNF complex recruitment to promoters of T₃-induced differentially expressed genes (**Figure 6D**). A majority of early upregulated genes have a SWI/SNF complex binding site within 10kb of the TSS; most have both ARID1A and PBRM1 peaks, while a minority had exclusively one or the other. There was a similar trend in the late upregulated genes. In contrast, a majority of downregulated genes, both early and late, did not have SWI/SNF binding near their promoter. However, among those that did there was a preference for ARID1A recruitment over PBRM1. Combined, these results suggest that SWI/SNF complexes participate in T₃-induced gene regulation near promoters of target genes, particularly in the context of upregulation. For upregulation of gene expression, both BAF and PBAF complexes are likely to be recruited to remodel the proximal promoter region, while BAF complexes may be preferred for remodeling of downregulated promoters.

DISCUSSION

Although it is now appreciated that the classic model for TR β interaction with chromatin is oversimplified, a consensus has yet to be reached on an updated model. Based on the data presented here, we propose a multi-modal regulation model where TR β has at least three distinct modes of binding and remodeling chromatin (**Figure 7**). In agreement with previous genome-wide studies^{4-6,8}, we observed significant shifts in TR β occupancy in the presence and absence of T₃. Unliganded TR β binds to chromatin with limited effects on chromatin accessibility, but much of this binding is lost upon T₃ treatment. Liganded binding sites represent the vast majority of TR β binding, and they are associated with significant changes in localized chromatin accessibility (**Figure 1A,B; Figure 2C**). Intriguingly, liganded binding sites show a clear increase in enrichment upon the addition of T₃, however most sites have some, albeit low, enrichment before T₃ is added (**Figure 1B**). This suggests that TR β interactions with this type of site are transient and are stabilized by ligand binding and recruitment of coregulators, consistent with a dynamic assisted loading model^{15,26}. Since they are numerous and broadly distributed across the genome, there are likely multiple functions of liganded binding sites that further studies may clarify. While some of these binding sites occur near proximal promoters and facilitate direct induction or repression of gene expression, many occur in distal regulatory elements and may regulate enhancers or coordinate higher order chromatin structure. Ligand-independent binding sites are defined by enrichment both in the presence and absence of T₃, and substantial induction of chromatin accessibility (**Figure 1A,B; Figure 2D**). While they have a clear functional importance for regulation of gene expression, these binding sites represent a small minority of the overall cistrome of TR β . Notably, ligand-independent binding sites have many of the characteristics originally described in the bimodal switch model such as their

proximity to transcriptional start sites, high frequency of full-length DR4 TREs, and high regulatory potential.

TR β binding sites are also characterized by differential recruitment of SWI/SNF chromatin remodelers. BRG1 alone is recruited to unliganded and liganded sites in the absence of T₃ (**Figure 4D**) suggesting that that it may be recruited to prime binding sites for TR β occupancy, a mechanism which has been clearly delineated for the glucocorticoid receptor²⁴. BAF and PBAF complexes, each with unique subunits which dictate their precise function, are recruited to TR β binding sites within promoters and likely contribute directly to the changes in chromatin accessibility and recruitment of other transcription factors to alter target gene expression. Both BAF and PBAF complexes have been implicated previously in hormone-dependent gene regulation^{27,28}, however the distinct functional role of each when they are recruited by a nuclear receptor to the same location remains unclear. BAF complexes are preferentially recruited by TR β to binding sites that occur in distal regulatory regions. This might be a mechanism by which TR β primes these binding sites or organizes higher order chromatin structure to promote persistent changes in gene expression. These multifaceted interactions with a single chromatin remodeling complex illustrate the importance of the proposed coregulator shift model⁸ over a complete coregulator switch.

As a deeper understanding of the variety of ways in which TRs regulate gene expression and coordinate a network of cofactors is developed, it is important that the models we use reflect their multidimensional function. We suggest a model for multi-modal regulation by TR β that has at least three distinct modes defined by their T₃-dependent occupancy, changes in accessibility, and differential recruitment of chromatin remodelers. Collectively, this study provides a next-generation model for TR β interactions with chromatin, and lays a foundation for further studies of TR β regulation of gene expression and recruitment of key cofactors in both normal cells and in disease models.

METHODS

Cell Culture and Hormone Treatments. Nthy-ORI cells (Sigma) were routinely cultured in RPMI 1640 growth media with L-glutamine (300 mg/L) (Sigma), sodium pyruvate and nonessential amino acids (1%) (Cellgro/Mediatech), supplemented with 10% fetal bovine serum (Peak Serum) and penicillin-streptomycin (200 IU/L) (Cellgro/Mediatech) at 37°C, 5% CO₂, and 100% humidity. For T₃ treatments, cells were hormone-starved for 24 hours in growth media substituted with phenol-red free RPMI 1640 and charcoal-stripped fetal bovine serum (Sigma) prior to the addition of 10nM T₃ or NaOH vehicle for the indicated time course. Nthy-ORI cell line was authenticated by the Vermont Integrative Genomics Resource at the University of Vermont with short tandem repeat profiles using the Promega GenePrint10 platform.

CUT&RUN.

Sample collection and Sequencing: CUT&RUN was performed as described¹³. Briefly, Nthy-ORI cells were harvested, washed, and bound to activated Concanavalin A coated magnetic beads (Epicyphe 21-1401). Cells were then permeabilized with Wash buffer (20mM HEPES pH 7.5, 150mM NaCl, 0.5 mM spermidine 0.05% digitonin). Permeabilized cells were then incubated with the indicated antibody (Supplemental Table 3) at 4°C with constant agitation overnight. Cells were washed twice more before incubation with recombinant p-AG MNase (Epicyphe 15-1016) at 4°C for 2 hours. Liberated DNA was purified, and libraries were prepared using the NEB Ultra FS II DNA Library Kit (NEB E6177) and amplified with 14 cycles of PCR. Amplified libraries were then purified with AMPure beads (Agencourt), quantified via Qubit (Life Technologies), and quality was assessed using the BioAnalyzer (Agilent) High-

Sensitivity DNA kit. CUT&RUN libraries were pooled and sequenced on the Illumina HiSeq 1500 with 100 bp paired-end reads.

Data Analysis: Quality scores across sequenced reads were assessed using FASTQC. Illumina adapters were removed using Trim-Galore. Paired-end reads were mapped to hg38 using Bowtie2, and peaks were called using MACS2. Consensus peak sets for downstream analysis were derived using IDR²⁹ using two replicates (Supplementary Figure 2A) per target and a cut-off of 0.05.

ATAC-seq.

Sample collection and Sequencing: ATAC-Seq was performed as previously described³⁰ using 50,000 Nthy-ORI cells with two biological replicates per condition. Libraries were generated using custom Nextera barcoded primers³⁰ and were amplified by PCR for a total of 10 cycles. Amplified libraries were then purified with AMPure beads (Agencourt), quantified using a Qubit (Life Technologies), and quality was assessed using the BioAnalyzer (Agilent) High-Sensitivity DNA kit. ATAC-seq libraries were then pooled and sequenced on the Illumina HiSeq 1500 with 100 bp paired-end reads.

Data Analysis: Quality scores across sequenced reads were assessed using FASTQC. Nextera adapters were removed using Trim-Galore. Paired-end reads were mapped to hg38 using Bowtie2, and peaks were called using MACS2. DiffBind³¹ was used to identify regions of differential accessibility. Consensus peak sets for downstream analysis were derived using IDR²⁹ using two replicates (Supplementary Figure 2) per target and a cut-off of 0.05.

RNA-seq.

Sample collection and Sequencing: Nthy-ORI cells were treated 10nM T₃ or vehicle for 6 or 24 hours prior to sample collection. Total RNA was extracted and purified using RNeasy Plus Kit (Qiagen) according to manufacturer's protocol. This was repeated to collect a total of three biological replicates per condition. Purity of the total RNA samples was assessed via BioAnalyzer (Agilent) and samples with an RNA integrity score >8 were used for library construction. rRNA was depleted from 1 µg of total RNA with the RiboErase kit (KAPA Biosystems). Strand-specific Illumina cDNA libraries were prepared using the KAPA Stranded RNA-Seq library preparation kit with 10 cycles of PCR (KAPA Biosystems). Library quality was assessed by BioAnalyzer (Agilent) to ensure an average library size of 300bp and the absence of excess adaptors in each sample. RNA-Seq libraries were pooled and sequenced on the Illumina HiSeq 1500 with 50 bp single-end reads.

Data Analysis: Quality scores across sequenced reads were assessed using FASTQC. All samples were high quality. For alignment and transcript assembly, the sequencing reads were mapped to hg38 using STAR. Sorted reads were counted using HTSeq and differential expression analysis was performed using DESeq2. Genes with a p-value of <0.05 and a log₂ fold change greater than 1 or less than -1 were considered differentially expressed (Supplemental Table 2).

Proximity Labeling by miniTurboID.

Cloning of 3xHA-miniTurbo-TRβ. 3xHA-miniTurbo-NLS_pCDNA3 vector was a gift from Dr. Alice Ting (Addgene plasmid # 107172; <http://n2t.net/addgene:107172> ; RRID: Addgene_107172)³². 3xHA-miniTurbo-NLS_pCDNA3 was linearized by PCR and THRB cDNA was inserted via HiFi Assembly Cloning (NEB E5520S) to create the 3X-HA-miniTurbo-TRβ vector. Site directed mutagenesis (NEB E00554) was used to create mutant 3X-HA-miniTurbo-TRβ-GS¹²⁵ and 3X-HA-miniTurbo-TRβ-PV vectors. Successful insertion of the THRB cDNA and

site-directed mutagenesis were confirmed by Sanger sequencing. Expression of fusion constructs from the cloned vectors was confirmed by Western blot (Supplemental Figure 3A).

Transfection and Biotin Labeling. Nthy-ORI cells were grown as a monolayer in DMEM-F12 (Cellgro/Mediatech) supplemented with 10% fetal bovine serum and penicillin-streptomycin (200 IU/L) in 15cm cell culture dishes. Cells were transfected at approximately 80% confluency with 20µg of plasmid DNA using 25µL Lipofectamine 3000 for 24 hours. BioID samples were simultaneously labeled using 250µM biotin and treated with 10nM T₃ or vehicle for 15 minutes. Labeling was stopped by placing cells on ice and washing three times with ice-cold PBS. Cells were detached from the plate and collected by centrifugation. The cell pellet was subjected to nuclear protein extraction using the NE-PER Protein Extraction Kit (ThermoFisher 78833) with the addition of Protease Inhibitor Cocktail (Thermo Scientific 781410) per the manufacturer's instructions.

Sample Preparation and Mass Spectrometry. To enrich biotinylated proteins, 1mg of nuclear extract was incubated for 30 minutes rotating at room temperature with 100uL of streptavidin-coated magnetic beads (Invitrogen 65001). The supernatant was removed, and the beads were washed three times with high salt RIPA buffer (100mM Tris pH 9.0, 500mM LiCl, 150mM NaCl, 1% Igepal/NP-40, 1% deoxycholic acid). Washed beads were then resuspended in Laemmli sample buffer and boiled for 10 minutes to denature and release the biotinylated proteins from the beads. The eluents were loaded onto 10% Tris-Glycine gels (Invitrogen XP00100BOX), and separated by SDS-PAGE. Gels were then silver stained (Pierce 24600) prior to band excision for mass spectrometry (Supplemental Figure 3B). LC-MS was performed using an LTQ-Orbitrap instrument (ThermoFisher).

Data Analysis: Data acquired by mass spectrometry was quantified using MaxQuant label-free quantification (LFQ) workflow, and LFQ values were used to calculate differential enrichment of identified proteins between experimental conditions using the DEP Bioconductor package³³ (Supplemental Figure 3C, Supplemental Table 1). Proteins with a p-value of <0.05 and a log₂ fold change greater than 1 or less than -1 were considered differentially enriched. Proteins found to be enriched in the empty vector control group were excluded from wildtype and mutant TRβ datasets and were not used for downstream analysis.

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Figure 1. CUT&RUN reveals three distinct modes of TR β binding in thyroid cells . A. Venn diagram illustrates peak overlap analysis and defines three modes of TR β occupancy. **B.** Heatmap demonstrating differentially bound regions classified as unliganded, ligand-independent, or liganded. **C.** Representative genome browser shots (fold enrichment over IgG) of differentially bound regions. **D.** Distribution of TR β binding sites annotated to proximal promoters (< 500bp from TSS), distal regulatory elements (500bp – 10kb from TSS), and intragenic regions. **E.** Distance from TR β binding sites to the nearest TSS. **F.** Fraction of TR β peaks containing a full-length DR4 thyroid hormone response element (TRE) or a TRE half-site.

Figure 2. T_3 induces changes in chromatin accessibility. A. Scatter plot highlights differentially accessible ATAC-seq peaks after 6 and 24 hours of T_3 treatment. T_3 induced accessibility at 7,754 peaks after 6 hours (light pink) and an additional 21,678 peaks after 24 hours (dark pink). 107 peaks were repressed by T_3 (blue). Differentially accessible peaks are defined as having a $\log_2FC \geq 1$ or ≤ -1 and an $FDR \leq 0.05$. ATAC-seq tag density is plotted near unliganded (B), ligand-independent (C), and liganded (D) TR β CUT&RUN peaks. E. Distribution of peak width of differentially accessible ATAC-seq peaks within 5kb of TR β binding sites. Statistical significance was determined by one-way ANOVA followed by multiple comparisons; * indicates $p < 0.001$.

Figure 3. The TR β interactome is altered by T $_3$ binding. A. Schematic of miniTurboID proximity labeling assay used to identify TR β -binding partners. B. Volcano plot depicts differentially enriched TR β binding partners (DEPs) defined as gained with T $_3$ treatment (75), lost with T $_3$ treatment (70), or stable (1,183). Differentially enriched proteins are defined as having a log $_2$ FC ≥ 1 or ≤ -1 and a p-value ≤ 0.05 . C. Venn diagrams show DEPs that interact with wildtype TR β and with TR β^{PV} (ligand-binding domain mutant), and TR β^{GS125} (DNA-binding domain mutant). D. GO Molecular Function enrichment of gained, lost, and stable TR β binding partners. E. Multisubunit chromatin remodeling complexes found to interact with TR β . F. Complex interaction scores of chromatin remodeling complexes.

Figure 4. BAF and PBAF complexes differentially interact with TR β . A. Diagram of BAF and PBAF complex subunits. Core subunits are colored in grey, BAF specific subunits are colored in blue, and PBAF specific subunits are colored in pink. Labels of subunits identified as TR β binding partners by miniTurboID proximity-labeling assay are bolded. B. Signal intensity of differentially enriched SWI/SNF subunits identified in miniTurboID proximity-labeling assay in the presence and absence of T $_3$. Significance (* $p < 0.05$) was determined by paired t-test. Box plots demonstrate differential CUT&RUN tag density of BRG1 (C), ARID1A (D), and PBRM1 (E) subunits at TR β binding sites. Significance (* $p < 0.05$) was determined by paired t-test.

Figure 5. BAF and PBAF complexes are differentially recruited to TR β binding sites. A. CUT&RUN tag density of BRG1, ARID1A (BAF-specific), and PBRM1 (PBAF-specific) near TR β binding sites classified as promoters (< 5kb from nearest TSS) or distal binding sites (> 5kb from nearest TSS). BRG1 and ARID1A are recruited to promoter and distal sites, PBRM1 is preferentially recruited to promoter sites. B. ATAC-seq tag density is increased upon T₃ treatment for 6 and 24 hours near promoter binding sites. C. ATAC-seq tag density is increased upon T₃ treatment for 6 and 24 hours near distal binding sites. D. Genome browser shots (fold enrichment over IgG) highlight differential recruitment of SWI/SNF complexes to a representative promoter and distal binding site.

Figure 6. TR β chromatin interactions are correlated with target gene expression. A. T_3 -induced and -repressed genes determined by RNA-seq after 6 and 24 hours of T_3 treatment in two independent biological replicates. Differentially expressed genes (DEGs) are defined as having a $\log_2FC \geq 0.5$ or ≤ -0.5 and an $p\text{-value} \leq 0.05$. B. BETA regulatory potential prediction of TR β direct targets among DEGs after 6 and 24 hours of T_3 treatment. Direct targets have a $\log_2FC \geq 0.5$ or ≤ -0.5 , $p\text{-value} \leq 0.05$, and a TR β peak within 10kb of the TSS. C. IPA analysis reveals differentially enriched biological functions after early and late T_3 treatment. D. Proportion of DEGs with ARID1A (yellow) or PBRM1 (red) binding exclusively, both ARID1A and PBRM1 (blue), or neither within 10kb of the promoter.

Figure 7. Multi-modal regulation model for TRβ chromatin interaction and recruitment of SWI/SNF complexes. A. BRG1 is recruited to unliganded TRβ binding sites for modest changes in chromatin accessibility. B. BAF and PBAF complexes are both recruited to ligand-independent TRβ binding sites near the TSS of target genes to facilitate broad changes in chromatin accessibility. C. BAF complexes are specifically recruited to liganded TRβ binding sites to facilitate T₃-induced changes in chromatin accessibility at distal regulatory regions.