NCoR1-independent mechanism plays a role in the action of the unliganded thyroid hormone receptor

Arturo Mendoza, Inna Astapova, Hiroaki Shimizu, Molly R. Gallop, Lujain Al-Sowaimel, S. M. Dileas MacGowan, Tim Bergmann, Anders H. Berg, Danielle E. Tenen, Christopher Jacobs, Anna Lyubetskaya, Linus Tsai, and Anthony N. Hollenberg

Division of Endocrinology Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Boston, MA 02115; Harvard Medical School, Boston, MA 02115; Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA 02115

Edited by Mitchell A. Lazar, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, and approved August 18, 2017 (received for review May 4, 2017)

Nuclear receptor corepressor 1 (NCoR1) is considered to be the major corepressor that mediates ligand-independent actions of the thyroid hormone receptor (TR) during development and in hypothyroidism. We tested this by expressing a hypomorphic NCoR1 allele (NCoR1ΔID), which cannot interact with the TR, in Pax8-KO mice, which make no thyroid hormone. Surprisingly, abrogation of NCoR1 function did not reverse the ligand-independent action of the TR on many gene targets and did not fully rescue the high mortality rate due to congenital hypothyroidism in these mice. To further examine NCoR1’s role in hypothyroidism, we deleted NCoR1 in the livers of euthyroid and hypothyroid mice and examined the effects on gene expression and enhancer activity measured by histone 3 lysine 27 (H3K27) acetylation. Even in the absence of NCoR1 function, we observed strong repression of more than 43% of positive T3 (3,3',5-triiodothyronine) targets in hypothyroid mice. Regulation of approximately half of those genes correlated with decreased H3K27 acetylation, and nearly 80% of these regions with affected H3K27 acetylation contained a bona fide TRβ1-binding site. Moreover, using liver-specific TRβ1-KO mice, we demonstrate that hypothyroidism-associated changes in gene expression and histone acetylation require TRβ1. Thus, many of the genomic changes mediated by the TR in hypothyroidism are independent of NCoR1, suggesting a role for additional modulators in hypothyroidism.

hypothyroidism | gene repression | TRβ1 | negative regulation | histone acetylation

Thyroid hormones (TH) exert pleiotropic effects in virtually every vertebrate species (1). Their concentration in the bloodstream is maintained relatively constant by the hypothalamic–pituitary–thyroid (HPT) axis (2). However, TH levels in peripheral tissues are finely tuned by a number of transporters and deiodinases (3). T3 (3,3',5-triiodothyronine) is the iodothyronine that binds to the TR receptors (TRs), which are ligand-dependent transcription factors (TFs) that are entirely responsible for the functional pleiotropy of TH. Two genes, THRB and THRA, express different TR isoforms in time- and tissue-specific fashion (4, 5). TRs bind to T3-responsive elements (TREs) in the regulatory regions of genes that are essential for normal function and development of organisms (6, 7). The classic paradigm of TH action dictates that on positively regulated genes the binding of T3 to the TR induces a conformational change, promoting recruitment of coactivators including SRC1/p160, CBP/p300, CARm1, and MEDI, that facilitate the assembly and activation of the general transcription machinery through acetylation and methylation of histones in the vicinity of the TRE (8). In contrast, in the absence of ligand, the TR recruits nuclear receptor corepressor 1 (NCoR1), forming a scaffold-binding surface for the multiprotein corepressor complex, which contains GPS2, TBLXI, TBLXR1, and HDAC3 (9). Histone deacetylation facilitates the formation of heterochromatin, ultimately leading to gene repression (10). In contrast to positive regulation, the mechanism by which TH induces the repression of target genes remains unclear, although in certain situations it appears that the coregulators may have opposite functions (7).

We have previously tested the classic model of coactivator and coactivator recruitment in TH action by developing a number of mouse models with disrupted or attenuated coregulator function. Animals in which NCoR1 function is disrupted through the expression of a hypomorphic allele (NCoR1ΔID) develop a syndrome of increased sensitivity to TH, with higher expression of positive gene targets in the presence of identical levels of circulating thyroxine (T4) and T3. This increased sensitivity is also seen at the level of the HPT axis, in that mice globally expressing NCoR1ΔID have lower circulating TH levels than WT mice but similar levels of thyroid-stimulating hormone (TSH) (11, 12). Interestingly, an identical syndrome has been identified in humans with mutations in TBLX1, a member of the NCoR1 complex (13). The ability of NCoR1 to mediate sensitivity to TH is further supported by the finding that levels of the coactivator SRC-1 also affect sensitivity, so that a balance between NCoR1 and SRC-1 dictates the response to T3 (14). Furthermore, the role of NCoR1 is specific, as we found that its paralog SMRT plays little role in TH signaling or in establishing the set point of the HPT axis assessed in liver-specific or whole-body SMRT-null mice, respectively (15).

Given that (i) our previous work had always examined the role of NCoR1 in the presence of some TH, and (ii) in propylthiouracil (PTU)-induced models of hypothyroidism, the functional absence of NCoR1-independent mechanism plays a role in the action of the unliganded thyroid hormone receptor

Thyroid hormone receptors (TRs) mediate the genomic actions of thyroid hormones. In the absence of T3 (3,3',5-triiodothyronine), the TR recruits a multiprotein repressor complex that decreases histone acetylation in the vicinity of target genes. Nuclear receptor corepressor 1 (NCoR1) is hypothesized to be the main corepressor that interacts with TR. Here we report that the deletion of NCoR1 does not prevent all gene repression and histone deacetylation across a variety of mouse models, whereas only the lack of TR was able to overturn the effects of hypothyroidism. Thus, we conclude that NCoR1 is not sufficient to mediate the actions of the unliganded TR; furthermore, our data suggest that alternative mechanisms of repression may be involved in the action of TRs.

Significance

Thyroid hormone receptors (TRs) mediate the genomic actions of thyroid hormones. In the absence of T3 (3,3′,5-triiodothyronine), the TR recruits a multiprotein repressor complex that decreases histone acetylation in the vicinity of target genes. Nuclear receptor corepressor 1 (NCoR1) is hypothesized to be the main corepressor that interacts with TR. Here we report that the deletion of NCoR1 does not prevent all gene repression and histone deacetylation across a variety of mouse models, whereas only the lack of TR was able to overturn the effects of hypothyroidism. Thus, we conclude that NCoR1 is not sufficient to mediate the actions of the unliganded TR; furthermore, our data suggest that alternative mechanisms of repression may be involved in the action of TRs.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The ChIP sequencing data have been uploaded to the National Center for Biotechnology Information Gene Expression Omnibus database (accession number GSE99475).

1To whom correspondence should be addressed. Email: thollenb@bidmc.harvard.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1706917114/-/DCSupplemental.
of NCoR1 in NCoR1ΔID animals led to the up-regulation of expression of some, but not all, positive TR targets, we decided to further test the role of NCoR1 in the complete absence of TH in Pax8−/− mice. These mice, which are born without a thyroid gland, have a high neonatal mortality rate due to the actions of unliganded TRs as they can be partially rescued by inactivating THRα1 (16). Here we demonstrate that NCoR1 plays little role in the physiologic effects of hypothyroidism in these mice. Furthermore, in most cases, ligand-independent gene repression is not abrogated. Based on these findings, we more formally tested the role of NCoR1 in hypothyroidism by completely ablating its presence in hepatocytes and testing its function in gene regulation and histone acetylation in the hypothyroid state. Our findings indicate that NCoR1 plays little role in the regulation of many T3 targets in hypothyroidism and that both the decrease and increase in histone acetylation mediated by the unliganded TR can be carried out independently of NCoR1. Thus, the TR may mediate repression or activation in the absence of hormone via novel signaling paradigms that remain to be identified.

Results

The Role of NCoR1 in Mice That Lack All Thyroid Hormone. We have shown previously that NCoR1 is involved in regulating T3 sensitivity, but its role in mediating the effects of the unliganded TR isoforms has not been determined. To further investigate the role of NCoR1 in the pathophysiology of complete hypothyroidism, we crossed mice that globally express the NCoR1ΔID allele with mice that lack Pax8, a critical TF required for the development of the thyroid gland. We used NCoR1ΔID mice, as it is not possible to develop complete NCoR1-KO mice during embryogenesis. As expected, Pax8−/− mice had a very high mortality due to congenital hypothyroidism, and 75% (15/20) of these mice died or showed signs of becoming moribund and had to be killed by P15 (Fig. 1A). In contrast, the neonatal mortality rate for WT mice was 8.4% (3/36) (Fig. 1A). If one NCoR1ΔID allele was present on a Pax8+/− background, the mortality rate was still 70%, and the introduction of two NCoR1ΔID alleles only decreased the mortality rate to 64% (the difference, 26/37 and 9/14, respectively, was not statistically significant). It is worth noting that the number of mice in all Pax8−/− groups was significantly lower than expected based on the Mendelian ratio, suggesting that a significant number of these animals died early and were not available to be genotyped (Fig. 1B). Indeed, there were at least 30 pups in the study that could not be genotyped and therefore were not included in the survival curve calculations. Taken together, these findings make it clear that the abrogation of NCoR1 function in the context of TR action could not rescue the high mortality rate of Pax8−/− mice, although a slight trend was present. Furthermore, the introduction of NCoR1ΔID had no significant effect on body weight or body length of Pax8+/− mice (Fig. 1 C and D). It remains possible that the complete deletion of NCoR1 might have allowed a more enhanced effect.

Given the putative role of NCoR1 in mediating ligand-independent repression of positively regulated target genes and potentially ligand-independent activation of negative targets, we examined the expression of T3-target genes in the pituitary in these mice. As expected, given the lack of circulating TH, Pax8−/− mice had repression of growth hormone (GH) mRNA expression and activation of TSHβ mRNA expression (Fig. 24). While expression of GH in Pax8+/− NCoR1ΔID animals was higher than in Pax8−/− animals, its expression was still greatly repressed compared with WT and NCoR1ΔID controls. Introduction of NCoR1ΔID had no significant effect on the regulation of the TSHβ subunit or prolactin gene expression in Pax8−/− mice. Similarly, we examined expression of other known T3 CNS targets including hairless (Hr), Lrm1, and Pcp2 in the cerebellum (Fig. 2B). Hr was repressed in Pax8−/− animals, and expression of NCoR1ΔID did not relieve this repression. While down-regulation of Lrm1 was less dramatic, it also was not affected by introduction of the NCoR1ΔID allele. In contrast, expression of Pcp2, which was mildly repressed in Pax8−/− mice, was almost rescued by the introduction of NCoR1ΔID. We next looked at T3-target genes in the liver and, as in the CNS and pituitary, there was little role for NCoR1ΔID in ligand-independent repression. Dio1, Thrsp, and Gpd2 were all repressed in Pax8−/− mice, and the introduction of NCoR1ΔID led to only a minor up-regulation of these genes but did not come close to completely overturning repression (Fig. 2C). Similarly, the activation of the negative target Gsta2 was unaffected by the presence of NCoR1ΔID (Fig. 2C). Thus, the lack of functional NCoR1 activity, as dictated by the presence of the NCoR1ΔID allele, does not appear to reverse repression of positive TR targets in the absence of the hormone. Furthermore, NCoR1 appears to play little role in ligand-independent activation on negative targets in this model.

Liver-Specific Ablation of NCoR1 Induces Hepatic Steatosis and Does Not Prevent Hypothyroidism-Induced Hypercholesterolemia. Given that we found only a small role for NCoR1 in complete hypothyroidism using the NCoR1ΔID allele, we next wanted to test
its role in hypothyroidism by completely deleting NCoR1 to rule out any ability of NCoR1ΔID to still interact with the TR. As discussed, we have previously analyzed liver-specific NCoR1ΔID mice in the hypothyroid setting and have shown that repression of positive T3 targets was overturned on only a minority of genes (16%) (17). Since NCoR1 global deletion is embryonically lethal, we chose to delete it only in hepatocytes (L-NCoR1–KO) using an albumin-Cre driver (18). As shown in the Western blot analysis in Fig. 3A, NCoR1 is effectively deleted in hepatocytes using this methodology with little effect on SMRT expression. We next rendered L-NCoR1–KO mice and their relevant controls hypothyroid using PTU (0.15%) and a low-iodine diet (PTU/LID) for 3 wk. Liver-specific ablation of NCoR1 did not affect serum T4 levels in the euthyroid setting, and all groups of animals were rendered similarly hypothyroid in the context of their suppressed T4 levels (Fig. 3B). Deletion of NCoR1 in hepatocytes did not significantly impact the mouse liver weight-to-body weight ratio, which was increased in hypothyroidism in all genotypes; however it induced an increase in hepatic triglyceride levels similar to that in control animals (Fig. 3D). Additionally, hypo- thyroidism did not reverse repression of T3-positive target genes. qPCR on the indicated hepatic genes was performed after mRNA extraction; n = 5 or 6 mice per genotype. Data are shown as mean ± SD; *P < 0.05, **P < 0.01; two-way ANOVA.

**Liver-Specific Ablation of NCoR1 Partially Regulates TH-Responsive Genes.** We next measured gene expression in L-NCoR1–KO and control animals. Based on the gene-expression profile, we grouped the NCoR1-regulated genes into two broad categories. (i) Type 1 genes were either up-regulated or expressed at similar levels to controls in the euthyroid state but were strongly repressed by hypothyroidism in either the presence or absence of NCoR1; this group included Dio1, Cyp3a16, Sult5a1, Pgd2, Bcl3, Cyp17a1, and Scl25a45 (Fig. 4A). (ii) In contrast, type 2 genes were up-regulated in both euthyroid and hypothyroid L-NCoR1–KO mice, and there was no suppression of expression in hypothyroid L-NCoR1–KO vs. euthyroid L-NCoR1–KO mice. This group of genes included Mel, Thra, Fasn, and Elovl3 (Fig. 4B). Interestingly, Elovl3 shows some degree of repression in hypothyroidism despite its activation in the absence of NCoR1. This indicates that L-NCoR1–KO mice, despite having T4 levels similar to those in control animals, had enhanced activation of certain genes. Importantly, this type of profile was previously observed in L-NCoR1ΔID mice (11).

To understand the role of NCoR1 in the regulation of type 1 and type 2 genes, we used histone 3 lysine 9 (H3K9) acetylation and histone 3 lysine 27 (H3K27) acetylation as a marker for enhancer activity at NCoR1-regulated genes. We examined changes in H3K9 and H3K27 acetylation at previously determined TRβ1-binding sites within 10 kb of the transcriptional start sites (TSS) of the Dio1 (type 1) and Thra (type 2) genes (Fig. 4C). Given the similarity in the three control groups in context of gene expression, we used NCoR1ΔID mice as the control group in our ChIP experiments. As shown in Fig. 4D, H3K9 acetylation near the Dio1 gene was not affected in L-NCoR1–KO mice in the euthyroid state. Similarly, in the absence of NCoR1, the hypothyroidism-associated decrease in H3K9 acetylation at the Dio1 locus was not changed. In contrast, H3K9 acetylation was increased in L-NCoR1–KO mice around both Thra sites in the euthyroid and hypothyroid settings, consistent with the observed increase in gene expression in the euthyroid and hypothyroid settings in L-NCoR1–KO mice. Similar to H3K9 acetylation, H3K27 acetylation around the Dio1 gene was strongly diminished in both L-NCoR1–KO and control mice in the hypothyroid setting, but H3K27 acetylation around the Thra gene was increased in L-NCoR1–KO mice in both the euthyroid and hypothyroid settings (Fig. 4E). Thus, consistent with our mRNA expression data, NCoR1 appears to regulate the basal level of histone acetylation of certain genes (i.e., Thra) while not being required to mediate the decrease in
histone acetylation of the Dio1 gene in hypothyroidism. Importantly, the hepatic deletion of SMRT had no effect on the expression of the genes examined in Fig. 4. Similarly, the deletion of SMRT had no effect on H3K27 acetylation of the Dio1 or Thrsp loci (Fig. S1) (15). Indeed, in mice that lack SMRT and express NCoR ΔID in hepatocytes, the pattern of expression is highly analogous to that found in L-NCoR1–KO mice and is consistent with a very little role for SMRT in TR action in hepatocytes.

**Repression in Hypothyroidism Is Mediated by TRβ1.** While our data in L-NCoR1–KO mice demonstrate an NCoR1-independent pathway for gene repression in hypothyroidism on certain targets, we wanted to definitively demonstrate that TRβ1 was responsible for repression of these targets. Thus, we deleted TRβ1 from hepatocytes using albumin-Cre (Fig. S2) and examined the regulation of T3-target genes in the hypothyroid, euthyroid, and hyperthyroid states. Importantly, thyroid function was similar in L-TRβ1–KO mice compared with control animals in all thyroid states studied (Fig. S3). Thyroid function was similar in L-TRβ1–KO mice compared with control animals in all thyroid states studied (Fig. S3). Hypothyroidism-induced repression of Dio1, Sult5a1, Ptgs2, Gpd2, Bcl3, and Slec25a45 is lost in L-TRβ1–KO mice (Fig. 5A). Additionally, these genes are no longer activated by T3, showing an essential role for the TRβ1 isoform in the regulation of these genes. Similarly, Thrsp, Fasn, and Me1 also lose repression in hypothyroidism and cannot be up-regulated in hyperthyroid L-TRβ1–KO mice. Interestingly, the absence of TRβ1 does not activate these genes in the euthyroid state, although it does in the hypothyroid state, as is consistent with a role for TRβ1/NCoR1 in mediating the basal level of expression of these genes. Interestingly, Cyp3a16, Elovl3, Cyp17a1, and, to a more limited extent, Dio1 appear to require TRβ1 for basal expression, so that their expression is significantly decreased in the euthyroid setting. Furthermore, all these genes require TRβ1 for T3-dependent regulation.

To demonstrate that TRβ1 action was required for the H3K27 acetylation changes seen in hypothyroidism, we performed ChIP on chromatin isolated from livers of L-TRβ1–KO mice and TRβ1/+/control mice. Indeed, at previously identified TRβ1-ChIP peaks that were used after ChIP with D. H3K9ac ChIP-PCR was run on chromatin of euthyroid and hypothyroid L-NCoR1–KO mice (n = 3 IP per genotype); anti-H3K9 acetyl (07-352; Millipore). (E) DNA libraries from euthyroid and hypothyroid WT and L-NCoR1–KO mice (n = 3 IP per genotype) were used as template for ChIP-qPCR. Anti-H3K27ac (catalog no. 39133; Active Motif) was used for immunoprecipitation (IP). Data are shown as mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; two-way ANOVA.

---

**Fig. 4.** Hypothyroidism represses the expression of T3-positive targets independently of NCoR1. Liver mRNA levels of the indicated type 1 genes (A) or type 2 genes (B) in the shown genotypes were measured by qPCR (n = 6 mice per genotype). (C) Dio1 and Thrsp TRβ1-ChIP peaks (6) visualized in genome browser. Arrows indicate the targeted acetylation sites upstream (+) or downstream (−) of TRβ1-ChIP peaks that were used after ChIP with D. H3K9ac ChIP-qPCR was run on chromatin of euthyroid and hypothyroid L-NCoR1–KO mice (n = 3 IP per genotype); anti-H3K9 acetyl (07-352; Millipore). (E) DNA libraries from euthyroid and hypothyroid WT and L-NCoR1–KO mice (n = 3 IP per genotype) were used as template for ChIP-qPCR. Anti-H3K27ac (catalog no. 39133; Active Motif) was used for immunoprecipitation (IP). Data are shown as mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; two-way ANOVA.
The primer list is given in Table S2. Three-way ANOVA was used as control (n = 4 IP per genotype). The primer list is given in Table S2.

Fig. 5. TRβ1 is required for T3-dependent gene regulation. (A) Liver mRNA levels of T3-target genes were measured by qPCR in the indicated conditions (euthyroid, Chow; hypothyroid, PTU/LID; hyperthyroid, PTU/LID + T3 (n = 5 or 6 mice per genotype). (B) ChIP-PCR was performed on the indicated genotypes. Primers targeted the area next to the previously reported TRβ-binding sites in repression in hypothyroidism, we focused on H3K27 acetylation data within 100 kb on either side of the TSS end, we performed differential binding analysis of the ChIP integrated H3K27 acetylation data in L-NCoR1 Δ/− mice with Agxt2l1 lost some T3 repression but not PTU up-regulation in L-TRβ1−/−KO mice (Fig. S4). These data suggest that negative regulation on these targets may be TRα1 dependent or may be mediated through a cell other than the hepatocyte in the liver.

Hypothyroidism Represses Gene Expression Independently of NCoR1. To examine the requirement of NCoR1 to regulate gene expression by TRs globally, we performed ChIP sequencing (ChIP-seq) for H3K27 acetylation in euthyroid and hypothyroid L-NCoR1−/−KO mice and NCoR−/−KO mice controls. Given the close phenotypic resemblance between L-NCoR1Δ1ΔD and L-NCoR1−/−KO mice, we integrated H3K27 acetylation data in L-NCoR1−/−KO mice with gene-expression profiles in L-NCoR1Δ1ΔD mice in the euthyroid and hypothyroid states, as previously determined (11). To that end, we performed differential binding analysis of the ChIP H3K27 acetylation data within 100 kb on either side of the TSS of target genes that were regulated in the hypothyroid state. For each gene, we looked for the histone site that demonstrated the largest significant change between the two conditions (Fig. 7A). Moreover, to delineate the role of TRβ1-binding sites in repression in hypothyroidism, we focused on H3K27 acetylation sites within 1 kb of previously determined TRβ1 ChIP peaks (6).
We found that 363 genes were significantly repressed in hypothyroid WT mice compared with euthyroid WT mice. Of these 363 genes, 198 (54%) had an H3K27 acetylation peak within 100 kb on either side of the TSS that was significantly decreased in hypothyroid animals compared with euthyroid animals (Fig. 7B). Remarkably, 76% of those H3K27 acetylation sites had an associated TRβ1-binding site (Fig. 7B). In the absence of NCoR1 function (in the presence of NCoR1ΔID), the number of repressed genes was decreased to 158, and 73 (46%) of these genes had decreases in H3K27 acetylation, 84% of which had an associated TRβ1-binding site (Fig. 7A). When the window for associated differentially regulated H3K27 acetylation and TRβ1 peaks was narrowed to 10 kb, 151 of the 198 repressed genes were included in WT mice, and 58 of the 73 genes were included in mice lacking NCoR1 function. Thus, repression in hypothyroidism is still present in more than 43% of genes in the absence of functional NCoR1, and in those that have changes in H3K27 acetylation consistent with repression, this repression is most likely associated with a TRβ1-binding site. This group of genes has a profile of expression and histone acetylation consistent with type one genes described above.

As has been previously demonstrated, ligand-independent activation in the hypothyroid state is more common than repression, even though the ability of unliganded TR to directly activate target genes has been recently questioned after performing genome-wide TR-binding studies (6, 19, 20). Remarkably, in WT mice, 551 of 615 activated hypothyroid genes had concordant changes in associated increased H3K27 acetylation consistent with repression, this repression is most likely associated with a TRβ1-binding site. Of these 165 genes, those that showed no difference in expression between euthyroid and hypothyroid L-NCoR1ΔID animals we have previously referred to as “type 2 genes.” Thus, the recruitment of NCoR1 by TRβ1 is likely responsible for controlling the basal expression of these genes or overall T3 sensitivity. Surprisingly, 181 genes were repressed in euthyroid mouse liver in the absence of functional NCoR1. One hundred of these were associated with decreases in H3K27ac, and 71% had associated TRβ1 sites (Fig. 8B), raising the possibility that on certain targets NCoR1 may activate gene expression via the TR. Finally, gene ontology Database for Annotation, Visualization and Integrated Discovery (DAVID) analysis revealed that type 1 and 2 genes enriched a variety of separate pathways (Table S1), consistent with differential signaling to modulate their expression.

Motif Enrichment Analysis in H3K27ac ChIP-Seq Reveals Putative DNA-Binding Partners for TR. Given that we show NCoR1-independent TRβ1 effects, we next examined differentially regulated H3K27 acetylation regions sequences for enrichment of JASPAR database motifs that might suggest TRβ1-associated TF-binding partners. Strikingly, regions associated with either up-regulated or down-regulated H3K27 acetylation peaks in hypothyroidism were enriched for different sets of TF-binding motifs (Fig. 9A). We further determined that co-occurrence of TF-binding motifs with TRβ1-binding sites within regions surrounding the up-regulated and down-regulated H3K27 acetylation peaks were also unique (Fig. 9B), suggesting that TRβ1 may interact with a specific set of secondary TFs to elicit positive or negative gene modulation in a ligand-dependent fashion. Importantly, the overall number of motifs was higher in the H3K27 acetylation signal that included a TRβ1 ChIP peak, in contrast to the H3K27 acetylation signal that does not included the TRβ1 ChIP peak (Fig. S5). In this context, motifs for ZNF263, SPI1, TCF3, and KLF5 were associated with TRβ1 in genes up-regulated in hypothyroidism, while motifs for Foxa2, Phox2b, and SHOX were among those most highly associated with TRβ1-binding sites in regions where H3K27 acetylation peaks were repressed by hypothyroidism (Fig. 9B). We looked further at Foxa2 (P = 5.30E-27, 28% co-occurrence), given
Fig. 7. Hypothyroidism can modulate gene expression in an NCoR1-independent fashion. (A) The heat maps depict changes in gene expression (microarray) and H3K27 acetylation (CHIP-seq) caused by hypothyroidism. (A1) Down-regulated genes in livers of control and L-NCoR1ΔID/KO mice. (A2) Up-regulated genes in livers of control and L-NCoR1ΔID/KO mice. The yellow-to-red scale indicates a log₂ fold change. Mice were fed a PTU/LID diet or chow diet (n = 3). (B) Top Row) The number and percentage of genes that have associated H3K27ac peaks with intersecting TRβ1-ChIP peaks near (within 1 kb) of the TSS. (Bottom Row) The number and percentage of genes that also have intersecting TRβ1-ChIP peaks near (within 1 kb) of the H3K27ac peaks.

Discussion

The classic model of TR action invokes a coregulator exchange, which is mediated by ligand binding to the TR on regulated target genes. Thus, in the ligand-independent state the recruited corepressor complex represses target genes, which are then activated by ligand through the dismissal of the corepressor complex and the recruitment of a coactivator complex. Importantly, repression and activation of target genes by T3 involves histone acetylation, as the corepressor complex is well known to deacetylate target histones, while the coactivator complex does the opposite (22, 23). Over the last number of years, using mouse models, we have challenged this classic model by demonstrating a primary role for the corepressor NCoR1 in mediating ligand sensitivity so that diminishing the amount of available NCoR1 enhances the response to a set amount of T3 (7, 24). However, the role of NCoR1 in ligand-independent repression has not been formally tested, as our previous models used a hypomor-
repression or activation of target-gene expression in congenital hypothyroidism and the lack of changes in growth rates. Thus, removing the function of the key TH-related corepressor does not appear to influence TR action in the complete absence of TH. This would be consistent with the ability of the TRs themselves to have strong ligand-independent functions in the absence of NCoR1. Indeed, mice lacking all TR isoforms have dramatically better survival rates than congenitally hypothyroid PAX8–/– mice (25). Still, this work does not rule out a potential role for SMRT during development or the possibility of an enhanced role for NCoR1, given that these experiments were done in the presence of NCoR1ΔID.

Given the likely minimal role of NCoR1 function in PAX8–/– mice, we next looked more carefully at the role of NCoR1 in more traditional models of adult hypothyroidism in which an antithyroid drug is used to induce hypothyroidism. Using L-NCoR1ΔID mice, we had previously determined that only 16% of the genes repressed in hypothyroidism were significantly activated by the expression of NCoR1ΔID, implying that the majority of genes remain repressed in hypothyroidism in the absence of NCoR1. To further interrogate this, we chose to fully delete NCoR1 to remove any chance that data seen with NCoR1ΔID reflect any remaining ability of its interacting with the TR. Importantly, the phenotype of L-NCoR1–KO mice was very similar to that found in L-NCoR1ΔID mice, with clear activation of lipogenic target genes in the euthyroid setting (22). In addition, deletion of NCoR1 could not prevent the hypothyroidism-induced rise in serum cholesterol seen in control mice, implying the potentially marginal role of NCoR1 in mediating the actions of the unliganded TR in hypothyroidism.

To substantiate this hypothesis, we first examined positive TR targets, which appeared to divide into two groups. In the first group there was significant repression in hypothyroidism that was not reversed in the absence of NCoR1. In the second group there was basal activation of expression in the absence of NCoR1, so that genes such as Thyroid increased their expression both in the euthyroid and hypothyroid settings. To prove that the repression seen in hypothyroidism was TRβ1-mediated, we disrupted its function in liver only. Strikingly, on these known targets histone acetylation was still dramatically reduced in the hypothyroid state in the absence of NCoR1 but not in the absence of TRβ1, supporting the notion that the unliganded or minimally liganded TR represses target-gene expression independently of NCoR1. A particularly relevant target is Dio1, the most highly regulated T3 target in the genome. Previous work has suggested that Dio1 is primarily regulated by enhanced TRβ1 binding in the presence of ligand by targeting open DNase hypersensitive sites in the first intron that align with TRβ1 binding (19). Clearly, from the data presented herein, TRβ1 is required for repression of Dio1 in hypothyroidism, and clearly neither deacetylation in the vicinity of TRβ1-binding sites nor repression requires NCoR1.

Indeed, NCoR1 remained nonessential when we explored this finding globally using H3K27 acetylation ChIP-seq across our mouse models. Forty-three per cent of positively regulated target genes remained repressed in hypothyroidism, with decreased H3K27 acetylation marks in the absence of NCoR1 when a large 100-kb window was used. Importantly, a great majority of the H3K27 acetylation sites were near known TRβ1 peaks, consistent with the hypothesis that the TR mediates repression independently of NCoR1 on many genes. The same remained true when the window around the transcription start site was reduced to 10 kb. Thus, the TRβ1 must somehow induce repression independently of NCoR1. We have previously shown that deletion of SMRT plays little role in reversing repression in hypothyroidism, and here we demonstrate that deleting SMRT plays no role in enhancing TRβ1 binding in hypothyroidism. To further interrogate NCoR1 but not in the absence of TRβ1-mediated, we disrupted its function in liver only. Strikingly, on these known targets histone acetylation was still dramatically reduced in the hypothyroid state in the absence of NCoR1 but not in the absence of TRβ1, supporting the notion that the unliganded or minimally liganded TR represses target-gene expression independently of NCoR1. A particularly relevant target is Dio1, the most highly regulated T3 target in the genome. Previous work has suggested that Dio1 is primarily regulated by enhanced TRβ1 binding in the presence of ligand by targeting open DNase hypersensitive sites in the first intron that align with TRβ1 binding (19). Clearly, from the data presented herein, TRβ1 is required for repression of Dio1 in hypothyroidism, and clearly neither deacetylation in the vicinity of TRβ1-binding sites nor repression requires NCoR1.

Indeed, NCoR1 remained nonessential when we explored this finding globally using H3K27 acetylation ChIP-seq across our mouse models. Forty-three per cent of positively regulated target genes remained repressed in hypothyroidism, with decreased H3K27 acetylation marks in the absence of NCoR1 when a large 100-kb window was used. Importantly, a great majority of the H3K27 acetylation sites were near known TRβ1 peaks, consistent with the hypothesis that the TR mediates repression independently of NCoR1 on many genes. The same remained true when the window around the transcription start site was reduced to 10 kb. Thus, the TRβ1 must somehow induce repression independently of NCoR1. We have previously shown that deletion of SMRT plays little role in reversing repression in hypothyroidism, and here we demonstrate that deleting SMRT plays no role in enhancing TRβ1 binding in hypothyroidism. To further interrogate NCoR1 but not in the absence of TRβ1-mediated, we disrupted its function in liver only. Strikingly, on these known targets histone acetylation was still dramatically reduced in the hypothyroid state in the absence of NCoR1 but not in the absence of TRβ1, supporting the notion that the unliganded or minimally liganded TR represses target-gene expression independently of NCoR1. A particularly relevant target is Dio1, the most highly regulated T3 target in the genome. Previous work has suggested that Dio1 is primarily regulated by enhanced TRβ1 binding in the presence of ligand by targeting open DNase hypersensitive sites in the first intron that align with TRβ1 binding (19). Clearly, from the data presented herein, TRβ1 is required for repression of Dio1 in hypothyroidism, and clearly neither deacetylation in the vicinity of TRβ1-binding sites nor repression requires NCoR1.

Indeed, NCoR1 remained nonessential when we explored this finding globally using H3K27 acetylation ChIP-seq across our mouse models. Forty-three per cent of positively regulated target genes remained repressed in hypothyroidism, with decreased H3K27 acetylation marks in the absence of NCoR1 when a large 100-kb window was used. Importantly, a great majority of the H3K27 acetylation sites were near known TRβ1 peaks, consistent with the hypothesis that the TR mediates repression independently of NCoR1 on many genes. The same remained true when the window around the transcription start site was reduced to 10 kb. Thus, the TRβ1 must somehow induce repression independently of NCoR1. We have previously shown that deletion of SMRT plays little role in reversing repression in hypothyroidism, and here we demonstrate that deleting SMRT plays no role in enhancing TRβ1 binding in hypothyroidism. To further interrogate NCoR1 but not in the absence of TRβ1-mediated, we disrupted its function in liver only. Strikingly, on these known targets histone acetylation was still dramatically reduced in the hypothyroid state in the absence of NCoR1 but not in the absence of TRβ1, supporting the notion that the unliganded or minimally liganded TR represses target-gene expression independently of NCoR1. A particularly relevant target is Dio1, the most highly regulated T3 target in the genome. Previous work has suggested that Dio1 is primarily regulated by enhanced TRβ1 binding in the presence of ligand by targeting open DNase hypersensitive sites in the first intron that align with TRβ1 binding (19). Clearly, from the data presented herein, TRβ1 is required for repression of Dio1 in hypothyroidism, and clearly neither deacetylation in the vicinity of TRβ1-binding sites nor repression requires NCoR1.

Indeed, NCoR1 remained nonessential when we explored this finding globally using H3K27 acetylation ChIP-seq across our mouse models. Forty-three per cent of positively regulated target genes remained repressed in hypothyroidism, with decreased H3K27 acetylation marks in the absence of NCoR1 when a large 100-kb window was used. Importantly, a great majority of the H3K27 acetylation sites were near known TRβ1 peaks, consistent with the hypothesis that the TR mediates repression independently of NCoR1 on many genes. The same remained true when the window around the transcription start site was reduced to 10 kb. Thus, the TRβ1 must somehow induce repression independently of NCoR1. We have previously shown that deletion of SMRT plays little role in reversing repression in hypothyroidism, and here we demonstrate that deleting SMRT plays no role in enhancing TRβ1 binding in hypothyroidism. To further interrogate NCoR1 but not in the absence of TRβ1-mediated, we disrupted its function in liver only. Strikingly, on these known targets histone acetylation was still dramatically reduced in the hypothyroid state in the absence of NCoR1 but not in the absence of TRβ1, supporting the notion that the unliganded or minimally liganded TR represses target-gene expression independently of NCoR1. A particularly relevant target is Dio1, the most highly regulated T3 target in the genome. Previous work has suggested that Dio1 is primarily regulated by enhanced TRβ1 binding in the presence of ligand by targeting open DNase hypersensitive sites in the first intron that align with TRβ1 binding (19). Clearly, from the data presented herein, TRβ1 is required for repression of Dio1 in hypothyroidism, and clearly neither deacetylation in the vicinity of TRβ1-binding sites nor repression requires NCoR1.

Indeed, NCoR1 remained nonessential when we explored this finding globally using H3K27 acetylation ChIP-seq across our mouse models. Forty-three per cent of positively regulated target genes remained repressed in hypothyroidism, with decreased H3K27 acetylation marks in the absence of NCoR1 when a large 100-kb window was used. Importantly, a great majority of the H3K27 acetylation sites were near known TRβ1 peaks, consistent with the hypothesis that the TR mediates repression independently of NCoR1 on many genes. The same remained true when the window around the transcription start site was reduced to 10 kb. Thus, the TRβ1 must somehow induce repression independently of NCoR1. We have previously shown that deletion of SMRT plays little role in reversing repression in hypothyroidism, and here we demonstrate that deleting SMRT plays no role in enhancing TRβ1 binding in hypothyroidism. To further interrogate NCoR1 but not in the absence of TRβ1-mediated, we disrupted its function in liver only. Strikingly, on these known targets histone acetylation was still dramatically reduced in the hypothyroid state in the absence of NCoR1 but not in the absence of TRβ1, supporting the notion that the unliganded or minimally liganded TR represses target-gene expression independently of NCoR1. A particularly relevant target is Dio1, the most highly regulated T3 target in the genome. Previous work has suggested that Dio1 is primarily regulated by enhanced TRβ1 binding in the presence of ligand by targeting open DNase hypersensitive sites in the first intron that align with TRβ1 binding (19). Clearly, from the data presented herein, TRβ1 is required for repression of Dio1 in hypothyroidism, and clearly neither deacetylation in the vicinity of TRβ1-binding sites nor repression requires NCoR1.
crossed with mice that express Cre recombinase under the control of albumin promoter albumin Cre+–. This line produced Cre expression in hepatocytes only [pCD-Tg[Alb-Cre21Mg)]. The experimental cohorts were generated by crossing mice with the genotypes NCoR1flox/flox-Alb-Cre+– and NCoR1flox/flox–. The resulting experimental mice included three WT littermate controls, NCoR1flox/flox–, NCOR1−/− Alb-Cre+–, NCoR1flox/flox– and L-NCOR1−/−, NCoR1flox/flox– Alb-Cre+–. The same breeding strategy was applied to generate liver-specific TPR1−/− (L-TRP1−/−) mice. The TRP1flox/flox line was obtained from the Gauthier laboratory, Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Lyon, France (28). Mice were maintained on a C57BL/6J genetic background.

PAX8-KO mice, a gift from laboratory of F. Flamant, Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Lyon, France (16) and Ahmed Mansouri, Department of Molecular Cell Biology, Max Planck Institute of Biophysical Chemistry, Göttingen, Germany (29), were crossed to NCoR1−/− mice and maintained in mixed background as previously described in ref. 12.

**Animal Experiments.** All mouse experiments were approved by the BIDMC IACUC. Mice were housed on a 12-h light/dark cycle and supplied with food and water ad libitum. At 6 wk of age, separated cohorts of mice were either kept on standard chow diet or were put on a PTU/LID diet (TD.95125; Envigo) for 3 wk. The PTU/LID + T3 cohort received daily i.p. injections of T3 (2 μg/mouse) for 3 wk. We used enzymatic colorimetric assay (Covasym; Beckman Coulter) to measure T3 in the sera of the experimental mice. The blood samples were collected from the tail vein of 10 to 12 mice per genotype. Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14). Total serum cholesterol was measured using standard-curve methods and were normalized to the level of cyclophilin (peptidyl isomerase A, Piia) mRNA.

**Microarray.** We used microarray data previously obtained from WT controls and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq.** ChIP for H3K27ac was performed on livers of WT and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq Analysis.** Sequenced reads were analyzed using a standard pipeline as described previously. Gene expression data were normalized to the level of cyclophilin (peptidyl isomerase A, Piia) mRNA.

**Microarray.** We used microarray data previously obtained from WT controls and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq.** ChIP for H3K27ac was performed on livers of WT and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq Analysis.** Sequenced reads were analyzed using a standard pipeline as described previously. Gene expression data were normalized to the level of cyclophilin (peptidyl isomerase A, Piia) mRNA.

**Microarray.** We used microarray data previously obtained from WT controls and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq.** ChIP for H3K27ac was performed on livers of WT and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq Analysis.** Sequenced reads were analyzed using a standard pipeline as described previously. Gene expression data were normalized to the level of cyclophilin (peptidyl isomerase A, Piia) mRNA.

**Microarray.** We used microarray data previously obtained from WT controls and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq.** ChIP for H3K27ac was performed on livers of WT and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq Analysis.** Sequenced reads were analyzed using a standard pipeline as described previously. Gene expression data were normalized to the level of cyclophilin (peptidyl isomerase A, Piia) mRNA.

**Microarray.** We used microarray data previously obtained from WT controls and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq.** ChIP for H3K27ac was performed on livers of WT and L-NCOR1−/− mice on chow or a PTU/LID diet (n = 3 per genotype). Each ChIP was performed with 100 μl of sera, washed with 0.9% NaCl, and centrifuged at 1,000 × g for 5 min. Serum TSH was measured by MILLIPLEX multianalyte profiling (MAP) (rat thyroid hormone TSH panel; EMD Millipore) (14).

**ChIP-Seq Analysis.** Sequenced reads were analyzed using a standard pipeline as described previously. Gene expression data were normalized to the level of cyclophilin (peptidyl isomerase A, Piia) mRNA.


