Transgenic Targeting of a Dominant Negative Corepressor to Liver Blocks Basal Repression by Thyroid Hormone Receptor and Increases Cell Proliferation*

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Unliganded thyroid hormone receptors (TRs) interact with corepressors and repress basal transcription of target genes in cotransfection and in vitro studies. Currently, little is known about the function of corepressors in vivo. We thus used a mouse albumin promoter to generate several transgenic mouse lines that overexpressed a dominant negative mutant corepressor, NCoRi, in liver. The transgenic mice had normal liver weight, appearance, and minimal changes in enzyme activity. To study the effects of NCoRi on transcription of hepatic target genes, we examined T₃-regulated gene expression of hypo- and hyperthyroid transgenic mice. In hypothyroid mice, hepatic expression of Spot 14, Bcl-3, glucose 6-phosphatase, and 5'-deiodinase mRNA was higher in transgenic mice than littermate controls whereas these genes were induced to similar levels in T₃-treated mice. Derepression was not observed for malic enzyme mRNA expression in hypothyroid mice. Thus, NCoRi selectively blocked basal transcription of several thyroid hormone-responsive genes but had no effect on ligand-mediated transcription. Additionally, compensatory increases in endogenous SMRT and NCoR mRNA were observed in hypothyroid transgenic mice. Interestingly, hepatocyte proliferation as detected by BrdUrd incorporation was increased in transgenic mice. The gene profile in transgenic mouse livers was studied by cDNA microarray, and several genes related to cell proliferation were induced. In summary, our studies show that NCoR plays important roles in mediating basal repression by TRs and may prevent cellular proliferation in vivo.

Thyroid hormone receptors (TRs) and retinoic acid receptors (RARs) can repress basal transcription in the absence of ligand and activate transcription upon ligand binding, in positively regulated target genes. TRs and RARs mediate basal repression through interactions with corepressors, such as NCoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors, Refs. 1, 2). NCoR and SMRT are both 270 kDa in size and their overall amino acid identity is 43% (3, 4). These corepressors have two nuclear hormone receptor interaction domains in the carboxyl terminus and three transferable repression domains in the amino terminus (5, 6). NCoR binds to Sin3, which in turn, recruits histone deacetylases (7–11). The formation of the NCoR-Sin3-HDAC complex by TR leads to hypoacetylation of local histones resulting in conformational changes in the nucleosome structure and decreased access of enhancers and components of the basal transcriptional machinery to the promoter region and transcriptional start site (7–10). In contrast, transcriptional activation occurs via ligand-dependent recruitment of p160 co-activators by TR, which then forms complexes that contain histone acetyltransferase activity (12). These complexes may then exchange with other complexes that share components with the RNA pol II transcriptional initiation complex (13, 14).

The NCoR-Sin3-HDAC complex not only mediates transcriptional repression by TR and RAR but also by other transcription factors such as Rev-Erb, COUP-TF, and MyoD as well as Mad/Max and Mad/Mxi dimers, (7–10, 15–17). Additionally, corepressors can interact with PML-RARα and the PLZF-RARα and LAZ3/BCL6, which are involved in acute promyelocytic leukemia and non-Hodgkin lymphomas, respectively (18–21). Recent evidence also suggests that NCoR and Sin3 may interact directly with the key components of the basal transcriptional machinery to inhibit basal transcription in an alternative repression pathway (22).

Almost all studies of NCoR function to date have been performed in in vitro transcription and cotransfection systems. Currently, little is known about the physiological and developmental roles played by NCoR in vivo. Hollenberg et al. (23) have shown that a variant form of NCoR, NCoRi, lacks the repression domains in the amino terminus but retains the nuclear receptor interaction domains. NCoRi also had dominant negative activity on TR-mediated basal repression in cotransfection assays. The liver has long been known to be a major target organ for TH, with more than 50 target genes identified recently by cDNA microarray (24). Thus, to examine the roles of NCoR in vivo, we created several transgenic mouse lines in which the dominant negative NCoRi was targeted to liver via an albumin promoter. Our studies showed that NCoRi selectively blocked basal repression of several TH-regulated target genes but had no effect on transcriptional activation. Additionally, hepatocellular proliferation was increased in the transgenic mice. Our study demonstrates that NCoR plays an important role in mediating basal repression by TRs in vivo and also may be involved in cellular proliferation in the liver.

MATERIALS AND METHODS

Construction of Liver-specific Expression Vectors for NCoRi—To construct a vector containing the albumin promoter and NCoRi sequence,
a 3.1-kilobase NCoRi fragment was obtained by EcoRI digestion from PKCR2-NCoRi (gift of A. Hollenberg, Beth Israel Hospital, Boston, MA, Ref. 23) and inserted into multiple cloning sites in the albumin promoter cassette PEGMLB-SVPA (gift from Dr. Jack Liaig, NIDDK, National Institutes of Health), resulting in PEGMLB-SVPA-NCoRi vector (Fig. 1A). The blots were probed with gel-purified FLAG (2×) sequence also was inserted into the amine terminus of NCoRi cDNA. All sequences were verified by sequencing.

Creation of Transgenic Mice—Alb-NCoRi (6.8-kilobase) fragment containing the mouse albumin promoter, NCoRi cDNA, and SV40 intron/poly(A) sequence (Fig. 1A) was liberated from the plasmid vector PEGMLB-SVPA-NCoRi by digestion with AatII and MluI, separated by electrophoresis on a 1% agarose gel and purified through a Qiagen gel extraction kit (Qiagen), following by Etupt column (Schleicher and Schuell). Microinjection was carried out by the NIDDK Transgenic Facility, National Institutes of Health.

Identification of Transgenic Mice by PCR Genotyping—Transgenic mice carrying Alb-NCoRi was identified by PCR genotyping of tail DNA using primers specific to albumin promoter and NCoRi cDNA. PCR amplification was carried out for 30 cycles using 200–400 ng of mouse tail DNA (Promega). Poly(A)trailer probe preparation, hybridization, and washes were carried out as described for Northern blotting (see below).

Relative of Thyroid Hormone Status in Mice—All animals used in these experiments were 8–12 weeks old. Hypothyroid mice were fed a low iodine (loI) diet supplemented with 0.15% propylthiouracil (PTU) purchased from Harlan Teklad Co. (Madison, WI) for 4 weeks (26). Serum TSH measurements (kindly measured by Dr. Samuel Refetoff, University of Chicago, Chicago, IL) showed that mice littermate control and transgenic mice treated with PTU were profoundly hypothyroid (TSH 27.0 mU/ml; 120 ± 13 milliunits/ml, respectively n = 4; α 0.005–0.034 milliunits/ml). After 4 weeks on this diet, hypothyroid mice were injected intraperitoneally with 100 μg of 1-T3 (Sigma) per 100 g mouse body weight in phosphate-buffered saline for 6 h before sacrifice and liver harvest. Control mice were injected with the same volume of phosphate-buffered saline alone for 6 h before sacrifice and liver harvest. Euthyroid mice were fed a normal diet. Control and transgenic mice were bled from their tails, and sera were analyzed by the endocrine laboratory, National Institutes of Health.

RNA Preparation and Labeling for Microarray—Total RNA was isolated from individual mouse livers by RNeasy kit (Qiagen) and further purified by TRIZOL reagent (Life Technologies, Inc.). 100 μg of total RNA was converted to cDNA by using SuperScript II RNA reverse transcriptase (Life Technologies, Inc.) as previously described (27). RNA isolated from a littermate control mouse liver was used to prepare cDNA labeled with Cy3-dUTP (Amersham Pharmacia Biotech) and RNA isolated from a transgenic mouse liver was used to prepare cDNA labeled with Cy5-dUTP (Amersham Pharmacia Biotech). Labeled cDNA was purified using MicroCon 30 filters (Amicon). Hybridization and cDNA microarray analysis were performed as described previously (24).

RNA Extraction, Probe Preparation, and Northern Blotting Hybridization—Total RNA was prepared from mouse livers using TRIZOL reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. Poly(A) RNA was isolated using poly(A)tract mRNA isolation kit (Promega). Poly(A) RNA (2 μg) was separated on 1% agarose-formaldehyde gel and then transferred to a nylon transfer membrane (Schleicher and Schuell). The blots were probed with gel-purified α-[32P]dCTP-labeled fragments and probes were purified using NICK column (AP-Biosham Pharmacia Biotech) according to the manufacturer’s instructions. mRNA signals were quantified using ImageQuant software (Molecular Dynamics) and normalized with corresponding thyroid hormone-insensitive 36B4 signals. Fold-induction was determined from transgenic mice signal values divided by control mice signal values within the same experiment.

Histological Examination and Measurement of Cell Proliferation—Mice were taken from mice and fixed in 10% neutral buffered formalin solution (Sigma). The specimens then were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin by American Histolab (Rockville, MD). To detect proliferating hepatocytes, mice were injected intraperitoneally with 5-bromo-2′-deoxyuridine (BrdUrd, 50 mg per kg of body weight, Roche Molecular Biochemicals). After 1 h, the mice were killed immediately, and liver biopsies were fixed in 10% neutral buffered formalin solution (Sigma).

RESULTS

To study basal repression in vivo, we constructed an expression vector containing the NCoRi cDNA and the mouse albumin promoter to target expression of NCoRi to the liver (25) (Fig. 1A). NCoRi is derived from the 3.1-kilobase cDNA that was originally isolated from a human placental library. The NCoRi protein contains the TR interaction domains and surrounding amino acids (1539–2453) but lacks the repressor domains (1–1538) shown that NCoRi has dominant negative activity on endogenous NCoRi. A, structure of liver-specific expression construct, Alb-NCoRi, used in preparation of transgenic mice. The size of each DNA fragment is shown below the corresponding structure. Restriction sites are also indicated below the construct. B, Northern blot analysis of poly(A) RNA (2 μg) from control and transgenic mice lines to determine relative NCoRi mRNA expression. mRNA signals corresponding to endogenous NCoRi or NCoRi transgene are indicated. mRNA ratio of NCoRi transgene and endogenous NCoRi are shown below the blot. Control probe (36B4) was used as a RNA-loading control. Position of endogenous NCoRi mRNA was confirmed using a 5′-probe specific for full-length NCoRi in a separate Northern blot. C, Northern blotting analysis of poly(A) RNA to demonstrate liver-specific expression of NCoRi in transgenic mouse line F210. Upper panel, autoradiography of Northern blot containing 2 μg of poly(A) RNA from liver, intestine, stomach, kidney, spleen, heart, and lung. Lower panel, autoradiography of control probe 36B4 used as a RNA-loading control.

staining of BrdUrd and TUNEL analysis was carried out by Molecular Histology Laboratories (Rockville, MD).
FIG. 2. Endogenous SMRT and NCoR gene expression in livers of transgenic mice. Northern blotting performed as described in “Materials and Methods.” All signals were normalized to control signal 36B4. Each bar represents the mean of four samples. Star indicates statistical difference between transgenic and littermate control mice, *p < 0.05. A, bar graph showing hepatic SMRT mRNA levels from hypothyroid and euthyroid mice from line F210 and littermate controls. B, bar graph showing endogenous hepatic NCoR mRNA levels from hypothyroid and euthyroid mice from line F210 and littermate controls. A probe against the 5’ region of NCoR was used to better visualize endogenous NCoR.

blotting. As shown in Fig. 1B, a 4-kilobase mRNA corresponding to NCoRi mRNA was detected from the transgenic mice but not their littermate controls. NCoRi mRNA was expressed 17–146 times higher than endogenous NCoR mRNA in these mouse lines in the euthyroid state. Moreover, endogenous NCoR mRNA did not increase in the transgenic lines (Figs. 1B and 2B). To determine whether the transgene was specifically expressed in liver, we examined the tissue distribution of NCoRi mRNA by Northern blot analysis and found NCoRi mRNA was detected only in the liver in the transgenic line 210 (Fig. 1C).

Mice from all the transgenic lines had normal appearance, size, and weight when compared with littermate controls (data not shown). Livers were normal in size and appearance. Small differences in serum alkaline phosphatase (transgenic 60 ± S.D. of 10 versus control 34.5 ± S.D. of 10, n = 4) and triglycerides (transgenic 41.25 ± S.D. of 0.9 versus control 78.5 ± S.D. of 24.9, n = 4) were observed between euthyroid transgenic and littermate control mice. No differences in serum glucose, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, total bilirubin were found between hypothyroid transgenic and littermate control mice.

SMRT is another nuclear corepressor expressed in various tissues (2, 28). Like NCoR, SMRT interacts with the unliganded thyroid hormone and retinoic acid receptors via conserved nuclear receptor interaction domains and strongly represses basal transcription in cotransfection studies. To determine whether expression of NCoRi changed the expression of endogenous SMRT and NCoR mRNA, we examined their expression in transgenic mice from line F210, which expressed the highest amount of NCoRi mRNA, and their littermate controls. Hepatic SMRT mRNA was slightly increased in the euthyroid transgenic mice (Fig. 2A). Because basal repression is mediated by unliganded TR in the hypothyroid state, we examined whether hepatic SMRT expression was increased in transgenic mice rendered hypothyroid after 4 weeks of treatment with PTU. SMRT mRNA was expressed at 3-fold higher levels in hypothyroid control mice than in euthyroid control mice suggesting that SMRT gene expression is negatively regulated by T3 in liver. Hepatic SMRT mRNA increased 7-fold in transgenic mice suggesting a compensatory increase in hypothyroid transgenic mice. Similarly, endogenous NCoR mRNA was increased in hypothyroid transgenic mice (Fig. 2B).

To study whether overexpression of NCoRi affected basal repression of target genes, we examined mRNA expression levels of six different thyroid hormone-responsive genes, Spot 14, 5’-deiodinase, bcl3, glucose 6-phosphatase, malic enzyme, and sialyltransferase in hypothyroid and hyperthyroid mice. With the exception of sialyltransferase, which is negatively regulated, all of these target genes are positively regulated (24). For these studies, transgenic mice from line F183 (which had a medium level of NCoRi mRNA expression) and their control littermates were used. We first examined hepatic spot 14 (S14) gene expression in hypothyroid transgenic mice, and found they exhibited 4-fold higher expression than their littermate controls (Fig. 3A), suggesting that NCoRi was able to derepress basal repression in the absence of ligand. In contrast, hepatic S14 mRNA expression was similar in hyperthyroid transgenic and littermate control mice, consistent with the notions that NCoR only interacts with TR in the absence of ligand and co-activator complexes are involved in ligand-dependent transcriptional activation. Similar results were found in several other thyroid hormone-responsive genes: bcl3, glucose 6-phosphatase, and 5’-deiodinase (Fig. 3, B–D). Of note, not all of the thyroid hormone-responsive genes studied were affected by overexpression of NCoRi. No difference in malic enzyme mRNA expression was observed between transgenic and control mice in the absence or presence of T3 (Fig. 3E). These findings suggest that NCoRi selectively blocked basal repression of several positively regulated thyroid-hormone responsive genes, but had no effect on their ligand-dependent transcriptional activation.

We also studied the role of NCoR on gene expression of a negatively regulated target gene, sialyltransferase. We previously used cDNA microarrays to identify this gene as negatively regulated by T3 in liver (24). In some negatively regulated target genes, there is ligand-independent activation by TR (29). Northern blotting analysis showed that ligand-independent activation in the hypothyroid state was decreased in the transgenic mice compared with littermate controls (Fig. 4). These findings result in decreased negative-regulation of sialyltransferase mRNA in the transgenic mice as the difference between mRNA expression in the hypothyroid state and the hyperthyroid state is less in the transgenic mice than wild-type control mice.

To investigate other effects of NCoRi expression on hepatic function, we injected control and transgenic mice intraperitoneally with 5-bromo-2’-deoxyuridine (BrdUrd). Proliferating cells that incorporated BrdUrd into their genomic DNA were visualized by staining liver sections with an antibody specific for BrdUrd (Fig. 5A). As shown in Fig. 5B, proliferating cells were increased by 120% in the highest expressing line (F210) and 50% in the medium expressing line (F183) when compared with control littermates. Additionally, TUNEL analyses was performed and did not show any significant apoptosis in the transgenic or littermate control mice (data not shown).
cDNA microarray analyses was undertaken to study the patterns of gene expression in the transgenic mouse livers. cDNAs obtained from hepatic RNA from two different transgenic lines were labeled with the fluorescent dye, cy5, whereas cDNA obtained from control mice RNA were labeled with a second fluorescent dye, cy3. The labeled cDNAs were hybridized on an array containing a total 4500 mouse gene elements, and their expression pattern was determined. We observed 28 genes that were up-regulated by more than 2-fold and 10 genes that were down-regulated by more than 60% in two different transgenic lines. The full results of this microarray study will be reported elsewhere. Interestingly, we found up-regulation of a number of genes that were either markers of proliferation or correlated with hepatocyte proliferation (Table I). Additionally, we observed a strong down-regulation of retinoblastoma (Rb) mRNA, which is a key regulator of $G_1$ to S phase transition. Northern blotting performed on RNA samples from each of the transgenic lines confirmed the up-regulation of these genes (Fig. 6).

**DISCUSSION**

Unliganded thyroid and retinoic acid receptors can repress transcription in cotransfection and *in vitro* systems by interacting with corepressors such as NCoR and SMRT. Previously, it was not known whether basal repression of target gene expression by TRs occurs in physiological conditions, and what its mechanism might be if it did occur. Our Alb-NCoR transgenic mouse model provides *in vivo* evidence that nuclear hormone corepressors are involved in the basal repression by TRs in absence of ligand.

Earlier *in vitro* studies showed that overexpression of NCoRi and several regions of NCoR involved in nuclear receptor interaction blockedRAR- and TR-mediated basal repression (23, 30). The mechanism for this derepression was dominant negative activity by the mutant NCoRi on wild-type NCoR activity. Our *in vivo* data are consistent with these *in vitro* findings, as overexpression of NCoRi in transgenic livers resulted in derepression of several positively regulated thyroid hormone-responsive genes but had no effect on ligand-dependent transcriptional activation. Additionally, NCoRi did not affect basal transcription of at least one thyroid hormone-responsive gene, malic enzyme, suggesting that specific corepressors may mediate basal repression of target genes. We also found that overexpression of NCoRi partially blocked the basal transcription of a negatively regulated target gene, sialyltransferase, in the absence of hormone. These results are consistent with the findings of Tagami et al. (29) who showed that NCoR can augment basal transcription of negatively regulated target genes in cotransfection studies.

The importance of basal repression in development is exemplified by recent studies with double knockout mice, which lack both TRα and TRβ (31, 32) These mice are viable, reproduce, and have a milder phenotype than mice with congenital or neonatal hypothyroidism. Thus the absence of TR produces a milder phenotype than the absence of thyroid hormone, suggesting basal repression of target genes may have deleterious effects. Because our studies on basal transcription were performed in hypothyroid mice, it is possible that basal repression of target genes in the hypothyroid state may contribute to the clinical phenotype observed in congenital hypothyroidism. Additionally, basal repression of target genes may have a normal physiological function as it is possible that transient hypothyroidism may occur in the fetus before its thyroid gland is competent (33). Basal repression also may occur in specific tissues, which have decreased transport or deiodination of $T_3$.

While this manuscript was in preparation, Rosenfeld and co-workers (11) reported a NCoR gene-deleted mouse, which died in *utero* with defects in erythrocyte, thymocyte, and CNS development (11). In co-injection studies of mouse embryo fibroblasts, they observed reversal of basal repression by retinoic acid receptor using an artificial direct repeat 5 reporter. Our results demonstrate that blockade of NCoR function can reverse basal repression of endogenous target genes in live animals and support a physiological role for corepressors.
We also observed that SMRT mRNA was negatively regulated by T3. This suggests that SMRT expression increases in the hypothyroid state when it is involved in basal repression of target genes. Moreover, there was a compensatory increase in endogenous SMRT and NCoR mRNA expression in hypothyroid transgenic mice. It currently is not known whether NCoR directly affects the transcription of these genes or whether derepression of other regulatory genes may alter their expression. Interestingly, a similar compensatory mechanism was observed for co-activators as TIF2 mRNA was increased in the SRC-1 knockout mouse (34).

Our data also showed that there was increased DNA synthesis in the hepatocytes from the transgenic mice as measured by BrdUrd incorporation. These findings are consistent with increased hepatocyte proliferation (35, 36); furthermore, we did not detect any change in apoptosis. In parallel with the increase in proliferation, we detected increased gene expression of several markers of cell proliferation in the livers of transgenic mice using cDNA microarray: alpha fetoprotein, MAP kinase phosphatase-1 (MKP-1), and cyclin A2, and decreased gene expression of a cell cycle repressor, Rb (Table I). Alpha fetoprotein (AFP) is a critical serum transport protein produced by the liver during embryogenesis (37) and is used clinically as a prenatal marker for a number of fetal abnormalities including spina bifida and Down’s syndrome. It also is expressed in hepatocellular carcinoma and is associated with dedifferentiation and proliferation of hepatocytes. MKP-1 is an immediate early gene that can be rapidly induced by mitogens, heat shock, or oxidative stress, and thus is induced in parallel with MAP kinases, which also are stimulated by mitogens (38). Interestingly, a recent study showed that MAP kinase stimulation phosphorylates SMRT and decreases its interaction with nuclear hormone receptors (39). These findings suggest that stimulation of MKP-1 mRNA could potentially enhance SMRT activity. Cyclin A2 mRNA is up-regulated at the beginning of S phase and has been used as a marker of hepatocyte prolifera-

**FIG. 4. Northern blot analysis of a negatively regulated target gene, sialyltransferase.** In the hypothyroid state, NCoRi effects on ligand-independent activation are examined. In the hyperthyroid state, NCoRi effects on ligand-dependent negative regulation are studied. NCoRi poly(A<sup>+</sup>) RNA preparation from line F183 and Northern blotting were performed as described in the legend to Fig. 3. Shown are the means and S.D. of at least four samples from control and transgenic mice under hypo- and hyperthyroid conditions. All signals were normalized to control signal 36B4 mRNA expression.

**FIG. 5. Effect of NCoRi on hepatic cell proliferation.** Control and transgenic mice from lines F210 and F183 were injected with BrdUrd, and hepatic slices were analyzed as described under "Materials and Methods." A, representative micrographs showing BrdUrd-labeled hepatocytes (red) in liver sections from adult transgenic mice (transgenic) and their littermate controls (control). B, bar graph showing relative scores of BrdUrd-labeled hepatocytes in 10,000 cells (n = 3–4).
tion (40, 41). Rb blocks the progression from G1 to S phase, and decreased expression or mutations of Rb correlate with cell proliferation and hepatocellular carcinoma (42, 43). Whereas these markers may not have a primary role in inducing proliferation, they nonetheless reflect the proliferative state of the transgenic livers.

Because this proliferation occurred in euthyroid mice, it is possible that this proliferation may be mediated by pathways that do not require thyroid hormone. Recently, it was shown that Mad/Max/Sin3/NCoR/HDAC complexes can repress transcription and potentially inhibit growth (7–10). Mxi also can recruit mSin3 (44). Thus Mad-Max and Mxi1-Max dimers can act as antagonists of Myc binding to Myc enhancer sites and repress transcription by recruiting corepressors (44, 45). Taken together, these findings suggest that overexpression of NCoRi, could disrupt the repression of Myc-mediated proliferation as well as other pathways repressed by NCoR.

Several novel target genes that are involved in cell proliferation were identified (Table I). Among them was cAMP response element modulator (CREM), which regulates cAMP-induced transcription. Recent studies have shown that CREM is increased during liver regeneration following partial hepatectomy (46). Moreover, CREM knockout mouse showed decreased cell proliferation and delayed liver regeneration following partial hepatectomy, confirming the important role of CREM in liver regeneration (47). Similarly, tumor necrosis factor receptor (TNFR) mRNA was induced in transgenic mice. Tumor necrosis factor has been shown to be important for liver regeneration and growth (48). Furthermore, recent studies on TNFR knockout mice showed they have markedly decreased DNA synthesis after partial hepatectomy (49). The mechanism for TNF-mediated regeneration likely involves STAT3-mediated transcription. Insulin-like growth factor binding protein-1 (IGFBP-1) mRNA also was increased in the transgenic mice. Insulin-like growth factors (IGFs) are potent mitogens that are important for growth and development. IGF-binding proteins (IGFBPs) are serum proteins that bind IGFs with high affinity. Recently, IGFBP-1 was identified as one of the most highly expressed immediate-early genes in liver regeneration (50, 51). It also is highly expressed in fetal rat liver suggesting that it may play a role in hepatic growth and development (50). Although IGFBPs can have growth inhibitory properties by competitively binding IGFs and preventing IGF interaction with IGF receptors, sequestration of IGF can enhance IGF-1 effects by allowing a slow release of IGF-1, and thereby protecting IGF receptors from down-regulation (52). It also is possible that IGFBPs can enhance IGF-1 activity independently of IGF-1 receptors (50). Although the precise mechanism by which NCoR represses the expression of these proliferative genes is not known, our findings demonstrate that NCoR has either direct or indirect effects on several key signaling pathways that affect hepatocellular proliferation.

Another potential contributor to hepatocyte proliferation is Bcl3 (53), which increased mRNA expression in the transgenic mice. Bcl3, an IκB protein, interacts directly with (NFκB) homodimers (54). Bcl3 also interacts with transcription coactivators and integrators such as SRC-1 and CBP/p300 (55, 56), and can function as a co-activator of retinoid X receptor and AP1-mediated transcription (53, 55, 56). Preliminary analysis of the bcl3 promoter showed that it contains potential Myc and thyroid hormone-response elements (57) suggesting that bcl3 could be a target gene of Myc/Max/Mad and TR. We recently showed that bcl3 is a target gene of thyroid hormone (24). Thus, the interaction of NCoR with members of both the nuclear hormone receptor and the Myc superfamilies raise the possibility that there may be co-operativity between the two pathways.

In conclusion, we have generated a transgenic mouse model in which a dominant negative corepressor, NCoRi, is targeted to liver. NCoRi selectively blocked basal repression of target genes by TRs suggesting that NCoR may mediate basal repression in certain physiological conditions. Moreover, NCoR also stimulated hepatocyte proliferation in euthyroid mice and induced several genes that may be involved in hepatocyte proliferation in transgenic mice. These findings suggest that NCoR may modulate transcription from TR-mediated and other signaling pathways. Recently, steroid hormone receptors bound to antagonists have been shown to recruit corepressors (58–60). RARs also can bind corepressors and mediate basal repression in the absence of ligand (1, 2). Thus, this system should be useful in characterizing the in vivo functions of NCoR for such antagonist-bound nuclear receptors as well as other nuclear hormone receptors such as RARs, which bind corepressors in the absence of ligand. It also may help define novel pathways for corepressor action.

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![FIG. 6. Northern blot analysis of hepatic genes induced in transgenic mice and associated with cell proliferation. Poly(A)⁺ RNA from livers of euthyroid transgenic mice from line F210 and their littermate controls were prepared and analyzed by Northern blot as in Fig. 3. Shown are the means and S.D. of at least four samples from transgenic and littermate control mice.](http://www.jbc.org/Downloaded from)
