MOLECULAR AND CELLULAR BIOLOGY, Apr. 2004, p. 3337–3346 Vol. 24, No. 8
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.8.3337–3346.2004

Recruitment of N-CoR/SMRT–TBLR1 Corepressor Complex by Unliganded Thyroid Hormone Receptor for Gene Repression during Frog Development

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Received 10 November 2003/Returned for modification 9 December 2003/Accepted 27 January 2004

The corepressors N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors) interact with unliganded nuclear hormone receptors, including thyroid hormone (T_3) receptor (TR). Several N-CoR/SMRT complexes containing histone deacetylases have been purified. The best studied among them are N-CoR/SMRT complexes containing TBL1 (transducin beta-like protein 1) or TBLR1 (TBL1-related protein). Despite extensive studies of these complexes, there has been no direct in vivo evidence for the interaction of TBL1 or TBLR1 with TR or the possible involvement of such complexes in gene repression by any nuclear receptors in any animals. Here, we used the frog oocyte system to demonstrate that unliganded TR interacts with TBLR1 and recruits TBLR1 to its chromatinized target promoter in vivo, accompanied by histone deacetylation and gene repression. We further provide evidence to show that the recruitment of TBLR1 or related proteins is important for repression by unliganded TR. To investigate the potential role for TBLR1 complexes during vertebrate development, we made use of T_3-dependent amphibian metamorphosis as a model. We found that TBLR1, SMRT, and N-CoR are recruited to T_3-inducible promoters in premetamorphic tadpoles and are released upon T_3 treatment, which induces metamorphosis. More importantly, we demonstrate that the dissociation of N-CoR/SMRT–TBLR1 complexes from endogenous TR target promoters is correlated with the activation of these genes during spontaneous metamorphosis. Taken together, our studies provide in vivo evidence for targeted recruitment of N-CoR/SMRT–TBLR1 complexes by unliganded TR in transcriptional repression during vertebrate development.

Thyroid hormone (T_3) receptors (TRs) are hormone-dependent transcription factors belonging to the superfamily of nuclear hormone receptors (12, 42, 46, 67). TR, which most likely functions as a heterodimer with 9-*cis*-retinoic acid receptor (RXR), also a member of the nuclear receptor superfamily, binds to T_3 response elements (TREs) in genes regulated by T_3 and, upon binding by T_3, activates the transcription of these genes. The binding of TREs by TR-RXR heterodimers is, however, independent of T_3 even in chromatin (49, 59, 67, 72), implying a role for unliganded TR in gene regulation. Indeed, various in vitro and in vivo studies have revealed that unliganded TRs repress target transcription, whereas in the presence of T_3, they enhance the transcription of these same genes (14, 29, 50, 67, 72, 80).

TRs regulate gene expression by recruiting TR-interacting cofactors (7, 10, 33, 47, 51, 73, 76, 80). In the absence of T_3, they bind to corepressors, a number of which have been identified (7, 80). The best studied among them are the highly related corepressors N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors), which were first identified as proteins capable of binding unliganded TR and retinoic acid receptor (RAR) and acting as transcription corepressors (9, 27).

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Both N-CoR and SMRT exist in multiple histone deacetylase (HDAC)-containing complexes (24, 35–37, 44, 68, 69, 77, 79), possibly reflecting the fact that numerous transcription factors in addition to TRs and RARs may use N-CoR and SMRT to repress target genes (22). A complex containing N-CoR, Sin3, and Rpd3 was found to be present in the frog oocyte (36), in agreement with earlier findings that both N-CoR and SMRT interact with the corepressor Sin3, which in turn binds to the HDAC Rpd3 or HDAC1/2 (2, 26, 41, 48). Another N-CoR or SMRT complex first identified in HeLa cells was found to contain HDAC3 and TBL1 (transducin beta-like protein 1) (24, 44). Subsequently, GPS2 (G-protein pathway suppressor 2) was also shown to be a component of this TBL1 or TBLR1 (TBL1-related protein) complex (77, 79). In addition, TBLR1 has also been shown to form a complex with N-CoR in the frog oocyte (66).

Studies with frog oocytes and tissue culture cells support the involvement of the TBL1-TBLR1-HDAC3 complex but not the Sin3-Rpd3 complex in transcriptional repression by unliganded TR (24, 36, 44, 55, 77). On the other hand, there has been no direct evidence that TR interacts with TBL1-TBLR1 in vivo, although TBL1-TBLR1 can be recruited to TR target genes (31, 77). In this study, we used two complementary systems to investigate the role of N-CoR/SMRT–TBLR1 complexes in TR function in the context of chromatin in vivo and during development. In the first, we made use of the frog oocyte system (71) to demonstrate in vivo association of TBLR1 with TR and the recruitment of N-CoR/SMRT–

TBLR1 complexes to a T_3-inducible promoter assembled into minichromosomes in vivo.

To determine whether such a mechanism functions in vivo on endogenous T_3 target genes during vertebrate development, we used *Xenopus laevis* metamorphosis as a developmental model system. Anuran metamorphosis involves the transformation of every organ and tissue of the tadpole. Different organs and tissues undergo vastly different changes, including de novo development of the limbs, complete resorption of the tail and gills, and drastic remodeling of other organs, and yet all are controlled by T_3 (11, 21, 60, 64, 78). This total dependence on T_3 makes anuran metamorphosis a unique model with which to study T_3 function in vertebrate development. On the basis of various studies in different laboratories, we have previously proposed a dual-function model for the role of TR in frog development (57). In premetamorphic tadpoles, TR-RXR heterodimers function as transcriptional repressors of T_3-inducible genes to promote animal growth and prevent premature metamorphosis. During metamorphosis, they act as transcriptional activators of these genes when T_3 becomes available, thus initiating metamorphic changes in different tissues. We show that TBLR1 is present in premetamorphic tadpoles when N-CoR/SMRT and TRs are expressed in the absence of T_3. Furthermore, TBLR1 is recruited to T_3-inducible genes, just like N-CoR/SMRT, and all are released upon T_3 treatment of the tadpoles, which induces precocious metamorphosis. More importantly, the N-CoR/SMRT–TBLR1 complexes at the TR target promoters are also released during natural metamorphosis when endogenous T_3 levels rise to initiate the tadpole-to-frog transformation. These results thus provide in vivo evidence to support a role for the N-CoR/SMRT–TBLR1 complex in gene repression by unliganded TR during vertebrate development.

MATERIALS AND METHODS

Plasmids. Plasmids pcDNA4-N-CoR-3′4k, which encodes the C terminus of the *Xenopus* N-CoR protein (N-CoR-C; amino acids [aa] 1151 to 2498) (58), and pCRT7-SMRT-C, which encodes the C terminus of *Xenopus* SMRT (GenBank accession no. AY498876; SMRT-C corresponds to human SMRT aa 2120 to 2507 [GenBank accession no. AF125672]), were generated through PCR cloning and used for in vitro translation (Promega). For expression and detection in frog oocytes, a Flag tag was added to the N terminus of *Xenopus* TRβA (75) by PCR with a primer containing the Flag sequence. The PCR products were cloned into the T7Ts expression vector (a gift from G. J. C. Veenstra, University of Nijmegen, Nijmegen, The Netherlands), which is based on the pGEM-4Z vector (Promega) and contains the 5′ and 3′ untranslated regions of the *X. laevis* β-globin gene flanking the multiple cloning sites. Plasmid pSP64-RXR, which encodes *Xenopus* RXRα, was described before (71). Dominant negative N-CoR with an N-terminal Flag tag (F-DN-RD1) was made by PCR cloning of the DNA fragment corresponding to the TBL1-interacting domain (aa 154 to 304) of *Xenopus* N-CoR (58). A nuclear localization signal sequence was also added during the PCR. The PCR primers were as follows: 5′-AGA TCT ACC GGT GCC ATG GAC TAC AAA GAC GAT GAC GAT AAA (Flag tag underlined) GGA TCC CCA AAG AAG AAG CGT AAG GTA (nuclear localization signal underlined) CTC GAG ATG TCT GGC CAA CCT GGA GAT-3′ and 5′-GCC GCC ACT AGT TCA ATC ATA GCG CTG ACA AAT GTT-3′. Another version, DN-RD1, which has a Myc tag instead of a Flag tag, was also made by PCR. The Myc sequence (5′-ATG GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG-3′) was used instead of the Flag sequence in the PCR primer. The pGL-TRE luciferase reporter vector (TRE-Luc) contains the T_3-dependent promoter of the *X. laevis* TRβA gene (3).

Antibody preparation and purification. Rabbit anti-Flag polyclonal antibody was purchased from Affinity BioReagents (Golden, Colo.). Mouse anti-Flag M2 monoclonal antibody was purchased from Stratagene. Rabbit anti-*Xenopus* N-CoR serum (58) was affinity purified with the glutathione *S*-transferase (GST)-tagged *Xenopus* N-CoR N-terminal fragment (aa 155 to 264). Rabbit anti-*Xenopus* TBLR1 (66) was affinity purified with the GST-tagged N terminus of TBLR1 (aa 1 to 211). Rabbit anti-*Xenopus* SMRT serum was generated against a synthetic peptide (KSKKQEMIKKLSTTNRSEQE) in the C-terminal nuclear receptor-interacting domain and was affinity purified with the same peptide. Briefly, *N*-hydroxysuccinimide-activated Sepharose 4 Fast Flow beads (Pharmacia) were conjugated with the synthetic peptide and washed sequentially with 10 mM Tris HCl (pH 7.5), 100 mM glycine (pH 2.3), 10 mM Tris HCl (pH 8.8), 100 mM triethylamine (pH 11.3), and 10 mM Tris HCl (pH 7.5). The antiserum was then mixed with the conjugated Sepharose beads and incubated for 2 h at 4°C. The beads were washed with 10 mM Tris HCl (pH 7.5), and the bound antibody was eluted with 100 mM glycine (pH 2.3).

IP assay. A 100-μl volume of protein A agarose beads was mixed with 25 μl of each affinity-purified antibody, and the mixture was incubated for 1 h at room temperature. After washing with 0.2 M triethanolamine (TB) at pH 8.5, 20 mM dimethyl pimelimidate cross-linker (Pierce) in TB was added to the beads and the mixture was incubated for 30 min. The beads were then washed sequentially with TB, TB plus 0.5% NP-40, 100 mM glycine (pH 2.3), Tris-buffered saline, and phosphate-buffered saline. Ten microliters of the resulting beads was used for each immunoprecipitation (IP) assay as described below.

Twenty *X. laevis* stage VI oocytes were lysed by pipetting in 400 μl of IP buffer (20 mM HEPES [pH 7.5], 5 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10 mM β-glycerophosphate, 150 mM NaCl, 0.1% NP-40, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail [Roche]). After centrifugation, the supernatant was subjected to IP. Antibody-cross-linked protein agarose beads (10 μl) were added to the oocyte lysate. After incubation for 4 h at 4°C, the beads were washed three times with IP buffer. Immunoprecipitated protein complexes were eluted with 100 mM glycine at pH 2.3 and subjected to Western blot analysis.

To express proteins in the oocytes, expression plasmids were used to make the corresponding mRNAs with T7 or SP6 in vitro transcription kits (mMESSAGE mMACHINE; Ambion). The mRNA (5.75 ng/oocyte) was microinjected into the cytoplasm of 20 oocytes. After incubation overnight at 18°C, the oocytes were lysed by pipetting in IP buffer. After centrifugation, the supernatant was used for IP with anti-Flag-M2 agarose beads (Sigma). Each lysate was incubated with appropriate beads for 4 h and washed three times with IP buffer. Immunoprecipitates were eluted with 100 mM glycine and immunoblotted with specific antibodies.

Transcription assay in the *Xenopus* oocyte system. The cytoplasm of stage VI oocytes from *X. laevis* was injected with the indicated mRNAs (1.15 to 5.75 ng/oocyte for TR and RXR, 1.15 to 11.5 ng/oocyte for F-DN-RD1). The luciferase reporter plasmid TRE-Luc (0.33 ng/oocyte) and the control vector phRG-TK (0.03 ng/oocyte; Promega) were coinjected into the germinal vesicle (nucleus) after mRNA injection. After overnight incubation at 18°C, oocyte lysates were assayed with a dual-luciferase assay kit (Promega). Six oocytes were lysed by pipetting in 90 μl of 1× lysis buffer from the dual-luciferase assay kit (Promega), and 7 μl of the lysate was used for each luciferase assay. Triplicate assays were performed at the same time, and the experiments were repeated three times. The relative expression of firefly luciferase from the reporter plasmid to *Renilla* luciferase from the control plasmid was determined and is reported here.

ChIP assay. The chromatin IP (ChIP) assay on oocyte samples for the recruitment of corepressor complexes and histone acetylation was done essentially as previously described (43, 66). Six oocytes were used for each assay. For Flag ChIP, anti-Flag-M2-agarose beads were used. Affinity-purified TBLR1 or N-CoR polyclonal antibody was used for TBLR1 or N-CoR ChIP. For acetylated histone H3 (Ac-H3) and Ac-H4, anti-Ac-H3 and anti-Ac-H4 antibodies (Upstate) were used. Salmon sperm DNA-protein A-agarose (Upstate) was used for ChIP of TBLR1, N-CoR, Ac-H3, and Ac-H4. After reversal of the DNA-protein cross-links, purification of the immunoprecipitated DNA was carried out with a PCR purification kit (QIAGEN). Buffer EB (40 μl) was used for elution, and 4 μl of eluted DNA was used for the PCRs: 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 20 s. Amplified DNA was loaded onto a 2% agarose gel and visualized by ethidium bromide staining after electrophoresis. The PCR primers used for the ChIP assay were as follows: TRE-Luc forward, 5′-TGC CTG TGT CTA TAC TGA TGG GAT-3′; TRE-Luc reverse, 5′-CAT TTT ACC AAC AGT ACC GGA ATG C-3′. These primers amplify regions containing the TRE to produce fragments of 190 bp.

Extraction of chromatin from tadpole intestines for ChIP was performed as previously described (59). The nuclei of the intestines were isolated from stage 54 tadpoles, stage 54 tadpoles with T_3 (5 nM) treatment for 24 h, and stage 61 tadpoles. The nuclei were cross-linked in buffer B (10 mM Tris-HCl [pH 7.5], 3 mM $CaCl_2$, 0.25 M sucrose, 1 mM dithiothreitol, protease inhibitor cocktail) with

1% formaldehyde for 20 min. After centrifugation at 6,000 × *g* for 5 min, the nuclei were lysed in 200 μl of sodium dodecyl sulfate lysis buffer (Upstate) for 10 min and sonicated to fragment the DNA (five times for 5 s on ice with a Branson Sonifier 450 [output 2, duty cycle 30]). Two microliters of each sonicated DNA sample was diluted 50 times in water for measurement of the DNA concentration by UV absorption. The DNA concentration was adjusted to 100 ng/μl with sodium dodecyl sulfate lysis buffer. The DNA samples were then further diluted with ChIP dilution buffer (Upstate) to 10 ng/μl. To preclear the samples, salmon sperm DNA-protein A-agarose beads (Upstate) were added and the mixture was incubated for 1 h. Equal amounts of those samples were aliquoted into 0.6-ml prelubricated polypropylene microcentrifuge tubes (slick tubes; PGC Scientific, Frederick, Md.) and used for IP with N-CoR antiserum, and affinity-purified SMRT and TBLR1 antibodies. After overnight incubation of the antibody and protein A-agarose beads with the precleared DNA supernatant, the mixture was washed. The bound DNA was eluted and purified as described for the ChIP assay on oocyte samples (66). The purified DNA was analyzed for the presence of specific DNA fragments by PCR. Each PCR, with or without [α-^{32}P]dCTP, was done at 95°C for 3 min, followed by 31 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 20 s. For detection of amplified DNA fragments with [α-^{32}P]dCTP, the DNA was analyzed on 6% Tris-borate-EDTA gels. After the gels were dried, DNA was detected with a phosphorimager. PCR samples not labeled with ^{32}P were loaded directly into a DNA bioanalyzer system (Agilent Technologies). The DNA was electrophoresed, quantified, and visualized as digital gel-like images.

PCR primers for the tadpole ChIP assay were as follows: TRβ promoter forward, 5′-TGT GTC TAT ACT GAT GGG ATG-3′; TRβ promoter reverse 5′-GAG GAA CTG AAG TAG CAG CG-3′; TH/bZIP promoter forward, 5′-TCT CCC TGT TGT GTA TAA TGG-3′; TH/bZIP promoter reverse, 5′-CTC CCA ACC CTA CAG AGT TCA-3′. These primers amplify the region containing the TREs to produce fragments of 186 and 284 bp, respectively.

RT-PCR analysis of gene expression in tadpoles. RNA was isolated from the intestines of tadpoles at stages 54, 58, 62, and 66 with Trizol (Invitrogen). The primers used for TBLR1 expression were 5′-GAG GTG TTT ATT TGT GCT TGG and 5′-TGC ACT TAA TAT GAA GTT GCC. The primers used for TRα, N-CoR, SMRT, and the internal control gene *rpl8* were previously published (58). All primers were designed to include more than one exon to avoid signals from potential genomic DNA contamination. Reverse transcription (RT)-PCRs were carried out with SuperScriptII (Invitrogen) for 30 min at 50°C for reverse transcription, followed by 24 to 28 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The primer concentrations were 0.04 μM for *rpl8* and 0.2 to 0.4 μM for the other genes, and 0.5 μg of RNA was added to each reaction mixture. In all cases, equal *rpl8* bands were obtained to confirm equivalent RNA amounts and uniform reaction conditions. All reactions were repeated at least three times with similar results.

RESULTS

Unliganded TR associates with TBLR1 in *Xenopus* oocytes. We have previously shown that TR-RXR heterodimers expressed in the frog oocyte through microinjection of their mRNAs into the oocyte cytoplasm can repress or activate a T_3-inducible promoter in the context of chromatin in the absence or presence of T_3, respectively (71). Our earlier studies have shown that unliganded TR is capable of recruiting both N-CoR and SMRT to its target promoter in chromatin in oocytes (28). To investigate whether they participate in repression by TR through TBLR1-containing complexes, we first analyzed the association of endogenous TBLR1 with endogenous SMRT and N-CoR in the oocyte. We carried out IP on oocyte extract with an antipeptide antibody against the receptor-interacting domain of *Xenopus* SMRT that specifically recognizes *Xenopus* SMRT but not N-CoR (Fig. 1A), anti-*Xenopus* TBLR1 (66), or anti-*Xenopus* N-CoR (58). Western blot analysis of the immunoprecipitates showed that both the anti-N-CoR (Fig. 1B, lane 2) and anti-SMRT (Fig. 1B, lane 3) antibodies were able to immunoprecipitate TBLR1 and that the anti-TBLR1 antibody was able to immunoprecipitate both N-CoR and SMRT (Fig. 1B, lane 4). These results indicate that

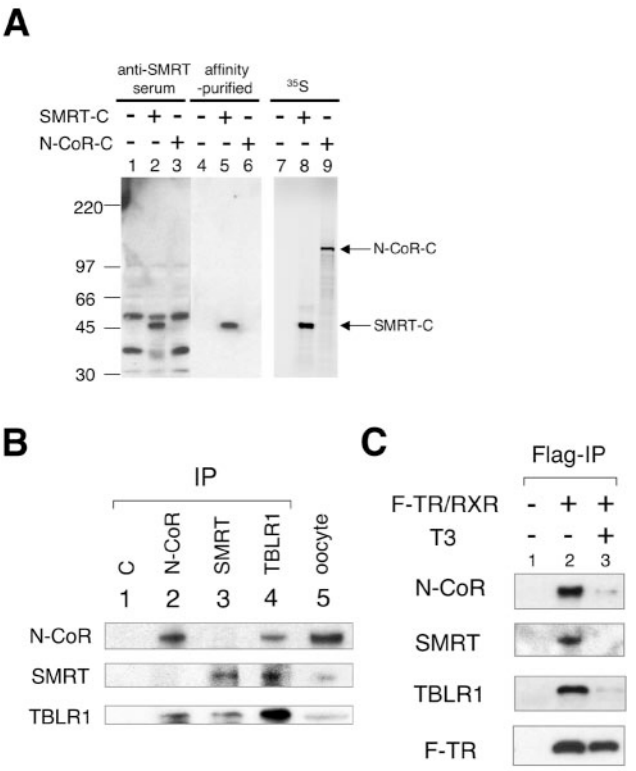

FIG. 1. Endogenous TBLR1 forms a complex with N-CoR or SMRT in the frog oocyte. (A) An anti-*Xenopus* SMRT antibody specifically recognizes SMRT but not N-CoR. In vitro-translated ^{35}S-labeled proteins were subjected to Western blot analysis with the crude (lanes 1 to 3) or affinity-purified (lanes 4 to 6) anti-*Xenopus* SMRT antibody or detection by ^{35}S autoradiography (lanes 7 to 9). Lanes: 1, 4, and 7, no template in the translation reaction mixture; 2, 5 and 8, template encoding the C terminus of *Xenopus* SMRT (SMRT-C); 3, 6, and 9, template encoding the C terminus of *Xenopus* N-CoR (N-CoR-C). The values on the left indicate the positions of the molecular weight markers. (B) TBLR1 interacts with endogenous N-CoR and SMRT in *Xenopus* oocytes. Twenty oocytes were lysed for IP with affinity-purified antibodies against the control (C) HA tag (lane 1), *Xenopus* N-CoR (lane 2), *Xenopus* SMRT (lane 3), and *Xenopus* TBLR1 (lane 4) after cross-linking of the antibodies to protein A beads. The immunoprecipitated proteins were eluted, and 20% of each eluted sample was subjected to immunoblotting with anti-*Xenopus* N-CoR, anti-SMRT, or anti-TBLR1 antibodies, as indicated. Five percent of the oocyte lysate before IP was used in lane 5 as a control. (C) Unliganded TR interacts with endogenous N-CoR, SMRT, and TBLR1 in oocytes. In vitro-transcribed mRNAs for F-TR/RXR (5.75 ng/oocyte) were injected into 20 oocytes (lanes 2 and 3), which were incubated overnight with (lane 3) or without (lanes 1 and 2) 50 mM T_3. After lysis, the whole lysate was subjected to IP with anti-Flag affinity beads. Eluted proteins (20%) were subjected to immunoblotting with anti-N-CoR, anti-SMRT, anti-TBLR1, and anti-Flag antibodies.

TBLR1 is complexed not only with N-CoR, as we have shown recently (66), but also with SMRT in the oocyte.

To investigate whether N-CoR/SMRT–TBLR1 complexes participate in the repression by unliganded TR-RXR, we determined whether unliganded TR interacts with TBLR1 in vivo. We microinjected in vitro-transcribed mRNAs encoding Flag-tagged TRβ protein (F-TR) and wild-type RXRα into the cytoplasm of oocytes to allow the synthesis of these proteins. After overnight incubation in the presence or absence of 50

nM T_3, the oocytes were lysed and subjected to an IP assay with anti-Flag affinity agarose beads. Western blot analysis showed that endogenous N-CoR, SMRT, and TBLR1 were all immunoprecipitated with F-TR in the absence of T_3 (Fig. 1C, lane 2). When T_3 was added to the oocyte culture medium, these corepressors were dissociated from TR (Fig. 1C, lane 3). These results indicate that F-TR interacts with N-CoR, SMRT, and TBLR1 in vivo.

Unliganded TR recruits TBLR1 to its target promoter in chromatin in gene repression. To study the potential role of the TR-TBLR1 interaction in gene repression in vivo, we microinjected in vitro-transcribed mRNAs encoding F-TR and wild-type RXRα into the cytoplasm of oocytes. Two hours later, we microinjected into the germinal vesicle (nucleus) two reporter plasmids, one containing a T_3-dependent promoter driving the expression of firefly luciferase (TRE-Luc) (Fig. 2A) and the other containing a control promoter driving the expression of *Renilla* luciferase (phRG-TK). After overnight incubation in the presence or absence of 50 nM T_3, the oocytes were lysed for the luciferase assay. As shown in Fig. 2B, the F-TR/RXR heterodimer repressed the T_3-dependent promoter in the absence of T_3 (lane 2) and activated it when T_3 was present (lane 3), in agreement with our earlier study with untagged TRβ and RXRα (71).

We next determined if N-CoR/SMRT–TBLR1 complexes are indeed recruited to the promoters by unliganded TR. Oocytes were injected and incubated in the presence or absence of T_3 as described above and subjected to a ChIP assay to determine the association of specific proteins with the target promoter (Fig. 2C and D). As expected, the ChIP assay with anti-Flag antibody confirmed that F-TR was bound to the target promoter constitutively (Fig. 2C and D, lanes 2 and 3). In the absence of ligand, TR recruited both N-CoR and TBLR1 to the promoter (Fig. 2C and D, compare lane 2 to lane 1). SMRT is also recruited to TR target promoters in the oocyte under these conditions (28). Accompanying this corepressor recruitment, the acetylation levels of both histones H3 and H4 were reduced concurrently (Fig. 2C and D). Upon ligand addition, all corepressors were dissociated from the promoter and histone acetylation levels were restored (Fig. 2C and D, lane 3). These data suggest that N-CoR/SMRT–TBLR1 corepressor complexes were recruited by unliganded TR-RXR to the target promoter, leading to histone deacetylation and gene repression.

Disruption of N-CoR/SMRT–TBLR1 complexes inhibits gene repression by unliganded TR. To study the role of N-CoR/SMRT–TBLR1 interaction in transcription regulation by unliganded TR, we investigated whether interfering with the association between N-CoR/SMRT and TBLR1 in vivo would alter gene regulation by TR. We have previously generated N-terminally Flag-tagged (F-DN-RD1) dominant negative *Xenopus* N-CoR (66) that contained the region corresponding to the TBL1-interacting domain of human N-CoR (24, 79). When the mRNA encoding F-DN-RD1 was injected into oocytes, the resulting protein interacted with endogenous TBLR1, as demonstrated by coimmunoprecipitation of TBLR1 with F-DN-RD1 by the anti-Flag antibody (Fig. 3A) (66).

To determine if dominant negative N-CoR interferes with the TBLR1-TR interaction, we generated a construct encoding dominant negative N-CoR with a Myc tag (DN-RD1) instead of a Flag tag and microinjected the corresponding mRNA together with the mRNAs for F-TR and wild-type RXR into oocytes. Following overnight incubation in the absence of T_3, the oocytes were lysed and subjected to IP with anti-Flag antibody to precipitate F-TR and associated proteins. Western blot analysis of the immunoprecipitates with the anti-Flag antibody confirmed that similar amounts of F-TR were immunoprecipitated in all of the samples, with different levels of DN-RD1 expression (Fig. 3B). Likewise, similar levels of endogenous full-length N-CoR were coimmunoprecipitated by the anti-Flag antibody, confirming that DN-RD1 did not interfere with the TR–N-CoR interaction. In contrast, with increasing levels of DN-RD1 expression, less endogenous TBLR1 was coimmunoprecipitated with unliganded TR (Fig. 3B). These results suggest that DN-RD1 functions as dominant negative N-CoR by competing for binding to endogenous TBLR1, thus disrupting the association of TBLR1 with endogenous N-CoR/SMRT, thereby reducing the association of TBLR1 with unliganded TR. These results further support the view that TBLR1 interacts with unliganded TR through N-CoR/SMRT.

The consequence of the disruption of the TBLR1-TR association on transcriptional repression by unliganded TR was investigated next. We microinjected increasing amounts of F-DN-RD1 mRNA together with mRNAs for TR-RXR, followed by nuclear injection of reporter DNA. After overnight incubation, the oocytes were harvested for luciferase assay. Again, unliganded TR-RXR repressed the target promoter. Expression of increasing levels of F-DN-RD1 gradually relieved the repression by unliganded TR-RXR (Fig. 3C, columns 1 to 4) but had no effect on the promoter activity in the absence of TR-RXR (Fig. 3C, columns 5 to 7), suggesting that TBLR1 or related proteins are critical for repression by unliganded TR-RXR.

TBLR1 is expressed in premetamorphic tadpoles when unliganded TR is presumed to repress T_3-inducible genes. To determine whether TBLR1 participates in gene repression by unliganded TR under physiological conditions, we turned to the remodeling of the tadpole intestine during frog metamorphosis. As mentioned in the introduction, earlier studies have provided strong evidence that unliganded TR functions as a transcriptional repressor in premetamorphic tadpoles to ensure tadpole growth (57). As a first step to study the function of TBLR1 during this process, we analyzed the expression profiles of TBLR1 in the intestines of *X. laevis* tadpoles during metamorphosis. The intestine has been studied extensively at the morphological, biochemical, and, more importantly, molecular levels. In particular, we have shown that N-CoR and SMRT are expressed and recruited to T_3-inducible promoters in the premetamorphic tadpole intestine when the hormone is absent (6, 58), making the intestine an ideal model for the study of the in vivo involvement of TBLR1 in TR function during development. Thus, we isolated total RNA from the intestines of premetamorphic (stage 54), metamorphic (stages 58 to 62), and postmetamorphic (stage 66) animals. RT-PCR analysis showed that TBLR1 was expressed throughout metamorphosis, with slightly lower levels at the climax of metamorphosis (stage 62) (Fig. 4). In comparison, SMRT and N-CoR were also expressed throughout metamorphosis, with the SMRT expression levels upregulated during metamorphosis (climax or stage 62), in agreement with earlier reports (58).

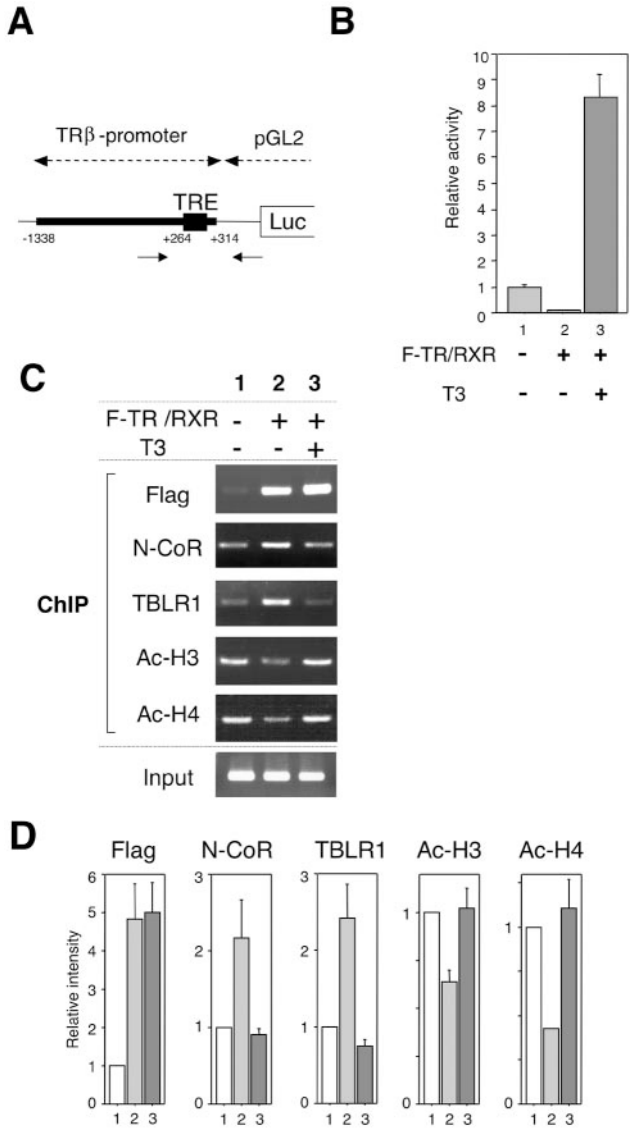

FIG. 2. Transcriptional repression by unliganded TR in the frog oocyte is associated with the recruitment of TBLR1. (A) Schematic representation of a luciferase (Luc) reporter vector (TRE-Luc) that contains the T_3-dependent *Xenopus* TRβ promoter driving the expression of the luciferase gene. The arrows at the bottom indicate the locations of the primers used for the ChIP assay. pGL2, pGL2-basic vector (Promega). (B) TR regulates transcription in a ligand-dependent manner in the oocyte. The mRNA for F-TR (1.15 ng/oocyte) was injected into the cytoplasm of 20 oocytes together with RXR mRNA (1.15 ng/oocyte), as indicated. Subsequently, the TRE-Luc reporter vector and phRG-TK luciferase control vector were coinjected into the germinal vesicles (nuclei) of the oocytes (lanes 1 to 3). The oocytes were incubated overnight with (lane 3) or without (lanes 1 and 2) 50 nM T_3. The oocytes were lysed and subjected to dual-luciferase assays. The relative activity of the reporter versus that of the control was plotted with the basal activity set to 1 (lane 1). (C) Unliganded TR recruits N-CoR and TBLR1 to the target promoter and induces histone deacetylation in vivo. Oocytes were injected and incubated as described for panel B. They were then processed for a ChIP assay with antibodies against Flag, N-CoR, TBLR1, Ac-H3, and Ac-H4. The presence of the TRE region of the promoter in the immunoprecipitated DNA was determined by PCR with primer pairs flanking the TRE (Fig. 2A). The PCR products were analyzed on a 2% agarose gel containing ethidium bromide. The DNA prior to IP was amplified as the "Input" control to show equal amounts of DNA in all samples. (D) Quantification of the ChIP data in panel C. The intensities of the bands as shown in panel C from two independent experiments were analyzed with BioMax 1D software (Kodak) (normalized against the input control). The resulting data are presented as the relative intensity against lane 1 for each antibody.

These results and the constitutive expression of TRα (Fig. 4) (40, 62, 71, 74) suggest that N-CoR/SMRT–TBLR1 complexes may participate in gene repression by unliganded TR in premetamorphic tadpoles.

TBLR1 is recruited to endogenous T_3-inducible genes in premetamorphic tadpoles. To directly investigate the involvement of TBLR1 in the regulation of T_3-inducible genes during development, we studied the binding of TBLR1 to the TRE regions of known T_3-inducible genes in developing tadpoles by using a ChIP assay. For this purpose, we chose to analyze the genes for TRβA and TH/bZip, two of the three *X. laevis* T_3-inducible genes whose TREs have been located (19, 45, 53) (Fig. 5A). As expected, the ChIP assay with anti-TR antibodies showed that TR was bound to the TRE regions of both genes in the intestine (Fig. 5B). T_3 treatment of the tadpoles did not change the binding of TR. Similarly, N-CoR and SMRT were also bound to both promoters in tadpoles. T_3 treatment of the tadpoles, however, led to the release of N-CoR (Fig. 5B), in agreement with earlier findings (6, 58). More importantly, we found that TBLR1 was recruited to both promoters in the tadpole intestine and released upon T_3 treatment of the tadpoles (Fig. 5B). Thus, TBLR1 and N-CoR/SMRT were bound to the promoters in the tadpole intestine in the absence, but not in the presence, of T_3.

To investigate whether the dissociation of corepressors plays a role during natural development, we carried out a ChIP assay on nuclei isolated from the intestines of premetamorphic tadpoles at stage 54 and tadpoles at the climax of metamorphosis (stage 61). Again, TR, TBLR1, N-CoR, and SMRT were present at the TR target promoters in the intestines of premetamorphic tadpoles (stage 54, Fig. 6). During metamorphosis (stage 61), when these target genes were induced (32, 61, 74), the corepressors N-CoR, SMRT, and TBLR1 were all dissociated from the promoters (Fig. 6, stage 61). These data thus support the idea that unliganded TR recruits N-CoR/SMRT–TBRL1 complexes to the promoters to repress their expression in premetamorphic tadpoles and the dissociation of the corepressors from the promoters by the rising concentrations of endogenous T_3 contributes to the activation of these target genes during metamorphosis.

DISCUSSION

Nuclear hormone receptors are ligand-dependent transcription factors. Some of them, including TR, have dual functions, repressing target genes in the absence of ligand and activating them when ligand is present. The unliganded receptors bind corepressors, such as SMRT and N-CoR, which form multimeric complexes containing HDACs, whereas in the presence of ligand, they interact with coactivator complexes, such as SRC-1, CBP-p300, or DRIP-TRAP complexes, many, but not all, of which have histone acetyltransferase activity (7, 8, 10, 24, 26, 30, 33, 34, 36, 44, 47, 48, 51, 52, 56, 73, 76, 80). While much

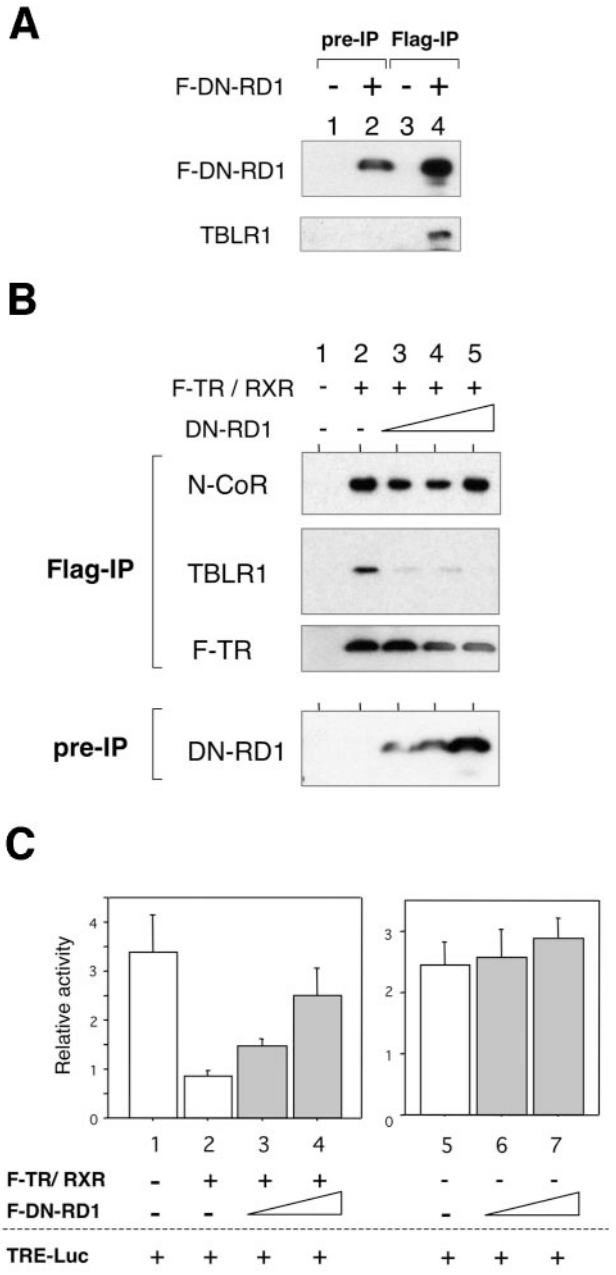

FIG. 3. A dominant negative form of *Xenopus* N-CoR inhibits TBLR1-dependent repression by unliganded TR in vivo. (A) F-DN-RD1 interacts with endogenous TBLR1 in oocytes. The mRNA for F-DN-RD1 (5.75 ng/oocyte) was injected into the cytoplasm of oocytes (lanes 2 and 4). After overnight incubation, the oocytes were lysed and subjected to IP with anti-Flag antibody. Pre-IP lysate (lanes 1 and 2) and IP samples (lanes 3 and 4) were subjected to immunoblotting. Detection was performed with anti-N-CoR, which recognizes F-DN-RD1, and anti-TBLR1 antibodies. (B) DN-RD1 disrupts the association of unliganded TR with endogenous TBLR1. The mRNAs for F-TR/RXR (5.75 ng/oocyte) and the dominant negative form of N-CoR with the Myc tag (DN-RD1) (2.3, 5.75, and 11.5 ng/oocyte in lanes 3 to 5, respectively) were injected into the cytoplasm of oocytes as indicated. After overnight incubation, the oocytes were lysed and used for anti-Flag IP. Pre-IP and IP samples were subjected to immunoblotting with anti-N-CoR, anti-TBLR1, and anti-Flag antibodies. Again, DN-RD1 was detected by the anti-N-CoR antibody. (C) Dominant negative N-CoR reverses the transcription repression by unliganded TR. A transcription assay with frog oocytes was performed as shown in Fig. 2B. The TRE-Luc reporter vector and the phRLG-TK control vector were injected into germinal vesicles (lanes 1 to 7). The mRNAs for F-TR/RXR (1.15 ng/oocyte) (lanes 2 to 4) and increasing amounts of F-DN-RD1 (1.15/oocyte in lanes 3 and 6 and 5.75 ng/oocyte in lanes 4 and 7) were injected into the cytoplasm. After overnight incubation, the oocytes were lysed and assayed for luciferase activity.

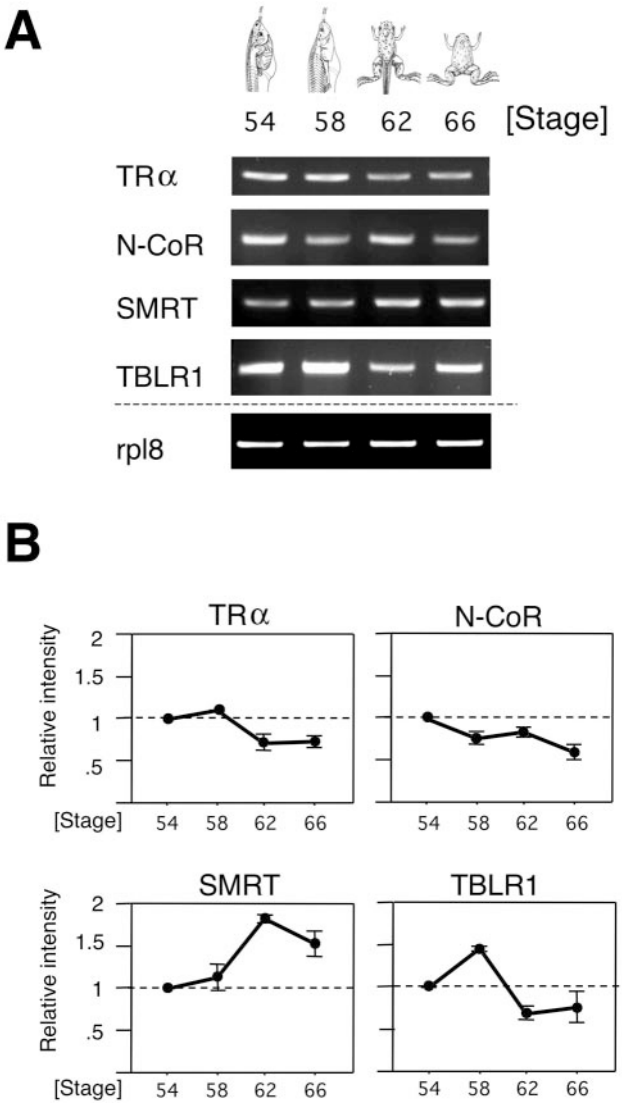

FIG. 4. Both TR and corepressors are expressed throughout metamorphosis in the intestine. (A) RT-PCR was carried out with primers specific to the indicated genes on total RNA isolated from the intestines of tadpoles at stages 54, 58, 62, and 66, encompassing the metamorphic period. The *rpl8* gene was used as the internal control (63). The data from three independent experiments were quantified by using BioMax 1D software (Kodak), and the relative band intensity is presented in panel B after normalization against the *rpl8* signal and setting of the signal at stage 54 to 1.

has been learned about the biochemical composition and molecular properties of these complexes, few studies have addressed their relevance in vivo, especially during vertebrate development. In this study, by using two complementary in vivo

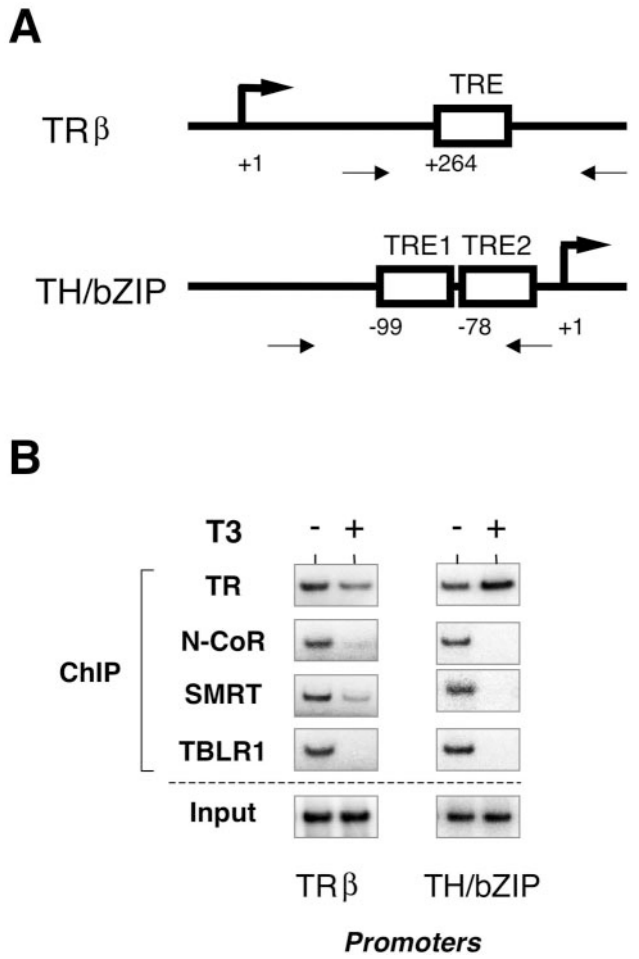

FIG. 5. N-CoR/SMRT–TBLR1 corepressor complexes associate with T_3-dependent promoters in the intestines of premetamorphic tadpoles. (A) Schematic representation of *Xenopus* TRβ and TH/bZip promoters, which are regulated by TR directly (19, 45, 53). Each promoter has one or two TRE sites, as indicated. The relative locations of the PCR primers used for the ChIP assay are shown as thin arrows. Transcription start sites are indicated as +1. (B) T_3 treatment of premetamorphic tadpoles induces dissociation of the corepressors from TR target promoters. A ChIP assay was carried out on intestines isolated from premetamorphic stage 54 tadpoles left untreated or treated with 5 nM T_3 for 24 h. Anti-TR, anti-N-CoR, anti-SMRT, and anti-TBLR1 antibodies were used for IP. The precipitated DNA was amplified by PCR in the presence of [^{32}P]dCTP with primers specific for the TRβ or TH/bZip promoter, as indicated, and the ^{32}P-labeled products were analyzed on a 6% polyacrylamide gel. After the gel was dried, the signals were detected with a phosphorimager. "Input" indicates equal amounts of sample DNA from tadpoles left untreated or treated with T_3. The experiments were done three times with similar results.

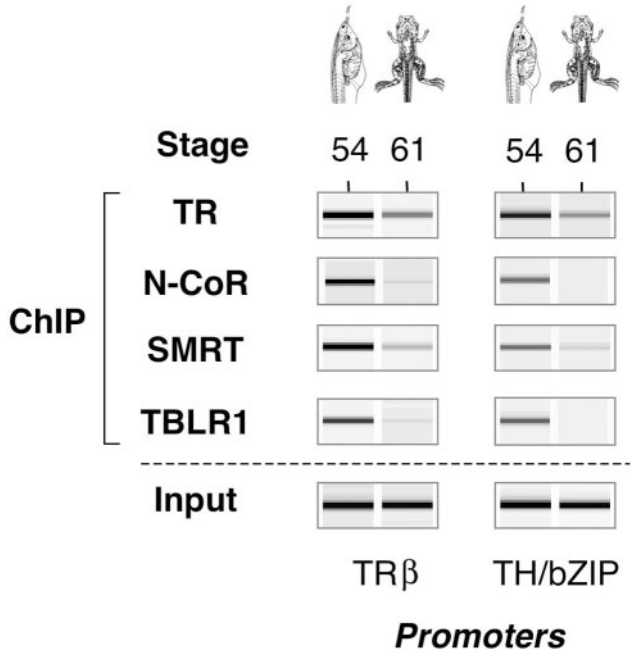

FIG. 6. Dissociation of N-CoR/SMRT–TBLR1 corepressor complexes from TR target promoters is correlated with T_3-dependent metamorphosis during natural development. Intestines from tadpoles at premetamorphic stage 54 and tadpoles at the climax of metamorphosis (stage 61) were isolated for ChIP assay. Anti-TR, anti-N-CoR, anti-SMRT, and anti-TBLR1 antibodies were used for IP. The precipitated DNA was amplified by PCR with primers specific for the TRβ or TH/bZip promoter, as indicated. The PCR products were analyzed with the DNA bioanalyzer system (Agilent Technologies). The digitized signals in each lane were converted into digital gel-like image as shown here. "Input" indicates equal amounts of sample DNA from tadpoles at different stages. The experiments were done three times with similar results.

systems, we demonstrated that (i) unliganded TR interacts with TBLR1 in vivo, likely through N-CoR/SMRT; (ii) N-CoR/SMRT and TBLR1 are recruited to TREs assembled in chromatin in vivo and to TREs located in the endogenous T_3-inducible genes during vertebrate development when T_3 is absent, leading to histone deacetylation; and (iii) N-CoR/SMRT–TBLR1 interaction is likely required for transcriptional repression in vivo. Our data thus support a role for N-CoR/SMRT–TBLR1–HDAC or related complexes in mediating transcriptional repression by unliganded TR during vertebrate development.

N-CoR/SMRT–TBLR1 complexes are directly recruited by unliganded TR to repress transcription in vivo. A number of earlier studies suggested that the highly related large corepressors N-CoR and SMRT mediate gene repression by unliganded TR and RAR through their interactions with the corepressor Sin3, which in turn binds to HDAC Rpd3 (HDAC1/2) (2, 26, 41, 48). In addition, biochemical studies and purification have suggested the existence of multiple N-CoR/SMRT–HDAC complexes, including an N-CoR–HDAC complex containing Sin3 and Rpd3 (24, 35–37, 44, 68, 69, 77, 79). Despite these implications, no evidence has been reported for in vivo association of unliganded nuclear receptors with an N-CoR/SMRT–Sin3–Rpd3 complex. Thus, the significance of Sin3-Rpd3-containing complexes in gene repression by unliganded TR in vivo remains questionable.

The best-characterized N-CoR/SMRT–HDAC complexes are those containing TBL1 or TBLR1 and HDAC3 first isolated from HeLa cells (24, 31, 44, 77, 79). In our previous studies of transcriptional repression by unliganded RARα and its oncogenic fusion proteins PML-RARα and PLZF-RARα (66), we showed that endogenous N-CoR and TBLR1 also form a complex with each other in the *Xenopus* oocyte and that

both interact with exogenously introduced unliganded RAR and the RAR fusion proteins. Furthermore, we showed that RARα and its fusion proteins recruit N-CoR and TBLR1 to target promoters and reduce histone acetylation, supporting a role for the N-CoR–TBLR1 complex in gene repression by unliganded RAR proteins.

Here we have extended our oocyte studies to show that endogenous TBLR1 interacts not only with N-CoR but also with SMRT. In addition, we provide direct in vivo evidence that unliganded TR, like unliganded RAR and its fusion proteins (66), interacts with TBLR1 in the oocyte. Furthermore, this TR-TBLR1 association is likely through N-CoR or SMRT because dominant negative N-CoR, which consists of the TBLR1-interacting domain of N-CoR, is able to inhibit the TR-TBLR1 association in vivo. Finally, our ChIP assay demonstrates that TBLR1 is recruited to TRE assembled into chromatin in vivo by unliganded but not T_3-bound TR. Concurrent with this recruitment, acetylation levels at the promoter are reduced, in agreement with the presence of HDAC activity in N-CoR/SMRT–TBLR1 complexes (24, 31, 44, 77, 79). Thus, our studies, together with earlier published work by us and others (24, 31, 44, 66, 77, 79), support the model in which unliganded nuclear receptors, at least the subfamily containing TR and RAR, recruit N-CoR/SMRT–TBLR1 complexes to deacetylate chromatin at promoters to mediate gene repression.

Role of N-CoR/SMRT–TBLR1 complexes in normal and pathological function of TR in vertebrate development. Transcriptional repression by unliganded TR has received a lot of attention in recent years because of increasing evidence implicating unliganded TR in developmental and pathological processes. In particular, mice lacking TRs have milder developmental defects than do hypothyroid mice (13, 17, 18, 20, 23, 70), suggesting that unliganded TRs affect mammalian development. In addition, a major cause of resistance to thyroid hormone syndrome in humans is mutation of the gene for TRβ, leading to the formation of dominant negative TRβs (dnTRβs) that behave like constitutively unliganded TRs (1, 5, 54, 76). Mice with dnTRβ mutations, which mimic humans with resistance to thyroid hormone syndrome, and dnTRα mutations have been analyzed (25, 38, 39, 65, 81). These animals show various degree of resistance to T_3, likely owing to constitutive gene repression by the dnTRs. On the other hand, in most of these studies with mammals, very little information is available on the expression of genes known to be regulated by T_3. In mice with a mutation introduced into either the TRα or the TRβ locus, resulting in the expression of dnTRα or dnTRβ, a number of known T_3 response genes in different tissues were found to have different expression levels compared to wild-type mice, but the results obtained with dnTRα and dnTRβ mice were quite different and the underlying mechanism remains to be determined (38, 39). Furthermore, it has yet to be shown whether these genes are directly or indirectly affected by the transgene, as no direct evidence is available to show if TR binds to these genes and/or recruits cofactors to their promoters in the animals.

In the model system that we are studying, the metamorphosis of the frog *X. laevis*, unliganded TR has also been suggested to repress target gene expression in premetamorphic tadpoles to allow animal growth and prevent premature metamorphosis (57). Furthermore, TR binds target genes in tadpoles even when T_3 is not present (59). We have previously shown that TR can recruit N-CoR and SMRT to endogenous promoters in premetamorphic tadpoles (6, 58). Our results here suggest that N-CoR/SMRT functions through N-CoR/SMRT–TBLR1 complexes in mediating transcriptional repression by unliganded TR. First, N-CoR, SMRT, and TBLR1 are all expressed in premetamorphic tadpoles when unliganded TR is presumed to repress gene expression. Second, by using a ChIP assay, we demonstrated directly that, in addition to N-CoR and SMRT, TBLR1 is also recruited to T_3-inducible promoters in premetamorphic tadpoles and that upon T_3 treatment, TBLR1 is released from the promoters.

More importantly, by analyzing the association of N-CoR, SMRT, and TBLR1 with endogenous TR target promoters during development, we provide the first example in which corepressor dissociation from target genes is correlated with hormone-dependent developmental activation of these endogenous target genes. Thus, our studies with different in vivo models, namely, the reconstituted frog oocyte system and the T_3-dependent metamorphosis model for vertebrate postembryonic development, provide two independent lines of in vivo evidence that strongly support the participation of N-CoR/SMRT–TBLR1 or related complexes in the repression of T_3-inducible gene transcription by unliganded TR in premetamorphic tadpoles. Such a role for TBLR1 in vertebrate development is also consistent with the finding that mutations in both TR and TBL1 are associated with deafness in mammals (4, 15, 16). Further molecular support for such involvement of N-CoR/SMRT–TBLR1 or –TBL1 or related complexes in the developmental function of nuclear receptors can be obtained in the future through analysis of the abundance of such complexes in different tissues and organs during development and their recruitment to various nuclear hormone receptor target genes in vivo.

ACKNOWLEDGMENTS

We acknowledge the support of A. Tomita and D. R. Buchholz by a JSPS (NIH) fellowship and an NRSA fellowship, respectively.

REFERENCES

1. **Adams, M., C. Matthews, T. N. Collingwood, Y. Tone, P. Beck-Peccoz, and K. Chatterjee.** 1994. Genetic analysis of 29 kindreds with generalized and pituitary resistance to thyroid hormone. J. Clin. Investig. **94:**506–515.
2. **Alland, L., R. Muhle, H. Hou, Jr., J. Potes, L. Chin, N. Schreiber-Agus, and R. A. DePinho.** 1997. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature **387:**49–55.
3. **Amano, T., K. Leu, K. Yoshizato, and Y.-B. Shi.** 2002. Thyroid hormone regulation of a transcriptional coactivator in Xenopus laevis: implication for a role in postembryonic tissue remodeling. Dev. Dyn. **223:**526–535.
4. **Bassi, M. T., R. S. Ramesar, B. Caciotti, I. M. Winship, A. De Grandi, M. Riboni, P. L. Townes, P. Beighton, A. Ballabio, and G. Borsani.** 1999. X-linked late-onset sensorineural deafness caused by a deletion involving OA1 and a novel gene containing WD-40 repeats. Am. J. Hum. Genet. **64:**1604–1616.
5. **Brucker-Davis, F., M. C. Skarulis, M. B. Grace, J. Benichou, P. Hauser, E. Wiggs, and B. D. Weintraub.** 1995. Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. Ann. Intern. Med. **123:**572–583.
6. **Buchholz, D. R., V. S.-C. Hsia, and Y.-B. Shi.** 2003. A dominant negative thyroid hormone receptor blocks amphibian metamorphosis by retaining corepressors at target genes. Mol. Cell. Biol. **23:**6750–6758.
7. **Burke, L. J., and A. Baniahmad.** 2000. Co-repressors 2000. FASEB J. **14:**1876–1888.
8. **Chen, H., R. J. Lin, R. L. Schiltz, D. Chakravarti, A. Nash, L. Nagy, M. L. Privalsky, Y. Nakatani, and R. M. Evans.** 1997. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell **90:**569–580.

9. **Chen, J. D., and R. M. Evans.** 1995. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature **377:**454–457.
10. **Chen, J. D., and H. Li.** 1998. Coactivation and corepression in transcriptional regulation by steroid/nuclear hormone receptors. Crit. Rev. Eukaryot. Gene Express. **8:**169–190.
11. **Dodd, M. H. I., and J. M. Dodd.** 1976. The biology of metamorphosis, p. 467–599. *In* B. Lofts (ed.), Physiology of the amphibia. Academic Press, Inc., New York, N.Y.
12. **Evans, R. M.** 1988. The steroid and thyroid hormone receptor superfamily. Science **240:**889–895.
13. **Flamant, F., and J. Samarut.** 2003. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. Trends Endocrinol. Metab. **14:**85–90.
14. **Fondell, J. D., A. L. Roy, and R. G. Roeder.** 1993. Unliganded thyroid hormone receptor inhibits formation of a functional preinitiation complex: implications for active repression. Genes Dev. **7:**1400–1410.
15. **Forrest, D.** 1996. Deafness and goiter: molecular genetic considerations J. Clin. Endocrinol. Metab. **81:**2764–2767.
16. **Forrest, D., L. C. Erway, L. Ng, R. Altschuler, and T. Curran.** 1996. Thyroid hormone receptor beta is essential for development of auditory function. Nat. Genet. **13:**354–357.
17. **Forrest, D., E. Hanebuth, R. J. Smeyne, N. Everds, C. L. Stewart, J. M. Wehner, and T. Curran.** 1996. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. EMBO J. **15:**3006–3015.
18. **Fraichard, A., O. Chassande, M. Plateroti, J. P. Roux, J. Trouillas, C. Dehay, C. Legrand, K. Gauthier, M. Kedinger, L. Malaval, B. Rousset, and J. Samarut.** 1997. The T3R alpha gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. EMBO J. **16:**4412–4420.
19. **Furlow, J. D., and D. D. Brown.** 1999. In vitro and in vivo analysis of the regulation of a transcription factor gene by thyroid hormone during Xenopus laevis metamorphosis. Mol. Endocrinol. **13:**2076–2089.
20. **Gauthier, K., O. Chassande, M. Plateroti, J. P. Roux, C. Legrand, B. Pain, B. Rousset, R. Weiss, J. Trouillas, and J. Samarut.** 1999. Different functions for the thyroid hormone receptors TRα and TRβ in the control of thyroid hormone production and post-natal development. EMBO J. **18:**623–631.
21. **Gilbert, L. I., J. R. Tata, and B. G. Atkinson.** 1996. Metamorphosis: post-embryonic reprogramming of gene expression in amphibian and insect cells. Academic Press, Inc., New York, N.Y.
22. **Glass, C. K., and M. G. Rosenfeld.** 2000. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. **14:**121–141.
23. **Gothe, S., Z. Wang, L. Ng, J. M. Kindblom, A. C. Barros, C. Ohlsson, B. Vennstrom, and D. Forrest.** 1999. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. EMBO J. **13:**1329–1341.
24. **Guenther, M. G., W. S. Lane, W. Fischle, E. Verdin, M. A. Lazar, and R. Shiekhattar.** 2000. A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. Genes Dev. **14:**1048–1057.
25. **Hashimoto, K., F. H. Curty, P. P. Borges, C. E. Lee, E. D. Abel, J. K. Elmquist, R. N. Cohen, and F. E. Wondisford.** 2001. An unliganded thyroid hormone receptor causes severe neurological dysfunction. Proc. Natl. Acad. Sci. USA **8:**3998–4003.
26. **Heinzel, T., R. M. Lavinsky, T. M. Mullen, M. Soderstrom, C. D. Laherty, J. Torchia, W. M. Yang, G. Brard, S. D. Ngo, J. R. Davie, E. Seto, R. N. Eisenman, D. W. Rose, C. K. Glass, and M. G. Rosenfeld.** 1997. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature **387:**43–48.
27. **Horlein, A. J., A. M. Naar, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Soderstrom, C. K. Glass, et al.** 1995. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature **377:**397–404.
28. **Hsia, V. S.-C., and Y.-B. Shi.** 2002. Chromatin disruption and histone acetylation in the regulation of HIV-LTR by thyroid hormone receptor. Mol. Cell. Biol. **22:**4043–4052.
29. **Hsia, V. S.-C., H. Wang, and Y.-B. Shi.** 2001. Involvement of chromatin and histone acetylation in the regulation of HIV-LTR by thyroid hormone receptor. Cell Res. **11:**8–16.
30. **Hu, X., and M. A. Lazar.** 2000. Transcriptional repression by nuclear hormone receptors. Trends Endocrinol. Metab. **11:**6–10.
31. **Ishizuka, T., and M. A. Lazar.** 2003. The N-CoR/histone deacetylase 3 complex is required for repression by thyroid hormone receptor. Mol. Cell. Biol. **23:**5122–5131.
32. **Ishizuya-Oka, A., S. Ueda, and Y.-B. Shi.** 1997. Temporal and spatial regulation of a putative transcriptional repressor implicates it as playing a role in thyroid hormone-dependent organ transformation. Dev. Genet. **20:**329–337.
33. **Ito, M., and R. G. Roeder.** 2001. The TRAP/SMCC/Mediator complex and thyroid hormone receptor function. Trends Endocrinol. Metab. **12:**127–134.
34. **Ito, M., C.-X. Yuan, S. Malik, W. Gu, J. D. Fondell, S. Yamamura, Z.-Y. Fu, X. Zhang, J. Qin, and R. G. Roeder.** 1999. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Mol. Cell **3:**361–370.
35. **Jepsen, K., and M. G. Rosenfeld.** 2002. Biological roles and mechanistic actions of co-repressor complexes J. Cell Sci. **115:**689–698.
36. **Jones, P. L., L. M. Sachs, N. Rouse, P. A. Wade, and Y. B. Shi.** 2001. Multiple N-CoR complexes contain distinct histone deacetylases. J. Biol. Chem. **276:**8807–8811.
37. **Jones, P. L., and Y.-B. Shi.** 2003. N-CoR-HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors. Curr. Top. Microbiol. Immunol. **274:**237–268.
38. **Kaneshige, M., K. Kaneshige, X.-G. Zhu, A. Dace, L. Garrett, T. A. Carter, R. Kazlauskaite, D. G. Pankratz, A. Wynshaw-Boris, S. Refetoff, B. D. Weintraub, M. C. Willingham, C. Barlow, and S.-Y. Cheng.** 2000. Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. Proc. Natl. Acad. Sci. USA **97:**13209–13214.
39. **Kaneshige, M., H. Suzuki, K. Kaneshige, J. Cheng, H. Wimbrow, C. Barlow, M. C. Willingham, and S.-Y. Cheng.** 2001. A targeted dominant negative mutation of the thyroid hormone α1 receptor causes increased mortality, infertility, and dwarfism in mice. Proc. Natl. Acad. Sci. USA **98:**15095–15100.
40. **Kawahara, A., B. S. Baker, and J. R. Tata.** 1991. Developmental and regional expression of thyroid hormone receptor genes during Xenopus metamorphosis. Development **112:**933–943.
41. **Laherty, C. D., W. M. Yang, J. M. Sun, J. R. Davie, E. Seto, and R. N. Eisenman.** 1997. Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression Cell **89:**349–356.
42. **Lazar, M. A.** 1993. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocrinol. Rev. **14:**184–193.
43. **Li, J., Q. Lin, W. Wang, P. Wade, and J. Wong.** 2002. Specific targeting and constitutive association of histone deacetylase complexes during transcriptional repression. Genes Dev. **16:**687–692.
44. **Li, J., J. Wang, J. Wang, Z. Nawaz, J. M. Liu, J. Qin, and J. Wong.** 2000. Both corepressor proteins SMRT and C-CoR exist in large protein complexes containing HDAC3. EMBO J. **19:**4342–4350.
45. **Machuca, I., G. Esslemont, L. Fairclough, and J. R. Tata.** 1995. Analysis of structure and expression of the Xenopus thyroid hormone receptor b gene to explain its autoregulation. Mol. Endocrinol. **9:**96–107.
46. **Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, et al.** 1995. The nuclear receptor superfamily: the second decade. Cell **83:**835–839.
47. **McKenna, N. J., R. B. Lanz, and B. W. O'Malley.** 1999. Nuclear receptor coregulators: cellular and molecular biology. Endocrinol. Rev. **20:**321–344.
48. **Nagy, L., H. Y. Kao, D. Charkaravarti, R. J. Lin, C. A. Hassig, D. E. Ayer, S. L. Schreiber, and R. M. Evans.** 1997. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell **89:**373–380.
49. **Perlman, A. J., F. Stanley, and H. H. Samuels.** 1982. Thyroid hormone nuclear receptor. evidence for multimeric organization in chromatin. J. Biol. Chem. **257:**930–938.
50. **Puzianowsak-Kuznicka, M., S. Damjanovski, and Y.-B. Shi.** 1997. Both thyroid hormone and 9-*cis* retinoic acid receptors are required to efficiently mediate the effects of thyroid hormone on embryonic development and specific gene regulation in *Xenopus laevis*. Mol. Cell. Biol. **17:**4738–4749.
51. **Rachez, C., and L. P. Freedman.** 2000. Mechanisms of gene regulation by vitamin D_3 receptor: a network of coactivator interactions. Gene **246:**9–21.
52. **Rachez, C., B. D. Lemon, Z. Suldan, V. Bromleigh, M. Gamble, A. M. Naar, H. Erdjument-Bromage, P. Tempst, and L. P. Freedman.** 1999. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. Nature **398:**824–828.
53. **Ranjan, M., J. Wong, and Y. B. Shi.** 1994. Transcriptional repression of Xenopus TR beta gene is mediated by a thyroid hormone response element located near the start site. J. Biol. Chem. **269:**24699–24705.
54. **Refetoff, S., R. E. Weiss, and S. J. Usala.** 1993. The syndromes of resistance to thyroid hormone. Endocrinol. Rev. **14:**348–399.
55. **Rietveld, L. E., E. Caldenhoven, and H. G. Stunnenberg.** 2002. In vivo repression of an erythroid-specific gene by distinct corepressor complexes. EMBO J. **21:**1389–1397.
56. **Ryu, S., S. Zhou, A. G. Ladurner, and R. Tjian.** 1999. The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. Nature **397:**446–450.
57. **Sachs, L. M., S. Damjanovski, P. L. Jones, Q. Li, T. Amano, S. Ueda, Y. B. Shi, and A. Ishizuya-Oka.** 2000. Dual functions of thyroid hormone receptors during Xenopus development. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **126:**199–211.
58. **Sachs, L. M., P. L. Jones, E. Havis, N. Rouse, B. A. Demeneix, and Y.-B. Shi.** 2002. N-CoR recruitment by unliganded thyroid hormone receptor in gene repression during *Xenopus laevis* development. Mol. Cell. Biol. **22:**8527–8538.
59. **Sachs, L. M., and Y.-B. Shi.** 2000. Targeted chromatin binding and histone acetylation in vivo by thyroid hormone receptor during amphibian development. Proc. Natl. Acad. Sci. USA **97:**13138–13143.
60. **Shi, Y.-B.** 1999. Amphibian metamorphosis: from morphology to molecular biology. John Wiley & Sons, Inc., New York, N.Y.
61. **Shi, Y.-B., and D. D. Brown.** 1993. The earliest changes in gene expression in

tadpole intestine induced by thyroid hormone. J. Biol. Chem. **268:**20312–20317.
62. **Shi, Y.-B., V. C.-T. Liang, C. Parkison, and S.-Y. Cheng.** 1994. Tissue-dependent developmental expression of a cytosolic thyroid hormone protein gene in Xenopus: its role in the regulation of amphibian metamorphosis. FEBS Lett. **355:**61–64.
63. **Shi, Y.-B., and V. C.-T. Liang.** 1994. Cloning and characterization of the ribosomal protein L8 gene from Xenopus laevis. Biochim. Biophys. Acta **1217:**227–228.
64. **Shi, Y. B., L. Fu, S. C. Hsia, A. Tomita, and D. Buchholz.** 2001. Thyroid hormone regulation of apoptotic tissue remodeling during anuran metamorphosis. Cell Res. **11:**245–252.
65. **Tinnikov, A., K. Nordstrom, P. Thoren, J. M. Kindblom, S. Malin, B. Rozell, M. Adams, O. Rajanayagam, S. Pettersson, C. Ohlsson, K. Chatterjee, and B. Vennstrom.** 2002. Retardation of post-natal development caused by a negatively acting thyroid hormone receptor α1. EMBO J. **21:**5079–5087.
66. **Tomita, A., D. R. Buchholz, K. Obata, and Y.-B. Shi.** 2003. Fusion protein of retinoic acid receptor a with promyelocytic leukaemia protein or promyelocytic leukaemia zinc-finger protein recruits N-CoR-TBLR1 corepressor complex to repress transcription in vivo. J. Biol. Chem. **278:**30788–30795.
67. **Tsai, M. J., and B. W. O'Malley.** 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu. Rev. Biochem. **63:**451–486.
68. **Underhill, C., M. S. Qutob, S. P. Yee, and J. Torchia.** 2000. A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and the corepressor KAP-1 J. Biol. Chem. **275:**40463–40470.
69. **Wen, Y. D., V. Perissi, L. M. Staszewski, W. M. Yang, A. Krones, C. K. Glass, M. G. Rosenfeld, and E. Seto.** 2000. The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc. Natl. Acad. Sci. USA **97:**7202–7207.
70. **Wikstrom, L., C. Johansson, C. Salto, C. Barlow, A. C. Barros, F. Baas, D. Forrest, P. Thoren, and B. Veenstrom.** 1998. Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor. EMBO J. **17:**455–461.
71. **Wong, J., and Y.-B. Shi.** 1995. Coordinated regulation of and transcriptional activation by Xenopus thyroid hormone and retinoid X receptors. J. Biol. Chem. **270:**18479–18483.
72. **Wong, J., Y. B. Shi, and A. P. Wolffe.** 1995. A role for nucleosome assembly in both silencing and activation of the Xenopus TR beta A gene by the thyroid hormone receptor. Genes Dev. **9:**2696–2711.
73. **Xu, L., C. K. Glass, and M. G. Rosenfeld.** 1999. Coactivator and corepressor complexes in nuclear receptor function. Curr. Opin. Genet. Dev. **9:**140–147.
74. **Yaoita, Y., and D. D. Brown.** 1990. A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. Genes Dev. **4:**1917–1924.
75. **Yaoita, Y., Y. B. Shi, and D. D. Brown.** 1990. Xenopus laevis alpha and beta thyroid hormone receptors. Proc. Natl. Acad. Sci. USA **87:**7090–7094.
76. **Yen, P. M.** 2001. Physiological and molecular basis of thyroid hormone action. Physiol. Rev. **81:**1097–1142.
77. **Yoon, H.-G., D. W. Chan, Z. Q. Huang, J. Li, J. D. Fondell, J. Qin, and J. Wong.** 2003. Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. EMBO J. **22:**1336–1346.
78. **Yoshizato, K.** 1989. Biochemistry and cell biology of amphibian metamorphosis with a special emphasis on the mechanism of removal of larval organs. Int. Rev. Cytol. **119:**97–149.
79. **Zhang, J., M. Kalkum, B. T. Chait, and R. G. Roeder.** 2002. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. Mol. Cell **9:**611–623.
80. **Zhang, J., and M. A. Lazar.** 2000. The mechanism of action of thyroid hormones. Annu. Rev. Physiol. **62:**439–466.
81. **Zhang, X.-Y., M. Kaneshige, Y. Kamiya, K. Kaneshige, P. McPhie, and S.-Y. Cheng.** 2002. Differential expression of thyroid hormone receptor isoforms dictates the dominant negative activity of mutant beta receptor. Mol. Endocrinol. **16:**2077–2092.