The thyroid (TR) and retinoid X receptors (RXR) belong to the nuclear receptor (NR) superfamily of ligand-mediated transcription factors. At the molecular level, TR activity is specifically modulated by interactions with the ligand 3,3',5 triiodo-l-thyronine (T3), RXR, DNA, and co-activators such as SRC1, occurring in concert or sequentially. Although binding sites for DNA and coregulators such as SRC1 are distinct and at distal regions of these receptors, cell-based and EMSA studies have suggested that these molecules can regulate binding of each other to the receptor. We present evidence of direct, DNA-dependent, communication between the DNA and ligand binding domains (DBD and LBD) that can allosterically regulate interactions with SRC1 and DNA, respectively, using isothermal titration calorimetry (ITC) and cell-based assays. Additionally, we note that interdomain communication is affected by RXR in RXR:TR. We also noticed a DNA-dependent cross-talk between RXR and TR within RXR:TR. Finally, we suggest that differences in transactivation on different TRE may be the consequence of different affinities between TRE and RXR:TR.

The nuclear receptor (NR) protein superfamily of transcriptional regulators have distinct domains that interact with ligands, co-regulatory proteins, and DNA response elements (RE) for diverse responses (1). Heterotrophic signaling within and across these domains is a recognized feature of NR activity with distant residues participating through inter and intramolecular pathways (2–6). For instance, ligand binding induces conformational changes in NRs, which result in docking surfaces for cofactor molecules that regulate gene transcription (Fig. 1a). Additionally, REs can provide both, platforms for nucleation over which the transactivation complex assembles and function as effectors that modulate NR transactivation (1, 7–9).

The thyroid hormone receptor is a non-steroidal NR that regulates gene expression primarily in response to the agonist ligand T3. The multiple domains of TR include an N-terminal transcriptional activation function 1 (AF-1), a central DNA binding domain, and a C-terminal ligand binding domain, encompassing the activation function 2 (AF-2) (10). Heterodimers of RXR and TR are the major functional form of TR, although functional TR monomers, homodimers, and multimers have been reported (8, 11, 12). Isoforms and oligomeric states of TR exhibit preferential binding to specific REs (8). These thyroid hormone response elements (TRE) have consensus AGGTCA (half-sites) arranged as direct repeats (DR), palindromes (Pal) or inverted palindromes (IP) with differing spacing between half-sites. T3 binding to the TR LBD switches the dormant TR into an active conformational state that can recruit coactivator proteins such as SRC1, SRC2, SRC3, TRAP, PGC-1, and TRBP (13, 14). Coactivator recruitment by TR is not only regulated by T3 but also by its oligomeric/RXR-bound state and by TRE (1). Coactivators can, in turn, modulate the affinity of TR for TRE (15, 16). This delicate regulation of macromolecular interactions is the basis of molecular association dynamics within the cellular environment, particularly with the REs, leading to controlled transactivation levels (17, 18). Thus far, studies of isolated TR LBD with coactivator and of DBDs of TR:RXR heterodimers with DNA have provided significant insights into mechanisms of biological response to TR activation (19, 20). However, cooperative interactions between multiple NR domains have also been reported to modulate transactivation suggesting additional layers of regulation (21).

We have identified direct, DNA-dependent interactions between the TR DBD, and LBD that form the basis of allosteric communication between these domains. Furthermore, to quantify the allosteric role of direct DBD LBD interactions, we have overexpressed the DBD-LBD of TR (residues 37–372, ΔAF1) and RXR (residues 131–463, ΔAF1) in E. coli and reconstituted the core TRE/RXR:9c:TR-T3/SRC1 transactivation complex (9c,9-cis retinoic acid) (Fig. 1b and supplemental Fig. S1). Here, we compare the recruitment of SRC1 (13-mer coactivator peptide) and TRE (minimal oligonucleotide fragment of DR4 and IP6) by TR and RXR:TR heterodimers using isothermal titration calorimetry. We establish that TRE/RXR:9c plays a regulatory role on TR activity and determine the role of each component within the TRE/RXR:9c:TR-T3/SRC1 complex.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—DBD, LBD, and DBD-LBD cDNA of chicken TR-α (N-terminal 36 amino acids deleted; kind gift from Dr. Barry M. Forman) were cloned into NdeI-BamHI of pET15b vector (Novagen Inc.) and human RXRα (N-terminal 130 amino acids deleted) was cloned into pET-SUMO vector (Invitrogen Inc.). Proteins were expressed in...
Escherichia coli BL21 (DE3) RIPL cells (kind gift from Dr. Martin Privalsky). Cells were grown for 20 h at 20 °C after isopropyl-1-thio-β-D-galactopyranoside induction at 0.6 OD. Harvested cells were lysed by sonication. Expressed protein from cell lysate supernatant was purified using Ni-NTA. Purified His-tagged DBD-LBDs of SUMO-RXR and TR were mixed in equimolar ratio for heterodimers. Tags were removed from TR monomers or RXR homodimers or RXR:TR heterodimers by thrombin digestion. Proteins were further purified using anion exchange and size exclusion chromatography (supplemental Fig. S1).

Isothermal Titration Calorimetry (ITC)—Gel filtration-purified TR monomers or RXR homodimers or RXR:TR heterodimers were directly used for ITC (VP-ITC MicroCalorimeter, MicroCalTM). Each titration contained 5–15 μM of the protein or protein-DNA complex in 20 mM Tris, pH 8, 125 mM NaCl, 5 mM MgCl₂, and 1 mM TCEP. TR DBD and LBD were prepared in 20 mM HEPES (pH 8), 125 mM NaCl, and 1 mM TCEP. 2 mM stocks of TRE (DR4: 5′-TAAGGTCAATTAA-GGTCATTTAAGGTCA-3′) and inverted palindromes (IP6) (5′-TTATGTACCCCCAGCTAGGTC- AAGGTTATGCCCCAGCTAGGTCATAAGGTTA-3′) and co-transfected with pSG5-cTR-α1 (50 ng) for TR-α1 expression (kind gift from Dr. Martin Privalsky). Assays were performed using Dual-Luciferase® reporter assay system (Promega). Cells were induced with 250 nm of 9-cis retinoic acid (MP Biomedicals) and/or 1 μM T3 (Acros). Control experiments with the DMSO and NaOH were performed. The plate was read using Synergy™ multidetection plate reader (Bio Tek® Instruments).

RESULTS AND DISCUSSION

We utilized ITC to compare the recruitment of an SRC1-derived LXXLL peptide and TREs by TR and RXR:TR heterodimers. Several studies have shown that ITC can be reliably utilized to characterize in vitro coactivator recruitment by NRs using the SRC1 peptide with the LXXLL motif (22, 23). All titrations were performed at 25 °C. Differences in the SRC1 affinity to the several complexes are also consistent at three temperatures (supplemental Fig. S2). The affinities (Kₐ) to the SRC1 peptide and TRE are used solely as a measure of interdomain communication within the TR monomers and RXR:TR heterodimers. Our results provide evidence for direct interactions between the DBD and LBD, thereby correlating recognition of REs with recruitment of SRC1.

Interdomain, DBD ⇔ LBD Interactions Are DNA-dependent—We have discovered that the TR DBD and LBD interact directly with each other, in a DNA-dependent manner, providing the basis for communication between the two domains discussed in sections below. The evolution of heat in a dose-dependent manner upon titrating isolated TR-T3 LBD into TR DBD suggests that there is an appreciable interaction between the two domains (Kₐ = 3.2 μM ± 0.8) (Fig. 2a). To examine the role of TRE on this DBD-LBD interaction TR-T3 LBD was titrated into preformed TR DBD/TRE (DR4) complex. To ensure that all the TR DBD was complexed with DNA, the TRE/DBD was prepared with 3-fold molar excess of the DNA adduct over the DBD. We noticed a substantial decrease in affinity of TR DBD and LBD in the presence of TRE, and the binding constants cannot be quantitatively analyzed (Fig. 2b). This suggests that there is a DNA-induced conformational change within the DBD that lowers its affinity for the LBD. Studies on the androgen receptor have indicated that DNA binding can influence coactivator recruitment, albeit through interactions between the AR N terminus and SRC1 (24). The recent structure of the DBD-LBD of the PPARγ-RXR heterodimers has determined that the PPARγ-LBD interacts with DBD of both, the PPARγ and the RXR, although the DNA-dependence of this interaction was not addressed (25). The data
TRE Binding to the DBD Can Influence Recruitment of SRC1 to the LBD—The interaction between the TR DBD and LBD reported above provides the basis for direct communication between the DBD and LBD. When the allosteric effect of DNA was quantified we noticed that TRE/TR\_H18528\_T3 can bind SRC1 with 3-fold greater affinity than TR\_H18528\_T3 (without TRE) (Fig. 3, a and a\_4 and Table 1: a, b, and c). It was also noticed that RXR:TR\_H18528\_T3 binds SRC1 with 3-fold greater affinity than TR\_H18528\_T3 alone (Fig. 3, a\_2 and a\_3 and Table 1, a and h). However, the TRE/RXR:TR\_H18528\_T3 complex can bind SRC1 with only 2-fold higher affinity than TR\_H18528\_T3 (Fig. 3, a1 and a2 and Table 1, a, i, and j). This indicates that contributions by the individual components of TRE/RXR:TR\_H18528\_T3 toward SRC1 binding by TR are 1) not additive, and SRC1 binding follows the general scheme: RXR:TR\_H18528\_T3/TRE/TR\_H18528\_T3/TRE/RXR:TR\_H18528\_T3 and 2) dependent on DNA such that TRE can have an attenuating effect on SRC1 binding by RXR:TR\_H18528\_T3.

We also examined the role of RXR ligand, 9c on SRC1 recruitment by TR (within TRE/RXR:9\_H18528:TR\_T3) especially because transactivation in response to both T3 and 9c is significantly lower than in response to T3 alone (Fig. 4a). As expected, TRE/RXR:9\_H18528:TR\_T3 binds SRC1 with lower affinity than TRE/RXR:TR\_T3 (Fig. 3, a\_1 and b\_4 and Table 1, i, j, l, and m). TRE and RXR together have been shown above to decrease the affinity of TR\_T3 (within TRE/RXR:9\_H18528:TR\_T3) for SRC1 and the presence of 9c reduces SRC1 affinity by the complex even further. Thus, while there are two SRC1 peptide binding sites on RXR:9\_H18528:TR\_T3 (± TRE), the stoichiometry of SRC1 binding (n = 1.5), and the overall affinity is less than sum of the individual SRC1 binding events on RXR:9\_H18528:TR\_T3 (Table 1, k, l, and m). With the binding data above, it is tempting to speculate that the lower transactivation with the combination of ligands T3 and 9c when compared with T3 alone may be a consequence of this damping effect of TRE/RXR:9\_H18528 on TR (Figs. 4 \_a and 3b\_4). Thus, we describe the RXR:TR\_T3 response to a combination of T3 and 9c as being negatively permissive in contrast to the permissive, non-permissive, or conditional response definitions applied to other RXR heterodimers (5). However, this difference in permissivity is isoform-specific because RXR:TR\_H18528\_H9252 heterodimers are reported to be non-permissive (5). 9c-induced dissociation of RXR:TR\_T3 has been suggested as an alternative mechanism for the negative permissivity of RXR:9\_H18528:TR\_H9252 (26). Our results argue against this model since we have consistently purified the RXR:TR\_T3, RXR:9\_H18528:TR\_T3, and RXR:9\_H18528:TR heterodimers by size exclusion chromatography without any evidence of dissociation of the heterodimers. Additionally, we do not detect dissociation of the TRE/RXR:TR complex upon saturation with T3 and/or 9c. Finally, ITC titrations indicate that affinity of RXR:TR heterodimers to TRE (DR4 and IP6) is unaltered by 9c and T3. (Table 2, a, b, g, and h).

Whereas the above results display a broad effect of TREs on RXR:TR, we also noticed distinct effects of TRE on RXR and TR within the RXR:TR heterodimers. For instance, RXR:TR\_T3 binds SRC1 with ~2-fold higher affinity than TRE/RXR:TR\_T3 (Fig. 3, a1 and a3; Table 1, h, i, j). On the other hand,
Allosterism in the Thyroid Receptor

**a**

[TRE/RXR:TR:T3](#)

![Diagram](image1)

- $K_d = 1.1 \mu M$

![Diagram](image2)

- $K_d = 1.8 \mu M$

![Diagram](image3)

- $K_d = 0.5 \mu M$

![Diagram](image4)

- $K_d = 0.5 \mu M$

**b**

[TRE/RXR-9c:TR](#)

![Diagram](image5)

- $K_d = 1.8 \mu M$

![Diagram](image6)

- $K_d = 2.4 \mu M$

![Diagram](image7)

- $K_d = 1.3 \mu M$

![Diagram](image8)

- $K_d = 1.6 \mu M$

**c**

[TR-T3](#)

![Diagram](image9)

- $K_d = 1 \mu M$

![Diagram](image10)

- $K_d = 0.5 \mu M$
TABLE 1
Thermodynamic parameters for SRC1 recruitment by TR monomers, RXR homodimers, and RXR:TR heterodimers in the presence of ligands or effectors indicated

| K_d (μM) | ΔH (kcal/mol) | −TΔS (kcal/mol) | ΔG (kcal/mol) | N |  |
|---------|---------------|-----------------|---------------|---|---|
| a TR-T3 (monomers) | 1.8 ± 0.02 | −16.3 ± 0.03 | 8.5 | −7.8 | 0.9 ± 0.1 |
| b DR4/4-T3 | 0.5 ± 0.03 | −5.5 ± 0.16 | 3.0 | −8.5 | 0.9 ± 0.2 |
| c IP6/1/10c:TR | 0.4 ± 0.14 | −6.5 ± 0.6 | 2.2 | −8.7 | 0.8 ± 0.1 |
| d RXR-9c (homodimers) | 1.3 ± 0.01 | −8.1 ± 0.2 | 0.1 | −8.0 | 1.8 ± 0.2 |
| e RXR-9c:TR | 2.4 ± 0.2 | −9.3 ± 0.9 | 2.3 | −7.0 | 1.0 ± 0.05 |
| f DR4/4-RXR-9c:TR | 1.8 ± 0.01 | −7.3 ± 0.13 | 0.6 | −7.9 | 1.0 ± 0.3 |
| g IP6/1/10c:TR | 1.7 ± 0.01 | −6.9 ± 0.15 | 1.0 | −7.9 | 1.0 ± 0.2 |
| h RXR-T3 | 0.5 ± 0.1 | −6.5 ± 0.07 | 1.9 | −8.4 | 0.9 ± 0.05 |
| i DR4/4-RXR-T3 | 1.1 ± 0.2 | −6.2 ± 0.2 | 1.9 | −8.1 | 0.8 ± 0.02 |
| j IP6/1/10c:TR | 1.2 ± 0.05 | −6.9 ± 0.4 | 1.0 | −7.9 | 0.8 ± 0.1 |
| k RXR-9c:TR | 1.4 ± 0.06 | −7.3 ± 0.08 | 0.6 | −7.9 | 1.4 ± 0 |
| l DR4/4-RXR-9c:TR | 1.6 ± 0.17 | −6.3 ± 0.42 | 1.6 | −7.9 | 1.6 ± 0.1 |
| m IP6/1/10c:TR | 1.6 ± 0.14 | −6.3 ± 0.06 | 1.6 | −7.9 | 1.5 ± 0.2 |

**FIGURE 3.** a, luciferase reporter assay showing ligand (T3 and 9c)-mediated RXR:TR transcriptional activity on DR4 and IP6 TRE in CV-1 cells. b, normalized ITC data showing relative affinity of SRC1 for RXR-9c:TR, RXR-T3, and RXR-9c:TR + TRE (DR4 and IP6). Data presented are averages of three independent experiments with error bars for S.D.

**FIGURE 4.** a, luciferase reporter assay showing ligand (T3 and 9c)-mediated RXR:TR transcriptional activity on DR4 and IP6 TRE in CV-1 cells. b, normalized ITC data showing relative affinity of SRC1 for RXR-9c:TR, RXR-T3, and RXR-9c:TR + TRE (DR4 and IP6). Data presented are averages of three independent experiments with error bars for S.D.

SRC1 Binding to the LBD Affects TRE Recruitment to the DBD—Interdomain communication in TR reported above can also occur in reverse i.e. conformational changes within the LBD can be transmitted to the DBD. Studies on the glucocorticoid receptor (GR) have shown that ligand binding can regulate the dynamic association of GR with DNA (18). To extrapolate the observations on GR to the RXR:TR system we monitored the effect that SRC1 binding to LBD has on the DBD of TR and RXR:TR. ITC titrations with preformed TR-T3/ SRC1 show that DR4 and IP6 bind this complex with 40 and 100% higher affinity, respectively, than either TR-T3 or unliganded TR (Fig. 3, c1 and c2 and Table 2, d–f and j–l). Thus, the TR DBD responds to changes in the conformation of the LBD and this behavior is similar to other monomeric and homodimeric NRs, such as the GR and androgen (AR) receptors. Our ITC titrations show that the affinity for TRE by RXR-9c:TR (Table 2, a–c and g–i). The role of RXR reported here is unique in that RXR (±9c):TR/TRE can override the allosteric effect of SRC1 on the DBD. There have been earlier reports suggesting that SRC1 can modulate DNA binding by interacting at once with both, AF1 and AF2 of some NRs such as the androgen receptor (15, 16, 27). Therefore, by utilizing the 13-mer LXXLL peptide in ITC titrations with the TR and RXR:TR DBD-LBD constructs, our data show that the SRC1 binding site in TRα is allosterically coupled to the DBD by an additional pathway that is independent of an external bridging link. Therefore, we conclude that binding of SRC1 to the LBD can remotely influence TRE binding to the DBD in TR monomers, but not in RXR:TR heterodimeric complexes further suggesting distinct activation modes for the various TR oligomeric states.

The fragility of the RXR:TR DBD-LBD constructs in these assays has only permitted a limited analysis of the thermodynamic parameters. While the affinities of TR ± T3 ± SRC1 for DR4 and IP6 are similar, the energetics of binding between TR and TRE (DR4 and IP6) predict that discrete mechanisms may drive these interactions. For instance, TRE binding to TR-T3 ±

---

**FIGURE 3.** Binding constants of SRC1 (a and b) and TRE (c) to TR monomers, RXR homodimers, and RXR:TR. The different complexes are ± T3 (a), ± 9c (b) with corresponding isotherms shown below. a, SRC1 binding to DR4/RXR:TR/T3 compared with TR-T3 (2), RXR:TR-T3 (3), and TRE/4:TR (4). b, SRC1 binding to DR4/RXR:9c:TR (1) compared with RXR-9c:TR (2), RXR-9c (3), and DR4/RXR:9c:TR (4). c, DR4 recruitment by TR-T3 (1) and TR-T3/SRC1 (2). The two TR monomers are shown to be separate, non-interacting molecules. Corresponding isotherms are shown on the right.
SRC1 is enthalpically less favorable but entropically more favorable compared with unliganded TR and follows the enthalpically favorable trend: unliganded TR > TR-T3 > TR-T3/SRC1 (Table 2, d–f and j–l). Interestingly, there are no significant differences in the affinities (K_d) or ΔG values of unliganded TR versus DR4 TRE for TR (Table 2, d, e, j, and k). Efforts to stabilize TR at different pH values and temperatures have been limited and this has prevented us from estimating the contribution of buffer ionization and the role of water molecules in binding. In the absence of this data or the associated three-dimensional structures, we speculate that the complexed T3/SRC1 upon binding DNA and consequently a more enthalpically favorable trend: unliganded TR 1.0, T3 0.07, T3/SRC1 0.05 (K_d) compared with RXR. There is growing evidence that TREs can also regulate NR transcriptional activity by directly affecting the assembly of the SRC1 recruitment (4). Therefore, it is very likely that two TR molecules bound to the TREs are independent monomers of TR, each binding one half-site on the REs, and it is insufficient to determine if there are any direct interactions between these TR monomers.

### Table 2

| Protein complexes | K_d (μM) | ΔH (kcal/mol) | ∆TΔS (kcal/mol) | ΔG (kcal/mol) | N |
|------------------|---------|---------------|-----------------|--------------|---|
| Recruitment of DR4 TRE |         |               |                 |              |   |
| a Unliganded RXR:TR | 0.05 ± 0.006 | −14.4 ± 1.7 | 4.5 | −9.9 | 0.8 ± 0.05 |
| b RXR:9c:TR-T3 | 0.06 ± 0 | −10.4 ± 0.2 | 0.5 | −9.9 | 0.9 ± 0.01 |
| c RXR:9c:TR-T3/SRC1 | 0.07 ± 0.02 | −14.9 ± 2.2 | 5.2 | −9.7 | 0.9 ± 0.2 |
| d Unliganded TR | 0.07 ± 0.007 | −15.1 ± 1.4 | 6.7 | −8.4 | 0.5 ± 0.02 |
| e TR-T3 | 0.07 ± 0.017 | −13.7 ± 0.5 | 5.3 | −8.4 | 0.5 ± 0.02 |
| f TR-T3/SRC1 | 0.05 ± 0.07 | −10.9 ± 0.2 | 2.3 | −8.6 | 0.5 ± 0.03 |
| Recruitment of IP6 TRE |         |               |                 |              |   |
| g Unliganded RX:TR | 0.1 ± 0.03 | −15.0 ± 0.1 | 5.5 | −9.5 | 1.0 ± 0.15 |
| h RXR:9c:TR-T3 | 0.1 ± 0.01 | −17.1 ± 1.4 | 2.1 | −9.6 | 0.9 ± 0.2 |
| i RXR:9c:TR-T3/SRC1 | 0.1 ± 0.01 | −15.8 ± 0.01 | 6.2 | −9.6 | 1.0 ± 0.1 |
| j Unliganded TR | 1.0 ± 0.02 | −9.6 ± 0.7 | 1.4 | −8.2 | 0.5 ± 0.03 |
| k TR-T3 | 1.0 ± 0.27 | −8.6 ± 0.7 | 0.1 | −8.2 | 0.5 ± 0.01 |
| l TR-T3/SRC1 | 0.5 ± 0.3 | −7.2 ± 0.4 | −1.4 | −8.6 | 0.4 ± 0.1 |

By comparison, TR monomers exhibit a 10-fold lower affinity for TREs (DR4 and IP6) over RXR:TR (Table 2). Thus, relative to TR, RXR:TR may have an optimal conformation for DNA binding, making RXR:TR the dominant form of TR transactivation.

**RXR Plays an Important but Supportive Role in TR Transactivation—**Transactivation of RXR:TR is primarily driven by TR-T3 and the consequent coactivator recruitment to RXR:TR-T3 complex. Transcriptional activity of RXR:TR is also elicited by 9c, but the response is significantly lower than with T3 (Fig. 4a). In vitro, affinity of SRC1 for RXR:TR-T3 (K_d = 0.05 ± 0.006 μM) compared with RXR:TR ± ligands ± SRC1 and IP6 (0.1 ± 0.03 μM) (supplemental Fig. S4 and Table 2, a–c and g–i). Taken together, the ITC data above suggest that differences in transactivation of RXR:9c:TR-T3 on DR4 versus IP6 may be a consequence of differences in affinity to these REs.
This is analogous to a phenomenon reported earlier that describes the reduction in ligand (LG69) binding by the RXR:TR-T3 complex and this decrease in ligand binding follows the trend, RXR>RXR:TR>RXR:TR-T3 (2). To compensate for the reported decrease in affinity for ligand by RXR, these titrations were performed at super-saturating levels of 9c (see “Experimental Procedures”). The subordination of RXR ligand-response is a recognized feature of RXR:NR heterodimers and has been postulated as a mechanism to prevent redundancy in NR-mediated signaling processes (29). Specific to this study, the suppression of RXR activity by TR (within RXR:TR) has been suggested to be a mechanism to limit 9c responsiveness of TR-regulated genes (2). However, the permissivity of RXR heterodimers also depends on the cell type and expression levels of RXR and coactivators (30).

Contrary to the effect of TR on RXR reported above, the increased affinity of TR for SRC1 in RXR:TR-T3 relative to TR-T3 suggests that RXR functions as an allosteric enhancer of TR activity without affecting T3 binding (11). Because REs for RXR (e.g. DR1) are different from those for TR and RXR:TR, these experiments are performed in the absence of DNA (31).

CONCLUSIONS

Our data established direct, DNA-dependent, interdomain communication within TR as an important allosteric mechanism regulating TR transactivation. The use of the 13-mer SRC1 peptide with a single NR-binding LXXLL motif allowed us to focus this study on direct interdomain interactions within the NRs without the additional complexity that may arise from allosteric pathways through SRC1 fragments that have multiple receptor-interacting sites. In separate experiments we noted that the SRC1 peptide utilized here with the RXR:TR DBD-LBD constructs in response to T3 and/or 9c exhibits a similar binding profile when a larger SRC1 fragment (SRC RID1–3, residues Asn-617 to Asp-769) that encompasses all the three receptor-interacting domains is titrated with isolated RXR:TR LBDs (data not shown). Data from published studies on NRs such as AR have shown that the N-terminal AF1 and C-terminal AF2 interact either directly or through intermediary cofactors (32–34). Additionally, mutagenesis and pull-down assays with full-length RXR:RAR and RXR:VDR have suggested that REs can influence co-activator binding and activity (4, 29). Recent structural studies on GR have provided further evidence for DNA induced conformational changes in DBD resulting in the modulation of GR activity (35). Moreover, in the recent DNA/RXR:PPARγ structure the DBD and LBD of PPARγ appear in close proximity, suggesting interactions and the potential for cross-talk between these domains (25). By limiting the binding assays here to only DBD-LBD constructs of RXR and TR we are able to characterize the role of direct interactions between the DBD and LBD in the allosteric regulation of TR. These direct interactions between the DBD and LBD are regulated by DNA binding, and this is most evident in TR monomers. DNA binding to the DBD appears to decrease its interactions with the LBD, which can consequently make stronger interactions with SRC1. It appears that the multidomain TR molecule is conformationally plastic with bi-directional regulation of SRC1 and CRE binding and with additional regulation provided by RXR. The relatively weak TRE/TR complex is strengthened with SRC1 (Fig. 3, c1 and c2). However, heterodimerization with RXR locks TR in a conformationally restricted state that limits interdomain cross-talk but enhances interactions with SRC1 and TRE at once making RXR:TR the dominant mode of transactivation (Fig. 3, a3 and supplemental Fig. S4). With TR alone, the presence of TRE enhances SRC1 recruitment and vice versa; thus TRE and SRC1 in TR monomers mimic the role of RXR within RXR:TR (Fig. 3, a4, c1, and c2). Our data also suggest that levels of RXR:TR transactivation observed in vivo on different TRE (DR4 and IP6) may be a consequence of differing affinities for these response elements (supplemental Fig. S4). Ultimately, changes in macromolecular affinities could alter the dynamics of assembly of NR transactivation complexes on DNA, which can be altered by ligands, co-regulators and p23 molecular chaperones, regulating the transcriptional activity of downstream genes (18, 36, 37). While studies with point mutations can have global effects and thus complicate the analyses (38), it is expected that three-dimensional structures of full-length TR and cofactors, will provide a strong basis for determining a molecular mechanism for allosteric processes within this molecule.

It is worth noting here that the complexity of NR regulatory mechanisms as suggested by the data presented make identification of targets that can modulate the activity of these proteins all the more difficult. There are many previous studies that point out the role of inter-domain allosterism and our findings suggest that these regulatory mechanisms may be more intricate than originally suspected. Allosteric sites on the AR LBD have been successfully manipulated to generate small-molecule regulators (39). Extended characterization of allosteric mechanisms within larger NR fragments can increase the potential of novel targets for drug design and discovery programs (40).

Acknowledgments—We thank Drs. Barry Forman, Martin Privalsky, Engin Serpersu, Edward Wright, Michael Goodson, and Cherian Zachariah for reading the manuscript.

REFERENCES

1. Lefstin, J. A., and Yamamoto, K. R. (1998) Nature 392, 885–888
2. Forman, B. M., Umesono, K., Chen, J., and Evans, R. M. (1995) Cell 81, 541–550
3. Herdick, M., Bury, Y., Quack, M., Uskokovic, M. R., Polly, P., and Carlberg, C. (2000) Mol. Pharmacol. 57, 1206–1217
4. Mouchon, A., Delmotte, M. H., Formstecher, P., and Lefebvre, P. (1999) Mol. Cell. Biol. 19, 3073–3085
5. Shulman, A. I., Larson, C., Mangelsdorf, D. J., and Ranganathan, R. (2004) Cell 116, 417–429
6. Shulman, I. G., Li, C., Schwabe, J. W., and Evans, R. M. (1997) Genes Dev. 11, 299–308
7. Ikeda, M., Wilcox, E. C., and Chin, W. W. (1996) J. Biol. Chem. 271, 23096–23104
8. Velasco, L. F., Togashi, M., Walfish, P. G., Pessanha, R. P., Moura, F. N., Barra, G. B., Nguyen, P., Reborg, R., Yuan, C., Simeoni, L. A., Ribeiro, R. C., Baxter, J. D., Webb, P., and Neves, F. A. (2007) J. Biol. Chem. 282, 12458–12466
9. Wood, J. R., Greene, G. L., and Nardulli, A. M. (1998) Mol. Cell. Biol. 18, 1927–1934
10. Yen, P. M. (2001) Physiol. Rev. 81, 1097–1142
11. Claret, F. X., Antakly, T., Karin, M., and Saatcioglu, F. (1996) Mol. Cell.

Allosterism in the Thyroid Receptor
Allosterism in the Thyroid Receptor

Biol. 16, 219–227
12. Mengeling, B. J., Lee, S., and Privalsky, M. L. (2008) Mol. Cell. Endocrinol. 280, 47–62
13. Lin, B. C., Hong, S. H., Krig, S., Yoh, S. M., and Privalsky, M. L. (1997) Mol. Cell. Biol. 17, 6131–6138
14. Moore, J. M., and Guy, R. K. (2005) Mol. Cell Proteomics. 4, 475–482
15. Diallo, E. M., Thompson, D. L., and Koenig, R. J. (2005) Protein Expr. Purif. 40, 292–298
16. Makowski, A., Brzostek, S., Cohen, R. N., and Hollenberg, A. N. (2003) Mol. Endocrinol. 17, 273–286
17. Krejci, A., and Bray, S. (2007) Genes Dev. 21, 1322–1327
18. Meijsing, S. H., Elbi, C., Luecke, H. F., Hager, G. L., and Yamamoto, K. R. (2005) Mol. Cell. Biol. 25, 3024–3031
19. Rastinejad, F., Perlmann, T., Evans, R. M., and Sigler, P. B. (1995) Nature 375, 203–211
20. Moore, J. M., Galicia, S. J., McReynolds, A. C., Nguyen, N. H., Scanlan, T. S., and Guy, R. K. (2004) J. Biol. Chem. 279, 27584–27590
21. Kraus, W. L., McInerney, E. M., and Katzenellenbogen, B. S. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 6314–6318
22. Darimont, B. D., Wagner, R. L., Apriletti, J. W., Stallcup, M. R., Kushner, P. J., Baxter, J. D., Fletterick, R. J., and Yamamoto, K. R. (1998) Genes Dev. 12, 3343–3356
23. Wright, E., Vincent, J., and Fernandez, E. J. (2007) Biochemistry 46, 862–870
24. Gonzalez, M. I., and Robins, D. M. (2001) J. Biol. Chem. 276, 6420–6428
25. Chandra, V., Huang, P., Hamuro, Y., Raghuram, S., Wang, Y., Burris, T. P., and Rastinejad, F. (2008) Nature 456, 350–356
26. Lehmann, J. M., Zhang, X. K., Graupner, G., Lee, M. O., Hermann, T., Hoffmann, B., and Pfahl, M. (1993) Mol. Cell. Biol. 13, 7698–7707
27. Bevan, C. L., Hoare, S., Claessens, F., Heery, D. M., and Parker, M. G. (1999) Mol. Cell. Biol. 19, 8383–8392
28. Hall, J. M., McDonnell, D. P., and Korach, K. S. (2002) Mol. Endocrinol. 16, 469–486
29. Kahlen, J. P., and Carlberg, C. (1997) Nucleic Acids Res. 25, 4307–4313
30. Castillo, A. I., Sanchez-Martinez, R., Moreno, J. L., Martinez-Iglesias, O. A., Palacios, D., and Aranda, A. (2004) Mol. Cell. Biol. 24, 502–513
31. Kersten, S., Gronemeyer, H., and Noy, N. (1997) J. Biol. Chem. 272, 12771–12777
32. He, B., Kemppainen, J. A., Voegel, J. J., Gronemeyer, H., and Wilson, E. M. (1999) J. Biol. Chem. 274, 37219–37225
33. Metivier, R., Penot, G., Flouriot, G., and Pakdel, F. (2001) Mol. Endocrinol. 15, 1953–1970
34. Metivier, R., Stark, A., Flouriot, G., Hübner, M. R., Brand, H., Penot, G., Manu, D., Denger, S., Reid, G., Kos, M., Russell, R. B., Kah, O., Pakdel, F., and Gannon, F. (2002) Mol. Cell 10, 1019–1032
35. Meijsing, S. H., Pufall, M. A., So, A. Y., Bates, D. L., Chen, L., and Yamamoto, K. R. (2005) Science 309, 407–410
36. Freeman, B. C., and Yamamoto, K. R. (2002) Science 296, 2232–2235
37. Klok, T. I., Kury, P., Elbi, C., Nagaich, A. K., Hendarwanto, A., Slagsvold, T., Chang, C. Y., Hager, G. L., and Saatcioglu, F. (2007) Mol. Cell. Biol. 27, 1823–1843
38. Greenspan, N. S., and Di Cera, E. (1999) Nat. Biotechnol. 17, 936–937
39. Estébanez-Perpiñá, E., Arnold, L. A., Arnold, A. A., Nguyen, P., Rodrigues, E. D., Mar, E., Bateman, R., Pallai, P., Shokat, K. M., Baxter, J. D., Guy, R. K., Webb, P., and Fletterick, R. J. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 16074–16079
40. Mao, C., Patterson, N. M., Cherian, M. T., Aninye, I. O., Zhang, C., Montoya, J. B., Cheng, J., Pottt, K. S., Hergenrother, P. J., Wilson, E. M., Nardulli, A. M., Nordeen, S. K., and Shapiro, D. J. (2008) J. Biol. Chem. 283, 12819–12830