Molecular Determinants Required for Selective Interactions between the Thyroid Hormone Receptor Homodimer and the Nuclear Receptor Corepressor N-CoR

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The unliganded nuclear receptor (NR) generally recruits the NR corepressor (N-CoR) and the silencing mediator of retinoid and thyroid hormone receptor via its direct binding to the extended helical motif within dual NR-interaction domains (IDs) of corepressors. Interestingly, N-CoR has a third ID (ID3) upstream of two IDs (ID1 and ID2) and its core motif (IDVII), rather than an extended helical motif, is known to be involved directly in the exclusive interaction of ID3 with the thyroid hormone receptor (TR). Here, we investigated the molecular determinants of the TR interaction with ID3 to understand the molecular basis of the N-CoR preference shown by the TR homodimer. Using a one- plus two-hybrid system, we identified the specific residues of N-CoR-ID2 and N-CoR-ID3 that are required for stable association of N-CoR with the TR homodimer. By swapping experiments and mutagenesis studies, we found that the C-terminally flanked residues of the core motif of ID3 contribute to the TR preference for N-CoR-ID3, suggesting that an extended three-turn helix might form within the ID3 via a C-terminal extension (IDVIITRQI) and participate directly in the TR-specific interaction. Structural modeling of the ID3 motif on TR-LBD is consistent with this conclusion. Notably, we identified a novel interaction between N-CoR-ID3 and orphan NR RevErb that is mediated by the residues crucial also in TR binding. These observations raise the intriguing possibility that NR homodimers such as TR and RevErb display preferential binding to the N-CoR corepressor via their specific interactions with ID3, which is normally absent from the silencing mediator of retinoid and thyroid hormone receptor.

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Introduction

Thyroid hormone (T3) plays a critical role in the development and adult functions of many organs and tissues.1 The effects of T3 are mediated by two types of receptor proteins that are encoded by the thyroid hormone receptor (TR) α and β genes, respectively. TRs bind to specific DNA sequences, known as thyroid hormone response elements (TREs), in the regulatory region of target genes. Although TR acts directly on gene transcription mainly as heterodimers with the retinoid X receptor (RXR), TR is able to bind to TREs as a monomer or homodimer.2 In the absence of T3 ligand, the unliganded TR is still bound to the TRE of target
genes and associates with corepressor proteins such as the nuclear receptor (NR) corepressor (N-CoR)/silencing mediator of retinoid and thyroid hormone receptors (SMRT) to repress gene transcription. In this regard, Marimuthu et al. tested over 100 mutations of the full-length human TR that span the surface of its ligand-binding domain (LBD) and identified a broad range of TR residues that are critical for optimal N-CoR binding. In contrast to this, the addition of T3 causes a conformational change in the LBD of TR, leading to release of the corepressors from the receptor and concomitant recruitment of various coactivator molecules, such as steroid receptor coactivators and the TR-associated protein 220 to achieve transcriptional activation.

The N-CoR/SMRT corepressor proteins perform crucial functions in various aspects of metazoan development, and have been implicated in some endocrine diseases. N-CoR gene-deletion has been shown to result in blockage at specific points in the central nervous system, erythrocytes, and thymocyte development in mice, and the cells of these mice exhibit impaired self-renewal and spontaneous differentiation into astroglia-like cells. Recently, a careful analysis of SMRT gene-deleted mice revealed that SMRT is crucial for the prevention of the retinoid-receptor-dependent induction of differentiation along a neuronal pathway. Additionally, the syndrome of thyroid hormone (RTH), which is impaired in physiological responses to the thyroid hormone, has been implicated in aberrant corepressor interaction. For example, a naturally occurring human RTH mutation, referred to as R429Q, is impaired in TR homodimerization and corepressor binding. N-CoR/SMRT has a modular structure consisting of three independent autonomous repression domains and two separate NR-interaction domains (IDs) that are located at their N-terminal and C-terminal regions, respectively. Corepressor-IDs have been shown to associate directly with the LBD surface of unliganded non-steroid receptors as well as antagonist-bound steroid receptors. SMRT is known to contain two IDs (ID1 and ID2) in general, whereas N-CoR contains a third ID (ID3) that is positioned upstream of the previously reported two IDs, C-terminally located ID1 and N-terminally located ID2. Recently, a variant form of Xenopus SMRT, which harbors a third ID (ID3), was shown to be generated via an alternative splicing mechanism and was implicated in the development of Xenopus. Each ID contains the corepressor-NR (CoRNR) box or the related extended-helix motif with the consensus sequence LXX/lHXXl/L (X is any amino acid) that is responsible for NR interactions. Interestingly, a particular NR interacts preferentially with the specific ID of corepressors, determining corepressor preference (N-CoR versus SMRT) or ID preference (ID1 versus ID2) of a given receptor. For example, the ID2 of N-CoR/SMRT interacts with the retinoic acid receptor (RAR), TR, RevErb, and the vitamin D receptor, whereas the AF2-deletion form of the RXR (RXRΔAF2) and the liver X receptor interact exclusively with the ID1 of corepressors. N-CoR stabilizes the TR homodimer when bound to DNA by preventing its dissociation from TREs. In particular, N-CoR-ID3 is known to interact exclusively with the TR via its core helical motif (IDVII) rather than the extended helical motif (three-turn helix) normally found in ID1 and ID2. Recently, Astapova et al. reported that the ID2 and ID3 domains of N-CoR are absolutely required for the TR interactions and that the specificity of N-CoR-TR interaction is determined by the amino acid sequence of ID3, but not by its distance from other IDs. Accordingly, in addition to the TR-ID2 interaction, the TR homodimer preferentially recruits N-CoR via its specific interaction with ID3. Regarding other NRs, we demonstrated the molecular basis of the corepressor-ID preference of NRs earlier by determining the residues within or outside the extended helix motifs of corepressor IDs that are involved in NR-specific interactions.

RevErbα, an orphan NR encoded on the opposite strand of the TRα, plays a pivotal role in the processes of adipocyte and muscle cell differentiation. RevErb has no known cognate ligand and constitutively represses transcription when bound as a homodimer to a specific subset of DR2 sites. In addition, RevErb lacks an AF-2 domain, which is required for ligand-dependent transcriptional activation of NRs, and this feature contributes to its constitutive repression of gene transcription. N-CoR is known to be required for transcriptional repression by RevErb, in that RevErb interacts with endogenous N-CoR and the repressive activity of RevErb is mediated by the N-CoR/histone deacetylase 3 complex. It was reported that RevErb interacts with two domains located in the C-terminus of N-CoR, but the detailed molecular basis of this interaction remains to be deciphered.

Here, we identified the specific amino acid residues of N-CoR-ID2 and N-CoR-ID3 that are involved in the preferential interaction with the TR homodimer through the one- plus two-hybrid screening. Moreover, we showed that the residues located in the C-terminal region of the CoRNR3 motif is involved directly in the preferred and strong interaction between ID3 and TR. Interestingly, the direct interaction of ID3 with RevErb is required also for RevErb-mediated repression, suggesting a plausible model to explain the N-CoR preference shown by NR homodimers such as TR and RevErb.

**Results and Discussion**

**ID preference of TRβ homodimer in N-CoR interactions**

As mentioned above, the TR homodimer interacts specifically with N-CoR via their direct association with ID3, which is not present in SMRT. As a first step to uncovering the molecular basis of this