Transcriptional repression by nuclear receptors: mechanisms and role in disease
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Abstract
Co-repressor proteins mediate transcriptional repression by nuclear receptors in the absence of ligand. The identification of a co-repressor-receptor interaction motif, and the finding that co-repressors and co-activators compete for the same site on the receptor, suggests a simple mechanism for the switch from repression to activation upon ligand binding. Defects in this mechanism result in dominant-negative receptors that repress transcription. Such receptors have been implicated in several clinically important diseases, including thyroid hormone resistance and diabetes mellitus.

Introduction
Many nuclear receptors repress transcription in the absence of ligand. This repression is mediated by co-repressor proteins that bind to the unliganded receptors. The co-repressors SMRT (silencing mediator for retinoid and thyroid receptors) and NCoR (nuclear receptor co-repressor) repress transcription to sub-basal levels, via the recruitment of histone deacetylase complexes [1-7]. Upon ligand binding, the co-repressor complexes are dissociated and various activation complexes are recruited to the liganded receptor. These include the p160 co-activators, such as ACTR (activator for thyroid and retinoid receptors) and SRC-1 (steroid receptor co-activator 1), which possess intrinsic histone acetyltransferase activity [8-10].

Crystal structures of the ligand-binding domains of a number of nuclear receptors have revealed in some detail the structural basis for how liganded receptors recruit co-activator proteins [11,12]. In contrast, we understand far less about the nature of receptor-co-repressor interactions. Understanding these interactions is important in relation not only to the ligand-dependent molecular switch, but also to clinical diseases that involve repression by nuclear receptors.

In this manuscript, we first discuss the mechanisms via which co-repressors interact with unliganded nuclear receptors and how ligand, along with co-activator proteins, causes release of the co-repressor. We will then discuss how mutations that result in defective release of co-repressors give rise to a dominant-negative phenotype that, in several cases, is a primary cause of clinical disease.

Mechanisms of nuclear receptor co-repressor interactions
Identifying minimal receptor interaction domains
We and several other labs have sought to understand the molecular basis for the interaction between co-repressors and nuclear receptors and, furthermore, to understand how this interaction relates to the interaction of receptors and co-activators. A first stage in this was to map the region within co-repressor proteins responsible for interaction with nuclear receptors.

Preliminary mapping studies had previously demonstrated that the C-terminal 513 residues of SMRT contain the region(s) responsible for in-
teraction with nuclear receptors [7,13]. More detailed mapping of this region using mammalian two-hybrid assays suggested that both NCoR and SMRT contain two independent receptor-interaction domains [14,15]. Proteolytic analysis of constructs of SMRT expressed in bacteria suggested that these two domains each comprise less than 30 amino acids. Further sequence analysis and two-hybrid assays (in yeast and mammalian cells) identified two minimal domains, ID1 and ID2, of 17 and 19 amino acids respectively (Figure 1). Proof that these very short peptides are sufficient for receptor interaction in vitro was demonstrated using peptide competition assays. Peptides with wild-type sequence were able to displace SMRT from the heterodimeric receptor, whereas peptides with mutations in the conserved hydrophobic amino acids did not displace SMRT.

The minimal ID1 and ID2 domains are each independently sufficient to mediate interaction with unliganded receptors, and to mediate release in response to ligand binding [15–17]. This finding suggested that if these interaction domains were to be fused to an activation domain, the resulting chimera could lead to a reversal of the normal signalling paradigm (i.e. unliganded activation and loss of activation on addition of ligand). We and others have demonstrated such an experiment.

Inspection of the sequences of the ID1 and ID2 domains, as well as secondary-structure prediction, suggests that they might form amphipathic α-helices [18]. This is strongly reminiscent of the finding that co-activator proteins interact with nuclear receptors using a conserved Leu-Xaa-Xaa-Leu-Leu (LXXLL) motif that forms a short amphipathic α-helix upon recruitment to the liganded nuclear receptors [19]. These findings led to the idea that co-repressor–receptor inter-

**Figure 1**

Identification of receptor interaction domains in co-repressors

(a) Schematic representation of the domain structure in SMRT. The numbering refers to the numbering of full-length human SMRT. (b) Mammalian two-hybrid assay illustrating the ligand-dependent association and release of the minimal receptor interaction domains with the RAR, GAL, GAL4 DNA-binding domain; LBD, ligand-binding domain; UAS, upstream activation sequence. (c) Alignment of co-repressor interaction domains. Regions predicted to be helical are overlined; grey-shaded boxes denote conserved hydrophobic residues. (d) Helical-wheel representations of conserved amphipathic helices for ID1 and ID2.
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(a) Graph showing the percentage of heterodimer bound to ACTR relative to different ligands.

(b) Bar graph indicating the effect of various mutants on luciferase activity.

(c) Detailed image of a protein structure with labeled residues.

(d) Another perspective of the protein structure.

(e) Side view of the protein structure with highlighted helices and coiled-coil regions.
actions might in some way be analogous to co-activator–receptor interactions and, indeed, that the two molecules might in some way compete for the same surface of the receptor.

Co-repressors and co-activators compete for overlapping binding sites

The idea that co-repressor and co-activator proteins might compete for overlapping surfaces of the receptor, with ligand determining which binds more tightly, provides an attractive model for the functioning of the ligand-activated switch of the receptor. To test this model, two types of experiment were performed: on the one hand, a direct competition assay and on the other, a mutational interference assay.

To test whether co-activators and co-repressors directly compete for overlapping surfaces on the receptor–ligand-binding domains, co-activator protein was immobilized on affinity resin. [In this case, glutathione S-transferase–ACTR was bound to glutathione–Sepharose.] This resin was then challenged with retinoic acid (RA) receptor (RAR)/retinoid-X-receptor (RXR) heterodimers in the presence or absence of SMRT and/or ligand. Under these conditions, the heterodimer interacts with co-activator in the absence of ligand. The presence of co-repressor strongly inhibits this interaction. Upon the addition of ligand, co-repressor is displaced and the heterodimer binds to the co-activator. These findings suggest that the co-repressor and co-activator interaction domains probably bind to overlapping surfaces on the receptors.

It had been shown previously that a cluster of five mutations in the thyroid hormone (RTH) receptor (TR) abolished interaction of the receptor with co-activator proteins [20]. These mutations were subsequently shown to comprise the co-activator interaction surface [11,12]. We tested whether these mutations might also perturb co-repressor binding (Figure 2b) using a mammalian two-hybrid assay. Strikingly, all five mutations abolished co-repressor binding. Thus it is apparent that residues critical for binding co-activators are also critical for co-repressor binding, providing further evidence that their binding sites on the ligand-binding domain must, at least partially, overlap [15].

We can conclude that there are four components to the molecular switch, as illustrated in Figure 2(e). In the absence of ligand, the receptor favours the binding of co-repressor. On the addition of ligand and co-activator, helix 12 of the receptor undergoes a conformational change such that co-repressor binding is no longer favoured, and it is displaced by incoming co-activator.

Role of nuclear receptor co-repressors in disease

It is becoming clear that the repression activities of nuclear receptors in the absence of ligand play crucial physiological roles in the cell. This is exemplified by the finding that receptors that are defective in co-repressor release exhibit a dominant-negative phenotype resulting in disease. The best-studied examples of this are discussed below.

Erythroleukaemia

The ability of the avian erythroblastosis virus (AEV) to induce erythroleukaemia and fibrosarcoma is mediated by the co-operative action of v-erbB and v-erbA oncogenes. v-erbA encodes a highly mutated viral homologue of the TR. Based on the observation that it lacks a C-terminal transactivation (AF-2) domain, it was hypothesized that simple loss of function accounted for its oncogenic activity. However, studies indicate that the v-erbA protein functions as a negative regulator, to block the transcriptional activity of its normal cellular counterpart TR, and also the RAR, at their respective target genes [21–23]. The biological significance of this suppressor function was underscored by the identification of AEV td359, a natural mutant virus, which is transformation-defective. This defective virus harbours a mutation (Pro1 Arg) in the hinge region of v-erbA. In AEV12, a viral revertant which has recovered transforming ability, this residue is
back-mutated to proline. The Pro$^{444}$Arg mutation disrupts transcriptional silencing and dominant-negative inhibition by v-erbA [24]. Its ability to repress transcription is now known to be mediated by constitutive recruitment of co-repressors (e.g. SMRT), and mutation of the residue equivalent to Pro$^{444}$ in TR abolishes co-repressor binding [7].

**Promyelocytic leukaemia**

Acute promyelocytic leukaemia, characterized by clonal expansion of cells blocked at the promyelocytic stage of differentiation, is typically associated with reciprocal chromosomal translocations involving the RAR$\alpha$ locus on chromosome 17 with either PML (for promyelocytic leukaemia) on chromosome 15 or PLZF (for promyelocytic leukaemia zinc finger) on chromosome 11. In each case, the translocation generates aberrant fusion proteins (PML–RAR$\alpha$, PLZF–RAR$\alpha$) consisting of the DNA- and ligand-binding domains of RAR$\alpha$ linked to domains from PML and PLZF proteins respectively. Both PML–RAR$\alpha$ and PLZF–RAR$\alpha$ inhibit normal retinoid signalling via the RAR/RXR pathway, and this action is linked to their oncogenic activity [25,26]. A unique feature of this disorder is that it is sensitive to RA therapy, which induces remission by promoting cellular differentiation. PML–RAR$\alpha$ patients achieve complete remission following treatment with pharmacological doses of RA; in contrast, PLZF–RAR$\alpha$ patients respond poorly. Recent studies have provided insights into the mechanism of such differential responsiveness. In the unliganded state, both fusion proteins recruit the co-repressor–histone deacetylase complex to mediate silencing of target gene transcription. The PML–RAR$\alpha$ fusion interacts via the receptor ligand-binding domain, such that high doses of RA release the histone deacetylase complex, relieving repression; in contrast, the PLZF–RAR$\alpha$ fusion also binds the co-repressor complex via a second site located in the PLZF moiety, and this interaction is resistant to RA [27–29].

**RTH resistance**

The syndrome of resistance to RTH is usually dominantly inherited, and characterized by elevated circulating RTH, together with impaired negative-feedback regulation of pituitary thyroid-stimulating hormone (TSH) production and peripheral refractoriness to RTH action. To date, over eighty different heterozygous mutations in the ligand-binding domain of TR$\beta$ have been identified in this disorder. The mutant receptors are transcriptionally impaired, usually because of a reduced ligand binding or failure to recruit transcriptional co-activators. In addition, when

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**Figure 3**

Mechanism of dominant-negative inhibition by mutant receptors

(a) The classic model for ligand-dependent release of co-repressor (CoR) and recruitment of co-activators (CoA). The CoR complex acts to silence basal gene transcription. CoR dissociation is followed by binding of CoA, which leads to target gene activation. (b) The consequences of dominant-negative receptor mutations (Mut). In comparison with wild-type (WT) receptor, the primary defect in mutant receptors is impaired ligand-dependent CoR dissociation or CoA recruitment. For most mutants, this functional alteration is a consequence of reduced ligand binding. However, a subset exhibited enhanced CoR binding and delayed CoR release in itself, with normal hormone binding. Mut receptor–CoR complexes compete with their WT counterparts for occupancy of promoter hormone response elements (HREs), resulting in inhibition of target gene expression.
co-expressed, the mutant receptors inhibit the function of their wild-type counterparts in a dominant-negative manner. Such dominant-negative inhibition is critical to the pathogenesis of RTH, since simple haploinsufficiency of TRβ in both humans and mice does not generate the resistance phenotype [30,31]. Studies indicate that the ability of mutant receptors to bind to DNA and heterodimerize with RXR is required for their dominant-negative activity. A further property exhibited by TR mutants is the ability to repress or 'silence' basal gene transcription, which contributes to their dominant-negative inhibitory potency. For example, non-3,3',5-tri-iodothyronine (T3)-binding receptor mutants exhibit constitutive silencing function, particularly when bound to target gene-response elements as homodimers, which cannot be relieved by ligand. Conversely, RTH mutants with impaired homodimerization properties are weaker dominant-negative inhibitors [32]. With the recent identification of co-repressors, Yoh et al. [33] have extended these observations, showing that some TR mutants either bind co-repressor more avidly when unliganded, or fail to dissociate from co-repressor following T3 binding. Furthermore, the introduction of artificial mutations that abolish co-repressor binding into a TR mutant background abrogates their dominant-negative activity [33]. In contrast with silencing of basal transcription observed with positively regulated target genes, unliganded TR has been shown to enhance the basal activity of negatively regulated (TRH, TSHβ) promoters, with ligand-dependent transrepression upon the addition of T3 [34]. Most recently, co-expressed co-repressors have been shown to augment such unliganded activation and an artificial mutant lacking co-repressor binding is impaired for this function [35]. In this context, an unusual TR mutant (Arg383His) exhibits delayed co-repressor release, and is selectively transcriptionally impaired with negatively regulated promoters [36]. Given the pivotal role of negatively regulated target genes in the pathogenesis of RTH, aberrant co-repressor recruitment or release may well prove to be the critical receptor abnormality in this disorder.

**Diabetes mellitus**

Finally, we have recently described similar defects in another nuclear receptor linked to human disease. Peroxisome-proliferator-activated receptor γ (PPARγ) promotes adipocyte differentiation and binds thiazolidinediones, which are a novel class of anti-diabetic agents that improve tissue insulin sensitivity. In three subjects with severe insulin resistance, type 2 diabetes and early-onset hypertension, we have identified heterozygous mutations in the PPARγ ligand-binding domain that disrupt transcriptional activation by impairing both ligand binding and co-activator recruitment [37]. Analogous to RTH, the mutant receptors are dominant-negative inhibitors of wild-type receptor action, and we have shown that they silence basal target gene transcription. From examination of the PPARγ X-ray crystal structure, it is apparent that both natural mutations destabilize helix 12, which mediates transactivation.

To understand further the mechanism by which they repress transcription, we have studied an artificial PPARγ receptor mutant harbouring helix 12 defects. This mutant is also a potent repressor and recruits SMRT or NCoR more avidly than wild-type PPARγ, but fails to release co-repressor following ligand binding [38].

**Conclusions**

In summary, these observations suggest that constitutive co-repressor binding by mutant nuclear receptors mediates transcriptional silencing, which interferes with signalling by their wild-type counterparts at target gene DNA-binding sites (Figure 3). This mechanism is the basis of their dominant-negative inhibitory action in different biological contexts, and we anticipate that other examples of such aberrant function in other nuclear receptors, linked to human disease, will be identified in the future. It is also likely that our increased understanding of the mechanisms through which co-repressors are normally associated with and dissociated from both wild-type and mutant receptors will lead to the development of pharmacological interventions that allow these interactions to be clinically controlled.

**References**

Structural aspects of agonism and antagonism in the oestrogen receptor

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Abstract

We have determined the three-dimensional struc-

Key words: activation function 2; ligand binding; steroid receptor; transcription factor.

Activations used: AF1, activation function 1; AF2, activation function 2; ER, oestrogen receptor; E2, 17β-estradiol; LBD, ligand-binding domain; NR, nuclear receptor; RAL, raloxifene.

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tures of both α- and β-forms of the ligand-binding domain of the oestrogen receptor (ER) in com-

plexes with a range of receptor agonists and antagonists. Here, we summarize how these structures provide both an understanding of the ER’s distinctive pharmacophore and a rationale for its ability to bind a diverse range of chemically distinct compounds. In addition, these studies provide a unique insight into the mechanisms that

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