Are corepressors always repressors?

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Abstract

In this review we summarize data on paradox actions of corepressors, acting under certain circumstances as activators of transcription. Putative mechanisms, including the role of splice variants, recruitment of coactivators by corepressors and the importance of chromatin structure and hormone response elements are discussed.

Introduction

The regulation of gene expression by transcriptional activation or repression is a general phenomenon present in all taxa. The mechanisms of these regulations are conserved in evolution.¹ In bacteria and archaea repressors or activators directly interact with the genes of interest whereas in eukaryotes more indirect mechanisms are evolved consistent with the chromatin environment of the genes, finally leading to a modulation of chromatin structure by enzymatic modifications of the core histone amino-terminal tails.^{2,4} This *histone code* is an essential feature in the distinct and specific regulation of transcriptional programmes.⁵ It is therefore not surprising that dysregulations in expression and function of these coregulators are connected to various diseases.⁶

In the present review we will mainly deal with corepressors of nuclear receptors (NR) which represent a huge family of proteins characterized by their ability to bind to transcription factors and to recruit various enzymatic complexes like histone deacetylases thus leading to a more compact chromatin and inhibition of transcription. Models of corepressor action, mainly of the two abundant corepressors NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) were recently reviewed.⁷ We therefore focus on *non-conventional* and unexpected actions of corepressors in this review.

Activating actions of corepressors

Originally corepressors were thought to be recruited to DNA by unliganded nuclear

receptors. But in addition to these *conventional* corepressors, *unconventional* ones were described which are specifically recruited by liganded nuclear receptors. As a third category multifunctional corepressors were described.⁷ An example of this category is the corepressor hairless, which can be either a conventional corepressor of unliganded thyroid hormone receptor or an unconventional one with the retinoic acid and the vitamin D receptor as well as with the retinoic acid related orphan receptor in an agonist-dependent fashion.⁸

As a fourth category of corepressor function it has been demonstrated that otherwise classical corepressors do not repress gene expression but may even lead to an activation. Examples for this action contradictory to their name are summarized in Table 1. This phenomenon was predominantly found for the corepressors SMRT and NCoR interacting with various nuclear receptors but also for different zinc-finger transcription factors. The outcome of the action of these factors obviously depends on the cellular environment, as also demonstrated in Figure 1. In COS-1 cells the androgen-induced transactivation is repressed by NCoR, whereas in the human prostate cancer cell line PC-3 it is increased. In both instances the specific class I and II histone deacetylase inhibitor Trichostatin A (TSA) leads to the expected results, a significant reduction of the inhibition in case of repressor function like in COS-1 cells (Figure 1A) but a significant reduction of the activation exerted by NCoR in prostate cancer cells (Figure 1B). The same results were also gained in experiments when NCoR is effectively silenced in COS-1 and the two prostate cancer cell lines PC-3 and LNCaP (Figure 2). If NCoR expression is reduced by a specific shRNA the hormone-induced transactivation is increased in COS-1 cells as expected, but reduced in the prostate cancer cell lines. If NCoR is overexpressed there is the *classical* repression effect in COS-1 cells, but activation in the prostate cancer cell lines (Figure 2A-C).

Possible mechanisms of the reversal of corepressor action

The question arises how these activating functions of corepressors are produced. At least three different modes are possible: i) Splice variants: In addition to the evolutionary gene duplication leading to SMRT and NCoR paralogs, there is also corepressor diversification by mRNA splicing. This leads to a series of corepressor protein variants with distinctive functional differences like distinguishable repression properties and/or recruitment of various transcription factors.²¹ If in a splice variant repression domains (located in the N-terminal and cen-

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tral regions) or *CoNR box motifs* (C-terminal, responsible for nuclear receptor binding) are lost or diminished, the repression function of a corepressor might be reduced, completely abolished or even reversed. This was demonstrated for example for an N-terminal-truncated NCoR variant and thyroid hormone receptor action,¹⁸ or for androgen dependent gene expression and SMRT β ,¹⁰ which lacks one of the classical repression domains and the newly described nuclear receptor binding site.²² A similar effect could also be shown for RAR α dependent gene expression in Jurkat cells;¹⁴ ii) Recruitment of coactivators by corepressors: Direct interactions of SMRT with the coactivator SRC-1,¹² and for NCoR with SRC1, -2, and -3 were described.²³ SMRT and SRC-3 bind directly in an estrogen receptor alpha (ER α)-independent way. Estrogen promotes SRC-3 binding to ER α . SMRT is thus recruited to the regulatory regions of the progesterone receptor and the cyclin D1 gene by estradiol. If SMRT is depleted this hormone-dependent gene expression is diminished, demonstrating that SMRT is required for full transcriptional activity of the ER α in breast cancer cells.¹² In case of NCoR, a trimeric complex consisting of the corepressor, the coactivator SRC and the unliganded thyroid hormone receptor was demonstrated.²³ The authors guess that the corepressor raises the local concentra-

Table 1. Activating actions of corepressors.

Transcription factor	Corepressor	Activation of transcription	Inhibition of transcription	Cell type	Remarks	References
Androgen receptor	SMRT, DAX-1 NCoR NCoR		+	HeLa cells COS-1 cells HeLa cells	Neither inhibition nor activation	Agoulunik <i>et al.</i> , 2003
Androgen receptor	NCoR, SMRT	+	+	PC-3, LNCaP COS cells		Laschak <i>et al.</i> , 2011
Estrogen receptor	SMRT	+	+	HeLa, MCF-7 cells HepG2 cells		Peterson <i>et al.</i> , 2007
Estrogen receptor	SMRT	+		HeLa, MCF-7 cells	Cooperative activation of estradiol dependent gene expression by the coactivator SRC-3 and SMRT	Karmakar <i>et al.</i> , 2010
Glucocorticoid receptor	SMRT	+	+	COS-1 cells	SMRT shows strong interaction with agonists and partial agonists	Ronacher <i>et al.</i> , 2009
Retinoic acid receptor	SMRT β	+		Jurkat, NB4-MRA1, U937 cells COS cells	Ligand-induced transactivation is influenced. SMRT α without effect	Coté <i>et al.</i> , 2004
Thyroid hormone receptor	NCoR, SMRT	+		Human embryonic kidney cells	Negatively regulated genes are activated, thyroid hormone abolishes this effect	Tagami <i>et al.</i> , 1997
Thyroid hormone receptor	NCoR, SMRT	+		HeLa, CV-1 cells	Activation from negative hormone response elements of TR	Berghagen <i>et al.</i> 2002
Thyroid hormone receptor	NCoR	+			Basal transactivation at negative T3 responsive elements is enhanced, T3 reverses this effect	Kim <i>et al.</i> , 2005
Thyroid hormone receptor	NCoR	+		<i>Saccharomyces cerevisiae</i>	Splice variants of NCoR coactivate unliganded TR. Thyroid hormone inhibits this effect	Meng <i>et al.</i> , 2005 and 2006
ZHX1 (zinc finger and homeobox transcription factor)	BS69	+	+	COS-7, CV-1 cells HEK cells		Ogata-Kawata <i>et al.</i> , 2007

Figure 1. Expression of an androgen-dependent reporter gene in COS-1 (A) and human prostate cancer cells PC-3 (B) under the influence of dihydrotestosterone (DHT) and the histone deacetylase inhibitor trichostatin A (TSA) with and without overexpression of the corepressor NCoR (own, unpublished results).

tion of the coactivator at target gene promoters leading to an enhanced transcriptional activation. For the androgen receptor it might be possible that corepressors recruit histone deacetylases. It has been shown that HDAC1 and HDAC3 are necessary for a full activation of androgen dependent genes.²⁴ Our results with the histone deacetylase inhibitor Trichostatin (Figure 1) reducing the NCoR effects also point in this direction; iii) Chromatin structure and hormone response elements: The thyroid hormone receptor can bind to either positive (pHRE) or negative hormone response elements (nHRE). In pHRE corepressors are bound to the nuclear receptor. Upon ligand binding there is a dissociation of this complex and a recruitment of coactivators. In an nHRE the activation of transcription occurs in the absence of hormone when nuclear receptor and corepressor are expected to form a complex. In such a situation a corepressor acts as a coactivator.^{15,16} Berghagen and coworkers mutated the corepressor binding site in the hinge region of the thyroid hormone receptor, inhibiting ligand-independent transcrip-

tional activity of the thyroid hormone receptor at an nHRE. Partial protease digestion experiments indicated that the conformation of the thyroid hormone receptor SMRT complex is different whether this complex binds to a pHRE or an nHRE.¹⁶

The importance of chromatin structure on the effect of a comodulator has also been demonstrated for the coactivator CBP. If this coactivator binds in an euchromatin region it acts as a repressor, but if it binds to heterochromatin it leads to an enhancement of transcriptional activity.²⁵

Conclusions

We here described the phenomenon that a corepressor might act as a coactivator, but there also exist examples for the opposite. For example, the coactivator KAP-1 (also known as TIF1 β or TRIM28) can also function as a corepressor,²⁶ suggesting that the classification of cofactors as corepressors or coactivators cannot be considered too rigidly.⁷

Figure 2. Effect of Flag-NCoR overexpression on AR transactivation in NCoR-silenced COS-1, PC-3 and LNCaP cells. Cells were seeded in 24-well plates and transfected with 400 ng geneticin-resistant shRNA NCoR or shRNA negative as indicated. After 24 h COS 1 (A) and PC-3 (B) cells were transfected with 200 ng ARE-luc, 62.5 ng pSG5 AR and 80 ng pGL4hR-luc (Ren) in RPMI 1640 containing G 418 (500 ng/mL). (C) LNCaP cells were transfected with 200 ng pGL3E-Pro (probasin) and 80 ng pGL4hR luc in RPMI 1640 containing G-418 (500 ng/mL). Additionally, 125 ng Flag-NCoR were co-transfected as indicated, DNA amount was balanced with pCMX-Gal4. Cells were treated with 10 nM DHT 24 h after transfection as indicated, 24 h later cells were harvested and luciferase activities were determined. Error bar = SD, n=3, *P<0.05. (D) COS-1, PC-3 and LNCaP cells were seeded in 25 cm² flasks and transfected with 4 µg shRNA NCoR and shRNA negative respectively. Successfully transfected cells were selected by cultivating the cells in RPMI 1640, 10 % FBS containing G-418 (500 ng/mL). Cells were harvested and lysed; proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane by Western blot. Expression of human NCoR and β-actin was detected by specific antibodies and corresponding HRP-conjugated secondary antibodies. (Own, unpublished results).

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