Are corepressors always repressors?

Andrea Hessenauer, Martin Laschak, Klaus-Dieter Spindler
University of Ulm, General Zoology and Endocrinology, Ulm, Germany

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. 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. This histone code is an essential feature in the distinct and specific regulation of transcriptional programmes. It is therefore not surprising that dysregulations in expression and function of these coregulators are connected to various diseases.

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. 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. 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 retinoid acid and the vitamin D receptor as well as with the retinoic acid related orphan receptor in an agonist-dependent fashion.

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. If in a splice variant repression domains (located in the N-terminal and central regions) or CoRNR 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 truncation. NCoR variant and thyroid hormone receptor action, or for androgen dependent gene expression and SMRTβ, which lacks one of the classical repression domains and the newly described nuclear receptor binding site. A similar effect could also be shown for RARα dependent gene expression in Jurkat cells. ii) Recruitment of coactivators by corepressors: Direct interaction of SMRT with the coactivator SRC-1, and for NCoR with SRC1, -2, and -3 were described. SMRT and SRC-3 bind directly in an estrogen receptor alpha (ERα)-dependent way. Estrogen promotes SRC-3 binding to ERα. 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 ERα in breast cancer cells. In case of NCoR, a trimeric complex consisting of the corepressor, the coactivator SRC and the unliganded thyroid hormone receptor was demonstrated. The authors guess that the corepressor raises the local concentration of the corepressor.

Correspondence: Klaus-Dieter Spindler, University of Ulm, General Zoology and Endocrinology, Albert-Einstein-Allee 11, D – 89069 Ulm, Germany.
Tel.: +49.0.731.5022583 - Fax: +49.0.731 5022581
E-mail: klaus-dieter.spindler@uni-ulm.de

Key words: corepressor/coactivator paradox, splice variants, coactivator recruitment, chromatin structure.

Acknowledgements: this work was supported by a grant from the Deutsche Forschungsgemeinschaft (HE 6078) to AH and the International Graduate School in Molecular Medicine to ML.

Conflict of interest: the authors report no conflicts of interest.

Received for publication: 15 July 2011.
Accepted for publication: 22 July 2011.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright A. Hessenauer et al., 2011
Licensee PAGEPress, Italy
Endocrinology Studies 2011; 1:e8
Table 1. Activating actions of corepressors.

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

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).
Published results). LNCaP cells were seeded in 25 cm² flasks and transfected with 4 µg shRNA NCoR and 1640 containing G-418 (500 ng/mL). Additionally, 125 ng Flag-NCoR were co-transfected with 200 ng pGL3E-Pro (probasin) and 80 ng pGL4hR luc in RPMI 1640 containing G 418 (500 ng/mL). (C) LNCaP 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). (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). 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).

**References**

31. Rambaud J, Desroches J, Balsalobre A, Drouin J. TIF1ß/KAP-1 is a co-activator of the orphan nuclear receptor NGFI-B/Neurotrophin.