


Corepressor/coactivator paradox: potential constitutive coactivation by corepressor splice variants

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The functional consequences of the interaction of transcriptional coregulators with the human thyroid hormone receptor (TR) in mammalian cells are complex. We have used the yeast, *Saccharomyces cerevisiae*, which lack endogenous nuclear receptors (NRs) and NR coregulators, as a model to decipher mechanisms regulating transcriptional activation by TR. In effect, this system allows the reconstitution of TR mediated transcription complexes by the expression of specific combinations of mammalian proteins in yeast. In this yeast system, human adenovirus 5 early region 1A (E1A), a natural N-CoR splice variant (N-CoR_I) or an artificial N-CoR truncation (N-CoR_C) coactivate unliganded TRs and these effects are inhibited by thyroid hormone (TH). E1A contains a short peptide sequence that resembles known corepressor-NR interaction motifs (CoNR box motif, CBM), and this motif is required for TR binding and coactivation. N-CoR_I and N-CoR_C contain three CBMs, but only the C-terminal CBM1 is critical for coactivation. These observations in a yeast model system suggest that E1A and N-CoR_I are naturally occurring TR coactivators that bind in the typical corepressor mode. These findings also raise the possibility that alternative splicing events which form corepressor proteins containing only C-terminal CBM motifs could represent a novel mechanism in mammalian cells for regulating constitutive transcriptional activation by TRs.

Received May 7th, 2006; Accepted September 1st, 2006; Published October 30th, 2006 | **Abbreviations:** CBM: CoNR box motif; CoR: corepressor; CoNR: CoR-NR interaction; E1A: human adenovirus type 5 early region 1A; ID: interacting domain; N-CoR: NR corepressor; NR: nuclear receptor; RD: repressor domain; SMRT: silencing mediator for retinoid receptors and TRs; TH: thyroid hormone; TR: TH receptor; TRE: TH response element; TSH: thyroid-stimulating hormone | Copyright © 2006, Meng et al. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use distribution and reproduction in any medium, provided the original work is properly cited.

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Exploitation of yeast as a model system for studying gene regulation by nuclear receptors

Nuclear receptor (NR) mediated gene activation or repression is regulated by the cellular context of transcriptional coactivators and corepressors (CoRs) [Cheng, 2000; Glass and Rosenfeld, 2000; McKenna and O'Malley, 2002]. Due to the complexity of endogenous NR coregulators in mammalian cells it is difficult to determine the precise mechanisms by which NR coactivators and corepressors function.

The budding yeast *Saccharomyces cerevisiae* contains the conserved eukaryotic general transcriptional apparatus and chromatin remodeling enzymes, but is devoid of nuclear receptors and their coregulators (see [Anafi et al., 2000; Meng et al., 2003; Walfish et al., 1997] and references therein). In this yeast model system it becomes possible to measure qualitatively and quantitatively the functions of exogenous cofactors by reconstituting desired combinations of factors in isolation from the bewildering effects of a myriad of endogenous competing factors.

Transcriptional coactivation by factors binding to unliganded NRs

Our previous studies have proved that two types of human thyroid hormone receptor (hTR) coactivation pathways can be reconstituted in the yeast *Saccharomyces cerevisiae*. Firstly, p160 proteins, such as GRIP1 and SRC1, which contain LXXLL motifs necessary for hormone dependent interaction with the TR [Heery et al., 1997; Torchia et al., 1997], require thyroid hormone (TH) and the SAGA adaptor protein complex to function [Anafi et al., 2000]. This pathway is akin to TR coactivation pathways that have been extensively studied in mammalian systems [Cheng, 2000; Glass and Rosenfeld, 2000] and functions through the intrinsic acetyltransferase activity of the SAGA adaptor complex [Anafi et al., 2000]. Secondly, human adenovirus type 5 early region 1A (E1A) protein behaves as a TH independent TR coactivator in yeast and does not require GCN5 or other components of SAGA [Meng et al., 2003]. Interestingly, coactivation of TR dependent transcription by E1A is lost upon addition of hormone through a ligand induced loss of E1A-TR interaction [Meng et al., 2003].

Hormone-dependent loss of E1A-TR interaction is reminiscent of TR interactions with nuclear receptor

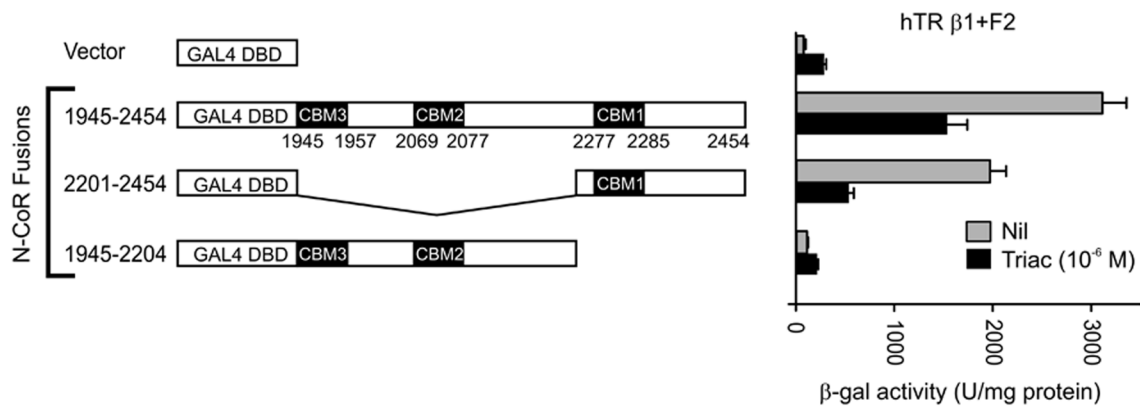


Figure 1. Analysis of the importance of the various CBMs for N-CoR_C coactivation in hTR dependent gene activation in yeast Left Side: A schematic depiction of the portions of N-CoR_C used in this study, which were fused to the GAL4-DNA binding domain. The amino acid residues present in each fragment are as indicated, as are the positions of the CBMs. Right Side: β-gal assays were performed with a yeast strain containing a β-galactosidase reporter gene regulated by single copy of the chicken lysozyme (F2) TRE and expressing hTRβ1, the indicated portions of N-CoR_C or empty vector. Cultures were treated with either vehicle (grey bars) or 10⁻⁶ M Triac (black bars). β-gal activity is expressed as Miller units per mg of protein. Data shown were pooled from three independent experiments and calculated as mean ± SE. Assay conditions as described previously [Meng et al., 2005; Walfish et al., 1997].

corepressors such as N-CoR and SMRT, which interact with unliganded TR and with other NRs in the absence of hormone or in the presence of antagonists [Cheng, 2000; Glass and Rosenfeld, 2000]. The corepressors contain NR interacting domains that share a core consensus motif: ΦXXΦΦ (where Φ is a hydrophobic residue and X is any amino acid) that comprise part of the extended consensus LXXI/HIXXXI/L CoRNR box motif (CBM) [Hu and Lazar, 1999]. These motifs are believed to encode an extended amphipathic helix which contacts the same hydrophobic pocket that binds LXXLL motifs, but is not dependent on the charge clamp and the AF2 helix [Hu and Lazar, 1999; Perissi et al., 1999]. This configuration has been confirmed in an X-ray crystal structure of peroxisome proliferator activated receptor α in complex with an antagonist and a SMRT CBM peptide [Xu et al., 2002]. Inspection of E1A identified a short sequence (LDQLIEEV amino acids 20-28) that strongly resembles a CBM and this was shown to be necessary for TR binding and coactivation, and to bind the same TR surface as corepressors [Meng et al., 2005; Meng et al., 2003]. Thus, a viral protein has co-opted a cellular protein interaction motif to target the unliganded TR. Although E1A binding to unliganded TR mimics that of corepressors, the end result is to confer constitutive coactivation function.

The interaction of E1A with TR in the absence of TH exemplifies the economical nature of adenovirus E1A to stoichiometrically affect target protein function [Frisch and Mymryk, 2002]. Clearly, if the purpose of the interaction of E1A with the unliganded TR is to activate transcription, there is no need for E1A to bind the TR in the presence of hormone and duplicate the function of existing cellular coactivators. This is highly reminiscent of the interaction of E1A with pRb, which regulates exit from the G1 phase of the cell cycle. In this case, E1A binds the hypophosphorylated form of pRb, which is actively involved in blocking G1 exit, but does not interact with inactive hyperphosphorylated forms of pRb [Mitnacht et al., 1994]. Presumably, the frugality of targeting only those

cellular proteins actively engaged in some activity preserves adequate levels of unbound E1A to serve all necessary cellular targets. However, the exact role of the interaction of the E1A protein with the TR is not clear. Inspection of the human adenovirus 5 genome failed to identify any consensus direct repeat TREs, but a number of consensus half sites are present. It remains to be determined if the TR can function through these half sites and whether the E1A proteins can influence any such activity, but it is tempting to speculate that they may play a role in controlling viral transcription and or replication in some fashion.

Given similarities between nuclear hormone receptors, it is also possible that E1A could engage in functionally important interactions with corepressor binding domains of other members of this family of transcription factors. Nevertheless, there is precedent for a role of thyroid hormone in regulating viral life cycle, as simian virus 40, human immunodeficiency virus type 1 and herpes simplex virus all contain functional TREs that control viral gene transcription [Desai-Yajnik et al., 1995; Desvergne and Favez, 1997; Park et al., 1993; Zuo et al., 1997].

These studies on the viral E1A protein also culminated in the novel observation that a nuclear receptor corepressor splice variant (N-CoR_I) or a C-terminal fragment of N-CoR (N-CoR_C) also act as hTR coactivators that bind in the repressor mode [Meng et al., 2005]. Both N-CoR_I and N-CoR_C lack the autonomous N-terminal repression domain found in the well characterized full length form of N-CoR. In the absence of the N-terminal domain, a paradoxical constitutive coactivation function can be observed which is dependent upon binding to hTR in a corepressor mode [Meng et al., 2005].

Mechanism of ligand independent transcriptional activation of NRs

N-CoR_I/N-CoR_C or E1A thus act as novel constitutive coactivators (CCs) through the interaction of their CBMs

with TR in the absence of TH [Meng et al., 2005]. Although, this mechanism appears entirely different from that of TH dependent coactivators (DCs), such as GRIP1 or SRC1 [Anafi et al., 2000; Meng et al., 2003], both types of coactivators nevertheless enhance transcription of their target genes. To further explore the mechanisms by which N-CoR_I/N-CoR_C function as activators, we constructed deletion mutants and determined that CBM1 is much more important than CBM2 and CBM3 for coactivation (Figure 1). If CBM1 was deleted, more than 90% of activity was lost. We also determined that CBM1 contains an intrinsic activation function using yeast one hybrid analysis (data not shown). Future studies will delimit the boundaries of this activation function and identify its target.

Although full-length N-CoR (FL-N-CoR) acts as a TR corepressor, N-CoR_I/N-CoR_C function as coactivators that bind and activate unliganded TR in a yeast coexpression system. This likely occurs because these splice variants of N-CoR lack the intrinsic N-CoR repressor domain and harbor a hitherto unrecognized coactivator function that lies in the vicinity of CBM1. When a p160 coactivator is coexpressed in a yeast model system [Anafi et al., 2000; Walfish et al., 1997] or is endogenously present in a mammalian cell, TH induces hormone dependent coactivation (DC; Figure 2, left panel). In contrast, when a p160 coactivator is either not present or expressed at a relatively low level, TH induces a hormone dependent repression (i.e. negative regulation) on the constitutive unliganded coactivation of N-CoR_I (CC; Figure 2, right panel).

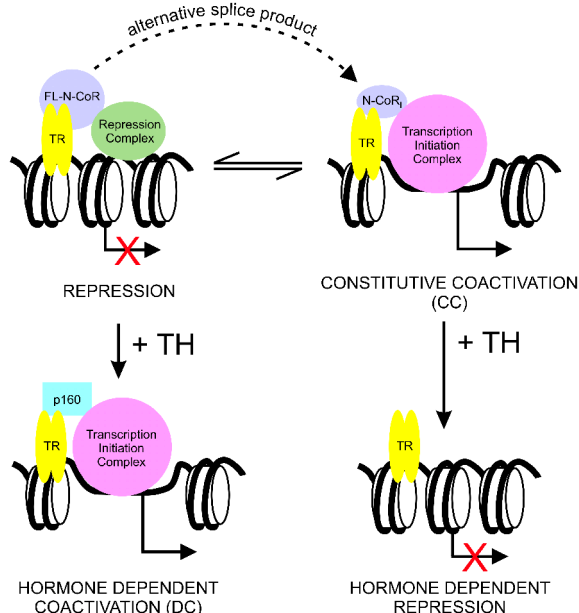


Figure 2. Schematic model in yeast of transcriptional regulation by corepressor or coactivator regulators Left Side: The transcriptional response of FL-N-CoR and a p160 coactivator in the absence and presence of TH. Addition of TH leads to hormone dependent coactivation (DC). Right Side: The constitutive coactivation (CC) of a natural splice variant N-CoR_I in the absence and presence of TH.

It must be acknowledged that experimental evidence for the physiological relevance of ligand independent

activation by N-CoR_I in mammalian cells is lacking. However, splice variants devoid of N-terminal repressor domains have been isolated in mammalian cells for N-CoR_I [Hollenberg et al., 1996] as well as s-SMRT [Chen and Evans, 1995; Ordentlich et al., 1999] and TRAC-1 [Sande and Privalsky, 1996]. Moreover, these splice variants have been observed to not only be devoid of repressor function but may also have a dominant-negative inhibitory effect on corepressor function [Chen and Evans, 1995; Hollenberg et al., 1996; Sande and Privalsky, 1996] and thereby serve as “anti-corepressors”. Thus, it remains a possibility that ratio differences in the cellular context of corepressor FL compared to their splice variants may result in either an overall anti-corepressor or perhaps a predominant coactivator influence on TR regulated genes.

Intriguingly, there is increasing recognition that splice variants of N-CoR and SMRT corepressors may be adapted and tailored by alternative mRNA splicing to different cells and to different developmental stages as a mechanism for regulating metazoan gene expression (see [Goodson et al., 2005a] and references therein). Moreover, differences in the expression of corepressors in different tissues and at various times of development suggest that organisms may adapt to their corepressor repertoire for distinct biological purposes [Jepsen et al., 2000]. Since a number of alternatively spliced variants have been shown to differ in their abundance at different times of development or in different tissues [Goodson et al., 2005b; Malartre et al., 2004; Short et al., 2005], further studies will be required to fully understand the biological properties of such sequences. It is likely that modules within N-CoR and SMRT regulate the repression or activation of target genes by recruiting domain specific transcriptional regulators (see [Goodson et al., 2005a] and references therein). Although the characterization of such regulators is still in its infancy, alternative splicing events could represent a mechanism by which cells can modulate varying degrees of corepression or coactivation of target genes.

It is possible that there are other mechanisms which could alter the ratio of full-length corepressors and their splice variants in mammalian cells. FL-N-CoR is targeted for proteasomal degradation by interaction with the mSiah2 ubiquitin ligase, which binds the N-terminal 160 amino acid residues [Zhang et al., 1998]. As this N-terminal region is absent in N-CoR_I, mSiah2 should preferentially degrade FL-N-CoR. This could alter the ratio of FL-N-CoR to N-CoR_I and potentially influence gene expression. mSiah2 expression is known to be cell-type specific [Zhang et al., 1998]. Interestingly, estrogen up-regulates mSiah2 expression leading to the subsequent degradation of FL-N-CoR [Frasor et al., 2005], suggesting the presence of a complex feedback pathway that could come into play under certain conditions. Future studies should focus on the identification and characterization of genes essential for CC or DC mediated gene activation by NRs. It is likely that some of these genes will play a role in regulating CoR function, whether by affecting their transcription, splicing, degradation or post-translational

modifications in normal, pathogenically infected or disease stage cellular contexts [Perissi and Rosenfeld, 2005].

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References

Anafi, M., Yang, Y. F., Barlev, N. A., Govindan, M. V., Berger, S. L., Butt, T. R. and Walfish, P. G. (2000) GCN5 and ADA adaptor proteins regulate triiodothyronine/GRIP1 and SRC-1 coactivator-dependent gene activation by the human thyroid hormone receptor *Mol Endocrinol* **14**, 718-32.

Chen, J. D. and Evans, R. M. (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors *Nature* **377**, 454-7.

Cheng, S. Y. (2000) Multiple mechanisms for regulation of the transcriptional activity of thyroid hormone receptors *Rev Endocr Metab Disord* **1**, 9-18.

Desai-Yajnik, V., Hadzic, E., Modlinger, P., Malhotra, S., Gechlik, G. and Samuels, H. H. (1995) Interactions of thyroid hormone receptor with the human immunodeficiency virus type 1 (HIV-1) long terminal repeat and the HIV-1 Tat transactivator *J Virol* **69**, 5103-12.

Desvergne, B. and Favez, T. (1997) The major transcription initiation site of the SV40 late promoter is a potent thyroid hormone response element *Nucleic Acids Res* **25**, 1774-81.

Frasor, J., Danes, J. M., Funk, C. C. and Katzenellenbogen, B. S. (2005) Estrogen down-regulation of the corepressor N-CoR: mechanism and implications for estrogen derepression of N-CoR-regulated genes *Proc Natl Acad Sci U S A* **102**, 13153-7.

Frisch, S. M. and Mymryk, J. S. (2002) Adenovirus-5 E1A: paradox and paradigm *Nat Rev Mol Cell Biol* **3**, 441-52.

Glass, C. K. and Rosenfeld, M. G. (2000) The coregulator exchange in transcriptional functions of nuclear receptors *Genes Dev* **14**, 121-41.

Goodson, M. L., Jonas, B. A. and Privalsky, M. L. (2005b) Alternative mRNA splicing of SMRT creates functional diversity by generating corepressor isoforms with different affinities for different nuclear receptors *J Biol Chem* **280**, 7493-503.

Goodson, M., Jonas, B. A. and Privalsky, M. A. (2005a) Corepressors: custom tailoring and alterations while you wait *Nucl Recept Signal* **3**, -

Heery, D. M., Kalkhoven, E., Hoare, S. and Parker, M. G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors *Nature* **387**, 733-6.

Hollenberg, A. N., Monden, T., Madura, J. P., Lee, K. and Wondisford, F. E. (1996) Function of nuclear co-repressor protein on thyroid hormone response elements is regulated by the receptor A/B domain *J Biol Chem* **271**, 28516-20.

Hu, X. and Lazar, M. A. (1999) The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors *Nature* **402**, 93-6.

Jepsen, K., Hermanson, O., Onami, T. M., Gleiberman, A. S., Lunyak, V., McEvilly, R. J., Kurokawa, R., Kumar, V., Liu, F., Seto, E., Hedrick, S. M., Mandel, G., Glass, C. K., Rose, D. W. and Rosenfeld, M. G. (2000) Combinatorial roles of the nuclear receptor corepressor in transcription and development *Cell* **102**, 753-63.

Malartre, M., Short, S. and Sharpe, C. (2004) Alternative splicing generates multiple SMRT transcripts encoding conserved repressor domains linked to variable transcription factor interaction domains *Nucleic Acids Res* **32**, 4676-86.

McKenna, N. J. and O'Malley, B. W. (2002) Combinatorial control of gene expression by nuclear receptors and coregulators *Cell* **108**, 465-74.

Meng, X., Yang, Y. F., Cao, X., Govindan, M. V., Shuen, M., Hollenberg, A. N., Mymryk, J. S. and Walfish, P. G. (2003) Cellular context of coregulator and adaptor proteins regulates human adenovirus 5 early region 1A-dependent gene activation by the thyroid hormone receptor *Mol Endocrinol* **17**, 1095-105.

Meng, X., Webb, P., Yang, Y. F., Shuen, M., Yousef, A. F., Baxter, J. D., Mymryk, J. S. and Walfish, P. G. (2005) E1A and a nuclear receptor corepressor splice variant (N-CoRI) are thyroid hormone receptor coactivators that bind in the corepressor mode *Proc Natl Acad Sci U S A* **102**, 6267-72.

Mittnacht, S., Lees, J. A., Desai, D., Harlow, E., Morgan, D. O. and Weinberg, R. A. (1994) Distinct sub-populations of the retinoblastoma protein show a distinct pattern of phosphorylation *Embo J* **13**, 118-27.

Ordentlich, P., Downes, M., Xie, W., Genin, A., Spinner, N. B. and Evans, R. M. (1999) Unique forms of human and mouse nuclear receptor corepressor SMRT *Proc Natl Acad Sci U S A* **96**, 2639-44.

Park, H. Y., Davidson, D., Raaka, B. M. and Samuels, H. H. (1993) The herpes simplex virus thymidine kinase gene promoter contains a novel thyroid hormone response element *Mol Endocrinol* **7**, 319-30.

Perissi, V. and Rosenfeld, M. G. (2005) Controlling nuclear receptors: the circular logic of cofactor cycles *Nat Rev Mol Cell Biol* **6**, 542-54.

Perissi, V., Staszewski, L. M., McInerney, E. M., Kurokawa, R., Krones, A., Rose, D. W., Lambert, M. H., Milburn, M. V., Glass, C. K. and Rosenfeld, M. G. (1999) Molecular determinants of nuclear receptor-corepressor interaction *Genes Dev* **13**, 3198-208.

Sande, S. and Privalsky, M. L. (1996) Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors *Mol Endocrinol* **10**, 813-25.

Short, S., Malartre, M. and Sharpe, C. (2005) SMRT has tissue-specific isoform profiles that include a form containing one CoRNR box *Biochem Biophys Res Commun* **334**, 845-52.

Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K. and Rosenfeld, M. G. (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function *Nature* **387**, 677-84.

Walfish, P. G., Yoganathan, T., Yang, Y. F., Hong, H., Butt, T. R. and Stallcup, M. R. (1997) Yeast hormone response element assays detect and characterize GRIP1 coactivator-dependent activation of transcription by thyroid and retinoid nuclear receptors *Proc Natl Acad Sci U S A* **94**, 3697-702.

Xu, H. E., Stanley, T. B., Montana, V. G., Lambert, M. H., Shearer, B. G., Cobb, J. E., McKee, D. D., Galardi, C. M., Plunket, K. D., Nolte, R. T., Parks, D. J., Moore, J. T., Kliewer, S. A., Willson, T. M. and Stimmel, J. B. (2002) Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha *Nature* **415**, 813-7.

Zhang, J., Guenther, M. G., Carthew, R. W. and Lazar, M. A. (1998) Proteasomal regulation of nuclear receptor corepressor-mediated repression *Genes Dev* **12**, 1775-80.

Zuo, F., Kraus, R. J., Gulick, T., Moore, D. D. and Mertz, J. E. (1997) Direct modulation of simian virus 40 late gene expression by thyroid hormone and its receptor *J Virol* **71**, 427-36.