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# Topic 1.2
# Nuclear receptor coregulators*

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*Abstract*: It has been postulated that nuclear receptors (NRs) regulate transcription via interactions with chromatin and the basal transcription machinery at the promoters of genes. Coregulators (coactivators or corepressors) are important in mediating these interactions and thereby modulating positive or negative receptor activity. A large number of putative coactivators have been isolated, several of which will be reviewed with respect to certain "criteria" initially proposed for coactivators. We will discuss, with reference to in vitro and in vivo experiments, the main steps in initiation that are influenced by coactivators: (1) initiation (e.g., SRC-1 family, CBP); (2) repetitive transcription (e.g., TRAPs/DRIPs); (3) RNA processing (PGC-1, etc); and (4) termination/turnover (E6-AP, etc). A variety of enzyme functions have been implicated in the coactivator complex including acetylase, methylase, ubiquitin ligase, kinase, and phosphatase activities. Moreover, coactivators and corepressors appear to exist in the steady-state cell as a series of multiprotein complexes referred to collectively as the "coregulatorsome". Different subcomplexes within the coregulatorsome may have different levels of preference for individual receptors or promoters, likely contributing to context-specific functions of NRs in target tissues.

## INTRODUCTION

Nuclear receptors (NRs) are transcription factors that respond to modulation by lipophilic ligands and other signaling pathways to regulate the expression of genes in a tissue- and context-selective manner [1,2]. Recent evidence suggests that they achieve this effect by interacting with a group of molecules collectively referred to as coregulators. NR coregulators are defined as cellular factors recruited by NRs that complement the activity of NRs. They are generally divisible into coregulators that promote positive receptor activity when recruited (coactivators) and those that mediate negative receptor activity (corepressors). Receptor activity is generally thought to involve the interaction of the receptor with specific promoter sequences (hormone response elements) to activate or repress target genes, although receptors can influence events outside the nucleus ("nongenomic" action). In this report, we summarize selected advances in the coregulator field to date. While recognizing the importance of corepressor function in receptor action, we will focus our discussion on coactivators.

Using a variety of experimental techniques, from in vitro experiments to cultured cells, to null deletions in living animals, our laboratory and others initially established a number of criteria for designation of a molecule as a coactivator. These included: enhancement of the transcriptional activity of a receptor, demonstrated by addition and subtraction in cultured cells; relief of "squelching", or transcriptional interference between receptors competing for a limited, common pool of transcriptional me-

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diators; and nuclear localization, reflecting the fact that many of these molecules appeared to be nuclear entities in most cells. Since then, an appreciation has grown of tissue-specific variations in coactivators' mode of action, their cellular localization, and their receptor/signaling pathway response preferences, all of which appear to be influenced, at least in part, by differential programming of these molecules by phosphorylation and other posttranslational modifications. The net result of this has been to broaden the compass within which NR coactivator functions are interpreted, and to define them simply as molecules that interact with and mediate NR functions. Due to space constraints, the reader is referred to more extensive reviews [3,4]

## COACTIVATORS: A TENTATIVE CLASSIFICATION

Coactivators can be divided into a series of functional groups which, while structurally distinct, are functionally coordinated during transcriptional initiation by NRs. Over the past several years, rapid progress has been made toward the goal of establishing the mechanistic principles that govern the interaction between receptor, coactivator, and promoter in the activation of target genes. A composite model of ligand-mediated receptor activation envisages at least four main steps, each appearing to involve the mediation of distinct categories of coactivators: initiation, repetitive transcription (or reinitiation), RNA processing, and termination/turnover.

In NR-mediated transcriptional initiation, coactivators considered to be among the first recruited by activated receptor are the SRC/p160s, CREB-binding protein (CBP), and p300 [3,4 and refs. therein]. (Note that this does not preclude the initial involvement of ATP-coupled chromatin domain-remodeling machines such as the SWI/SNF complex, components of which have been shown to be required by individual receptors for efficient transcriptional initiation in certain experimental systems—see also refs. [3,4].) It was in the SRC/p160 family that an amphipathic helix conserved on the surface of most coactivators was demonstrated (the LXXLL motif or NR box) [5], which substantially determines the interaction between ligand-activated receptors and these molecules. The interface between ligand-dependent receptor motifs and the LXXLL motif has been the subject of intense study as a possible target for manipulation of NR pharmacology, and as a flexible, informative basis for ligand screening assays.

The physiological importance of SRC/p160s has been implied by knock-out studies of genes encoding these coactivators. Although the phenotypes of these knock-outs are largely subtle in nature, they provide clues as to their functions. SRC-1 knock-out mice show a partial resistance to hormones and a reduced growth and development of various steroid target organs [6]. SRC-3 knock-out mice show reduced growth and female reproduction, and lack of mammary gland development. In addition, mouse embryonic fibroblasts or liver cells derived from these SRC-$3^{-/-}$ mice are reported to be insensitive to growth stimulation by IGF-1 or growth hormone [7,8]. The participation of SRC-3 in cell growth is further supported by its role in various cancers—indeed, the correlation between SRC-3 expression and cancers is striking. It has been demonstrated that SRC-3 is amplified in 5–10 % of breast tumors and 7–8 % of ovarian cancer samples [9].

SRC/p160s and CBP/p300 contain acetyltransferase activity that targets specific lysines in nucleosomal histones to generate a transcriptionally permissive environment at NR-regulated promoters. The histone targeting specificity and the relative contribution of SRC/p160s and CBP or p300 to histone modification at various promoters may to some extent determine differential patterns of gene expression in various tissues, and in the same tissue in response to different signals. More recently, it has been shown that this acetyltransferase activity is used to choreograph specific protein–protein interactions during receptor-mediated assembly of the preinitiation complex [10].

The sequential model of NR-mediated transcriptional initiation suggests that following initial recruitment of SRC/p160s and CBP to effect modification of local histones and other proteins, members of the TRAP/DRIP complex directly contact components of the basal transcription machinery. The evolutionary conservation of various guises of this complex, from the yeast Mediator through to human

SMCC and others, is reflected in the fact that targeted deletion of the receptor-interacting subunit (TRAP220) results in embryonic lethality [11]. This phenotype stands in contrast to the less severe phenotypes of the SRC-1 and p/CIP/SRC-3 null mice, which exhibit a variety of partial aberrations in specific NR- and non-NR-mediated signaling pathways. PGC-1, a coactivator originally thought to be a receptor-specific coactivator for PPARγ, has been shown to interact with a variety of receptors, as well as with components of the RNA splicing machinery [12]. These studies raise the intriguing possibility that, through PGC-1 and similar coregulators, NRs might specify tissue- and ligand-specific edits of individual primary RNAs, further contributing to the functional diversity of NR signaling.

To continue the theme of employing posttranslational modifications to achieve efficient transcriptional initiation, the role of targeted ubiquitination in tagging components of the various complexes for removal and recycling is attractive from the point of view of refurnishing the promoter for subsequent rounds of transcriptional reinitiation. The E3 ubiquitin ligase E6-AP was the first ubiquitin ligase to be shown to coactivate NRs [13], and though the enzyme and transactivation functions of E6-AP are separable, the fact that the 26S proteasome is required for efficient ER-mediated transcriptional activation points to a fundamental role for protein turnover in receptor-mediated transactivation.

## ROLE OF KINASE-BASED SIGNALING PATHWAYS

Studies on characterized NRs indicate that ligand-independent modulation through posttranslational modification is a common currency in control of protein–protein interactions between NR and "non-NR"-regulated pathways. While intricate patterns of reciprocal auto- and heterophosphorylation have long been known to mediate the functions of membrane-associated receptors, recent studies suggest that such modifications also directly influence protein–protein interactions in NR-regulated pathways. In addition, enzyme functions of coregulators themselves appear to target receptors, histones, and coregulators themselves, to modulate their molecular interactions [reviewed in 3,4]. It seems that coactivators can, depending upon the phosphorylating kinase, be commandeered by any one of a number of such pathways, with their ultimate promoter specificity being determined by the specific "phosphosignature" they bear. Evidence suggests that these modifications may be able to effect changes in coregulator concentration in individual intracellular compartments, alter functional specificity, or determine the final transcriptional complexes into which they are recruited. The term "coregulatorsome", coined to account for the myriad protein–protein interactions in which coactivators participate, likely represents a heterogeneous continuum of complexes, whose composition and promoter specificity are under constant scrutiny and revision according to a variety of parameters (Fig. 1). Discrimination by receptors between these complexes likely occurs on a trial-and-error basis according to the specific requirements of the promoter at a defined point in time (see refs. [3,4] for review).

## ROLE OF LIGANDS

Ligands have been shown to be capable of influencing the pharmacokinetics of the interaction between the receptor AF-2 and the coregulator NR box (references), hinting at a possible basis for the endocrine activity of many exogenous, nonphysiological ligands. To illustrate this, it is becoming apparent that the type of agonist bound to a specific receptor is an important determinant of its affinity for a particular subset of coactivator complexes, thereby ultimately influencing the biological response to the ligand. In the case of the estrogen receptor (ER) and vitamin D receptor (VDR), ligands and ligand derivatives may elicit their distinct biological responses through effecting differential interactions of ligand-bound VDR with coactivators (reviewed in ref. [14]). Similar findings have been observed with PPAR ligands that specify distinct patterns of SRC recruitment by members of the PPAR family. The potential role of selective estrogen receptor modulators (SERMs) in influencing ER function in a tissue-specific manner has been the subject of much recent interest, and it appears that selective coregulator recruitment may contribute in part to the custom pharmacology of many of these compounds [15–17].

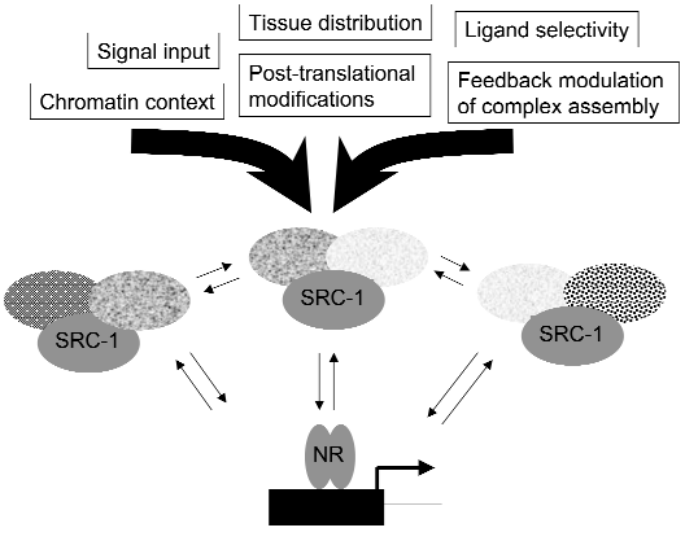


**Fig. 1** Order and disorder in transcriptional complex assembly by nuclear receptors. A variety of factors influence the composition of coregulator complexes recruited by NRs (boxes). The "coregulatorsome" is likely defined by a spectrum of complexes in continuous flux, which are incorporated into the final transcriptional complex according to the specific requirements at a given time point (heterogeneity in SRC-1 complexes is shown as an example).

## COREPRESSORS

In addition to potentiation of gene expression, many NRs possess a silencing, or repression function [18]. As was anticipated, this silencing function requires the participation of corepressors, of which SMRT and NCoR have been the most intensively studied (for a review, see ref. [3]). Corepressors were initially identified as receptor-interacting proteins in the absence of ligand, with this interaction being uncoupled in the presence of ligand. Just like their coactivator counterparts, corepressors play important roles in both health and disease. For example, both SMRT- and NcoR-mediated repression have been shown to play a role in promyelocytic and myelogenous leukemias as well as thyroid hormone resistance. Mutation of NCoR in mice results in defects in CNS, erythrocyte, and thymocyte development [19]. The association of corepressors with histone deacetylases [20] suggests that one of the primary functions of corepressors is to transform local chromatin structure to an inactive conformation by deacetylating histones. Remarkably, corepressors utilize an amphipathic helix related to the NR box (referred to as the "CoRNR" motif) that recognizes unliganded NRs in a manner that is inhibited by the activation helix of AF2 (H12) [21]. The mechanisms underlying coactivator and corepressor interactions with liganded and unliganded receptors, respectively, are therefore surprisingly similar, given their radically opposed transcriptional consequences.

## FUTURE RESEARCH DIRECTIONS

It is likely that animal models will soon be developed in which the contribution of coactivators to the organization of genes along select metabolic pathways can be more closely scrutinized. These animal models may hold the key to examining in a more "physiological" context the functional relationships between SRMs and their ability to influence gene expression in a tissue-specific manner. Moreover, the potential for combination SRM/NR box peptide therapeutics holds much promise for the future, given its potential for further fine-tuning the tissue-specificity of SRM function. For example, the administration of a specific modulator along with a peptide that might inhibit a specific receptor conformation might abolish an unwanted effect while retaining a desirable effect of the SRM. In conclusion, a full ap-

preciation of coregulator biology will involve characterization of both ligand-dependent and -independent activation pathways, and their role in determining the interactions that contribute to the regulation by NRs of complex spatiotemporal patterns of gene expression.

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