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The roles of protein–protein interactions and protein methylation in transcriptional activation by nuclear receptors and their coactivators☆

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Abstract

Hormone-activated nuclear receptors (NR) bind to the promoters of their target genes and recruit coactivator proteins to help activate transcription. The p160 coactivators bind directly to activated NRs and recruit secondary coactivators CBP/p300 with protein acetyltransferase activity and CARM1 with protein methyltransferase activity. To further investigate the components of the p160 coactivator complex and their mechanisms of action, we have used two guiding assumptions. First, the coactivators constitute a signal transduction pathway that convey the signal from DNA-bound NRs to the transcription machinery. Second, each coactivator has signal input and signal output domains that facilitate signal transduction. These assumptions were used to address the mechanism by which CARM1 and the N-terminal region of p160 coactivators transmit activating signals to the transcription machinery. The p160-binding activity of CARM1 is in the same centrally located structural domain as the methyltransferase activity; the p160-binding domain anchors CARM1 to the target gene promoter and thereby serves as its signal input domain. CARM1 has two signal output mechanisms: the protein methyltransferase activity, which methylates histones and other proteins in the transcription initiation complex; and a strong autonomous activation function in the C-terminal region. We identified a protein, CCCP, which binds to the C-terminal region of CARM1 and cooperates synergistically with CARM1 to enhance NR function. We also defined the N-terminal region of p160 coactivators as another signal output domain, which binds a novel coactivator called coiled-coil coactivator (CoCoA). CoCoA acts synergistically with p160 coactivators to enhance NR function.

Keywords: Nuclear receptor; Coactivator; Signal transduction; Protein methylation; CARM1; p160 coactivator

1. Introduction

Activation of transcription from the promoter of a gene involves the binding of transcriptional activator proteins to specific DNA sequences in the promoter region which serve as enhancer elements. The DNA-bound transcriptional activator protein either brings with it or subsequently recruits several different complexes of coactivator proteins which locally remodel the chromatin structure in the promoter region and help to recruit and activate RNA polymerase II and its associated basal transcription machinery [1–3].

1.1. Multiple NR coactivators contribute in complementary ways to transcriptional activation

The nuclear receptors (NR) are one family of transcriptional activator proteins for which there has recently been rapid progress in defining the coactivators which mediate transcriptional activation. The approximately 50 members of the mammalian NR family include the receptors for steroid hormones, thyroid hormone, Vitamin D, and retinoic acid as well as receptors for other hormones and a group of orphan receptors for which there is no ligand or the ligand is currently unknown [4–6]. There is strong evidence for the participation of multiple complexes of coactivator proteins in chromatin remodeling and in recruitment and activation of RNA polymerase II by NRs. Each complex accomplishes a specific set of tasks which contribute to the overall process of transcriptional activation of target gene promoters by NRs [2,3,7]. The SWI/SNF coactivator complex

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and other similar complexes have an ATP-dependent nucleosome remodeling activity which contributes to the local alteration of chromatin structure in the promoter of the target gene. The p160 coactivator complexes, which are the subject of this article, also contribute to chromatin remodeling, but through a different mechanism that involves catalysis of covalent histone modifications such as acetylation and methylation. The p160 coactivator complexes may also use protein–protein interactions to help (directly or indirectly) to recruit or activate the basal transcription machinery. The TRAP/DRIP complex interacts directly with RNA polymerase II and apparently directly participates in the recruitment and activation of the polymerase. Although substantial progress has been made recently in defining the subunit composition of these coactivator complexes and the specific roles played by each subunit in the process of transcriptional activation, it is likely that additional subunits and many more mechanistic details remain to be discovered.

1.2. Guiding assumptions for identifying NR coactivators and their mechanisms of action

One effective strategy for studying the mechanism of transcriptional activation by NRs and their coactivators has been to define the specific functional domains of each protein and investigate the mechanism by which each functional domain contributes to the activity of the protein. For example, NRs have a DNA-binding domain (DBD) which recognizes a specific enhancer element, a hormone-binding domain which binds a specific hormone and thereby induces the active conformation of the NR, and (generally) two activation domains by which the hormone-activated and DNA-bound NR recruits coactivators to the target gene promoter [2,4,5]. Many coactivators, including the p160 coactivators, were discovered by virtue of their binding to NR activation domains [2,3,7].

For defining relevant coactivators and studying their mechanisms of action, we have used two guiding assumptions (Fig. 1). First, each coactivator or coactivator subunit occupies a specific position in a signal transduction pathway which transmits an activating signal from the enhancer element-bound NR to the transcription machinery. For convenience, we think of the NR as being at the upstream end of the pathway and RNA polymerase II as being at the downstream end. Second, two types of functional domains will be found in most or all coactivators: one or more signal input domains to receive the signal from the upstream pathway components with which they interact; and one or more signal output or activation domains to convey the signal to downstream components of the pathway (Fig. 1). Input domains allow a coactivator to be recruited to the promoter, by direct binding to the NR or to an upstream coactivator which is bound directly or indirectly to the NR. The contribution of the output or activation domain to the transcriptional activation process may be by catalysis of an

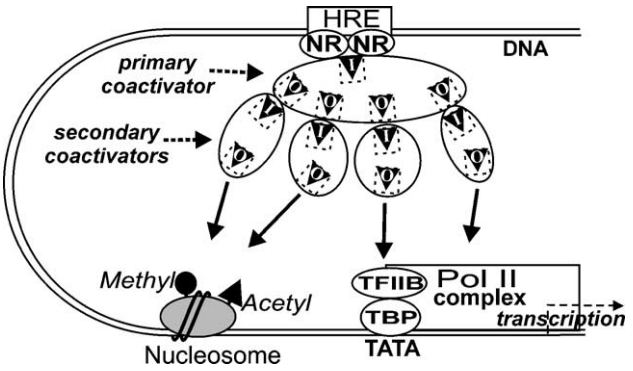

Fig. 1. NR coactivators as components of a signal transduction pathway. NRs bound to an enhancer element or hormone response element (HRE) in a target gene promoter recruit complexes of coactivators to accomplish the transcriptional activation process. The coactivator subunits in each complex form a signal transduction pathway and transmit the activating signal from the NR to the transcription machinery by performing specific tasks. Domains that anchor a coactivator subunit to the promoter by binding to upstream components are signal input domains (triangles labeled *I*). Domains that transmit the activating signal by binding to or enzymatically modifying downstream components of the pathway are signal output domains (triangles labeled *O*). Coactivators that bind directly to NRs are called *primary coactivators*. Coactivators that do not bind directly to NRs but are recruited to the promoter by binding to other coactivators are called *secondary coactivators*. The ultimate downstream targets of the activating signal include: the chromatin, which is structurally remodeled by alteration in *nucleosome* structure and addition of covalent modifications (e.g. *acetyl* and *methyl* groups) to histones; and recruitment of RNA polymerase II and its associated basal transcription machinery (*Pol II complex*), including basal transcription factors such as *TFIIB* and *TBP*, which binds to *TATA* box sequences in the basal promoter region.

enzymatic reaction (e.g. acetylation or methylation of histones or other proteins) or may be through a protein–protein interaction which results in recruitment or activation of another downstream coactivator or component of the transcription machinery. We use the name primary coactivator for those that bind directly to NRs and can enhance NR function in transient transfection assays without the assistance of other coactivators; we refer to as secondary coactivators those that are recruited to the promoter primarily through contact with an upstream coactivator and can only enhance NR function in cooperation with that upstream coactivator.

1.3. Questions addressed in this article

Currently, two secondary coactivators have been shown to be part of the p160 coactivator complex and collaborate with p160 coactivators to mediate transcriptional activation by NRs: the histone acetyltransferase CBP (or the related protein p300), and the histone methyltransferase CARM1 [2,3]. The binding sites for CBP/p300 and CARM1 are on the C-terminal end of p160 coactivators and serve as signal output domains. The NR-binding domains, which serve as the signal input domains, are located in the central region and at the C-terminal ends of the p160 polypeptide chains (Fig. 2). This article will focus on defining additional

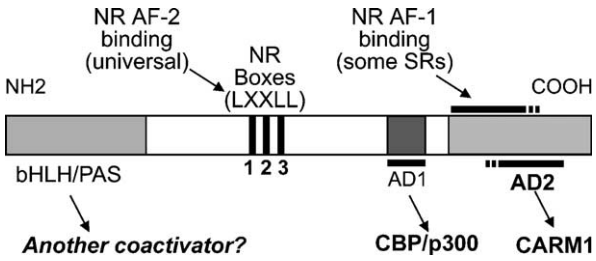

Fig. 2. Functional domains of p160 coactivators. Major functional domains of the p160 coactivators are connected to their upstream or downstream binding partners by the *arrows*, which indicate the direction of signal transduction. *NH2*, amino terminus; *COOH*, carboxy terminus; *AD*, activation domain of p160 coactivators; *AF*, activation functions or domains of NRs.

components of the p160 coactivator complex and the mechanisms by which p160 coactivators and CARM1 contribute to the transcriptional activation process. We will address two questions: (1) What are the signal input and output domains of CARM1, and how do the signal output domains function? (2) Does the N-terminal region of p160 coactivators, which is the most highly conserved part of the p160 coactivator family but has no currently known role in coactivator function, serve as an additional signal output domain?

2. Guiding examples from previous work: signal input and output domains of p160 coactivators and CBP/p300

2.1. Direct NR-binding recruits p160 coactivators to the promoter, and p160 activation domains AD1 and AD2 transmit the signal downstream

The p160 coactivators and other currently know components of the p160 coactivator complex can be used to illustrate how the above assumptions, about the NR coactivator signaling pathway and the existence of specific signal input and output domains on coactivators, apply to our current state of knowledge and can be used again to advance our understanding of additional NR coactivators and their mechanisms of action. The p160 coactivators, which include SRC-1, GRIP1/TIF2, and pCIP/ACTR/AIB1/RAC3/TRAM1, are 160 kDa proteins with two different domains which bind directly to NRs [2,3]. Three so-called NR box motifs, each comprising an amphipathic alpha-helix containing the sequence LXXLL (L = leucine, X = any amino acid), are located in the central region of the p160 polypeptide chain. The LXXLL motifs bind the AF-2 activation domain of NRs, which forms a conserved hydrophobic cleft on the surface of the hormone-binding domain of all NRs which serve as transcriptional activators [8,9]. The C-terminal region of p160 coactivators also can bind to the AF-1 activation domain of at least some steroid receptors [10] (Fig. 2). These NR-binding domains serve as signal input domains by anchoring p160 coactivators to the promoter and connecting p160 coactivators with the upstream end of the signaling pathway. So far two activation or signal output domains, AD1 and AD2 in the C-terminal region of p160 coactivators, have been defined. AD1 is a binding site for the secondary NR coactivator CBP and its relative p300, which are histone acetyltransferases [11]. AD2 may bind many proteins, among which is the protein arginine methyltransferase (PRMT) CARM1 which also serves as a secondary coactivator [12,13] (Fig. 2). There is considerable evidence that these three coactivators cooperate in contributing to the process of transcriptional activation by NRs. The p160 coactivators can form a ternary complex with CBP/p300 and CARM1; under carefully defined conditions where limiting levels of the NRs are expressed, these three coactivators function synergistically to enhance NR function in transient transfection assays [14]; and by chromatin immunoprecipitation assays all three coactivators were found to be recruited to steroid hormone-regulated promoters in a hormone-dependent manner [15–17].

2.2. CBP and p300 are anchored to NR-regulated promoters through their p160-binding domain, while their protein acetylation and multiple protein–protein interaction domains provide signal output

CBP and p300 have binding sites for many different DNA-binding transcriptional activator proteins and also for some coactivators, including the p160 coactivators [2,11,18,19]. For DNA-binding transcriptional activators such as CREB and AP-1, CBP/p300 bind directly to the transcriptional activator and thus function as primary coactivators. However, when CBP/p300 cooperate with most (but perhaps not all) NRs, there is considerable evidence that the binding of CBP/p300 to p160 coactivators is primarily responsible for recruiting them to the promoter, and they thus function as secondary coactivators for most NRs [13,20]. Thus, CBP and p300 have multiple potential signal input domains, and different signal input domains can be used in different promoter contexts. The specific C-terminal site in CBP/p300 which binds to p160 coactivators thus serves as their signal input domain in NR-mediated transcriptional activation. CBP/p300 can potentially contribute to transcriptional activation through two different types of activation or signal output domains. The histone acetyltransferase activity acetylates histones, which contributes to chromatin remodeling, and acetylates other proteins in the transcription machinery which contributes in ways that are still being determined [16,21]. The many protein interaction domains of CBP and p300 may also contribute to transcriptional activation by serving as signal output domains. For example, CBP/p300 bind the basal transcription factors TFIIB and TBP and thus may directly help to assemble and activate the basal transcription machinery on the promoter [11,21]. Thus, once it is recruited to the promoter through

its signal input domain, a single coactivator may contribute to transcriptional activation through multiple signal output domains (see example in Fig. 1).

3. Signal input and output domains of CARM1

3.1. The GRIP1-binding domain recruits CARM1 to the promoter

CARM1 shares a large homologous domain with other members of the protein arginine methyltransferase family. This homologous domain contains the methyltransferase activity and is also responsible for formation of homo-dimers or larger homo-oligomers [22]. Each member of the PRMT family also has a unique N-terminal domain, and CARM1 also has a unique C-terminal domain. In spite of their homology in the methyltransferase domain, each member methylates a unique set of protein substrates [23,24]. We used a series of deletion and point mutations to define further the various functional domains of CARM1 and, in particular, to determine which of them served as signal input and output domains to mediate CARM1s coactivator function. Since CARM1 functions as a NR coactivator only in the presence of a co-expressed p160 coactivator with an intact C-terminal CARM1-binding domain [12,13], the p160-binding domain of CARM1 must function as its signal input domain which is responsible for recruiting CARM1 to the promoter. Analysis of deletion mutants indicated that the p160-binding domain was located in the same conserved region that contains the methyltransferase activity [25] (Fig. 3). Since this large domain is one structural unit [22], additional deletions within this region could not be used to assign the p160-binding site to smaller subregions of this conserved domain.

3.2. Methylation of histone H3 and possibly other proteins by CARM1 contributes to the transcriptional activation process

To define the signal output domains of CARM1, the CARM1 deletion and point mutants were tested for coactivator function in transient transfection assays using low-NR

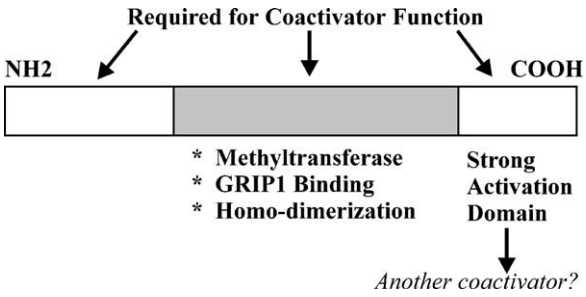

Fig. 3. Functional domains of CARM1. Locations of specific CARM1 functions are indicated within the central domain, which is conserved throughout the protein arginine methyltransferase family, or the unique N-terminal (*NH2*) or C-terminal (*COOH*) domains.

conditions, under which p160-CARM1-p300 synergy is observed; under these conditions, very little or no enhancement of NR function is observed unless all three coactivators are co-expressed by transient transfection [14]. We defined a point mutation which eliminated the methyltransferase activity of CARM1 without affecting the ability of CARM1 to bind p160 coactivators. Under the p160-CARM1-p300 synergy conditions, this mutation eliminated most of the NR coactivator function of CARM1 [14]. This indicates that the methyltransferase activity of CARM1 serves as a key signal output domain for the coactivator function of CARM1. Another indication that the methyltransferase activity of CARM1 is important for its synergy with p160 coactivators and CBP/p300 came from our demonstration that other members of the PRMT family, which methylate different protein substrates than CARM1, cannot function synergistically as coactivators with CBP/p300 [14]. What proteins are methylated by CARM1 during the transcriptional activation process? CARM1 can methylate histone H3 in vitro [12] and in vivo [15], and we demonstrated by chromatin immunoprecipitation assays that methylation of histone H3 occurs locally in steroid hormone-regulated promoters as part of the hormone-induced transcriptional activation process [15]. Thus, histone H3 is an important methylation target of CARM1. CARM1 methylation of CBP/p300 was also recently reported to contribute to NR-mediated transcriptional activation [26], and methylation of other currently undefined proteins could also contribute to the transcriptional activation process.

3.3. The unique N- and C-terminal regions of CARM1 apparently function as signal output domains

Deletion mutants of CARM1 were used to test whether other CARM1 domains, in addition to the methyltransferase and p160-binding domains, are also important for coactivator function. Deletion of the N-terminal region (amino acids 1–120) or the C-terminal region (amino acids 501–608) of CARM1 left the methyltransferase (signal output) and p160-binding (signal input) activities intact but essentially eliminated the coactivator function of CARM1 [25]. These results indicate that the N- and C-terminal domains of CARM1, along with the methyltransferase domain, are essential to achieve effective signal output for the coactivator function of CARM1 (Fig. 3). Since the unique N- and C-terminal regions of CARM1 have no known enzymatic function, we assume that they contribute to signal output through protein–protein interactions with downstream components of the signaling pathway, i.e. they may anchor other coactivators to the p160 coactivator complex or may interact directly with components of the chromatin or basal transcription machinery. The C-terminal region of CARM1 harbors a strong autonomous transcriptional activation function, i.e. when fused to Gal4 DBD it can efficiently activate transcription of a reporter gene controlled by Gal4 enhancer elements in transient transfection assays in

mammalian cells [25]. Neither the unique N-terminal domain nor the methyltransferase domain of CARM1 exhibits any autonomous activation function. It is interesting to note that the lack of autonomous transcriptional activation activity by the CARM1 methyltransferase domain corresponds with our observation that the methyltransferase activity of CARM1 alone is not sufficient to allow CARM1 to achieve efficient signal output as a coactivator [25].

3.4. A coactivator that binds the C-terminal activation domain of CARM1 and synergistically cooperates with CARM1 as a coactivator

To search for potential downstream targets, we performed a yeast two-hybrid screen of a mouse 17-day embryo cDNA library, using the C-terminal region of CARM1 as bait. Here, we report preliminary results obtained with a full length cDNA clone of one of the identified binding partners, which we will call CARM1 C-terminal coactivator partner (CCCP) (C. Teyssier, M.R. Stallcup, unpublished data). CCCP bound to the C-terminal domain of CARM1 in yeast two-hybrid, mammalian two-hybrid, and GST pull down assays, indicating a direct interaction that can occur in vitro and in vivo. In transient transfection assays with NRs, CCCP exhibited little or no coactivator activity by itself or when co-expressed with a p160 coactivator or with CARM1. However, when a p160 coactivator, CARM1, and CCCP were all expressed together, there was a dramatic synergy which produced NR-dependent reporter gene activity that was up to 20 times higher than that achieved with any combination of two of the three coactivators. Thus, our preliminary results suggest that CCCP is a good candidate for a coactivator that is downstream from the C-terminal signal output domain of CARM1, and thus may be an important mediator of CARM1 coactivator function, part of the p160 coactivator complex, and part of the NR coactivator signal transduction pathway (Fig. 4).

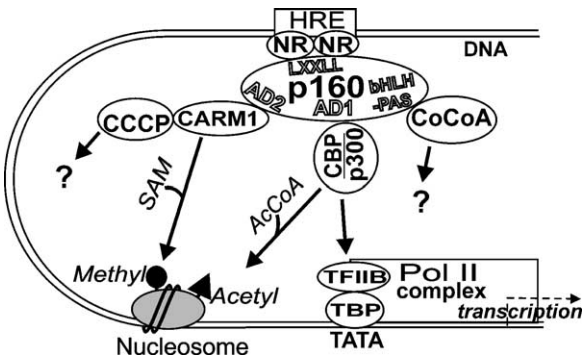

Fig. 4. A working model of the p160 coactivator complex. The coactivators discussed in this article as part of the p160 coactivator complex are shown with *arrows* to indicate their downstream targets. Major input and output domains of the p160 coactivator are labeled with *hollow letters* and arranged next to their upstream or downstream protein partners. The cofactors acetyl-CoA (*AcCoA*) for acetylation and S-adenosylmethionine (*SAM*) for methylation of histones are shown with the appropriate coactivators.

4. AD3, a new signal output domain in the N-terminal region of p160 coactivators, binds a novel coactivator

4.1. The N-terminal region of p160 coactivators may function as a signal input or signal output domain in different contexts

As described earlier, the two known NR interaction or signal input domains of p160 coactivators and the two known activation or signal output domains are all located in the central and C-terminal regions of the p160 coactivators (Fig. 2). However, the N-terminal region of these proteins, which contains one basic-helix–loop–helix (bHLH) domain and two Per-ARNT-Sim (PAS) domains, is the most highly conserved part among the members of this protein family, with 60% amino acid sequence identity over more than 300 amino acids [27]; and yet its role in NR coactivator function is unknown. Other transcriptional activator proteins containing bHLH-PAS domains include the aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (ARNT), and hypoxia inducible factors (HIF) [28]. The bHLH-PAS domains are believed to function as protein-interaction domains, although some bHLH domains can also function in DNA-binding and dimerization. In fact, several other proteins have already been shown to bind to the N-terminal regions of p160 coactivators, including some DNA-binding transcriptional activators (e.g. MEF2C and TEF4) [29,30] and some coactivators (e.g. hMMS19 and BAF57) [31,32]. Such a large domain may be expected to have multiple protein interaction surfaces and thus multiple functions. The p160 coactivators are known to enhance transcriptional activation by many different classes of transcriptional activator proteins, in addition to NRs and including those identified as binding partners for the p160 bHLH-PAS domain [29,30]. Thus, DNA-binding transcriptional activators such as MEF2C and TEF4 apparently recruit p160 coactivators by binding to the N-terminal bHLH-PAS domain. The discovery that other coactivator proteins (i.e. hMMS19 and BAF57) can also bind to the p160 N-terminal region suggests that this domain may also function as a signal output domain. Our preliminary results (J.H. Kim, M.R. Stallcup, unpublished data) as well as some published studies [33] suggest that the p160 N-terminal region is indeed necessary for p160 coactivator function with NRs, but whether hMMS19, BAF57, or any other binding partners are required downstream targets of this signal output domain has yet to be directly demonstrated.

4.2. CoCoA, a novel coactivator that binds to the AD3 activation domain in the N-terminal region of p160 coactivators

To search for additional protein interaction partners of the bHLH-PAS domain of p160 coactivators, we performed a yeast two-hybrid screen of a mouse 17-day embryo cDNA library, using the N-terminal 479 amino acids of GRIP1 as

bait. One of the interacting partners identified in this screen was a previously uncharacterized 691-amino acid protein, which we named coiled-coil coactivator, or CoCoA (J.H. Kim, H. Li, M.R. Stallcup, submitted). As the name suggests, CoCoA contains a coiled-coil domain, located approximately in the center of the polypeptide chain. Coiled-coil domains consist of two-to-five alpha helices bundled into a super-helical conformation and are thought to function as protein interaction domains [34]. The coiled-coil domain was shown in vitro and in vivo to be responsible for the binding of CoCoA to the GRIP1 N-terminal region. To test whether CoCoA may help to mediate the coactivator function of GRIP1, we first demonstrated that the GRIP1 N-terminal domain fused to the Gal4 DBD had a weak autonomous activation function in mammalian cell transient transfections; co-expression of CoCoA dramatically stimulated this activity. CoCoA also cooperated synergistically with GRIP1 to enhance NR-mediated transcriptional activation in transient transfection assays. Mutants of GRIP1 lacking the N-terminal region still retained partial coactivator function but failed to cooperate with CoCoA, whereas GRIP1 mutants lacking the C-terminal AD2 domain but containing an intact N-terminal domain retained the ability to cooperate synergistically with CoCoA as NR coactivators (J.H. Kim, H. Li, M.R. Stallcup, submitted). Thus, CoCoA is recruited to the promoter as a secondary coactivator through the binding of its coiled-coil domain to the N-terminal region of p160 coactivators (Fig. 4). These results further suggest that the N-terminal region of p160 coactivators contains as a third activation or signal output domain, which we call AD3.

4.3. Signal input and output domains of CoCoA

Since the coiled-coil domain of CoCoA apparently functions as a signal input domain, we used deletion mutants of CoCoA to define possible signal output domains. We determined that the ability of CoCoA to enhance transcriptional activation by the GRIP1 N-terminal domain fused to Gal4 DBD depended on the coiled-coil domain as well as the C-terminal domain of CoCoA, suggesting that the C-terminal domain may function as a signal output domain for CoCoA. By fusing various fragments of CoCoA to the Gal4 DBD, we demonstrated in mammalian cell transient transfection assays that the C-terminal region of CoCoA contains an autonomous activation function (J.H. Kim, H. Li, M.R. Stallcup, submitted). Thus, we propose that CoCoA is another important component of the coactivator signal transduction pathway which mediates transcriptional activation by NRs (Fig. 4); that CoCoA may be part of the p160 coactivator complex and binds to p160 coactivators through its coiled-coil signal input domain; and that CoCoA propagates the activation signal at least partly through interaction of its C-terminal signal output domain with a currently unidentified downstream coactivator or component of the transcription machinery. Additional studies using chromatin immunoprecipitation and functional knock-out or knock-down techniques will be important for further testing the involvement of CoCoA, as well as CCCP, in NR-mediated activation of native, stably integrated genes.

4.4. Summary

We have used guiding assumptions of a coactivator signal transduction system in which each component has specific domains for interacting with upstream and downstream components of the pathway. These assumptions lead to an effective strategy for defining key functional domains of coactivators, new coactivator components of the pathway, and the mechanisms by which each coactivator contributes to the NR-mediated transcriptional activation process.

Acknowledgements

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