The Expanding Cosmos of Nuclear Receptor Coactivators

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About 200 coactivators play a central role in promoting gene expression mediated by nuclear receptors. This diverse group of proteins are key integrators of signals from steroid hormones and have been implicated in cancer and other diseases.

Introduction
Nuclear receptors (NRs) comprise a superfamily of conserved transcription factors that are activated by their steroid hormone ligands and play essential roles in diverse biological processes. For example, the estrogen, progesterone, and androgen receptors are important in reproduction; glucocorticoid receptors in glucose metabolism and stress; the thyroid hormone receptor in oxidative metabolism; and PPARs in lipid and energy metabolism (Mangelsdorf et al., 1995). Coactivators are molecules recruited by ligand bound activated NRs (or other DNA binding transcription factors) that elicit enhanced gene expression. In contrast to NRs, which are structurally conserved, their coactivators are diverse, both structurally and in the way they contribute to the transcriptional process, namely through a diverse array of enzymatic activities such as acetylation, methylation, ubiquitination, and phosphorylation or as chromatin remodelers. NR coactivators are essential effectors of the biological activities of NRs and their ligands (Xu et al., 1999). Although the focus of this essay is NR coactivators, conceptually they work in a manner similar to general coactivators for other transcription factors.

There is little doubt that the counterparts of coactivators, corepressors, are equally important to the cell (Glass and Rosenfeld, 2000). Corepressors interact with NRs that are not bound to ligand and repress transcription. Corepressor-associated proteins such as histone deacetylases enforce a local chromatin environment that opposes the transcription-promoting activities of coactivators (such as histone acetyltransferase). Through their opposing actions, a balance exists between coactivators and corepressors that defines the magnitude and nature of responses to NR ligands.

Coactivators and Transcriptional Regulation
Initial experiments in yeast produced a picture of coactivators as “transcriptional adaptors” (Ptashne and Gann, 1990). These adaptors were predicted to provide a bridge between DNA binding transcription factors and the general transcription machinery. This simple scenario of coactivator action turned out to be much more complex.

Coactivators are predicted to have many activities in addition to the initiation of transcription, such as mRNA transport from the nucleus, mRNA translation, and posttranslational modifications of the synthesized protein. That coactivators possess stratified actions in the entire process of transcription/translation reflects the fact that they do not act alone but rather as part of multiprotein complexes. These multisubunit entities, containing many individual enzymatic activities, represent a complex machine that is able to concentrate and link diverse enzymes, and the processes that they regulate, together in one place. In this way, the coactivator complex executes the coactivator’s final agenda—that is, to see a particular gene expressed as a mature functional protein.

After identification of the first NR coactivator—the steroid receptor coactivator SRC-1 (Onate et al., 1995)—it was predicted that there might be a small family of coactivators (perhaps five to ten) that carried out the bridging role between transcription factor and transcriptional machinery. There are now ~200 published NR coactivators that work with ~48 NRs. Of these, ~50–70 have been characterized by more than one laboratory and have been definitively shown to be NR coactivators. Clearly, we are far from identifying the totality of authentic NR coactivators (http://www.nursa.org) or their specific functions in the cell.

Transcription is a highly dynamic and orderly process involving many subreactions (multiple steps of initiation, elongation, splicing, and termination). Given that so many NR coactivators have been identified, there is certainly no shortage of them to participate in the wide variety of transcription subreactions. But why would a cell possess such a cumbersome transcriptional apparatus? The answer may lie in the fact that mammals are substantially more complex than organisms such as yeast, worm, and the fruit fly, which have far fewer NR coactivators. For instance, only a single NR coactivator (Taiman/dAIB1) has been identified in fruit flies so far.

Coactivator activity results in particular physiological consequences.
For instance, the PGC-1 coactivator is expressed when an organism needs to alter its metabolic program in response to exercise or cold temperatures (Lin et al., 2005). Work in mice lacking the coactivators SRC-1 and SRC-2 reveals their importance in carbohydrate and lipid metabolism (Picard et al., 2002). Thus, although at first glance they appear to act only in transcriptional control, coactivators are important for modulating the expression of a wide array of physiologically important groups of genes.

A Cacophony of Activities

For transcription to proceed, there need to be histone modifications (such as acetylation and methylation), ATPase-dependent chromatin remodeling, initiation of transcription, elongation, alternative RNA splicing and mRNA processing, and termination. The focus of coactivator enzymatic activities in these processes has centered on the posttranslational modification of histones and chromatin. However, it is becoming clear that NRs and their coactivators are also subject to posttranslational modification. For instance, ligand-dependent sumoylation of PPARγ mediates the repression of inflammatory response genes (Pascual et al., 2005). The posttranslational targeting of NRs and their coactivators is important because these modifications influence the expression of functionally related groups of genes.

For cessation of transcription, RNA polymerase must dissociate from the gene and reinitiation of transcription must be curtailed. As part of the cessation of transcription, coactivators and their NRs are modified by ubiquitination and degraded by the proteasome. In addition to transcriptional termination, the ubiquitin proteasome degradation system is likely to be important in clearing “used” coactivator complexes from the promoter, allowing for subsequent steps in sequential transcription to ensue. Here, the ubiquitin proteasome degradation system plays a positive role in transcription prior to a subsequent duty in transcription termination (Reid et al., 2003). This theory may account for the large number of coactivators that are E2 and E3 ligases, such as E6-AP, RPF-1, Ubch7, and p300. One can envisage the recruitment of a process of coactivators—for example, during transcription initiation, there would be SRCs and p68; chromatin remodelers such as BRG-1 and other ATPase-dependent chromatin remodelers; and histone modifiers (histone acetyltransferases and methyltransferases) such as p300/CREB, SRCs, and CARM-1. Later during transcription, elongation would be mediated by P-TEFβ followed by alternative splicing of mRNA by PGC-1, CAPER, and CoAA. Finally, transcriptional-complex remodeling or the termination of its activities would be accomplished by E6-AP, SSA, and TRIP1 (Metivier et al., 2006). There is no doubt that newly identified coactivators will continue to be a prime source for the discovery of new molecular events in transcriptional reactions.

Coactivators: Integrators of the Cellular State

Primary or core coactivators—those that interact directly with NRs—exist in steady-state complexes with secondary or co-activator partners (Stallcup et al., 2003) (see Figure 1). The coactivator core complex is...
composed of a tightly bound invariant group of proteins, whereas the more loosely bound co-activators associate with the core complex in a dynamic, regulated manner. Perhaps a higher-order “complex of complexes” also forms, enabling coactivator intercomplex communication and efficient integration of signaling pathways such as those required for metabolism, growth, and inflammation. The fact that coactivators belong to distinct complexes may explain how more than 200 different coactivator proteins individually contribute to cell regulation in a coherent manner.

Because coactivators exist as multiprotein complexes, a member of a single coactivator complex can serve as a rate-limiting conduit to control the actions of the whole complex. For example, the phosphorylation status of SRC-3 defines its association with other members of the complex, such as p300 and CBP histone acetyltransferase or CARM1 methyltransferase (Wu et al., 2005). This attendant signaling feature afforded by coactivator complexes suggests that coactivators may be integrators of multiple cell signaling systems. Activation of membrane receptors and signaling cascades may allow the genome to sense the impact of the total environment on the cell. Given that coactivators can organize the expression of “functional groups” of genes involved in the execution of a specific regulatory regime (such as genes involved in metabolism, growth, or cytokine action), they are prime targets for posttranslational modification and modulation by kinase cascades (Wu et al., 2005).

Phosphorylation of coactivators by kinases, such as IKKα and CDK2, modulates NR-mediated transcription by altering the affinity between different coactivators and their NRs, influencing which transcription factors they are able to coactivate (Wu et al., 2005; Narayanan et al., 2005). Other modifications, such as methyl- or acetylation, can promote the dynamic dissociation of coactivator-complex components (Xu et al., 2003; Lee et al., 2005). In the end, final occupation of binding sites on protein partners is a product of both their cellular concentration and their affinity for each other. In the case of SRC-3, phosphorylation of a specific combination of residues defines which transcription factors this coactivator is able to activate, suggesting that there may be a “phosphorylation code” (Wu et al., 2005). The selectively phosphorylated coactivator can be conscripted to preferentially implement the expression of genes downstream of a particular growth-factor signaling cascade. Binding of coactivators to NRs generally occurs through LXXLL interaction motifs in the coactivator. Many coactivators possess several different receptor-interacting LXXLL motifs, enabling them to bind to different combinations of NRs. Complexity is also afforded through these LXXLL motifs by amino acid residues that flank these sequences. In some cases, these flanking residues are also subject to posttranslational modifications, allowing for the dynamic control of this NR-interacting motif.

**Histone Modification: Directed versus Distributed Regulation**

Coactivator-mediated histone modifications play an important role in regulating the transcription of a particular gene, but the biological impact is limited usually to that target gene. Coactivators, however, can direct their enzymatic action toward other coactivator proteins (Xu et al., 2003; Lee et al., 2005). Conceptually, cross-posttranslational modification of one coactivator by another would allow the affected coactivator (and the coactivator complex that it resides in) to act in an altered manner on a “global scale” similar to the far-reaching biological effects that kinases exert on SRC-3 function. Although the histone modification code may define the transcriptional state of individual genes, coactivator modification codes (acylation, methylation, phosphorylation) may define the transcriptional state of broad groups of functionally related genes and may control coactivator preferences among NRs and other transcription factors (see Figure 1).

**Transcriptional Dynamics: Remodeling, Removal, Reinitiation**

That many coactivators contribute to NR-dependent gene expression suggests the presence of a dynamic force that acts as a propulsion system for these transcriptional machines. In a simple system involving only a few proteins, thermal Brownian-driven association and dissociation would be sufficient to allow for the necessary proteins to associate, perform their enzymatic roles, and dissociate, allowing other proteins to then be recruited to do their jobs. Although Brownian forces are likely to play some role, such a simple physical force is unlikely to be sufficient for transcription to ensue. An orderly procession of coactivator proteins must associate with the promoter for efficient transcription (An et al., 2004). Many other proteins interact with the promoter in an orderly sequential fashion (Reid et al., 2003), such that additional organizing processes must be involved to actively disrupt and rearrange these coactivators.

So what are some of the motive forces that allow for orderly remodeling capabilities? Protein degradation via the proteasome is one force that makes this procession possible. Because protein degradation mediated by the ubiquitin-proteasome system is a highly regulated and specific process, it is capable of selectively removing coactivator proteins after they have fulfilled their roles in transcription, clearing the way for subsequent coactivator associations with the promoter. The ubiquitin-proteasome system is itself remarkably complex, as evidenced by the large number of ubiquitin ligases responsible for the directed targeting of ubiquitin to proteasome substrates, making it the largest class of enzymes in mammalian cells. Another group of proteins that may play essential roles in coactivator dynamics are the ATP-driven protein chaperones. An example of a protein that alters coactivator protein conformation is Pin1, a prolyl isomerase that catalyzes the cis-trans isomerization of proline residues in SRC-3 (see Fig-
Coactivators play important roles in diverse pathological processes, such as cancer, inherited genetic diseases, metabolic disorders, and inflammation. The cancer cell, dedicated to relentless growth, is certainly a master at accumulating high levels of “growth coactivators” such as SRC-3/AIB1, thereby assuring a preferential rate of expansion (Anzick et al., 1997). Germline mutations affecting E6-AP result in the inherited genetic disease Angelman syndrome, (Matsumura et al., 1997). Polymorphisms in PGC-1 may lead to increased susceptibility to type II diabetes (Lin et al., 2005). Finally, variations in the expression of coactivators among different individuals may be associated with phenotypic differences among humans. There is little doubt that we have much to learn about the biologically diverse roles of NR coactivators and that we have only scratched the surface of this expansive coactivator cosmos.

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