

Combinatorial Control of Gene Expression by Nuclear Receptors and Coregulators

Review

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The nuclear receptor (NR) superfamily of transcription factors regulates gene expression in response to endocrine signaling, and recruitment of coregulators affords these receptors considerable functional flexibility. We will place historical aspects of NR research in context with current opinions on their mechanism of signal transduction, and we will speculate upon future trends in the field.

Preface

The coordinated expression of gene networks in numerous physiological, developmental, and metabolic processes can be ascribed in large part to a superfamily of ligand-inducible transcription factors, the nuclear receptors (NRs). Abundant evidence has identified NRs as mediators of the transcriptional response to a variety of ligands: acting as signaling conduits in steroid regulation of reproductive processes and thyroid/retinoid regulation of development; as metabolic barometers in the regulation of bile acid and cholesterol biosynthesis; and as prominent etiological factors in diseases ranging from breast and prostate cancer to diabetes and obesity. In addition to offering a brief historical summary of the functional characterization of NRs, this review will emphasize the evaluation of concepts and models that more recent research efforts have generated to place in context future goals toward which the field is currently striving.

In the Beginning

In most instances, the observation that a hormone bound specifically to tissue preparations preceded by several decades the purification of a NR and the cloning of a NR cDNA. The initial characterization of steroid hormone action focused upon the striking proliferative properties of these hormones in their target tissues. The concept of high-affinity, tissue-specific, steroidophilic factors—receptors—as mediators of hormone function gained credence from pioneering tissue binding studies (reviewed in Jensen and Jacobson, 1962). Subsequent attempts to define a chronology for the effects of these molecules in the oviduct, uterus, and other target tissues established cell- and ligand-specific increases in mRNA and protein synthesis as primary events in their action (O'Malley and McGuire, 1968) and substantiated a basic linear model of steroid hormone action from ligand to target gene product (Means et al., 1972). Biochemical purification strategies used radiolabeled ligands to considerable effect to overcome the sparse cellular levels of many NRs, and by the late 1970s a steroid receptor,

the progesterone receptor (PR), had been purified to homogeneity (Schrader et al., 1977). Studies using purified receptor fractions, particularly those of PR and the glucocorticoid receptor (GR; Wrangé et al., 1984), helped to sketch a model of receptor activation in which steroid binding induced dimerization and increased affinity of the receptor for specific *cis*-acting DNA sequences (hormone response elements, or HREs; Payvar et al., 1982) to effect an increase in RNA production—a model which has survived essentially intact to this day. By the end of the decade, cDNAs had been cloned that encoded first GR (Hollenberg et al., 1985) followed by the estrogen receptor (ER), PR and receptors for androgens (AR), thyroid hormone (TR), all-*trans* and 9-*cis* retinoic acid (RAR and RXR), and vitamin D (VDR) (reviewed in detail in Evans, 1988). At this point, the field was primed to bring a substantial body of experimental evidence to bear upon the structural and functional dissection of NRs.

NRs: A Superfamily Portrait

While a structural relationship among receptors for steroid ligands had been presumed, it was only after comparison of the deduced encoded amino acid sequences of the cloned receptor cDNAs that the concept of an evolutionarily related superfamily of transcription factors—the NR superfamily—was validated. NRs have been historically divided into type I receptors (essentially the classical steroid receptors), which undergo nuclear translocation upon ligand activation and bind as homodimers to inverted repeat DNA half sites, and type II receptors (TR and RAR, among others), which are often retained in the nucleus regardless of the presence of ligand and usually bind as heterodimers with RXR to direct repeats. The recognition of the NR superfamily prompted a series of experiments in which degenerate oligonucleotide sequences, based upon conserved regions of NR cDNAs, were used in low-stringency screening experiments to identify clones that were then subjected to sequence analysis. Beginning with the estrogen receptor-related receptors (ERRs; see Evans, 1988), these experiments established the existence of previously unidentified cDNAs encoding proteins that contained domains resembling those present in characterized NRs. Since ligands for these proteins had not been previously identified and the prediction of ligand structure from their primary LBD sequences was (and remains) a practically impossible task, they were designated “orphan NRs” (ONRs) and are categorized in the type III class within the NR superfamily (Giguere, 1999; Mangelsdorf and Evans, 1995). While scope limitations preclude a detailed discussion of ONRs in this review, the regulatory influences that impinge upon type I and II NRs also apply generally to the ONRs.

In the next several years, following the cloning of expressible NR cDNAs, the transient transfection-based HRE-reporter assay became the workhorse for laboratories pursuing a molecular rationale for NR action (Evans, 1988). The advent of molecular biology afforded investigators the opportunity to mix and match the au-

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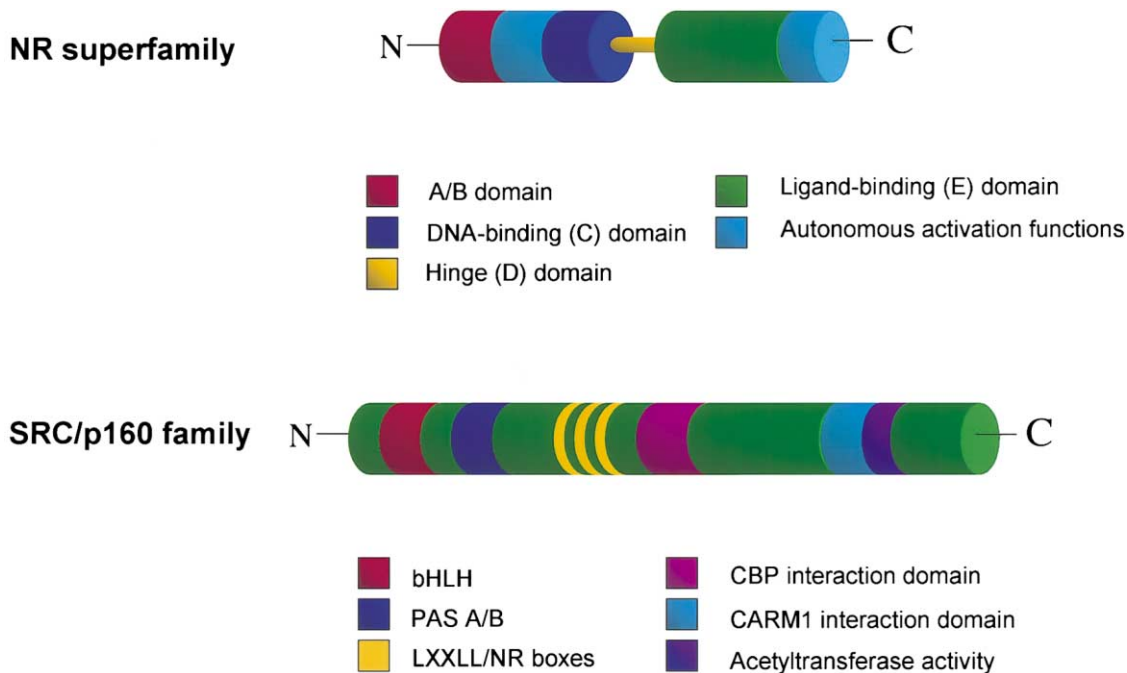


Figure 1. Shared Functional Domains of NR Superfamily and SRC/p160 Family Members

(Top) General structure of NRs. AF-1 is embedded in the N terminus of type I NRs and AF-2 in the C terminus of all NRs. Intramolecular communication between the two functions is thought to be involved in coregulator function. The A/B domain is prominent in type I NRs and is considerably foreshortened in type II receptors.

(Bottom) General structure of the SRC/p160 family. The CBP interaction domain and the CARM1 interaction domain overlap with the transferable activation domains 1 and 2 of the SRC/p160 family, respectively.

Autonomous functional modules of the receptors, to generate specific domain and point mutations, and to probe the specificity of the *cis*-acting HREs, all in the context of a single functional assay of receptor action. The identification of signature-shared regions—a conserved zinc finger-based DNA binding domain (DBD) and a C-terminal ligand binding domain (LBD), containing regions mediating ligand binding and dimerization—added molecular detail to the biochemical description of receptors as hormone-inducible DNA binding factors (Figure 1A; reviewed in Evans, 1988; Tsai and O’Malley, 1994). Moreover, the development of a cell-free system that recapitulated receptor activity *in vivo* facilitated the functional analysis of receptor domains (Bagchi et al., 1992). In addition to the regions mentioned above, constitutive (AF-1) and ligand-dependent (AF-2) activation functions were identified, along with repression domains (reviewed in Tsai and O’Malley, 1994). The schizoid functionality of NRs, alternating between activation and repression in response to specific molecular cues, is now known to be attributable in large part to their recruitment of a diverse group of ancillary factors, the coregulators. Although the remainder of this review will focus largely on coregulators, the more recent cloning of NRs such as ER β (Kuiper et al., 1996), which has a pharmacological activation profile distinct from that of ER α (Katzenellenbogen et al., 2000), suggests that the pursuit of novel NR functions remains an important area in this field.

Enter Coregulators

The discovery of NRs had its roots in decades of historical endocrinology and pathology, and prior to their char-

acterization there was abundant empirical evidence that pointed to their existence. This stands in contrast to the rapid characterization over the last several years of NR coregulators. As early as three decades ago, aspects of NR action were being ascribed to the interaction of receptors with hypothetical non-DNA “nuclear acceptor” molecules (Spelsberg et al., 1971; Yamamoto, 1985). While not calling coregulators by name, this thesis generated speculation for a role for intermediary factors in NR action. Tangible evidence for the recruitment by activated receptors of factors other than their presumptive bedfellows—RNA polymerase and the basal transcription machinery—came initially from yeast experiments based on transcriptional interference (squelching; Gill and Ptashne, 1988) and subsequently from squelching noted between cotransfected receptors in reporter assays in mammalian cells (Meyer et al., 1989). The tissue-selective transactivation properties of autonomous receptor activation (Nagpal et al., 1992) and later repression (Baniahmad et al., 1995) functions further reinforced the notion of intermediary factors in NR function. Building upon these initial molecular approaches, biochemical strategies provided the first evidence that ligand binding resulted in the recruitment by ER of associated molecules in mammalian cells (Cavallès et al., 1994; Halachmi et al., 1994). Within the same decade, cDNAs encoding close to thirty of these molecules were cloned (see below), representing a rapid accumulation of information that is yet to be organized into a coherent model of their biological significance. Table 1 summarizes recently described properties of selected coregulators. They are (broadly) divisible into coactivators, which

Table 1. Selected Nuclear Receptor Coregulators

Coregulator	Selected Recent Reports
Coactivators	
RIP140	Initially defined as a coactivator (Cavallès et al., 1995); may also function as a corepressor (Windahl et al., 1999).
SRC-1/NCOA-1	Targeted by MAP kinases (Rowan et al., 2000).
TIF2/GRIP-1/SRC-2	Initially identified as a coactivator, also mediates promoter-dependent corepression (Rogatsky et al., 2001).
p/CIP/RAC3/ACTR/AIB-1/ TRAM-1/SRC-3	Present in IκK complex; phosphorylated by IκK; null deletion preferentially impacts growth factor mediated-physiology (Xu et al., 1998, 2000; Wang et al., 2000).
CBP/p300	Methylation by CARM-1 uncouples interaction with CREB (Xu et al., 2001), acetylates ACTR/SRC-3 to uncouple its interaction with NR (Chen et al., 1999b).
TRAPs/DRIPs	Disruption of TRAP220 subunit results in embryonic lethality (Ito et al., 2000).
PGC-1	Transduces GR- and CREB-mediated hepatic gluconeogenesis (Herzig et al., 2001; Yoon et al., 2001); coordinates transcription and RNA processing (Monsalve et al., 2000); sequestration by a corepressor reversed by MAPK-mediated phosphorylation (Knutti et al., 2001).
CARM-1	Recruited by SRC-1 to potentiate transcriptional coactivation (Chen et al., 1999a); related to another protein methyltransferase, PRMT-1 (Wang et al., 2001); see also CBP/p300.
PRIP/ASC-2/AIB3/ RAP250/NRC	Contains NR box; possible bridging factor between CBP/p300 and DRIP-130, a component of the DRIP complex; gene identical to one overexpressed in breast cancer (Caira et al., 2000; Lee et al., 1999; Mahajan and Samuels, 2000; Zhu et al., 2000).
GT-198	Broad-spectrum coactivator whose gene localizes to breast cancer susceptibility locus; phosphorylated by a variety of kinases in vitro (Ko et al., 2002).
SHARP, CoAA, p68, p72	Coactivators containing RNA-binding domains (Endoh et al., 1999; Iwasaki et al., 2001; Shi et al., 2001).
Corepressors	
SMRT	Subcellular distribution induced by MAP kinase-mediated phosphorylation (Hong and Privalsky, 2000); distributed among a variety of repressor complexes (reviewed in Rosenfeld and Glass, 2001).
NCoR	Found in a variety of repressor complexes (reviewed in Rosenfeld and Glass, 2001); functions with a specific HDAC to mediate transcriptional activation at a subtype of retinoic acid HRE (Jepsen et al., 2000).
REA	Selective ER corepressor; competes with SRC-1 for binding to liganded ER (Montano et al., 1999).

Although many other coregulators exist, constraints upon space and reference number limit this table to a brief update on selected recent reports. For a more in-depth discussion of earlier studies, the reader is encouraged to refer to previous reviews (McKenna et al., 1999; Rosenfeld and Glass, 2001).

mediate the potentiating functions of activated receptors, and corepressors, which transduce the attenuating functions of nonactivated receptors. More recent evidence indicates that coregulators serve as stages upon which subplots of functional complexity, in the form of combinatorial interactions and specific posttranslational modifications, are acted out during the intricate programs of transcriptional regulation in which NRs participate.

Coactivators

The functional autonomy of the receptor LBD permitted its adaptation to the yeast two-hybrid protein-protein interaction assay, and a pioneering screen identified an array of TR-interacting factors in a HeLa cDNA library (Lee et al., 1995). The cloning in rapid succession of mammalian cDNAs encoding SRC-1/NCOA-1 (Onate et al., 1995; Kamei et al., 1996), GRIP-1/TIF2/SRC-2 (Hong et al., 1996; Voegel et al., 1996), and p/CIP/RAC3/ACTR/AIB-1/TRAM-1/SRC-3 (Torchia et al., 1997; Anzick et al., 1997; Chen et al., 1997; Li et al., 1997; Takeshita et al., 1996) identified a family of ligand-recruited NR coactivators, the SRC/p160 family. The designation of the three members of this family (and other proteins) as NR coactivators was initially predicated upon their nuclear localization, their ability to interact with and amplify ligand-dependent functions of NRs on HRE-linked reporter genes, and their capacity to relieve NR squelching (Onate et al., 1995). The subsequent observation (in mice in which the coactivator gene had been deleted) of a

partial resistance to steroid hormones (Xu et al., 1998, 2000; Wang et al., 2000) confirmed their coactivator function in a physiological context. As with NRs, the modular construction of coactivators facilitated the localization of autonomous activation domains believed to mediate interactions with the basal transcription apparatus—see review in McKenna et al. (1999) and Rosenfeld and Glass (2001).

The SRC/p160 family (Figure 1B) is structurally and functionally distinguishable from other molecules that fulfilled some or all of the experimental properties that initially defined NR coactivators—the acetyltransferases CBP (Kamei et al., 1996) and p300 (Chakravarti et al., 1996); members of the TRAP/DRIP complex (Fondell et al., 1996; Rachez et al., 1998); the ubiquitin ligase E6-AP (Nawaz et al., 1999); the ATP-coupled chromatin remodeling SWI/SNF complex (Fryer and Archer, 1998; Yoshinaga et al., 1992); the RNA coactivator, SRA (Lanz et al., 1999); the protein methylases CARM-1 and PRMT-1 (Chen et al., 1999a; Wang et al., 2001); and members of the basal transcription machinery, in particular TBP and the TAFs (Naar et al., 2001). A recurring structural feature of the protein coactivators is an α-helical LXXLL motif, or NR box (Heery et al., 1997), present from a single to several copies in many coactivators, which is implicated in their ligand-dependent recruitment by the LBD-embedded activation function (AF-2) of NRs. Moreover, several functional properties are common across different groups of coactivators. Acetyltransferase activity, for instance, with which co-

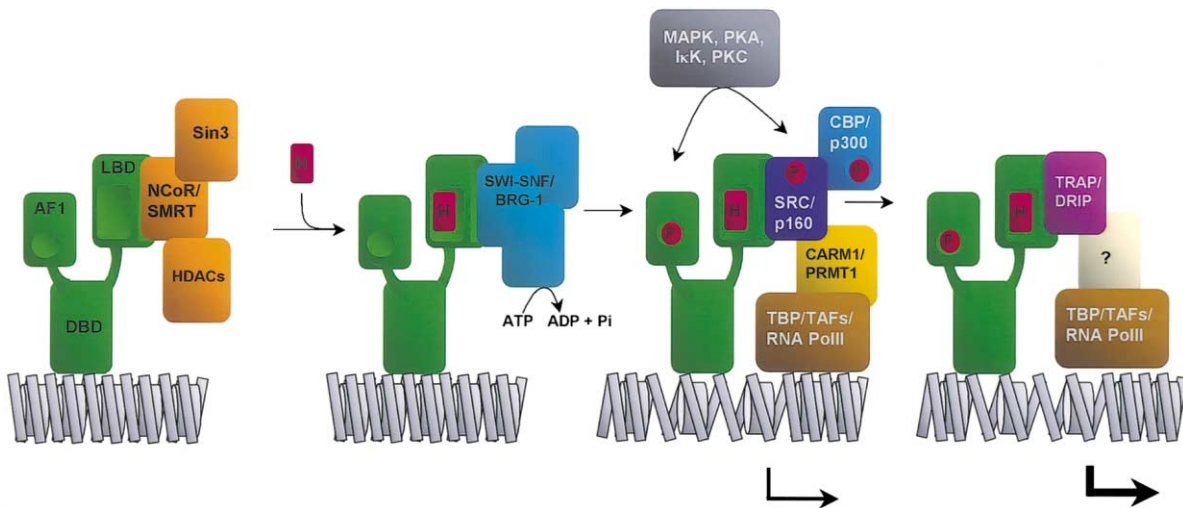


Figure 2. Model of Combinatorial NR-Mediated Transcriptional Initiation

Initial binding of ligand results in dissociation of corepressors and recruitment of SWI/SNF chromatin remodeling machines to modify chromatin domains. Binding of SRCs and CBP results in local acetyltransferase activity and disruption of local nucleosomal structure. Kinase-mediated signaling pathways may communicate directly with NR-regulated promoters. AF-1 phosphorylation might serve to further consolidate ligand-dependent NR-SRC interactions or to recruit SRCs directly to the promoter in the absence of ligand. TRAP/DRIP directly contacts components of the basal transcription machinery to effect transcriptional initiation, and certain TAFs may afford some additional input into promoter-specific NR transcription. The extent of overlap in binding of complexes to the promoter is currently unclear. Local coactivator requirements may vary—for example, a promoter in a readily accessible chromatin context may not require significant chromatin remodeling or histone acetyltransferase activity for assembly of a preinitiation complex.

activators are thought to target histones and other proteins to fashion a transcriptionally permissive environment at the promoter (Figure 2), is possessed by CBP (Bannister and Kouzarides, 1996), PCAF (Yang et al., 1996), and members of the SRC family (Chen et al., 1997; Spencer et al., 1997).

Corepressors

Repression by NRs in many ways closely mirrors the manner in which they effect transcriptional activation. Analogous to coactivators, NR corepressor (N-CoR; Horlein et al., 1995) and silencing mediator of retinoid and thyroid receptors (SMRT; Chen and Evans, 1995) are recruited by NRs in the absence of ligand or in the presence of NR antagonists such as Tamoxifen and RU486. Moreover, recognition by transcriptionally inert NRs of corepressors is mediated by amphipathic helical peptides called “CoRNR boxes” (Hu and Lazar, 1999), which are similar to the previously characterized coactivator NR boxes. In addition to their structural similarities, corepressors are functionally comparable to coactivators. Histone deacetylation, for example, appears to antagonize coactivator acetyltransferase activity, although corepressors, lacking intrinsic deacetylation domains, require recruited factors such as Sin3 and histone deacetylases (HDACs) to achieve this (Heinzel et al., 1997; Nagy et al., 1997).

NRs, Coregulators, and Genes: A Complex Relationship

The goal of understanding the stringent spatiotemporal coordination of gene expression that NRs effect in response to diverse developmental, physiological, and metabolic cues can be approached on at least two lev-

els: (1) the molecular events that direct cyclical interactions at individual promoters, and (2) the global factors that contribute to combinatorial gene expression on the wider promoter-, ligand-, and cell type-specific levels. The initial characterization of coregulators, colored to some extent by the reporter assays in which their properties were first manifest, emphasized their propensity to amplify or silence transcription when overexpressed with receptor. Given the well-documented tissue selectivity of NR action, the realization that these coactivators have, apart from a few exceptions (such as FHL-2; Muller et al., 2000), a relatively broad tissue distribution pattern, was food for thought. The first hints of a mechanistic rationale for the puzzling plurality of NR coregulators were provided by studies describing promoter-specific coregulator requirements (Korzus et al., 1998; Puigserver et al., 1998). More recently, promoter identity has been shown to effect functional inversion among coregulators, such that corepressors can become coactivators (Jepsen et al., 2000) and vice versa (Rogatsky et al., 2001; Xu et al., 2001). In addition, variations in ligand-specified recruitment of coactivators (Katzenellenbogen et al., 2000) and/or dissociation of corepressors may be a supplementary source of signaling flexibility at NR-regulated promoters. To further muddy the waters, corepressors may be capable of binding to liganded receptor to sensitize the transcriptional response to ligand (Montano et al., 1999), a fact conceivably attributable, at least in part, to the structural symmetry between the NR box and the CoRNR box.

The interface between receptor AF-2 elements and the coregulator NR box has been the subject of intense study as a potentially rewarding target for peptide-based manipulation of NR pharmacology (Norris et al.,

1999). Our laboratory (Smith et al., 1997) and others (Graham et al., 2000) have speculated that coregulators may play an important role in interpreting the tissue specificity of many NR ligands and selective receptor modulators (SRMs) such as Tamoxifen and Raloxifene. This model remains clinically relevant as SRMs continue to be identified and characterized and as the role of NRs and coregulators in metabolic and neoplastic diseases emerges. Detailed crystallographic dissections of NR LBDs and the AF-2/NR box complex (Feng et al., 1998; Nolte et al., 1998; Shiau et al., 1998) have identified a ligand-induced hydrophobic cleft and an adjacent ionic clamp as principal determinants of the interaction of AF-2 with the NR box. A model is emerging in which ligand-specified variations in the AF-2 relief map, along with covalent modifications effected by other signaling molecules, can generate a tremendous diversity in the capacity of a receptor to recruit coregulators on a promoter- and cell-type specific basis.

Given, then, the multiplicity of regulatory strata that exist to tweak receptor action, it can be appreciated that without the abundance of coregulators identified to date, the remarkable context dependency of NR transcriptional regulation would be significantly compromised. It has become clear from work in our laboratory and others that coregulators are organized *in vivo* into complexes (Figure 2) that are primed for recruitment by NRs in response to appropriate cues (Fondell et al., 1996; McKenna et al., 1998; Rachez et al., 1998). Intriguingly, many of these complexes share subunits (reviewed in Rosenfeld and Glass, 2001), suggesting that perhaps we are viewing in these biochemical complexes a molecular bucket brigade in which subunits are traded and switched out, a mechanism that may contribute to cyclical assembly of coregulator complexes (Freeman and Yamamoto, 2001; Shang et al., 2000). Recent work has elucidated the mechanisms that regulate the fine-tuning composition of these complexes, such that a selective transcriptional response to any one of a variety of afferent signals, or an aggregate response to multiple stimuli, can be effected.

Regulating the Coregulators

Coactivators appear to be subject to modulation by selective repressor molecules, such as RIP140 (Cavallès et al., 1995; Windahl et al., 1999), that bind to them and antagonize their function. Perhaps more importantly, a variety of targeted posttranslational modifications exert considerable control over the functional relationships between NRs, their coregulator complexes, and their cognate gene networks. No type of covalent modification, it seems, is unworthy of a role in modulating coregulator function. Some of these, such as acetylation (Chen et al., 1999b) and ubiquitination (Lonard et al., 2000), appear to alter the half-life of molecules in the complex; some, such as phosphorylation, can specify the compartmentalization or activity of a coregulator within the cell (Hong and Privalsky, 2000); and others, such as methylation, may potentiate coregulator function by targeting histones to complement the acetyltransferase activity of other coregulators (Chen et al., 1999a; Wang et al., 2001).

Many modifications originate in a variety of kinase-

mediated cellular signaling pathways, and while the ability of protein kinases to modulate NR function is familiar ground, there is growing evidence that NR coregulators themselves are prime targets for control by these pathways. This modulation can take the form of enhancement of coregulator enzymatic activities such as acetylation (Ait-Si-Ali et al., 1998); promoting the recruitment of other coregulators (Font de Mora and Brown, 2000); or the dismantling of inhibitory coregulator complexes (Knutti et al., 2001). In addition, liganded NRs recruit cytoplasmic members of kinase signaling cascades such as the MAP kinase (Boonyaratankornkit et al., 2001) and PI3K pathways (Simoncini et al., 2000)—examples of the historical “nongenomic” component of receptor action—effectively challenging the definition of coregulators as nuclear entities with functions restricted to NR-regulated promoters. The identification of coregulators as components of these diverse signaling pathways reinforces the notion that they may serve as general control panels for integrating multiple afferent stimuli into an appropriate cellular response. While speculation exists as to the mechanistic consequences of kinase-mediated modification, it may influence the combinatorial recruitment of coactivator into active transcriptional complexes at distinct promoters (Figure 3).

Recent studies support the notion that kinase-mediated modification might be a mechanism for directly altering the tissue- and promoter-specific functionality of coregulators. For example, the coactivator PGC-1 was initially characterized as a dedicated UPC-1 coactivator in brown fat cells (Puigserver et al., 1998), raising the intriguing possibility of the existence of promoter-specific coactivators. More recent data suggests, however, that in liver cells, targeting of PGC-1 by the cAMP signaling axis facilitates its potentiation of CREB- and GR-mediated induction of genes encoding key gluconeogenic enzymes, such as glucose-6-phosphatase, resulting in increased glucose output (Herzig et al., 2001; Yoon et al., 2001). Conceivably, in these cases a tissue/signaling pathway-specific pattern of PGC-1 phosphorylation is directing its communication with complexes controlling the transcriptional output of the gluconeogenic promoters, although the precise mechanism is unclear. These data are a signal reminder of the complex interplay between parallel signaling conduits and the role that coregulators play in mediating this crosstalk.

Among coregulators, SRC family members and CBP appear to be particularly prone to modification by kinase-mediated pathways, presumably permitting these signaling pathways to directly influence events at a broad range of NR-regulated promoters (Figure 2). Intuitively, the modular construction of coactivator and corepressor complexes can create an array of templates that constantly evolves and morphs in response to a variety of stimuli. It is this plasticity that likely contributes to the capacity of NRs and coregulators to integrate and execute complex programs of gene expression. An intriguing scenario is that coactivators and corepressors are programmable, and through specific sequences of posttranslational modifications, they can serve as memory cards to sensitize specific promoters and/or cell types for subsequent transcriptional responses. Indeed, in support of this assertion, certain coactivators are known to have a low rate of turnover within the cell. The

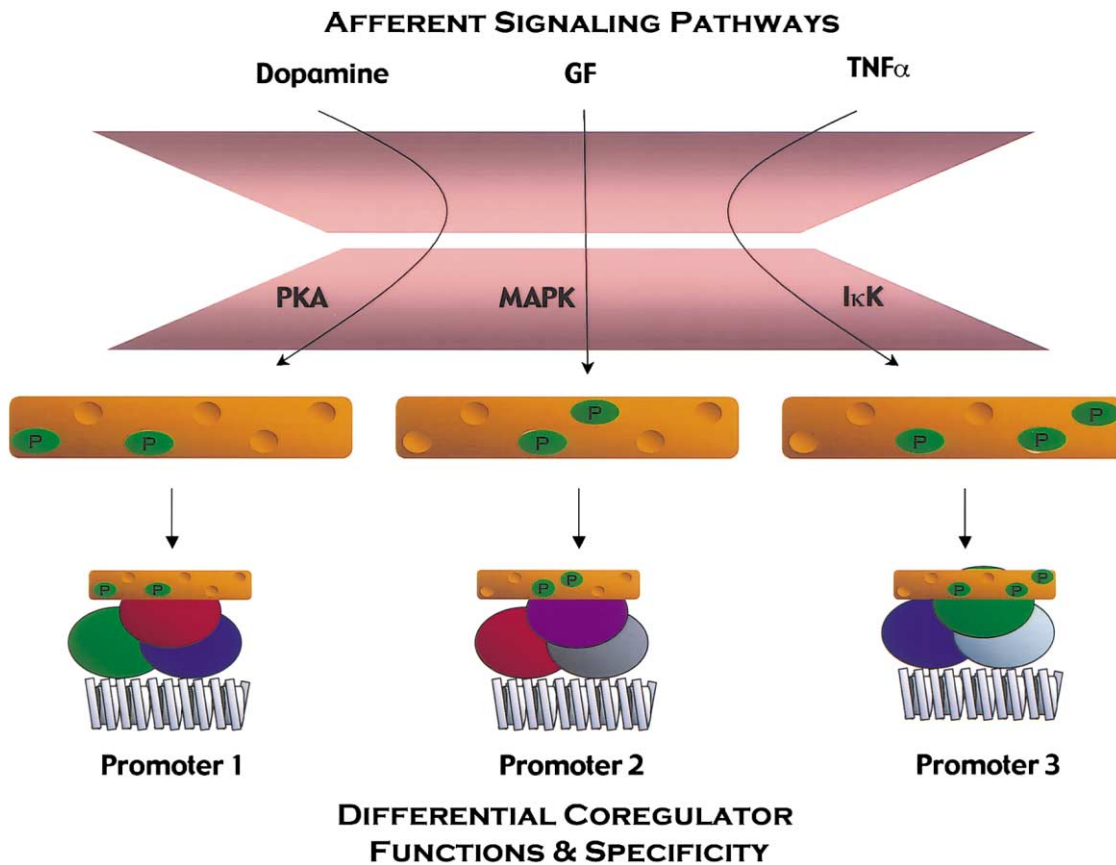


Figure 3. Does the Squeaky Wheel Get the Grease?

Apportioning of coregulator function among distinct kinase signaling pathways may occur on a supply and demand basis, according to unique phosphorylation codes that determine the functional specificity of the coregulator for distinct NRs and promoters. Abbreviations: GF, growth factors; PKA, protein kinase A; and MAPK, MAP kinases.

results of experiments that will probe tissue-specific variations not only in coregulator complex composition but potentially in the patterns of posttranslational modification of these complexes in response to endocrine signals (Figure 3) are awaited with anticipation. At any rate, coregulator posttranslational modification is emerging as an elegant mechanism whereby a relatively small number of factors can govern a broad array of transcriptional responses.

Physiology of Coregulators

The list of physiological implications of coregulators is expanding rapidly, and it is likely not an overstatement to suggest that these molecules seem to be the missing links to explanations for many cellular phenomena. For example, the cellular balance of coactivators and corepressors affords a smooth and tightly controlled induction curve for NR-mediated gene expression. Increased coactivator would likely provide a more rapid transcriptional response and has been shown to shift the hormonal induction curve to the left (Chen et al., 2000). This might explain, at least in part, some of the well-known variability in the degree of individual human responses to steroid hormones. Since some of the same coactivators are used in interphase gene regulation and in regulation of cell cycle control factors, there exists a link for communication between those two processes. Viruses

such as adenovirus and papillomavirus appear to capture coregulators frequently when they infect cells (Lundblad et al., 1995; Ait-Si-Ali et al., 1998), thereby giving them control of important components of the cellular transcriptional machinery. Moreover, genetic diseases attributable to coregulators have been reported (Petrij et al., 1995), which often give rise to skeletal and CNS abnormalities and altered hormonal response.

Finally, we speculate that the coactivator/corepressor levels in different individuals may explain in part the remarkable differences in individual phenotypes observed throughout the human population. Although structural gene allelism is an important factor, the differences are of such magnitude, and the genes of individuals so similar, that other factors must come into play. Given that coactivators act at the amplification step in gene expression, a few percent difference in the inherited coactivator levels (or allelism of coactivators) in the population could underlie major alterations in hormone-mediated development of, for example, the musculo-skeletal and organ systems.

Looking to the Future

In contrast to the strides taken toward clarifying coactivator mechanism at the promoter, issues concerning the physiological and metabolic roles of coactivators and their tissue-specific functionality are only beginning

to be elucidated. The problem has been compounded by the nature of assays routinely employed in the field, such as cell transfection and *in vitro* protein-protein interaction assays, which do not readily lend themselves to meaningful comparison. While these approaches have made tangible progress in the mechanistic characterization of coactivator function, they permit only tentative extrapolation when the biological functions of intact coregulators, and coregulator complexes, are considered. Assays employed to considerable effect more recently have been those that interrogate the patterns of recruitment of receptors and coregulators to DNA in living cells, such as chromatin immunoprecipitation (ChIP; Shang et al., 2000), and the real-time imaging techniques that afford an insight into the cellular dynamics of these factors (McNally et al., 2000; Stenoien et al., 2001). A caveat in attempting to reconcile data generated by these techniques, however, is that while ChIP is a composite freeze-frame of promoter occupancy in millions of cells, real-time imaging focuses on a chronology of events in a single cell.

Much remains to be explored in coregulator biology over the next decade, and the field is aptly poised to build upon the current store of knowledge of their mechanistic properties. A primary objective is the definition of a reasonably complete list of coregulators and their enzymatic functions, with a subsequent goal of understanding and cataloging the target genes responsive to combinations of individual coactivators and corepressors. At that point, we can then assign regulated gene sets to coregulators and begin to elucidate the presumptive metabolic (Herzig et al., 2001; Yoon et al., 2001) and developmental (Ito et al., 2000) roles of coregulators and their coordinate activation of target gene sets that have evolved to perform specific functions. Such information would go a long way to substantiate a role for coactivators as overall coordinators for the efficient expression of gene sets controlling metabolic functions.

We have suggested that the heterogeneity of coactivators can be explained in part by their organization into multifunctional complexes. But is their function simply to enhance the transcriptional potency of NRs—or is a complex array of other functions yet to be uncovered? Putting aside the likely consideration that coregulator functions outside the nuclear compartment will be discovered, it is almost certain that the heterogeneity is dedicated in part to subsequent downstream transcription-coupled reactions required to effect gene expression. A great deal of the current experimental work in our field has been devoted to elucidating the mechanisms by which NRs and coactivators initiate and reinstate transcription. This is undoubtedly of great importance, but if a gene is activated maximally for transcription, it will be of little import to expression of the function of that gene if elongation, RNA splicing, 5'- and 3'-RNA processing, mRNA packaging and transport, and translation, become rate limiting. The fact that NRs might act as templates for assembly of factors that enhance these subsequent reactions should not be surprising, and evidence suggests that coactivators are likely to be the factors that mediate many of these downstream NR-related events (Monsalve et al., 2000). This concept is reasonably consistent with current thinking within the transcription field, which purports that the major steps leading to the formation of mature mRNAs are not carried

out in isolation but are coordinately regulated, such that initiation and reinitiation of transcription are coupled to 5'-capping, polyadenylation, and RNA splicing (Lewis and Tollervey, 2000, and references therein). In addition to the C-terminal domain of RNA polymerase II, NRs may serve as additional platforms to target the myriad factors required for optimum gene expression, thereby justifying the need for a large and diverse cohort of coactivators.

It is widely anticipated that medical therapy and pharmaceutical development will benefit significantly from the plethora of recent molecular data. Future clinical strategies should clarify the extent to which the reported relationships between altered coactivator (or corepressor) levels and tumor progression and treatment influence the course of these diseases (Anzick et al., 1997; Lavinsky et al., 1998; Graham et al., 2000). It is logical that high coactivator levels could afford tumors a selective gene expression advantage for proliferation, but definitions both of the signaling pathways that interact with and modulate coactivator function (Figure 3) and the biological consequences of this modification are of major current importance. The concept of membrane receptor pathway and nuclear pathway collaboration in the cell is accepted, but the mechanisms by which MAP kinases, protein kinase A, I κ K, and stress-activated pathways specifically influence coactivator function is insufficiently defined at present. It can be reasonably speculated, however, that overexpression of a coactivator (e.g., p/CIP/AIB1/SRC-3) in the presence of high levels of growth factors, growth factor receptors, or MAP kinase activity, could likely result in the development of a very aggressive cancer.

Finally, it is likely that the recent coregulator discoveries and the information on their mechanisms of action will expedite the development of new pharmaceutical leads. It is already clear that the existence of coregulators greatly impacts the tissue-specific pharmacology of mixed antagonist/agonist drugs such as the SRMs. Evidence exists that the specific coregulator levels in cellular or cell-free systems contribute to the relative antagonist versus agonist activity of a ligand on NR activation (Smith et al., 1997). Although X-ray structures of the LBD and coactivator interaction surfaces have been solved (Nolte et al., 1998; Shiao et al., 1998), it may be that the full impact of these structural studies on new drug development will not be realized until the full-length NRs are crystallized and complexes of N-terminal-interacting and C-terminal-interacting coregulators are determined.

Conclusion

Combinatorial control of gene expression by NRs requires their recruitment of functionally distinct coregulator complexes (Figure 2), which appear to communicate with each other through intricate sequences of post-translational modifications. It has become clear that a definition of coregulators that restricts their roles to amplifying or silencing the transcriptional output of NR-regulated promoters is insufficient to account for their elaborate functionality. The collective efforts of laboratories in this field have identified more highly complex and intricate subplots in this narrative than was initially anticipated, and it is increasingly appreciated that li-

gand, promoter, receptor, and coregulator contribute to spatiotemporally distinct patterns of gene expression in a wide variety of biological processes. While gaps in our appreciation of NR and coregulator biology still remain, we are rapidly approaching a point at which coherent functional and mechanistic models for NR action can be constructed in the wider context of cellular signaling and transcriptional regulation.

Acknowledgments

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