A REVIEW OF NUCLEAR RECEPTOR GENE ACTIVATION THROUGH COFACTOR PROTEIN INTERACTIONS

BY

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Abstract

Humans are faced with a vast number of pathological conditions. Some the most prevalent and deadly disease states include cancer, obesity, and diabetes. A large amount of scientific research and development has gone into determining the underlying cause of these pathologies. Certain aspects of the aforementioned diseases have been linked to the aberrant expression and activity of selected gene-expression-programs. Gene expression is regulated by proteins known as transcription factors. One of the largest transcription factor families is the nuclear receptor (NR) superfamily. The ability of NRs to drive gene activation is directed by specific interacting proteins called cofactors. This review will highlight new discoveries regarding the mechanistic role of NRs and their associated protein cofactors in regulating gene activation and in the progression of cancer, diabetes, and obesity.

Introduction

Several types of nuclear receptors exist in cells which are responsible for the transcription of genes. NRs have been divided into classes based on the receptor-ligand interaction or the receptor's structure. Structurally, NRs are made up of an N-terminal activation function domain (AF-1), a DNA binding domain (DBD), a hinge region, and the C-terminal which contains the ligand binding domain (LBD) which contains a second activation function domain, (AF-2). These attributes are found in the all NRs.

NRs can be categorized into two main classes based on the mechanism of action and sub-cellular location. NRs can be broken down into types of receptor classes that include Type I, Type II, and orphan receptors. Type I NRs feature the classic steroid hormone receptors glucocorticoid receptor (GR), estrogen receptor (ER), and androgen receptor (AR). This type of receptor goes through a nuclear translocation upon hormone binding and associates with a consensus sequence on DNA as a homodimer. This nuclear binding is found to recruit coactivator proteins to aid in the gene expression.

Type II receptors include retinoic acid receptor (RAR), retinoid X receptor (RXR), thyroid hormone receptor (TR), and vitamin D3 receptor (VDR) among others. This class of receptors resides in the nucleus, and regardless of a ligand binding event, will heterodimerize with other NRs on

their binding sites. In the absence of ligands, Type II receptors are usually associated with corepressor proteins. This association inhibits or represses gene expression. When a ligand is bound to Type II receptors, the corepressor proteins are disassociated, which allows for gene expression to proceed.

A third family of NRs, designated orphan receptors, was identified through the use of low stringency screening methods and conserved sequences of NR cDNAs. These orphan receptors share a high structural homology with other NRs but do not have an intrinsic physiological "parent" ligand. The orphan receptors interact with both corepressors and coactivators but not in a clearly defined role as the other two classes.

All three classes of NRs modulate gene expression through interactions with cofactors. NRs react to the cells' environment and rely on cofactors to drive proper gene expression. The cofactors interact with NRs to enhance their ability to activate or repress transcription. It is becoming increasingly clear that these cofactors connect many diverse biological processes with NR-mediated transcription in a cohesive, but poorly understood communication network.

Cofactors represent a diverse group of proteins that serve to enhance NR-mediated transcription primarily by binding to the ligand-activated receptor. Cofactors can be broken into two groups, the corepressor and the

coactivator. The corepressor was originally defined by its ability to inhibit transcription while the coactivator promoted the initiation of transcription. Unlike NRs, which adhere to a common structural theme, cofactors are highly diverse and have expanded to more than 300 in number. They can serve as adapters between the receptor and the general transcription machinery.

Two main ideas about cofactor functions have emerged regarding the relationship between cofactors and NR interactions. The first idea that became apparent was that enzymatic activities and structures are required for transcription. Enzymatic activities and structures are not only required for transcriptional function but also for modification of the integral components of the multiprotein complexes involved in transcription. These complexes also have been shown to be involved in modifications of the components of the basic transcriptional apparatus, specifically gene promoters. For example, many cofactors interact with the NR ligand binding domain (LBD) through a highly conserved NR box, or short amino acid motif. This NR box is made up of a LXXLL motif, (where L represents leucine, and X corresponds to any amino acid), that forms an amphipathic alpha helix [1, 2]. The combination of the specific LXXLL motifs and flanking sequences influence the structural patterns of usage by different NRs [3].

The second idea about cofactor function to emerge is that many regulated transcription factors use a precise, almost step wise, sequence of functional actions by multiple protein complexes for mediating gene expression. The biological actions of post translational modifications (PTMs) permit cofactors to perform in a scheduled fashion. Many of the sequential PTMs are responsible for the specific gene expression responses to biological signals. These modifications are needed for gene activation or gene deactivation. Yet, for these modifications to start gene expression, the interaction of cofactors with NRs requires a recognition interface between the proteins. This primary recognition interface is between the LXXLL motif and the NR.

Transcriptional Dynamics of NRs through the LXXLL Motif

The regulatory sequences of NR-target genes are normally incorporated into the tightly bound chromatin structure that is independent of gene expression. This configuration allows for an agonist binding event to trigger a conformational change in structure of the NRs. The area in which this conformational change occurs is in the NR region of the AF-2. This leads to the recruitment of cofactors through the LXXLL motif and promotes transcriptional activation.

The structural causes for signal dependent cofactor interactions have been intensively studied with regards to nuclear receptors and the interactions of the LXXLL motif. The nuclear receptor LBD consists of three inner core alpha helices and two outer core helices. The central core of these three helices is packed between two additional layers of helices which form the ligand-binding cavity. An additional helix required for ligand-dependent transcriptional activation resides at the C terminus of the LBD and assumes different positions depending on the presence or absence of ligands [4]. This activation helix assumes a different configuration in the presence of agonists. This agonist inspired helical configuration is called a “charge clamp" [5].

In the presence of an agonist, the AF-2 helix is stabilized in the active conformation that forms the charge clamp pocket and facilitates the binding of the coactivator helix. In contrast, the binding of an antagonist keeps the AF-2 helix out of the active position, resulting in a larger pocket that destabilizes coactivator binding. The AF-2 helix consequently serves as a ligand sensor to regulate NR functions involved in gene expression through interaction with the LXXLL motif.

Several cofactors have been shown to contain the LXXLL motif, with single or multiple copies of the sequence forming the NR interacting domain. The number of the LXXLL motifs also varies considerably among cofactors and is likely to account for the observed differences in binding to selected NRs. The motif, or a form of it, is involved in regulating the interaction of NRs with both coactivators and corepressors.

Corepressors that have this motif include the nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT). Both NCoR and SMRT interact with unliganded nuclear receptors through an elongated LXXLL motif, e.g. LXX I/H IXXX I/L, (I represents isoleucine and H represents histidine). This elongated LXXLL motif is alternatively referred to as the Cornr-box [6, 7]. In the absence of agonist binding, this extended helix can fill the same hydrophobic

pocket in the NR that is occupied by the LXXLL motif due to the displacement of the AF-2. However, the extended helices of NCoR/SMRT are too long to be accommodated by this pocket when the AF-2 assumes the charge clamp configuration in response to ligand binding. Therefore, ligand binding reduces the affinity of corepressors that have the Cornr-box motif. This increases the affinity for the shorter LXXLL sequence containing coactivators [6, 7].

Other NR corepressors are recruited by the LXXLL motif in a ligand-dependent manner. For example, ligand-dependent nuclear corepressor (LCoR), receptor interaction protein 140 (RIP140), repressor of estrogen receptor activity (REA), and the preferentially expressed antigen in melanoma, (PRAME), are each recruited to nuclear receptors in a ligand dependent manner through interaction with LXXLL helices [8-11]. The LXXLL motifs, which may be used in a nuclear receptor specific fashion, can permit allosteric effects to modulate the efficacy and the stability of coactivator function [12].

The cofactor motif LXXLL functions to aid in the regulation of the gene expression of NR target genes. Interaction of this cofactor motif is not the only way that cofactors are able to regulate NR responses. Cofactors are able to respond to endocrine signals as well as regulate the stability,

localization, and PTMs of NRs. For this to occur, many cofactors have a shared characteristic in their enzymatic activities that promote and direct transcription through PTMs. For instance, in the p160 family or cofactors known as steroid receptor coactivators (SRC-1, 2 and 3) PTMs are integral to many of the activities required for gene expression. SRC-1 and 3, along with cAMP response binding element (CREB)-binding protein (CBP), and p300 contain histone acetyltransferase (HAT) activity that targets histones or other proteins at NR-regulated gene promoters for acetylation.

Post-translational Modifications of Cofactors

Histones are a major site for regulation of gene expression by cofactors. The targeting of histones by cofactors with PTMs causes the genes to activate or deactivate. However, histones are not the only targets of cofactor PTM actions. Cofactor PTMs lead to distinct biological responses. These responses affect the histones embedded in the chromatin and transcriptional expression [13-16].

PTMs can also lead to the epigenetic modification of DNA. Modifications of histones can be directed to a specific gene locus and will influence the expression of an individual gene. This influence happens through the PTM's ability to modulate multiple gene sets that are targeted by a wide variety of transcription factors. These physical interactions direct the

role of cofactors to assemble a variety of gene products to organize different functions towards a physiological goal. It is apparent that the DNA, chromatin proteins, transcription factors, cofactors, and signaling enzymes all communicate with each other. This communication occurs through reversible covalent modifications which provide a complicated, but critical signal integration of individual proteins that direct cell dynamics.

Biological complexity and environmental signal integration can be seen in many cofactors. For instance, SRC-3 can be modified by a small ubiquitin-related modifier protein or SUMOylated (at aa 723 and 786) and is phosphorylated at threonine/serine residues (at aa T24, S505, S543, S857, S860, and S867). After phosphorylation events at serine 505/509 in a glycogen synthase kinase 3 (GSK3)-dependent manner, SRC-3 becomes monoubiquitinated at amino acids 723 and 786, and is able to function as a potent and specific transcriptional activator [15, 17-21]. The ubiquitination sites are gradually polyubiquitinated during subsequent rounds of transcription, ultimately leading to its degradation by the 26S proteasome [22]. Yet, other phosphorylation sites in SRC-3 are targeted by other kinases and are necessary for SRC-3 to form different multiprotein complexes and coactivate more of its targeted transcription factors [19, 23]. In SRC-3, other PTMs such as methylation or acetylation lead to cofactor complex disassembly, which along with proteasome-mediated degradation, contributes

to coregulator component interactions by altering its transcriptional properties [24, 25].

Ubiquitin and SUMOylation mediated processes have functions in cofactor activity involved with proteasome-mediated degradation. For example, ubiquitin plays a critical role in regulating cofactor activity which induces proteasome-mediated degradation of the protein by directly altering its transcriptional properties. SUMOylation is involved with the subcellular localization of cofactors and affects the stability and activity of cofactors through proteasome-mediated degradation. The further roles of SUMOylation and ubiquination in regards to cofactors and PTMs are currently under investigation. Other forms of PTMs and cofactor interactions, such as phosphorylation, have been studied for a long time. In the case of SRC-3 six phosphorylation sites are necessary for activation of different NRs. Phosphorylation of these six sites in SRC-3 can also activate other cofactors. However, not all of these sites are required for coactivation of other cofactors. Different combinations of site-specific phosphorylation of SRC-3 are necessary for regulation of endogenous genes involved in inflammation or transformation. Biochemical studies support the concept that modulation of SRC-3 phosphorylation alters its interactions with potential other cofactor partners, allowing the partners to function as a regulated integrator for diverse signaling pathways [15, 20, 21]. This is seen when phosphorylation of several

residues of SRC-3 are required for its effective interaction with the cAMP response element binding protein (CREB) binding protein (CBP) [20, 24, 26-28].

Two closely related cofactors, CBP and p300, can directly interact with NRs through the receptor interaction domain at the N-terminal. This domain appears to be non-essential for transcriptional activation *in vitro* [29]. The CBP/p300 cofactors are mainly recruited indirectly with SRC-1and 3 to NR target genes. These cofactors through HAT activity serve as assembly points for multiple activation complexes of ligand-dependent activation of NRs [30].

Many proteins interact with multiple cofactors, and these cofactor-cofactor interactions enhance transcriptional activity. An example of this type of interaction was seen with cofactor interactions and the glucocorticoid receptor interacting protein 1 (GRIP1) [31]. The p160 family showed that these interactions occur through the coiled-coil coactivator A (CoCoA) and is bound to the basic helix loop helix (bHLH) in the period circadian protein/Ah receptor nuclear translocator protein/single-minded protein (PAS) domain of GRIP1. This binding interaction of p160s and GRIP1 was shown to enhance the transcriptional activation of NRs [28]. This type of activity further showed the importance of other cofactor-cofactor interactions and

transcriptional activity. The discovery that coactivator associated arginine methyltransferase 1 (CARM1) can interact with GRIP 1 helped to uncover other pathways involving the p160s. These two proteins interact on the AF2 domain and demonstrate activity of histone methylation into NR-activation networks [31].

CARM1 exhibits intrinsic protein arginine methyltransferase activity. This activity is responsible for modifying Arg 17 on histone H3, and these two factors together boost the activation of transcription. The increase in activation occurs when recruited hormone activated transcriptional complexes are interacting with the p160 proteins. Protein arginine methyltransferase 1 (PRMT1), another type of arginine specific protein methyltransferase, acts on histone H4. PRMT1 was also found to be a coactivator for NRs [16, 32]. PRMT1, like CARM1, binds to the C-terminal activation domain of the p160 coactivators, and this interaction combines to synergistically stimulate transcription by NRs with CBP/p300 in transient transfection assays [16, 32]. CARM1 and GRIP1 specifically associate with a large tandem array of mouse mammary tumor virus (MMTV) promoters in a ligand-dependent manner to enhance gene expression. However, lysine methylation at K4 in histone H3, which is often associated with transcriptionally competent chromatin, is not affected by hormone treatment. This work suggests that arginine specific histone methylation by CARM1 is an important step in transcriptional

activation at NR target genes [33]. CARM1 has been shown to regulate a number of nuclear hormone receptors and the use of the chromatin immunoprecipitation assay (ChIP), coupled with promoter arrays, can be helpful in understanding the larger role of arginine methylation in activating NR target genes.

Numerous studies support the idea that arginine methylation correlates with transcriptional activation. An interesting feature of this modification is the retention of a positive charge, as opposed to acetylation, which neutralizes the positive charge of lysine. Whether methylated arginine alters the higher order of the chromatin structure or serves as a marker for recruitment of additional proteins is unknown. Even so, it has become clear that different histone modifications by cofactor proteins are an integral part of gene regulation. It has been established that the interactions between histone methylation by CARM1 and PRMT1 and histone acetylation by CBP/p300 are important for the expression of NR target genes. Although methylation of Arg3 by PRMT1 eases further acetylation events of H4 tails by p300. These ordered cooperative functions of PRMT1 and CARM1 are not restricted to NR pathways, as these interactions have also been observed in transcriptional activation by p53 [16, 32].

CBP acetylates SRC-3 through its receptor interaction domain. This discovery provided a new idea of transcriptional regulation through acetylation [34]. This idea suggests that a built-in mechanism may exist that enables the hormone response to be attenuated in both a ligand and receptor-dependent fashion. Yet, methylation of CBP by CARM1 has a different outcome. CARM1-mediated methylation of CBP in the CREB binding domain (KIX) stops interaction with CREB, resulting in the inhibition of CREB-dependent transcription. This differs from the normal action of CARM1 and CBP where these two coactivators act together to increase the coactivated transcriptions by NRs. In these examples, CARM1 appears to be a molecular switch that selectively blocks cAMP signaling while it potentiates NR-mediated transcription [16]. Subsequent studies have revealed that CBP/p300 can be methylated by CARM1 at other sites, which are important for the coactivator communications which will activate NRs.

Many PTMs serve to enhance the role of cofactors for the activation of NRs. Post translational events such as, methylation, acetylation and phosphorylation direct the transcriptional activity for intricate signaling pathways required for gene expression. Taken together, an increasing number of cofactor modifications are emerging, which affect complex assembly and mediate a broad array of transcriptional responses. The cofactors are the assembly point for multiple protein complexes and precipitate the

transcriptional events involved in gene expression. There are instances when cofactors are able to reverse their normal function in certain circumstances.

Modifications which Cause Role Reversals in Some Cofactors

Some cofactors can function as both coactivators and corepressors. One such example is GRIP1, which normally functions as a coactivator of nuclear receptors. Yet, GRIP1 can function as a corepressor in combination with a bound estradiol receptor [35]. When this occurs there are allosteric influences in the DNA-binding hormone response element which regulate the ability of GRIP1 to bind unique parts of GR. These allosteric events are able to change its role from coactivator to corepressor [36]. Two corepressors, SMRT and NCoR, have illustrated a role reversal in certain situations. SMRT and NCoR can function as coactivators in specific promoter cofactor interactions. This demonstrates that these cofactor proteins can enhance or repress gene expression in a gene specific manner [37]. One example of role reversal is seen in SMRT. When SMRT is bound to TRα it undergoes a conformational change in a manner that causes an increase in the reaction of hormone response elements. Changes in the structures of NCoR and SMRT have been reported to impact the biological actions of various proteins as well. These changes suggest that PTMs have the ability to change the transcriptional effects of these corepressors [38].

Cofactor functions can be modulated by conformational changes as seen with SMRT. Cofactors can also be regulated by PTMs. The modifications highlight the complex integrations of upstream signals. In certain cases PTMs can alter the function of a corepressor such that it becomes a coactivator. An example of PTM role reversal is seen in the corepressor, RIP140. This role reversal occurs to RIP140 when it is conjugated to vitamin B6 through arginine methylation and phosphorylation. These PTMs modify RIP140's repressive function through direct competition with coactivators for agonist bound receptors. RIP140 is able to directly oppose the transcriptional activity of agonist ligands [39, 40]. This opposition allows for an alternative way of transcription that is distinct from the SMRT role reversal [41].

Role reversals are seen in the steroid receptor RNA activator (SRA) in certain situations. A role reversal in SRA is based on the phosphorylation of two specific proteins. These proteins may function in a manner to control whether SRA positively or negatively influence gene transcription through phosphorylation [42]. The example of SRA shows that a functional role reversal in a coregulator's action can be driven by its PTMs status. The action of PTMs on cofactors illustrates coregulator dynamics that are present in the cell [13, 14]. Cofactors perform role reversals through conformational

changes or PTMs and are an important mechanism involved in the molecular biology of gene expression.

Cofactor PTMs as a Mechanism of Gene Expression in Molecular Biology

Gene expression is achieved by the regulation of transcription and is needed for cell survival. The regulation of transcription is accomplished through timing events from multiple signaling pathways. The translation of the signals from the environment to gene expression is guided by PTMs in several types of transcriptional events. A single PTM, such as phosphorylation, to a cofactor can be responsible for the effects of proper timing of gene expression, an immune response, and degradation transcription machinery operation [43]. . This section is on timing so you need to introduce and focus on timing in this paragraph. Give your reader some idea of what will be discussed in this section.

Phosphorylation events define SRC-3 association with other members of the transcription complex, such as p300 and CBP or CARM1 [15]. The diverse physiological functions of SRC-3 can be attributed to its multiple phosphorylation sites. The six phosphorylation sites allow for the incorporation of multiple cellular signaling pathways to proceed with protein expression in the same time that the signals are being received [21]. The timing of the phosphorylation of a specific sequence on SRC-3 defines which

transcription factors this coactivator is able to activate and the time of activation [15]. This implies that the selectively phosphorylated factor is forced to preferentially implement the expression of genes downstream of a particular signaling cascade. PTMs such as these casts a light on the role cofactors have at being the directors of multiple cell signaling systems. Activation of membrane receptors and signaling cascades then allow the genome to sense the impact of the total environment on the cell.

PTMs of coactivators can enhance the timing of events in transcription. Covalent modifications including phosphorylation, acetylation, sumoylation, ubiquitination, and poly(ADP ribosyl)ation of coactivators such as CBP are critical aspects of a stepwise timing function [13, 15, 16, 32]. This stepwise function is seen when the CREB activates the transcription of target genes. The activation occurs through direct interactions with the KIX domain of the coactivator CBP in a phosphorylation-dependent manner [44-46]. The complex is formed first by the phosphorylated kinase inducible domain (pKID) of CREB. The pKID undergoes a coil-to-helix folding transition upon binding to KIX. This binding event causes a conformational change which forms two helices in pKID. One helix of pKID is amphipathic and interacts with a hydrophobic groove defined by the structure of KIX. The second pKID helix contacts a different segment of the KIX where a critical phosphate group of pKID forms a hydrogen bond to the side chain of the Tyr 658 in the

KIX structure. This combination provides a model for phosphorylation-dependent interactions between other transactivation domains and their targets in a stepwise manner [44-46].

Cofactors can organize the expression of functional groups of genes involved in the implementation of a specific regulatory regime, such as the inflammatory response. Members of the NF-κB family regulate a large number of genes involved in immune responses, specifically inflammation. This inflammation is mediated by IkappaB Kinase (IKK). These kinases are able to modulate the corepressor, SMRT, by phosphorylation [47]. IKK phosphorylates SMRT, permitting ubiquitination and export from the nucleus, and this appears to occur in a cycling mode [48]. Separately, IKK independent of cofactor function can cause S10-H3 phosphorylation and also controls acetylation of K14-H3. These PTMs suggest specialized functions of inflammatory cytokines are required for the regulation of inflammation at specific times [49].

The stepwise building of multiprotein complexes is important for the proper transcriptional events. It is critical to note that the degradation of these complexes is needed for future transcriptional events to proceed. Protein degradation is mediated through the ubiquitin proteasome (Ub) and constitutes a new concept in which transcription is fine tuned. Ub, along with

ubiquitinating enzymes and the proteasome, have been implicated in transcriptional regulation, which is sometimes independent of any degrading function.

Early reports suggested the Ub conjugating enzyme (Ubc9) interacted with GR [50]. Several NRs, such as GR, androgen receptor, and PPARs, have subsequently been found to be modified by SUMO 1. These modifications are mediated by Ubc9, and often coincide with the repression of NR transcriptional activity. Furthermore, if SUMO 1 modification is blocked by mutations on Ubc9 or the corresponding NRs, Ubc9 can act as a coactivator for NRs. This indicates that Ubc9 modulates NR activity regardless of its ability to catalyze SUMO-1 conjugation through degradation of protein complexes [51]. Degradation of proteins is needed to modulate the transcriptional activation for gene expression. For gene expression to occur, the chromatin structure has to be remodeled in exact locations. The activation of NR target genes requires direction of ATP-dependent chromatin remodeling complexes at these sites.

ATP Dependent Chromatin Remodeling Interaction with NRs

The chromatin structure in the eukaryotic nucleus creates barriers for transcription. Changes in chromatin, such as the disruption or reassembly of nucleosomes, are mediated by large multiprotein modules called chromatin-

remodeling complexes. ATP dependent chromatin remodeling complexes use energy from ATP hydrolysis to increase the mobility of nucleosomal DNA. This regulates a variety of cellular processes including transcription, DNA replication, DNA repair, and recombination. These complexes can be divided into 4 families according to the identity of their core ATPase subunits. Two of the most studied family members with NR interactions are the switch/sucrose non-fermentable (SWI/SNF) and imitation SWI (ISWI) [52]. The understanding of the mechanisms of chromatin remodeling and the biochemistry of individual functions of these different subunits is increasing rapidly.

ATP-dependent chromatin-remodeling complexes have been tied to transcriptional regulation by steroid receptors for more than 15 years [53, 54]. Initial reports indicated that GR and ER interact with the SWI/SNF remodeling complex [55]. The targeting of SWI/SNF is thought to be achieved through the interaction of DNA-binding transcription factors, cofactors, or general transcription machinery. In addition, certain subunits of SWI/SNF with bromodomains are known to readily bind to acetylated histone tails. Different SWI/SNF components have been shown to act in an intermediary fashion with critical interactions between ER and mammalian SWI/SNF subunits. Researchers have looked at the context of NR cofactor

complexes and found multiple interactions were involved in the recruiting and stabilization of SWI/SNF on NR target gene promoters [55].

Depending on the step of transcription, one or more subunits might play dominant roles in docking SWI/SNF. The ChIP assay has made it possible to determine the specific recruitment of different factors and complexes by NRs, of which RAR/RXR and ERs have been extensively studied [56, 57]. Also, the use of immunofluorescence technology has allowed investigators to observe the dynamics of NR and chromatin interactions. One such example of this chromatin interaction was with the GR. The GR interacts rapidly with hormone response elements in living cells. Experiments have shown that GR first binds weakly to glucocorticoid response elements located throughout nucleosomes. These nucleosomes target the SWI/SNF complex and result in histone reorganization [55, 58]. The transiently remodeled chromatin creates a higher affinity cavity for additional GR binding. The resulting chromatin reverts to the basal state through displacement reactions. This suggests that the interaction between receptors and chromosomal regulatory elements during chromatin remodeling is not only a complicated process, but that it is also a reversible process.

In addition to the SWI/SNF complex, other chromatin remodeling complexes such as ISWI, and nucleosome remodeling and deacetylation

(NURD), play a crucial role in NR function. ISWI has been found to be the earliest remodeling complex recruited by activated retinoid receptors on a reconstituted chromatin template [57]. Activation of minichromosomes assembled with the MMTV promoter requires the progesterone receptor to bind to ISWI, but not SWI/SNF. This event illustrates a chromatin remodeling event which helps to provide access by another transcription factor [53, 59]. The NURD complex, however, can be recruited to the ER by metastasis-associated gene 1 (MTA1). The consequence of NURD recruitment is the repression of ligand-dependent transcription through the ER. ER dependent transcription by the NURD complex happens because it contains at least two histone deacetylase complexes [16].

Histone exchange and displacement can be mediated by the transcription elongation complex and by ATP-dependent remodeling enzymes [60]. This has been seen *in vivo* throughout the *S. cerevisiae* genome where a partial depletion of histones H3 and H4 tetramers have been observed [61]. Histone depletion phenomenon is also observed with the progesterone receptor (PR). Receptor-bound progesterone recruits SWI/SNF to the promoter in the cell after progesterone treatment. SWI/SNF displaces histone H2A and H2B from nucleosomes containing the receptor binding sites, but not from adjacent nucleosomes. The main reason for this displacement is within the actual DNA sequence. It appears that the SWI/SNF promotes nucleosome

sliding on assembled ribosomal DNA without the displacement of H2A and H2B. This information suggests the remodeling by SWI/SNF depends on the actual nucleotide sequence in the nucleosomes [62]. Given that NR-activated genes respond quickly to ligand treatment, the actively transcribed genes may be identified by histone variants. Identification of histone variants is an important step in understanding the roles of NR driven transcription through remodeling proteins. Abnormalities in Transcription and actual gene expression are an underlying cause in many of the disease states seen in many humans today.

Cofactors and Diseases

Many human diseases can be associated with faulty expression of cofactors or the incorrect interactions of transcriptional events. This is seen in aggressive breast, uterine, ovarian, and prostate cancers. In these types of cancers estrogen and androgen are both known to be powerful mitogenic factors. In certain situations these factors can activate coregulators to promote unchecked cellular growth. The p160 proteins, specifically SRC-3, are closely involved in the uncontrolled promotion of cancer. SRC-3 was first identified as the coregulator amplified in breast cancer 1 (AIBC1) [63]. Many other cofactors have been found to be over expressed in cancers while other cofactors are involved in oncogenesis [8, 63-65].

Cofactor interactions are revealing themselves in other human pathologies in addition to cancer. Studies have identified peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1 α) as a cofactor that has multiple partners to interact for activation or repression. PGC-1, originally identified as a PPARγ interacting coactivator in brown adipose tissue, has now been demonstrated to coactivate many NRs and several other transcription factors [66, 67]. PGC-1α has recently been shown to be methylated by PRMT1 [68]. The timing of many PGC-1α actions are potentiated by PRMTl-mediated methylation. Understandably, mutations on PGC-1α methylation sites compromise its ability to activate transcription. For instance, polymorphisms in PGC-1α are reported to be linked to diabetes, polycystic ovarian syndrome, and other metabolic syndromes [69, 70]. PGC-1α is a key coactivator in the regulation of metabolic function [71]. Its expression is highly induced in brown adipose tissue and muscle during exercise, fasting, or cold exposure [66]. PGC-1α coactivates PPARγ, as well as other NRs, and it was shown to have a central role in metabolic function when knocked out in mouse models [72, 73]. The related protein, Peroxisome proliferator-activated receptor gamma coactivator 1 beta (PGC-1β), also functions as a metabolic coactivator. PGC-1β knockout mice have defects in fat metabolism and mitochondrial function [74, 75].

Problems with obesity are directly related to energy metabolism. members of the p160 family function in energy metabolism through the NR PPARγ. PPARγ is essential for adipoctye differentiation and correct energy homeostasis. SRC-1 and SRC-2 have been shown to occupy opposite roles in energy metabolism in mice knockout studies. In these knockout studies, a relationship with PPARγ was established. Knockout mice of SRC-1 are prone to obesity due to decreased energy expenditure, whereas SRC-2 knockout mice are leaner due to the reduced transcriptional capacity of PPARγ. Also, SRC-2 knockout mice have a distinct increase in the PGC-1α/SRC-1 interaction. This interaction enhances the thermogenic actions of PGC-1α in brown adipose tissue through PPARγ interactions in metabolism [76].

SRC-3 is involved in energy metabolism by promoting the formation of white adipose tissue. SRC-3 knockout mice possess a decreased adipose tissue mass [77]. Through knockout animal studies it was determined that SRC-3 is able to enhance CAAT enhancer binding protein β (C/EBPβ) mediated transcription of PPARγ. While our understanding in this area grows, the complete interactions involved in energy homeostasis are still unknown.

PPARγ is known to be directly involved with the cofactor PGC-1α in the differentiation of adipose tissue and energy metabolism. Other cofactors,

such as RIP140, are also involved in energy metabolism. RIP140 can repress the transcription of a variety of genes involved in fat and carbohydrate metabolism. Loss of RIP140 in knockout mice causes a leaner phenotype, resistance to obesity, and increased insulin sensitivity [78].

Conclusions

The complex coordination of gene expression by NRs in response to diverse physiological, metabolic, and environmental cues requires the recruitment of functionally distinct cofactors and chromatin remodeling complexes. The combination of these proteins in transcriptional regulation has been shown to be receptor dependent, ligand specific, and promoter to gene-specific. The cell and tissue expression of cofactors have a dramatic impact on gene expression. For example, PGC-1α is expressed in highly oxidative organs, such as the heart, muscle, brown fat, kidney, and liver. Animal studies that have used cold or fasting as experimental conditions show PGC-1α expression is dramatically induced. This induction shows the ability of PGC-1α to regulate the metabolic process. Many of the p160 family of proteins and PGC-1α have been shown to potentiate transcription by NR coactivators [76, 79]. Yet, it is important to observe that some coactivators appear to regulate a subset of NRs while others are more general in function.

Our initial understanding of cofactor function was simplistic as to center only on their role in the activation or repression of gene expression. Cofactor PTMs show the complex nature of the interactions by turning on and off genes. PTMs influence the specific nature of the transcriptional response when exposed to distinct signaling conditions. At this time it is impossible to determine the exact widespread effects these modifications will have on a biological system. It stands to reason that broad NR functions will follow the same pathways in similar systems for fine tuning the transcriptional expression in biological models. Studies from knockout mice and ChIP assays show a more complete role for cofactors in the recruitment of numerous proteins to the transcription complex. Coactivators are organized *in vivo* into complexes that are primed for recruitment by NRs. Studies have shown and support the sequential coactivator recruitment model. In this model, ATP-dependent remodeling factors and histone modification enzymes act in sequence to fine-tune NR action. PTMs of cofactors add an additional control by altering the affinity of the modified cofactor to the target NRs or to other cofactors. This alteration changes the magnitude of transcriptional output of NR-regulated promoters. Changes in cofactor expression, modification status, and enzymatic activities are often linked to disease states.

Pharmaceutical research is being conducted to aid in the control of disease states. Many of these pathological conditions involve pathways that

are responsible for gene expression through cofactor interactions. People suffering from diabetes or obesity could benefit from drugs that target specific cofactor interactions. A main stumbling block of this research is the body's energy homeostasis. There is no shortage in the possible drug targets for the treatment of diabetes and obesity. However, a subtle change to the delicate balance of energy metabolism triggers a defense from the body itself. This defense comes from the body's perception of starvation and reduces the energy expenditure to compensate for the change.

Further research into the interactions of cofactor involvement could lead to a better awareness of the pathology of some cancers. Estrogens are a key steroid that act through the ER and are important regulators of breast cancer growth. The receptor controls gene expression through the recruitment of transcriptional cofactors. These transcriptional coafactors are involved with the protein--protein interactions that allow for the uncontrolled growth of breast cancers. This illustrates the importance of the interactions of the ER with cofactors in breast cancer pathology. The development of improved selective NR modulators will be useful for the prevention and treatment of cancer as well as other diseases.

There are still major gaps in our understanding of the complexity of NR signaling events. Many questions are being asked by researchers in the

field now. What are the mechanisms of action of an individual coactivator? How are the engagement of complexes and the order of recruitment controlled on a promoter and cell-type-specific basis? Providing a wide view of the transcriptional landscape will be accomplished by the use of ChIP assay. This assay provides a depiction of the transcriptional methods, where the sequential or random recruitment of specific protein complexes can be analyzed. These experiments will reveal the specific transcription activation and the proteins involved and potentially aid the development of novel therapies that will benefit treatment of diabetes, obesity and cancer.

Bibliography

1. Voegel, J.J., et al., *The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways.* EMBO J, 1998. **17**(2): p. 507-19.
2. Heery, D.M., et al., *A signature motif in transcriptional co-activators mediates binding to nuclear receptors.* Nature, 1997. **387**(6634): p. 733-6.
3. Darimont, B.D., et al., *Structure and specificity of nuclear receptor-coactivator interactions.* Genes Dev, 1998. **12**(21): p. 3343-56.
4. Brzozowski, A.M., et al., *Molecular basis of agonism and antagonism in the oestrogen receptor.* Nature, 1997. **389**(6652): p. 753-8.
5. Li, Y., M.H. Lambert, and H.E. Xu, *Activation of nuclear receptors: a perspective from structural genomics.* Structure, 2003. **11**(7): p. 741-6.
6. Hu, Y.C., et al., *Functional domain and motif analyses of androgen receptor coregulator ARA70 and its differential expression in prostate cancer.* J Biol Chem, 2004. **279**(32): p. 33438-46.
7. Webb, P., et al., *ERbeta Binds N-CoR in the Presence of Estrogens via an LXXLL-like Motif in the N-CoR C-terminus.* Nucl Recept, 2003. **1**(1): p. 4.
8. Cavailles, V., et al., *Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor.* EMBO J, 1995. **14**(15): p. 3741-51.
9. Delage-Mourroux, R., et al., *Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA.* J Biol Chem, 2000. **275**(46): p. 35848-56.
10. Epping, M.T., et al., *The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling.* Cell, 2005. **122**(6): p. 835-47.
11. Fernandes, I., et al., *Ligand-dependent nuclear receptor corepressor LCoR functions by histone deacetylase-dependent and -independent mechanisms.* Mol Cell, 2003. **11**(1): p. 139-50.
12. McInerney, E.M., et al., *Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation.* Genes Dev, 1998. **12**(21): p. 3357-68.
13. Jenuwein, T. and C.D. Allis, *Translating the histone code.* Science, 2001. **293**(5532): p. 1074-80.
14. Rosenfeld, M.G., V.V. Lunyak, and C.K. Glass, *Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response.* Genes Dev, 2006. **20**(11): p. 1405-28.
15. Wu, R.C., C.L. Smith, and B.W. O'Malley, *Transcriptional regulation by steroid receptor coactivator phosphorylation.* Endocr Rev, 2005. **26**(3): p. 393-9.

16. Xu, W., H. Cho, and R.M. Evans, *Acetylation and methylation in nuclear receptor gene activation.* Methods Enzymol, 2003. **364**: p. 205-23.
17. Pascual, G., et al., *A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma.* Nature, 2005. **437**(7059): p. 759-63.
18. Wu, H., et al., *Coordinated regulation of AIB1 transcriptional activity by sumoylation and phosphorylation.* J Biol Chem, 2006. **281**(31): p. 21848-56.
19. Wu, R.C., et al., *SRC-3 coactivator functional lifetime is regulated by a phospho-dependent ubiquitin time clock.* Cell, 2007. **129**(6): p. 1125-40.
20. Wu, R.C., et al., *Regulation of SRC-3 (pCIP/ACTR/AIB-1/RAC-3/TRAM-1) Coactivator activity by I kappa B kinase.* Mol Cell Biol, 2002. **22**(10): p. 3549-61.
21. Wu, R.C., et al., *Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic reponses to multiple cellular signaling pathways.* Mol Cell, 2004. **15**(6): p. 937-49.
22. Nuber, U., S.E. Schwarz, and M. Scheffner, *The ubiquitin-protein ligase E6-associated protein (E6-AP) serves as its own substrate.* Eur J Biochem, 1998. **254**(3): p. 643-9.
23. Reid, G., et al., *Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling.* Mol Cell, 2003. **11**(3): p. 695-707.
24. Feng, Q., et al., *Signaling within a coactivator complex: methylation of SRC-3/AIB1 is a molecular switch for complex disassembly.* Mol Cell Biol, 2006. **26**(21): p. 7846-57.
25. Lee, Y.H., et al., *Regulation of coactivator complex assembly and function by protein arginine methylation and demethylimination.* Proc Natl Acad Sci U S A, 2005. **102**(10): p. 3611-6.
26. Chen, J.D. and R.M. Evans, *A transcriptional co-repressor that interacts with nuclear hormone receptors.* Nature, 1995. **377**(6548): p. 454-7.
27. Chen, Y.H., J.H. Kim, and M.R. Stallcup, *GAC63, a GRIP1-dependent nuclear receptor coactivator.* Mol Cell Biol, 2005. **25**(14): p. 5965-72.
28. Kim, J.H., H. Li, and M.R. Stallcup, *CoCoA, a nuclear receptor coactivator which acts through an N-terminal activation domain of p160 coactivators.* Mol Cell, 2003. **12**(6): p. 1537-49.
29. Yang, X.J., et al., *A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A.* Nature, 1996. **382**(6589): p. 319-24.
30. Torchia, J., et al., *The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function.* Nature, 1997. **387**(6634): p. 677-84.
31. Chen, D., et al., *Regulation of transcription by a protein methyltransferase.* Science, 1999. **284**(5423): p. 2174-7.

32. An, W., J. Kim, and R.G. Roeder, *Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53.* Cell, 2004. **117**(6): p. 735-48.
33. Ma, H., et al., *Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter.* Curr Biol, 2001. **11**(24): p. 1981-5.
34. Chen, H., et al., *Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300.* Cell, 1997. **90**(3): p. 569-80.
35. Cvoro, A., et al., *Distinct roles of unliganded and liganded estrogen receptors in transcriptional repression.* Mol Cell, 2006. **21**(4): p. 555-64.
36. Rogatsky, I., et al., *Alternate surfaces of transcriptional coregulator GRIP1 function in different glucocorticoid receptor activation and repression contexts.* Proc Natl Acad Sci U S A, 2002. **99**(26): p. 16701-6.
37. Berghagen, H., et al., *Corepressor SMRT functions as a coactivator for thyroid hormone receptor T3Ralpha from a negative hormone response element.* J Biol Chem, 2002. **277**(51): p. 49517-22.
38. Goodson, M., B.A. Jonas, and M.A. Privalsky, *Corepressors: custom tailoring and alterations while you wait.* Nucl Recept Signal, 2005. **3**: p. e003.
39. Gupta, P., et al., *Regulation of co-repressive activity of and HDAC recruitment to RIP140 by site-specific phosphorylation.* Mol Cell Proteomics, 2005. **4**(11): p. 1776-84.
40. Huq, M.D., et al., *Vitamin B6 conjugation to nuclear corepressor RIP140 and its role in gene regulation.* Nat Chem Biol, 2007. **3**(3): p. 161-5.
41. Cohen, R.N., et al., *The nuclear corepressors recognize distinct nuclear receptor complexes.* Mol Endocrinol, 2000. **14**(6): p. 900-14.
42. Zhao, X., et al., *Pus3p- and Pus1p-dependent pseudouridylation of steroid receptor RNA activator controls a functional switch that regulates nuclear receptor signaling.* Mol Endocrinol, 2007. **21**(3): p. 686-99.
43. McKenna, N.J. and B.W. O'Malley, *Combinatorial control of gene expression by nuclear receptors and coregulators.* Cell, 2002. **108**(4): p. 465-74.
44. Radhakrishnan, I., et al., *Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: a model for activator:coactivator interactions.* Cell, 1997. **91**(6): p. 741-52.
45. Meyer, T., D.B. Starr, and J. Carlstedt-Duke, *The rat glucocorticoid receptor mutant K461A differentiates between two different mechanisms of transrepression.* J Biol Chem, 1997. **272**(34): p. 21090-5.
46. Impey, S., et al., *Phosphorylation of CBP mediates transcriptional activation by neural activity and CaM kinase IV.* Neuron, 2002. **34**(2): p. 235-44.

47. Jonas, B.A. and M.L. Privalsky, *SMRT and N-CoR corepressors are regulated by distinct kinase signaling pathways.* J Biol Chem, 2004. **279**(52): p. 54676-86.
48. Hoberg, J.E., F. Yeung, and M.W. Mayo, *SMRT derepression by the IkappaB kinase alpha: a prerequisite to NF-kappaB transcription and survival.* Mol Cell, 2004. **16**(2): p. 245-55.
49. Anest, V., et al., *A nucleosomal function for IkappaB kinase-alpha in NF-kappaB-dependent gene expression.* Nature, 2003. **423**(6940): p. 659-63.
50. Gottlicher, M., et al., *Interaction of the Ubc9 human homologue with c-Jun and with the glucocorticoid receptor.* Steroids, 1996. **61**(4): p. 257-62.
51. Callewaert, L., et al., *Differential effect of small ubiquitin-like modifier (SUMO)-ylation of the androgen receptor in the control of cooperativity on selective versus canonical response elements.* Mol Endocrinol, 2004. **18**(6): p. 1438-49.
52. Becker, P.B. and W. Horz, *ATP-dependent nucleosome remodeling.* Annu Rev Biochem, 2002. **71**: p. 247-73.
53. Johnson, C.N., N.L. Adkins, and P. Georgel, *Chromatin remodeling complexes: ATP-dependent machines in action.* Biochem Cell Biol, 2005. **83**(4): p. 405-17.
54. Muchardt, C. and M. Yaniv, *ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job.* J Mol Biol, 1999. **293**(2): p. 187-98.
55. Fry, C.J. and C.L. Peterson, *Chromatin remodeling enzymes: who's on first?* Curr Biol, 2001. **11**(5): p. R185-97.
56. Dilworth, F.J. and P. Chambon, *Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription.* Oncogene, 2001. **20**(24): p. 3047-54.
57. Dilworth, F.J., et al., *ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR In vitro.* Mol Cell, 2000. **6**(5): p. 1049-58.
58. Dostert, A. and T. Heinzel, *Negative glucocorticoid receptor response elements and their role in glucocorticoid action.* Curr Pharm Des, 2004. **10**(23): p. 2807-16.
59. Di Croce, L., et al., *Two-step synergism between the progesterone receptor and the DNA-binding domain of nuclear factor 1 on MMTV minichromosomes.* Mol Cell, 1999. **4**(1): p. 45-54.
60. Belotserkovskaya, R. and D. Reinberg, *Facts about FACT and transcript elongation through chromatin.* Curr Opin Genet Dev, 2004. **14**(2): p. 139-46.
61. Lee, C.K., et al., *Evidence for nucleosome depletion at active regulatory regions genome-wide.* Nat Genet, 2004. **36**(8): p. 900-5.
62. Vicent, G.P., et al., *DNA instructed displacement of histones H2A and H2B at an inducible promoter.* Mol Cell, 2004. **16**(3): p. 439-52.

63. Anzick, S.L., et al., *AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer.* Science, 1997. **277**(5328): p. 965-8.
64. Bannister, A.J., R. Schneider, and T. Kouzarides, *Histone methylation: dynamic or static?* Cell, 2002. **109**(7): p. 801-6.
65. Martini, P.G. and B.S. Katzenellenbogen, *Modulation of estrogen receptor activity by selective coregulators.* J Steroid Biochem Mol Biol, 2003. **85**(2-5): p. 117-22.
66. Puigserver, P., et al., *A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis.* Cell, 1998. **92**(6): p. 829-39.
67. Puigserver, P. and B.M. Spiegelman, *Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator.* Endocr Rev, 2003. **24**(1): p. 78-90.
68. Teyssier, C., et al., *Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation.* Genes Dev, 2005. **19**(12): p. 1466-73.
69. Ek, J., et al., *Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus.* Diabetologia, 2001. **44**(12): p. 2220-6.
70. Hara, K., et al., *A genetic variation in the PGC-1 gene could confer insulin resistance and susceptibility to Type II diabetes.* Diabetologia, 2002. **45**(5): p. 740-3.
71. Spiegelman, B.M. and R. Heinrich, *Biological control through regulated transcriptional coactivators.* Cell, 2004. **119**(2): p. 157-67.
72. Lin, J., C. Handschin, and B.M. Spiegelman, *Metabolic control through the PGC-1 family of transcription coactivators.* Cell Metab, 2005. **1**(6): p. 361-70.
73. Yoon, J.C., et al., *Suppression of beta cell energy metabolism and insulin release by PGC-1alpha.* Dev Cell, 2003. **5**(1): p. 73-83.
74. Lelliott, C.J., et al., *Ablation of PGC-1beta results in defective mitochondrial activity, thermogenesis, hepatic function, and cardiac performance.* PLoS Biol, 2006. **4**(11): p. e369.
75. Vianna, C.R., et al., *Hypomorphic mutation of PGC-1beta causes mitochondrial dysfunction and liver insulin resistance.* Cell Metab, 2006. **4**(6): p. 453-64.
76. Feige, J.N. and J. Auwerx, *Transcriptional coregulators in the control of energy homeostasis.* Trends Cell Biol, 2007. **17**(6): p. 292-301.
77. Jeong, J.W., et al., *The genomic analysis of the impact of steroid receptor coactivators ablation on hepatic metabolism.* Mol Endocrinol, 2006. **20**(5): p. 1138-52.
78. Leonardsson, G., et al., *Nuclear receptor corepressor RIP140 regulates fat accumulation.* Proc Natl Acad Sci U S A, 2004. **101**(22): p. 8437-42.

79. Tcherepanova, I., et al., *Modulation of estrogen receptor-alpha transcriptional activity by the coactivator PGC-1*. J Biol Chem, 2000. **275**(21): p. 16302-8.