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Mediator-dependent Nuclear Receptor Functions

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Abstract

As gene-specific transcription factors, nuclear hormone receptors are broadly involved in many important biological processes. Their function on target genes requires the stepwise assembly of different coactivator complexes that facilitate chromatin remodeling and subsequent preinitiation complex (PIC) formation and function. Mediator has proved to be a crucial, and general, nuclear receptor-interacting coactivator, with demonstrated functions in transcription steps ranging from chromatin remodeling to subsequent PIC formation and function. Here we discuss (i) our current understanding of pathways that nuclear receptors and other interacting cofactors employ to recruit Mediator to target gene enhancers and promoters, including conditional requirements for the strong NR-Mediator interactions mediated by the NR AF2 domain and the MED1 LXXLL motifs and (ii) mechanisms by which Mediator acts to transmit signals from enhancer-bound nuclear receptors to the general transcription machinery at core promoters to effect PIC formation and function.

Keywords

Nuclear Receptor; Mediator; MED1; LXXLL motif; Coregulators

1. Introduction

Nuclear receptors (NRs) comprise a large family of transcription factors that regulate expression of specific genes during development, cell differentiation, reproduction and homeostasis [1, 2]. A typical NR consists of structural domains that include a relatively less conserved N-terminal activation domain (AF1), a highly conserved central DNA-binding domain, a hinge region and a conserved C-terminal ligand binding domain (LBD) that contains a second and very strong activation domain (AF2) [3, 4]. Many NRs regulate target gene expression in a ligand-dependent manner and typical ligands include steroids (e.g., ER and GR ligands), non-steroids (e.g., TR and VDR ligands) and products of lipid metabolism (e.g., PPAR ligands) [3, 4]. The NR superfamily also includes many orphan NRs, such as HNF4 and ERRs, whose ligands are not yet identified [5]. NRs function as homo- or hetero-dimers bound to specific DNA sequences on target gene promoters and generally regulate gene expression in a ligand-dependent manner through concerted and stepwise recruitment of various transcription coregulators (including both coactivators and corepressors).

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The NR coactivators include (i) factors that effect chromatin modifications, such as the ATP-dependent chromatin remodelers and various factors (e.g., histone acetyl- and methyl-transferases) that directly modify histone tails through their intrinsic enzymatic activities, (ii) peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 and steroid receptor coactivator (SRC) family members that serve as scaffolds to recruit histone-modifying (and potentially other) factors, and (iii) factors, such as the Mediator, that act more directly on the general transcription machinery and RNA polymerase II (for reviews see [3, 4, 6-11]).

The NR corepressors, which actively repress target gene expression, include the related NR corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) proteins. These factors associate with NRs in unliganded states and recruit histone deacetylase (HDAC) complexes to NR target gene promoters to repress transcription [4, 12]. Other NR corepressors, notably receptor interacting protein 140 (RIP140), interact with NRs and repress their target gene expression in a ligand-dependent manner. The underlining repression mechanisms for RIP140 are thought to involve direct interactions with C-terminal binding proteins (CtBPs), which in turn recruit HDACs [13], as well as competitive interactions with coactivators with overlapping binding sites on liganded NRs [12].

The Mediator, mentioned above, is a large multisubunit complex that was originally identified as an activity in yeast [14] and mammalian [15] cell extracts that effected activator-dependent transcription in systems reconstituted with RNA polymerase II and cognate initiation factors. Mediator was first purified, and shown to interact with RNA polymerase II, in yeast [16, 17]. The mammalian complex was first purified through an intracellular ligand-dependent association with TR α (and thus called the TRAP complex) and simultaneously shown to be a TR α coactivator [18]. Similar or identical complexes were subsequently reported as the VDR-interacting DRIP complex [19], the SREBP-interacting ARC complex [20], SRB/Med-containing cofactor complex (SMCC) [21], the E1A-interacting human Mediator complex [22], the CRSP complex [23], positive cofactor 2 (PC2) [24], mammalian mediator [25] and negative regulator of activated transcription (NAT) [26]. The yeast and metazoan complexes share many conserved subunits and now are commonly referred as Mediator [27]. Combined biochemical, genetic and structural studies have revealed that Mediator is organized into discrete head, middle and tail modules, with some subunits (MED1 and MED26) located at the junction of the middle and tail modules [28]. Initial functional studies established that Mediator functions as a coactivator for a variety of DNA-binding activators (reviewed in [28, 29]). Subsequent analyses revealed that mammalian Mediator functions not only to effect activator-dependent transcription, but also to stimulate activator-independent basal transcription [30, 31] and to suppress transcription through the dissociable CDK8-kinase submodule [4, 28]. It is noteworthy that several isolates of Mediator were co-purified with NRs and that Mediator has been found to be a crucial, and general, coactivator for ligand-dependent NR functions. In this review, we will discuss functions of Mediator as an integration center for NR signaling pathways in response to a variety of signal inputs and how Mediator processes these signals to transcription outputs. We discuss, first, how NRs anchor the Mediator complex to target gene promoters; second, types of cell signals that affect this process; and, finally, models of how Mediator transmits the signals from NRs to the general transcription machinery to effect PIC formation and function.

2. MED1 acts as a key component to mediate strong ligand-dependent interactions between Mediator and NRs

Since the cloning of its cognate cDNA, the MED1 subunit of Mediator has been demonstrated to interact strongly with most tested NRs in a ligand-dependent manner and,

consequently, to facilitate strong NR-Mediator interactions. These strong, well-characterized interactions between MED1/Mediator and NRs depend upon the MED1 LXXLL motifs and the NR AF2 domain and, importantly, are necessary for maximal NR-dependent transcription in reconstituted cell free systems. Remarkably, however, they were found to be unnecessary for most normal physiological functions, including the adipogenic differentiation pathway that was first shown to require MED1 [32, 33]. These results have suggested a conditional requirement for these strong NR-Mediator interactions and functions, perhaps under conditions of dietary stress, as well as the existence of alternative, potentially redundant, pathways for Mediator recruitment. In this regard, there are recent reports of NR/cofactor interactions with the conserved MED1 N-terminal domain that is essential for adipogenesis. These issues and their implications are discussed below.

2.1 MED1 LXXLL motif- and NR AF2-dependent interactions between Mediator and NRs

Along with associated functional studies, the isolation of human Mediator through an intracellular T₃-dependent interaction with TR α [18] and through an *in vitro* 1,25(OH)₂D₃-dependent interaction with VDR [19] revealed an important new function for NR AF2 domains and greatly enhanced our understanding of how NRs regulate target gene expression in response to cognate ligands. Subsequent studies revealed that TR α and VDR [19, 34, 35], as well as many other NRs that include TR β , ER α/β , PPAR α/γ , GR, AR, RAR α and RXR α [34, 36-42], interact with MED1 in a ligand-dependent manner. Corresponding functional studies have shown MED1-dependent activation of NRs both in cell-based assays [34, 36, 38-40, 43] and, especially, in cell-free systems reconstituted with MED1-deficient Mediator [32, 44].

Besides the *in vitro* studies, *in vivo* functional studies with mouse models and derived mouse embryonic fibroblasts (MEFs) with deletions or mutations in the endogenous *Med1* gene also have demonstrated the importance of MED1 in NR-mediated biological processes. The deletion of *Med1* in mice results in embryonic lethality at E11.5 days [43, 45], demonstrating an important role of MED1 in transcription during development but not for cell viability per se. Further analysis of MEFs derived from *Med1*^{-/-} mouse embryos revealed that MED1 is essential for some NR pathways (e.g., PPAR γ -mediated adipogenesis), but not for other developmental pathways such as MyoD-induced myogenesis [38]. These results reflect selective functions of MED1 in certain NR pathways and further indicate that MED1 regulates transcription in a gene-specific manner. Because of the embryonic lethality of the *Med1* null mice, several conditional *Med1* knockout lines and *Med1* knockin lines with mutated MED1 have been reported. Analyses of these mouse lines further documented important functions of MED1 in NR pathways. These include (besides PPAR γ -mediated adipogenesis [38]), PPAR α -mediated oxidation of fatty acids [46], ER α -dependent mammary gland development [33], GR- and CAR-mediated hepatic steatosis [47], TR α -mediated thermogenesis through uncoupling protein-1 (*UCP-1*) up-regulation [48] and skeletal muscle functions in the regulation of insulin signaling and energy expenditure [49]. Therefore, MED1 functions as a key component to anchor the Mediator complex to NRs on target gene promoters for ligand-induced NR functions.

MED1 contains two LXXLL motifs [34, 36], a signature motif that is shared by a variety of cofactors (including SRC family members, PGC-1 family members, p300/CBP and RIP140) and employed for their binding to NRs [50, 51]. Studies with isolated wild type and mutant MED1 and NR domains have shown ligand-induced MED1-NR interactions dependent upon MED1 LXXLL motifs and an intact AF2 domain [34, 52, 53]. Importantly, studies using reconstituted Mediator complexes containing mutations in the MED1 LXXLL motifs have shown that these motifs are essential both for strong ligand-dependent interactions of Mediator with NRs and for optimal NR-mediated transcription *in vitro* [32, 44] and *in vivo* [33]. Therefore, the AF2 domains of NRs and the LXXLL motifs of MED1 are critical

components that mediate ligand-dependent interactions between NRs and MED1-containing Mediator complexes. In relation to the structural basis of these interactions, crystal structures have been solved for many NR LBDs, in both the unliganded (apo) and liganded (holo) states and, as well, for several ternary complexes containing LBDs, cognate ligands and LXXLL peptides from SRCs and MED1 [54-57]. The AF2-containing LBDs of all NRs studied to date bear a similar three-layered α -helical sandwich structure with a central ligand-binding site in which the ligand is buried. The H12 helix of the AF2 motif extends away from the LBD core in the unliganded state, but, upon ligand binding, is folded against the LBD, with two conserved hydrophilic residues (glutamine and lysine) forming a charged clamp that mediates interactions with the α -helix LXXLL motif of coactivators [55-57]. These observations have important implications for the overall NR-mediated activation pathways.

Similar to what has been observed for SRC LXXLL motifs [54, 58], *in vitro* binding assays have shown some preferential binding of different NRs to each of the two MED1 LXXLL motifs. Generally, steroid hormone receptors, such as ER, show preferred binding to the first LXXLL motif, whereas non-steroid hormone receptors, such as TR α and VDR, show preferred binding to the second LXXLL motif [34, 52, 53]. However, transcription assays using cell-free systems and cell-based assays have demonstrated a requirement for both LXXLL motifs for efficient NR-mediated transcription [32, 44]. This is consistent with the results from structural studies indicating that each LBD within an NR dimer binds an LXXLL motif peptide and suggests that multiple LXXLL motifs within a single coactivator may act cooperatively -- either to facilitate stable binding of a single coactivator molecule to a heterodimeric receptor complex or to facilitate subsequent function [55, 57]. The cooperative binding of two LXXLL motifs within one coactivator may also explain the observation that the spacing and flanking sequences of LXXLL motifs are important determinants for coactivator binding to NRs [52, 54, 58, 59].

2.2 Conditional requirements for the strong MED1 LXXLL motif-dependent NR-Mediator interactions

Previous studies with *Med1*^{-/-} MEFs demonstrated that MED1 is essential for PPAR γ -stimulated adipogenesis and PPAR γ -mediated gene activation in MEFs [38]. However, subsequent studies revealed that the evolutionarily conserved N-terminal region, which mediates interactions of MED1 with the core Mediator complex but lacks the two LXXLL motifs, is sufficient to rescue both PPAR γ -stimulated adipogenesis and PPAR γ -target gene expression in *Med1* null MEFs [32]. Furthermore, although MED1 LXXLL mutant knockin mice show deficient functions in ER α -dependent pubertal mammary gland development and associated ER α target expression, these mice are generally healthy and, therefore, not deficient in many NR signaling pathways [33]. As Mediator appears to be generally required for activator functions [28, 60], these results strongly suggest the existence of alternative MED1 LXXLL-independent pathways for Mediator recruitment to NR-activated genes. Possibilities, discussed further below, include pathways that involve (i) NR interactions with the N-terminus of MED1, (ii) NR interactions with other Mediator subunits or (iii) interactions with Mediator of other enhancer-bound factors that act in conjunction with NRs on NR target genes (Figure 1).

Our observation that the strong MED1 LXXLL-dependent NR-Mediator interactions are not generally required for NR-dependent functions *in vivo* raises interesting questions regarding the normal functions of these interactions and may also have important therapeutic implications. Thus, more recent studies have shown that MED1 LXXLL mutant mice are not only generally healthy and viable [33], but also show enhanced glucose tolerance and insulin sensitivity and resistance to weight gain on a high fat diet (M. Ito and R. Roeder, unpublished observations). This has led to speculation that the strong MED1 LXXLL-

dependent NR-Mediator interactions may have evolved, and are conditionally required, for special NR functions – including, for example, enhanced PPAR γ functions under conditions such as a feast-fast environment where the organism may need to efficiently store energy (as fat) when food is plentiful [61]. An alternative explanation is that the various pathways are simply redundant and have evolved to effect biological robustness -- such that the MED1 LXXLL pathway might be essential in the absence of the alternative pathways. In either case, the results also suggest the NR AF2-MED1 LXXLL interface as a potential therapeutic target “cf. [62]”.

2.3 MED1 N-terminal domain-dependent interactions of Mediator with NRs

Consistent with an alternative Mediator recruitment pathway involving the MED1 N-terminus, Stallcup and colleagues [63] identified a coactivator, CCAR1, that interacts not only with CoCoA, a coactivator that interacts with NR-bound SRC coactivators, but also with the MED1 N-terminus. Importantly, CCAR1 was shown to function as a coactivator for ER α and GR, and depletion of CCAR1 was found to significantly reduce ligand-dependent recruitment of MED1/Mediator to ER α - and GR-target gene promoters. Therefore, MED1/Mediator could be targeted to NR target genes through a MED1 N-terminal domain interaction with CCAR1, which in turn interacts with CoCoA-SRC-NR complexes on responsive promoters [63] (Figure 1). In addition to these indirect interactions of NR AF2 domains with the MED1 N-terminal domain, the AF-1 domain of the NR4A1-3 subfamily (Nur77, NURR1 and NOR-1) of orphan NRs has been reported to interact directly with the MED1 N-terminus [64]. Although of potential importance for Mediator recruitment, the functional significance of this interaction for regulation of NR4A1-3 target genes remains to be established.

These results, and others described below, suggest alternative, LXXLL-independent, pathways for physical and functional interactions between NRs and Mediator, although the MED1-LXXLL-independent interactions appear to be considerably weaker than the MED1-LXXLL-dependent interactions. In this regard, it should also be noted that, apart from pathways of Mediator recruitment through interactions of distinct Mediator subunits with NRs, Mediator may also be recruited to NR target genes through direct interactions with other DNA-binding transcription factors that act synergistically with NRs on specific genes. For example, C/EBPs have been shown to function cooperatively with PPAR γ in the activation of adipogenic genes [65-67] and recently have been reported to interact with the N-terminal domain of MED1 [68] and with MED23 [69]. Therefore, such interactions may also serve as the basis for alternative pathways for Mediator recruitment and function on NR target genes (Figure 1).

2.4 Regulation of interactions between Mediator and NRs

Protein phosphorylation, with consequent modification of protein-protein interactions, provides an effective way for cell signals to regulate gene expression. In this regard, the MED1 subunit of the Mediator has been reported to be phosphorylated by the MAPK family [70, 71]. More recently, this phosphorylation has been shown to promote association of MED1 with the Mediator complex and to modestly stimulate TR α -mediated gene activation [72]. Similarly, some NRs have also been found to be phosphorylated, with consequent effects on Mediator interactions. Thus, phosphorylation of human RXR α at serine-260 by the Ras-Raf-MAPK pathway was reported to significantly disrupt 1,25(OH) $_2$ D $_3$ -induced recruitment of MED1 to RXR α -VDR bound to cell cycle regulatory genes without affecting RXR α dimerization with VDR [73]. As RXR α phosphorylation at serine-260 is known to cause resistance to the anti-proliferative effects of 1,25(OH) $_2$ D $_3$ in several cancer cell lines, the above results may at least partially explain this resistance [73]. In contrast, phosphorylation of GR at serine-211 was shown to enhance binding to the MED14 subunit

of the Mediator and to stimulate transcription of MED14-dependent genes such as insulin-like growth factor binding protein 1 (*IGFBP1*) and interferon regulatory factor 8 (*IRF8*) [74]. Therefore, phosphorylation of both MED1 and NRs could differentially regulate Mediator recruitment in response to specific cell signals.

3. Interactions of NRs with other subunits of Mediator

In addition to MED1, other subunits of the Mediator complex also have been reported to play important roles in anchoring the Mediator complex to NRs (Table 1). These pathways and their roles in NR functions are discussed below.

3.1 A role for MED14 in Mediator-NR interactions

MED14 was first reported to interact with the GR AF-1 domain and to coactivate GR in transfection assays [39]. Recently, MED14 also was found to interact with PPAR γ and to be necessary both for Mediator recruitment to PPAR γ target gene promoters and for normal activation of a subset of PPAR γ target genes [75]. All of the above-mentioned interactions between MED14 and NRs are mediated through the N-terminal AF1 domain of NRs and, consistent with these observations, are independent of GR and PPAR γ ligands. However, GR, and PPAR γ also have been shown to interact with MED1 through their C-terminal AF2 domains. Therefore, these receptors may recruit and/or modulate the functions of Mediator, through interactions with different subunits of Mediator. This may reflect mechanisms through which NRs differentially, and perhaps synergistically, regulate their target gene expression in response to different physiological inputs. Indeed, expression of some GR target genes is dependent on MED14 but not MED1, while expression of other GR target genes is dependent on both MED1 and MED14, thus indicating differential and synergistic roles of MED14 and MED1 in regulating GR-mediated gene activation [76]. This also is apparent for PPAR γ , as MED14 was also found to be required only for a subset of PPAR γ -target genes [75] while expression of many other PPAR γ -target genes during adipogenesis is dependent on MED1 [38].

3.2 MED25-mediated interactions between Mediator and NRs

Med25 was cloned both as a gene that is overexpressed in prostate cancer [77, 78] and as a gene that encodes a Mediator subunit that interacts with the herpes simplex transcriptional activator VP16 [79, 80]. More recently, MED25 has been linked to the function of NRs [81, 82]. Consistent with the presence of an LXXLL motif in the C-terminus of MED25, Lee et al [81] reported a ligand-dependent MED25-RAR α interaction involving the MED25 LXXLL motif and RAR α AF2 domains and, further, that this interaction is important for recruitment of MED1/Mediator to the *RAR* β 2 promoter [81]. The function of MED25 in NR-mediated recruitment of Mediator is further evidenced by studies of the role of HNF4 α in drug and lipid metabolism [82]. In these studies, MED25 was found to be critical for the assembly of an HNF4 α coactivator complex (including Mediator), through a direct and specific interaction between the MED25 LXXLL motif and HNF4 α , and to regulate the expression of a subset of HNF4 α target genes that are specifically involved in drug and lipid metabolism [82].

Apart from being unique to vertebrate Mediator complexes, MED25 differs from other Mediator subunits, such as MED1 and MED14, in that it has a number of defined motifs. Thus, beyond the C-terminal LXXLL motif, MED25 contains an N-terminal VWA (A domain of the von Willebrand factor) domain that interacts with SRC-1 and with MED1/Mediator, a central PTOV/ACID domain that interacts with VP16 and p300/CBP, and two SD (synasin I) domains whose function is still unclear [78]. Because of its unique structure and its reported interactions with both CBP and Mediator, MED25 is believed to function

mechanistically in a manner distinct from MED1. Thus, given the further demonstration of its essential role in the synergistic recruitment of CBP and MED1/Mediator to the *RAR β 2* promoter in response to ligand, it may act in association with promoter-bound *RAR α* to facilitate both chromatin remodeling (via p300/CBP) and PIC formation (through Mediator recruitment) [81].

3.3 MED15 as a master regulator of lipid metabolism and detoxification

Recently, Mediator subunit MED15 has emerged as a key coregulator of lipid homeostasis in conjunction with SREBP-1 α , which regulates expression of cholesterologenic and lipogenic genes through the recruitment of cofactors that include Mediator and the p300/CBP histone acetyltransferases [20, 83]. As previously demonstrated for p300/CBP [83], MED15 was found to contain a KIX domain that mediates an interaction of MED15/Mediator with the activation domain of SREBP-1 α and thus facilitates activation of SREBP-1 α target genes [20, 84]. A KIX domain is also found in the MED15 homologue MDT-15 in *C. elegans* [84] and in the Gal11p Mediator subunit in yeast [85]. Consistent with the role of MED15 in lipid homeostasis in mammalian cells, MDT-15 has been shown to have an important role, in conjunction with SREBP-1 homolog SBP-1, in lipid homeostasis in *C. elegans* [84]. Interestingly, not only SREBP-1 α , but also orphan NRs NHR-49, a PPAR α /HNF4 α analog in *C. elegans*, and Oaf1, a zinc finger cluster transcription factor that is also a PPAR α analog in yeast, have been shown to specifically interact with the KIX domains of MDT-15 and Gal11p in a fatty acid-stimulated manner [85, 86]. These results further indicate the importance of the MED15/Mediator complex in regulating lipid homeostasis through NRs. Indeed, NHR-49:MDT-15 and Oaf1:Gal11p functional pairs have been demonstrated to be necessary for expression of fatty acid metabolic genes, for maintenance of regular fatty acid composition, and for maintenance of normal life span and cell growth [85, 86].

Also of major interest, a genome wide *mdt-15* RNAi-based study in *C. elegans* has shown that the predominant genes that are down-regulated by knockdown of *mdt-15* include not only new and previously identified lipid metabolism genes, but also genes that are involved in xenobiotic detoxification pathways [87]. These results confirm the important role of MDT-15 in lipid metabolism, and further suggest new MDT-15/Mediator functions in detoxification and multidrug resistance pathways. A later study with zinc finger cluster transcription factors *Pdr1p* and *Pdr3p*, analogs of the mammalian pregnane X receptor (PXR) in yeast and fungi, provided such evidence [88]. Thus, these factors were found to function through a PXR-like pathway with their C-terminal domains (LBD) binding to the KIX domain of MED15/Gal11p in a xenobiotic-stimulated manner [88].

4. Pathways for NR-mediated recruitment of Mediator to natural chromatin templates -- a multi-step cofactor exchange model

The normal cellular environment imposes a number of constraints to interactions of Mediator with NRs, either on or off the natural chromatin template. These include (i) different subcellular compartmentalization of Mediator and some NRs; (ii) the compact structure of the repressed chromatin template and, especially, (iii) other cofactors that interact with a common site on NRs. Below, we discuss mechanisms by which Mediator is recruited to NRs in the face of these constraints.

4.1 Access of Mediator to NRs

A subset of NRs are localized in the cytoplasm, in association with chaperone proteins, in the unliganded state. Upon ligand binding and consequent dissociation of chaperones, these NRs translocate to the nucleus, bind to target gene promoters/enhancers and recruit coactivators, including Mediator, that are constitutively localized in the nucleus. This

pathway is best exemplified by steroid receptors such as GR and ER [89]. In contrast, a large group of NRs, exemplified by TRs, PPARs and RARs, are constitutively localized in the nucleus but, in the absence of ligands, may be bound to target genes in association with corepressor complexes such as NCoR and SMRT [90, 91]. The associated corepressors further recruit HDAC complexes that, through histone deacetylation, actively maintain chromatin in a repressed state that prevents the recruitment of coactivators including Mediator [10, 90, 91]. As discussed below, these constraints are overcome by ligand-mediated events.

4.2 General pathway for cofactor exchange on promoter-bound NRs

NR cofactors include a number of prominent corepressors (the above-mentioned NCoR/SMRT, as well RIP140) and coactivators (SRC, PGC-1 and p300/CBP families) that, like Mediator, interact through LXXLL (or extended LXXLL) motifs with overlapping NR AF2 domains, raising questions regarding potentially competitive interactions and factors that govern their distinct and orderly functions. Given the above-mentioned functions of NCoR/SMRT, demonstrated co-activator functions of SRC proteins through recruitment of histone acetyltransferases (including p300/CBP) and protein arginine methyltransferases [10, 92], the “downstream” function of Mediator through direct interactions with the general transcription machinery [28], and demonstrations of the orderly, temporal recruitment of individual factors during gene activation [93, 94], a general cofactor exchange model has been proposed [34, 90, 95] (Figure 2). This model invokes (i) binding of unliganded NRs to target sites, with co-recruitment of corepressors and associated HDACs and consequent histone deacetylation and transcription repression, as mentioned above, (ii) ligand-mediated conformational changes in NR AF2 domains that lead to dissociation of corepressors followed by corecruitment of SRC coactivators and interacting histone modifying factors, (iii) HAT/PRMT/HKMT-mediated acetylation and methylation of adjacent nucleosomal histones and potentially other factors, leading to an open chromatin structure, (iv) exchange of SRCs and associated factors for Mediator and (v) Mediator-dependent functions in PIC formation and function. This model is consistent with in vitro studies indicating that Mediator facilitates NR-dependent transcription of naked DNA templates in the absence of histone modifying co-activators, whereas NR-dependent transcription of chromatin templates requires such factors along with Mediator [18, 96]. Given that Mediator and SRCs show independent (but competitive) ligand-dependent interactions with NRs, a major question is what determines the ordered pathway. One possibility is that there exists an equilibrium between chromatin/promoter-bound NR-Mediator and NR-SRC complexes, but that the Mediator complexes are inactive (unable to proceed to PICs) without prior nucleosomal histone modifications and chromatin remodeling (discussed further in [95]). In support of this notion, an important study with an ER α mutant that interacts with Mediator but not with SRCs has shown an intracellular (but non-functional) interaction of Mediator with an ER α target gene in the absence of SRC recruitment and histone acetylation, although the observation of a delay in the steady state level of promoter-associated Mediator also indicated a chromatin modification-facilitated recruitment of Mediator [97]. Beyond nucleosome modification/destabilization by SRC-associated factors, other driving forces for SRC-Mediator exchange could be stabilizing interactions of Mediator with components of the PIC [28] and HAT-mediated modification of SRCs [98]. It also could be, especially in light of the presence of free, highly stable NR-Mediator complexes in nuclear extracts [18] and the rapid intracellular exchange of chromatin-bound NRs [99], that the exchange pathway might involve some preformed NR-coactivator complexes.

4.3 Other pathways for cofactor exchange on NRs

Beyond the general pathway presented above, other more specialized pathways are also evident. First, the pathway for NRs (e.g., steroid receptors) that do not interact with NCoR/

SMRT corepressors may involve initial interactions with SRC coactivators. Second, reports of direct ligand-dependent interactions of NRs with p300/CBP suggest the possibility of SRC-independent recruitment for these factors, especially in cases where the NR-p300 interactions are stronger than the NR-SRC interactions (discussed in [100]). Similar considerations apply to other histone modifying factors, such as SAGA [101, 102] and MLL3/4 complexes [103] (Z. Tang and R.G. Roeder, unpublished observations), that show direct ligand-dependent interactions with NRs. Third, the inducible, cell-specific PGC-1 family of cofactors show direct, ligand-dependent interactions with many NRs [6] and may recruit p300 either directly [100] or potentially in conjunction with SRCs [104]. Interestingly, PGC-1 α also was shown to interact directly with MED1/Mediator and to enhance both PPAR γ /p300-dependent histone acetylation and transcription from chromatin templates and PPAR γ /Mediator-dependent transcription from DNA templates [100]. Apart from indicating a new Mediator interaction, these results strongly suggested that PGC-1 α might facilitate the transition between early chromatin remodeling and subsequent transcription events.

Our recent studies have indicated that PGC-1 α may fulfill this chromatin remodeling-transcription transition through a modified pathway involving a series of dynamic and concerted interactions with NRs and MED1/Mediator [48]. In the proposed pathway, PGC-1 α binds to a chromatin-bound NR through a ligand-dependent AF2-LXXLL domain interaction and subsequently recruits p300 for localized histone acetylation. After chromatin remodeling, NR-bound PGC-1 α is then displaced by Mediator through a stronger ligand-dependent interaction between the MED1 LXXLL motif and the NR AF2 domain. However, PGC-1 α is retained and further stabilized within the promoter complex through an interaction between C-terminal domains in MED1 and PGC-1 α . The stabilized PGC-1 α may then cooperate with Mediator, by an as yet unknown mechanism, to stimulate PIC formation and/or function in transcription initiation [48] (Figure 2, dotted PGC-1 α pathway). In this model, the sequence of events emphasizes key roles for dynamic MED1 and PGC-1 α interactions in coupling chromatin remodeling and transcription initiation events in NR functions [48]. Given the enormous number and diversity of reported NR co-regulators [10, 11], there no doubt will be many other alternative recruitment pathways that ultimately modulate Mediator recruitment and function.

5. Mechanisms by which Mediator regulates NR-target gene transcription

Given documented interactions of Mediator with RNA polymerase II, it has long been assumed that Mediator acts mainly to facilitate PIC formation. However, post-initiation roles have also been documented and, more recently, Mediator has been implicated in the recruitment of chromatin remodeling factors. A comprehensive review of the role of Mediator in PIC formation and function in regulation through chromatin has been published recently [28]. Hence, here we will focus specifically on mechanisms by which Mediator transmits the input signals from the ligand-bound NRs to the transcription machinery outputs.

5.1 Mediator facilitates chromatin looping

In response to corresponding activation signals, NRs bound to target gene response elements engage in cofactor interactions that lead to the assembly and function of preinitiation complexes on corresponding target gene core promoters (as discussed above). As emphasized by gene-specific chromatin immunoprecipitation (ChIP) and, especially, by genome wide ChIP-seq analyses, many NR binding sites are located far from corresponding core promoters (reviewed in [99]). Importantly, however, these types of analyses also have colocalized NRs, Mediator, coactivators and RNA polymerase II both to distal enhancers and to core promoter regions, thus suggesting that enhancers may be brought into the

vicinity of corresponding core promoters through chromatin looping [99, 105-107]. The chromatin looping model for NR targets is supported by direct evidence from studies of T₃-induced and MED1-dependent juxtaposition of the distal TR α -bound TRE and the basal promoter elements of the *Crabp1* gene [108] and by chromosome conformation capture (3C) analyses of AR and VDR target genes [106, 109] and, more recently, the AR-targeted *UBE2C* oncogene [110]. These studies also demonstrated important roles for MED1 in facilitating the chromatin looping events.

Most recently, Young and colleagues reported that Mediator and cohesin co-occupy both the enhancer and core-promoter regions of many actively transcribed genes, as measured by ChIP-seq assays, and that Mediator and cohesin function cooperatively to activate gene transcription in mouse embryonic stem cells [107]. Further studies indicated direct physical interactions between Mediator and the cohesin complex, including the cohesion loading factor, as well as requirements for both Mediator and cohesin for DNA looping events in ES cells [107]. Apart from providing further evidence for the importance of Mediator in chromatin looping events, these results also provide new insights into the underlying mechanism; and it will be interesting to see if the cohesin-related mechanism is relevant to NR-activated genes.

5.2 Mediator may directly link chromatin remodeling and PIC formation

As discussed earlier, NR coactivator PGC-1 α appears to link p300-dependent chromatin remodeling to Mediator-dependent transcription through dynamic interactions with NRs, p300 and MED1/Mediator [48, 100]. More direct roles for Mediator in linking chromatin remodeling and transcription have been indicated by studies of Carey and colleagues [111], who showed that p300 and initiation factor TFIID interact directly and competitively with VP16-bound Mediator. Thus, in their model, the promoter -VP16-Mediator complex initially recruits p300 to form a chromatin remodeling complex. Then, following histone acetylation and p300 auto-acetylation, Mediator-bound p300 is subsequently displaced by TFIID, facilitating PIC formation [111]. Therefore, perhaps in special cases Mediator may function to provide a platform for exchange of p300 and TFIID and, thus, to couple chromatin remodeling with PIC formation. Documented functional synergies between Mediator and p300 in HNF4- and ER α -mediated transcription [41, 96] are also consistent with the possibility of more direct roles for Mediator in the coordination of chromatin remodeling and transcription, although direct interactions of Mediator with chromatin remodeling factors remain to be identified in these cases.

5.3 Mediator provides a platform for the recruitment of corepressor complexes

In addition to its coactivator functions, Mediator also has been demonstrated to function directly in transcriptional repression through the core Mediator-associated CDK8 kinase module that contains MED12, MED13, cyclin C and CDK8 [4, 28]. The mechanisms by which the kinase module-associated Mediator represses transcription can be broadly divided into kinase-dependent and kinase-independent mechanisms. The kinase activity of CDK8 can phosphorylate DNA binding factors [112, 113] and general transcription factors [114], with the former phosphorylation events directing the DNA binding factors to degradation pathways and the latter affecting PIC formation. In other studies, the kinase module was reported to physically block the association of RNA pol II with Mediator independent of its kinase activity [28], indicating a possible kinase-independent inhibitory mechanism, and to effect repression of the RAR target gene *RAR β 2* [115]. The latter study of Reinberg and colleagues demonstrated a retinoic acid-dependent, PARP1-mediated transition from an inactive Mediator complex (containing the repressive CDK8 module) to an active Mediator complex (lacking the CDK8 module), although the actual basis for the repression by the kinase module was not fully investigated. More recently, Mediator has been implicated in

the epigenetic silencing of neuronal genes through a mechanism that involves direct interactions of Mediator with promoter-bound RE1 silencing transcription factor (REST), subsequent recruitment of the H3K9 methyltransferase G9a through interactions with both REST and Mediator subunit MED12, and interactions of the repressive H3K9methyl marks with other factors [116]. Therefore, Mediator can function both as a coactivator and, through the dynamic association of the kinase module, as a corepressor in response to different signals.

Recently, we reported that MED1 has opposing effects on the transcription of the brown fat-specific gene *UCP-1* gene. Thus, whereas MED1/Mediator activates *UCP-1* expression in cooperation with PGC-1 α in brown adipocytes [48], deletion of MED1 in skeletal muscle was found to activate expression of *UCP-1*, other BAT-specific genes, and genes specific to type I/IIA (slow twitch) muscle fibers [49]. These results strongly suggest that MED1/Mediator acts as a powerful suppressor, in muscle, of genetic programs related to energy expenditure. Although the underlying suppression mechanisms are still unknown, the metabolic phenotypes of corepressor *RIP140* null mice [117] and transgenic mice that overexpress PGC-1 α in muscle [118] are similar to that of the muscle-specific *Med1* knockout mice. Moreover, RIP140 has been reported to repress *UCP-1* expression in white adipose tissue through interactions with LXR α and ERR α [119, 120]. Considered together, these observations raise the interesting possibility that Mediator may provide an exchange platform for gene- and cell-specific coactivators (e.g., PGC-1 α) and corepressors (e.g., RIP140) to differentially regulate gene transcription in different tissues or in response to different NR signal inputs.

6. Concluding remarks

Since the identification of human Mediator as a functional NR-associated co-activator over 15 years ago, Mediator has been implicated in the function of a large number of nuclear receptors, in diverse physiological processes, in organisms ranging from yeast to human. Whereas in vitro studies provided key initial insights into mechanisms underlying Mediator-dependent NR functions, complementary cell-based and genetic analyses have led to the discovery and characterization of alternative pathways for NR signaling through Mediator. These analyses have suggested the possibility of conditional requirements for the conventional, highly robust MED1 LXXLL motif-dependent pathway, as well as redundant pathways that operate through other subunits or MED1 domains and that may contribute to biological robustness. Functional and mechanistic studies of Mediator in conjunction with other NR co-regulators have also suggested new roles for Mediator not only as a coactivator that interfaces with the general transcription machinery after initial NR-induced chromatin remodeling steps, but also as a coactivator that links chromatin remodeling and transcription steps. New gene- and tissue-specific functions of MED1/Mediator as a repressor of NR signaling pathways have also emerged. Finally, the favorable metabolic phenotype of mice with tissue-specific MED1 ablation (muscle) or with MED1 mutations that eliminate the strong NR-Mediator interactions also suggest therapeutic potential for the targeted manipulation of NR-Mediator interactions. Our future, more comprehensive understanding of the role of Mediator in NR signaling pathways will depend upon continued biochemical studies to elaborate specific and increasingly diverse mechanisms and corresponding cell-based and genetic analyses to establish the physiological relevance of these mechanisms and to elaborate new gene- and NR-specific functions of Mediator and its numerous subunits.

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- We discussed gene-specific Mediator functions and mechanisms
- The conventional nuclear receptor-Mediator pathway is conditionally required.
- New alternative Mediator-NR pathways are elucidated.
- Physiological processes linked to nuclear receptor-Mediator interactions are summarized.
- How Mediator transmits signals from nuclear receptors to the general transcription machinery

Figure 1. Alternate pathways for Mediator recruitment to NR target genes

The blue arrow indicates the major, and original, pathway that was established by biochemical studies and that is necessary for maximal NR-mediated transcription. Alternative pathways, suggested in part from cell-based assays and necessitated by the lack of a general requirement for the NR AF2-MED1 LXXLL interaction pathway, are indicated by red arrows. For details see text.

Figure 2. Multistep cofactor exchange model for target gene activation by NRs

The figure indicates a very general pathway most applicable to those receptors that show ligand-independent interactions with the NCoR/SMRT corepressors. For other receptors that do not interact with NCoR/SMRT, the pathway may begin with ligand-dependent binding of SRCs and interacting cofactors such as the p300/CBP histone acetyltransferases (HATs) and CARM1 and PRMT1 protein arginine methyltransferases (PRMTs). In other variations, p300 and potentially other HATs and histone lysine methyltransferases (HKMTs) may be recruited by direct ligand-dependent interactions with NRs or by other NR-associated cofactors. In another special case, PGC-1 may interact with NRs in a ligand-dependent manner to facilitate p300 recruitment and function, with subsequent retention in the NR-enhancer/promoter complex (after MED1/Mediator-induced dissociation from NRs) through a direct interaction with MED1/Mediator. The indicated exchanges involve interactions of cofactors with overlapping surfaces on the NR AF2 domain -- with the SRC interaction being dependent upon ligand-induced AF2 conformational changes that result in prior NCoR/SMRT dissociation and with the MED1/Mediator-SRC exchange and subsequent Mediator functions in PIC formation being dependent upon HAT/PRMT/HKMT-mediated nucleosomal histone modifications. Not indicated are known requirements for ATP-dependent chromatin remodeling factors, other histone modifying factors and a variety of other coregulators. For further details see text.

Table 1

Characteristics of interactions between Mediator subunits and NRs

Mediator subunit	Interacting NRs	Mediator subunit domains involved	NRs domains involved	Ligand effects
MED1	Most NRs like TR [34], VDR [35], ERα / β [37], PPAR [34, 36, 38], RAR [34], RXR [34], AR , GR [39, 40], HNF4 [41] et. al.	LXXLL motifs	AF-2	Ligand-dependent
	ERα [63], GR [63], NR4A (NUR77, NURR1, NOR-1)[64]	N-terminus		Ligand-independent
MED14	GR [39], HNF4 [41], ERα [121], PPARγ [75]		AF-1	Ligand-independent
MED25	RAR [81], HNF4α [82]	LXXLL motif	AF-2	Ligand-dependent
MED15	NHR-49 [86], Oaf1 [85], Pdr1 [88], Pdr3 [88]	KIX domain	LBD	Ligand-dependent