

Nuclear receptor coactivators as potential therapeutic targets: the HATs on the mouse trap

Review Article

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Abbreviations: HAT, histone acetyl transferase; NR, nuclear receptor; LBD, ligand binding domain; RAR, retinoic acid receptor; RXR, retinoic X receptor; PPAR, peroxisome proliferator activated receptor; SRC1, steroid receptor coactivator 1; NIDDM, non-insulin dependent diabetes mellitus; AF-2, activation function-2; RIP140, receptor interacting protein 140; TIF2, transcription intermediary factor 2; RAC3, receptor associated coactivator 3; ARA70, androgen receptor activator 70; GRIP1, glucocorticoid receptor interacting protein 1; TR, thyroid receptor; ER, estrogen receptor; AIB1, amplified in breast cancer 1; CBP, CREB binding protein; P/CAF, p300/CBP associated factor; GNAT, GCN5 related N-acetyltransferase; AcCoA, acetyl-coenzyme A.

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Summary

The recent past has seen an immense burst in our understanding of nuclear receptor (NR) signaling. Key achievements have been the structure determination of the ligand binding domain and the identification of coregulators which mediate the transcriptional effects of NRs. Both types of studies have now converged on the description of the NR coactivator interface at the atomic level, which, together with the elucidation of the structure of two coactivator related histone acetyl transferases (HATs) points towards previously unknown targets for drug design potentially leading to novel types of non-ligand antagonists of NR function.

I. Introduction

Nuclear receptors are ligand inducible transcription factors that are implicated in virtually any genetic program, such as development, differentiation, control of proliferation, homeostasis and apoptosis (for recent review on NR signaling see Mangelsdorf et al., 1995; Chambon, 1996; Heine and Gronemeyer, 1998). They act directly or indirectly on the expression of a variety of target genes by (i) modifying the chromatin environment of the promoter, (ii) altering the activity of the basal transcription machinery, and (iii) mutually interfering with other transcriptional signaling pathways. As key regulatory molecules they have attracted much attention for therapeutic treatment, also in view of the fact that misregulation of nuclear receptor signaling is apparently directly related to the generation of a number of diseases (Carapeti et al., 1998; Chen et al., 1997; Fenaux et al., 1997; Taki et al., 1997). Synthetic ligands that partially or

completely block NR activity are already routinely used in the treatment of several endocrine cancers or as contraceptives, and recent improvements in the specificity and efficacy predict that NR ligands will acquire an even broader spectrum of applications in medicine, like for example in the treatment of osteoporosis via differential regulation of the activity of the estrogen receptors alpha and/or beta. Furthermore, through the insights into orphan nuclear receptor signaling, novel targets for synthetic ligands emerge. For instance it may be possible to treat obesity and NIDDM (non-insulin dependent diabetes mellitus) via regulating the activity of the nuclear receptor PPAR (Mukherjee et al., 1997), and there is still a great number of orphan receptors with potentially very important physiological functions (Mangelsdorf et al., 1995; Chambon, 1996) which have not yet been analysed in detail on a functional level.

NR activity is negatively regulated by corepressors and positively by coactivators. A common mechanism of NR

coregulator action seems to be the covalent modification of histones (reviewed in: Torchia et al., 1998), although other non-histone targets may exist as well (Imhof and Wolffe, 1998; Imhof et al., 1997; Gu and Roeder 1997) (**Figure 1**). NR-recruited coactivator complexes contain HAT activities that acetylate the nucleosomal template thereby overcoming a barrier to enhanced transcription, while corepressor complexes are capable to reverse this effect by deacetylating and consequently condensing the chromatin template (Kuo and Allis, 1998; Imhof and Wolffe, 1998). Through direct and indirect, often ligand-independent, interactions with basal transcription factors, NRs are thought to modulate the activity of the pre-initiation complexes (Mangelsdorf et al., 1995; Chambon, 1996). Positive or negative interference with other signal transduction pathways, referred to as signal transduction crosstalk, is still poorly understood but may result from several different mechanisms such as coregulator sharing/squelching, direct interactions between the involved transcription factors and regulation of posttranslational modification.

The cloning and characterization of NR coregulators has shed some light on the mechanisms by which NRs exert their multiple effects on chromatin and, possibly, the basal transcription machinery (Chambon, 1996; Heine and Gronemeyer, 1998). With detailed mechanistic information at hand new dimensions of specific therapeutic interference into the highly complex phenomenon of NR transcription regulation emerge. The very recent structural definition of the NR coactivator interface and the insights we gain from the elucidation of the structures of histone acetyl transferases will undoubtedly nucleate new drug development approaches.

II. NR coactivator specificity

The cloning and analysis of NR coregulators was followed by definition of the short and structurally defined coactivator signature LxxLL (where x is any amino acid) motifs (or NR boxes) embedded in a short α -helical peptide, which are necessary and sufficient for ligand dependent interaction with the transcriptional activation function-2 (AF-2) located in the ligand binding domain (LBD) of NRs (Torchia et al. 1998). The LBDs of different NRs share a common fold that has been compared to a mouse trap since the binding of a specific ligand results in a conformational change (springs the trap) involving repositioning of several helices to form the coactivator binding site (see Moras and Gronemeyer, 1998). The fact that some coactivators contain multiple LxxLL motifs (up to nine in RIP140), all of which appear to be functional at least in terms of *in vitro* binding to NRs, had brought up the intriguing question of whether this multiplicity reflects redundancy or a means of conferring specificity to the interface.

Initial observations that different coactivators display NR preferences (Voegel et al., 1998; Ding et al., 1998; Kalkhoven et al., 1998; Hayashi et al., 1997; Leers et al., 1998) have gained ground through the studies on the binding of NR boxes to holo LBDs by Darimont et al. (1998). Residues adjacent to the core LxxLL motif indeed encode domains with specificity for different NRs and the definition of specificity-conferring residues, directly N-terminal to the LxxLL motif (M. Parker, pers. communication), substantiates this observation. The emerging picture here points towards two levels of NR coactivator specificity. First, highly related coactivators from the TIF2/SRC1/RAC3 family display NR preferences, since for example the mouse homologue of TIF2 binds well to the androgen receptor, whereas SRC1 binds very poorly (Ding et al. 1998). Second, different LxxLL motifs found on one coactivator are only partially redundant for their binding to a specific NR.

The NR box 2 of TIF2 is probably the major contact site for the estrogen receptor since mutations in this site have the greatest effects on transcriptional stimulation and binding (Voegel et al., 1998; Leers et al., 1998). Though the other NR boxes are able to compensate *in vitro* to some extent loss of a critical motif (Voegel et al., 1998; Leers et al., 1998), it is conceivable to assume that *in vivo* redundancy is much less important. Another level of complexity is introduced by the fact that different isoforms of the same coactivator (SRC1a vs. SRC1e) differ in their ability to activate a single NR (Kalkhoven et al. 1998). These isoform-specific effects are attributable to a fourth NR box found in SRC1a which is not present in the differentially spliced form SRC1e, or other coactivators from this family. In turn, SRC1e is the major SRC1 isoform to mediate thyroid hormone response (Hayashi et al. 1997).

Concluding, it seems very likely that the redundancy of LxxLL boxes reflects the need to accommodate *in vivo* different NRs with different variations of the same theme. Thinking along these lines it is even tempting to speculate that coactivators are not at all promiscuous, as suggested by initial *in vitro* binding studies, in their choice of NRs. This view is supported by the cloning of an apparently androgen receptor specific coactivator (ARA70, Yeh and Chang, 1996), and helps to explain the multiplicity of different coactivators for NRs. If coactivators have NR specificity, why then do they need several LxxLL motifs? An appealing answer to this question comes from the structure of the complex between the PPAR γ LBD homodimer and a SRC1 peptide encompassing two of the three NR boxes (Nolte et al. 1998). The complex has a molar composition of two PPAR γ LBDs per SRC1 peptide molecule, and the crystal structure reveals that each LxxLL motif participates in the binding to one of the LBDs. Both motifs make identical contacts to the hydrophobic clefts of the respective monomers (Nolte et al. 1998). Although there is yet no confirmation that these

data indeed reflect simultaneous binding of entire coactivators to both subunits of a NR homo- or heterodimer, the crystallographic data are supported by in vitro binding studies indicating that, in solution, SRC1 is also able to bind both partners in a retinoic acid receptor

(RAR)-retinoic X receptor (RXR) heterodimer (Westin et al., 1998).

Figure 1. A schematic representation of a nuclear receptor homo- or hetero-dimer bound to DNA with a coactivator or coactivator-complex and potential interactions with the basal machinery and the nucleosomal template. Note that either one or both partners in the dimer might be ligand-bound and contribute to coactivator recruitment. The LxxLL nuclear receptor interaction motifs in the coactivator are indicated, as well as a histone acetyltransferase active center, which might target histones, general transcription factors or other non-histone targets. For simplicity direct interactions between the nuclear receptors and the basal machinery as well as the possibility of multiple coactivator/-complexes have been omitted. For further explanations refer to the text.

The view that NR coactivators might contact both partners in NR homo- or hetero-dimers, which represent the biologically active states of NRs, helps to explain remaining obstacles associated with NR transactivation. Westin et al. (1998) and Nolte et al. (1998) for example provide with their findings a possible explanation of how RXR ligands can potentiate the effect of RAR ligands in the RAR-RXR heterodimer, namely by cooperative recruitment of coactivators. Here, the RXR partner, in presence of a specific ligand, further stabilizes the interaction with the RAR-recruited coactivator. However, it has to be kept in mind, that there is no in vivo evidence for cooperative recruitment of coactivators to heterodimers where both partners are ligand-bound. The possibility remains that RXR recruits a second coactivator to the heterodimer. Furthermore, Westin et al. (1998) still

assume in their model that RXR in the heterodimer is neither able to bind its cognate ligand nor to recruit a coactivator unless RAR is also ligand-bound. This is in disagreement with several studies that unequivocally demonstrate that RXR is indeed able to bind its ligand and subsequently recruit a coactivator to a heterodimer with an unliganded RAR in vitro (Chen et al., 1998; Kersten et al., 1996; AB, unpublished).

III. The structure of the interface

Until recently, it was unclear whether the holo (ligand-bound) LBD surface provides multiple coactivator binding sites allowing cooperative binding of two or more NR boxes present in one single coactivator (di-, tri-partite interface) or even the cooperative recruitment of two coactivator molecules at the time. Three recently solved crystal structures of holo-NR LBD coactivator peptide

complexes [SRC1-PPAR γ , (Nolte et al. 1998); TIF2/GRIP1-TR β , (Darimont et al. 1998); and TIF2/GRIP1-ER α , (Geoffrey Greene, pers. Communication)] display a ratio of one NR box per LBD, making the hypothesis of a multipartite interface between NRs and coactivators unlikely. This view is further supported by scanning surface mutagenesis of the TR β LBD, and subsequent monitoring of coactivator (in this case SRC1 and GRIP1, the mouse homologue of TIF2) binding and transactivation properties (Feng et al. 1998).

This study delineates a hydrophobic cleft on the holo LBD that is in part composed of residues from helix H12 which had already been known to undergo a major transitional relocation upon ligand binding (“springing the trap”) and in numerous mutational studies been implicated in transactivation (Moras and Gronemeyer 1998). In this case ligand binding is directly linked to coactivator binding since the repositioning of helix H12 on the one hand is induced by the ligand which makes contacts to several residues in this helix, and on the other hand then contributes with two more residues to the hydrophobic cleft that accommodates the coactivator.

From the mutational analysis it is furthermore apparent, that the hydrophobic cleft is flanked by charged residues at either side making up a so-called “charge clamp” which probably contributes to (i) defining the orientation of the NR box towards the LBD and (ii) making contacts to residues outside of the core LxxLL motif to achieve the above mentioned specificity for different NR boxes (Feng et al. 1998). In both co-crystal structures the LxxLL motifs are accommodated in a hydrophobic cleft on the respective LBDs that is very similar to the one defined by the surface scanning mutagenesis, highlighting once more the validity of the mouse trap model based on the common fold of NR LBDs (Moras and Gronemeyer 1998).

The fact that the LBD cavity is almost completely filled by one coactivator NR box argues against the possibility that multiple NR box LBD contacts are made. Furthermore, it is obvious from both structural studies that the charged residues at either end of the hydrophobic cleft are contacting additional residues in the NR box. In this respect it will be very interesting to see whether a good correlation between the N-terminal specificity encoding amino acids (Malcom Parker, pers. communication) and the identity of the charged residues at the corresponding parts on the LBD surface can be made. In good agreement with the mutational studies defining the LxxLL motif (reviewed in: Torchia et al. 1998), is also the fact that the leucine residues of the NR box indeed contribute in two ways to its identity. First, they confer structural identity to the encompassing peptide resulting in the formation of this amphipathic α -helix, and second, they form the hydrophobic surface that matches the hydrophobic cleft on the NR LBD. It is also worth to note that only residues in

the immediate vicinity of the NR boxes make contacts to the LBDs in the PPAR γ co-crystal (Nolte et al. 1998). This reduces the importance of other residues in the nuclear receptor interacting domains to providing an overall structure that is promiscuous to the formation of the interface. Noteworthy, the intervening residues between the two NR boxes in the PPAR γ SRC1 co-crystal seem to have little structural identity (Nolte et al. 1998), which is in keeping with the low sequence conservation found between members of this coactivator family. In contrast, the spacing between the different NR boxes is rather conserved, making the finding that SRC1 can bind to both homo- or hetero-dimeric NRs likely to be a general feature of this coactivator family.

IV. Implications for NR ligand design

The structural clues obtained in these studies will certainly have an impact on the development of new synthetic NR ligands. For the efficient development of synthetic NR ligands two new aspects should be considered:

First, although it has been known for some time that the natural ligands for NRs make, among others, also direct contacts to the helix H12 of the LBD, the fact that helix H12 contributes directly to the coactivator binding surface, and that this surface potentially displays coactivator specificity, should be incorporated in the rational of ligand design. For instance, one approach would be to screen for synthetic partial agonists that influence only slightly helix H12 positioning; these molecules, instead of abrogating coactivator binding altogether, would change coactivator affinity. In contrast to classical antagonists which are non-permissive for the relocation of helix H12 on the LBD surface, and therefore for the formation of the coactivator binding site, such ligands might shift the relative affinities for different coactivators. This might prove effective for disorders where a complete block of NR activity is not feasible since it generates secondary effects that might promote disease but where it is desirable to down-regulate NR activity. An example could be to target breast cancer with an amplification/ overexpression of the AIB1/RAC3 coactivator (Anzick et al. 1997) with such ligands. If high amounts of AIB1 trigger unusual estrogen receptor activity in these cells, it would certainly be advantageous to selectively lower the affinity of estrogen receptor for AIB1, while preserving other functions performed by the receptor in combination with other coactivators, e.g. the antiproliferative effects probably exerted via the general mediator CBP (reviewed in Heine and Gronemeyer, 1998). The ultimate aim would be to obtain synthetic ligands that selectively impair specific combinations of NR coactivator pairs. This would allow to overcome a limitation that classical synthetic ligands for NRs have: they are specific for one receptor but restricted in their

action to the receptor molecule itself and are not coactivator-specific.

Second, a search for combinatorial ligands could be prompted by the fact that one coactivator molecule seems to contact both partners in a heterodimer composed of receptors with different natural ligands. It will be of interest to develop combinations of ligands that do not have major effects on a single NR activity, but when administered together block coactivator association to a specific heterodimer combination; for example, such drugs could block RAR-RXR heterodimer signaling but not RXR homodimers or RXR heterodimers with partners other than RAR. Both strategies for ligand development account for the increasing combinatorial complexity in NR signaling by aiming to restrict their action to very specific NR functions. Interestingly, considerable progress has been made on the development of RXR dimer-selective ligands that specifically affect either PPAR-RXR heterodimers (Mukherjee et al. 1997) or RXR homodimers (Lala et al. 1996).

V. The NR coactivator interface as drug target

The recent gain in understanding of NR coactivator function at the molecular level sets the grounds for new strategies of pharmacological interference within NR signaling pathways. The fact that the interface between NR and coactivator is composed of very defined features, namely an amphipathic α -helical chain containing the LxxLL motif of the coactivator and a hydrophobic cleft plus "charge clamp" on the surface of the LBD of the NR (Darimont et al. 1998; Nolte et al. 1998; Feng et al. 1998), together with the fact that the coactivator α -helix is structured in solution (as little as 8 amino acids are sufficient for ligand-dependent interaction with NRs, reviewed in Torchia et al., 1998) raise the possibility to disrupt such interactions with small synthetic molecules. On basis of the structural information that we have now, it is feasible to screen combinatorial peptides containing the core LxxLL motif for high affinity binding to the hydrophobic groove on the LBD. These peptides might prove to be effective in inhibiting the interaction between NRs and coactivators in the cell and therefore abrogate NR transactivation. In this respect, since the LxxLL motif confers structural identity to the encompassing peptide, it is tempting to envision hybrid proteins containing LxxLL motifs that act as dominant negative coactivator mimics while being more stable than peptides *in vivo*. Alternatively, peptidomimetics might open a path leading to overcoming obstacles associated with the delivery and stability of peptides in the organism (Kieber-Emmons et al. 1997). The fact that combinatorial compound libraries can be created by medicinal chemistry and molecular biology make it feasible to attempt generating drugs that mimic the structure of the LxxLL peptides, and therefore

prove effective in blocking the NR coactivator interface. In this respect it is interesting to note that peptidomimetics have already been used very successfully to mimic a peptide hormone (Livnah et al. 1996).

VI. The shape of the HAT

Thinking further along these lines, one could ask whether it is feasible to interfere with the activity of a given coactivator for NRs rather than regulating the activity of the NR itself. Given that NR coactivators seem to play a pivotal role also in non-NR driven transcription e.g. the general mediator and nuclear receptor coactivator CBP/p300 (Yao et al. 1998), inactivating the whole molecule e.g. by antisense approaches will have deleterious side effects on other signaling pathways. Hence, it seems reasonable to concentrate on either blocking specific interaction domains (as discussed in the paragraph above), or blocking the endowed enzymatic activities that most coactivators possess. In this respect obviously the histone acetyl transferase (HAT) activities, that stimulate transcription (Martinez-Balbas et al. 1998; Zhang et al. 1998) not only by acetylating histones but also non-histone protein targets (reviewed in Kuo and Allis 1998; Bayle and Crabtree 1997), are of high importance. As mentioned in the Introduction, NRs recruit HAT activities in order to render the chromosomal target promoter prone to transcriptional activation. Recently, the structures of two histone N-acetyltransferase enzymes that are highly related to the nuclear receptor coactivators P/CAF and GCN5 have been solved (Dutnall et al., 1998; Wolf et al., 1998). Both the yeast HAT1 and the *Sarratia marcescens* aminoglycoside 3-N-acetyltransferase share common features encoded in a canonical GCN5-related N-acetyltransferase (GNAT) core motif (Dutnall et al., 1998; Wolf et al. 1998; Neuwald and Landsman, 1997). This motif confers cofactor (coenzyme A) binding, encodes the active center of the enzymes and contributes to substrate recognition and binding. Differences between members of the HAT family in the core region reflect probably different substrate specificities (reviewed by Kuo and Allis, 1998). The alignment of the GNAT motif with the structures obtained in both crystallographic approaches allows now a rigid analysis of structure & function relationships of different residues within the enzymatic core of different HATs. Together with biochemical analysis this will allow a more precise definition of HAT substrates and their mode of recognition.

Since acetyl coenzyme A (AcCoA) is an abundant metabolic key intermediate it might be difficult to specifically interfere with HAT function at the level of competitive AcCoA inhibitors. Efforts could be directed to developing either allosteric effectors of HAT activities, or suicide inhibitors that covalently modify the active center. The structural information will be of great help to accomplish these tasks. However, most promising seems

to be the approach of blocking specific HAT activities by targeting the substrate recognition site with competitive inhibitors. Based on their data as well as molecular modeling Dutnall and coworkers suggested a complementary fit model for the recognition of histone tails by the HAT enzyme (Dutnall et al., 1998). As discussed above for the NR coactivator interface, peptidomimetics might also lead to the development of synthetic inhibitors of HATs with high bioactivity able to selectively associate with the substrate recognition site of the enzyme thereby blocking its function. Of special interest is the fact that different HAT enzymes have different substrates (Kuo and Allis, 1998), some of non-histone nature (Bayle and Crabtree 1997), and display considerable substrate specificity (Kuo and Allis, 1998; Imhof and Wolffe, 1998). For example, p53 is regulated in its DNA binding activity through acetylation by p300 (Gu and Roeder 1997) and the general transcription factors TFIIIE β and TFIIIF can be acetylated by P/CAF or p300 (Imhof et al. 1997). Again, in view of the differential effects that closely related coactivator-HATs like CBP and p300 (Yao et al. 1998; Kawasaki et al. 1998) or CBP and P/CAF (Puri et al. 1998) have, it will be of prime importance to define drugs that are capable of selectively interfering with a specific function of a given HAT rather than blocking the whole enzyme family.

VII. Concluding remarks

The importance of nuclear receptors in cell fate has been elucidated and understood for a long time. The fact that nuclear receptors represent "master genes" makes them attractive targets for drug research in disease therapy. Since their mode of action is highly complex the emerging details from investigations on nuclear receptor coregulators not only decipher an amazing transcription network that controls spatial and temporal expression of target genes, but also promote the identification of potential candidate functions for pharmacological interference. To this end several new options have been sketched here, focussing on the coactivator rather than on the nuclear receptor itself. If such approaches prove to be effective this might mark the beginning of a post-ligand era for NR drugs.

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Note added in proof:

While this manuscript was in press the work cited as Darimont et al, 1998 has been published. The full citation is as follows:

Darimont BD, Wagner RL, Apriletti JW, Stallcup MR, Kushner PJ, Baxter JD, Fletterick RJ, Yamamoto KR (1998) Structure and specificity of nuclear receptor-coactivator interactions. *Genes Dev* 12, 3343-3356.

In the same issue another study dealing with the NR coactivator interface has been published, that substantiates and extends the idea of specificity determining residues adjacent to the LxxLL motif:

McInerney EM, Rose DW, Flynn SE, Westin S, Mullen TM, Kronen A, Inostroza J, Torchia J, Nolte RT, Assa-Munt N, Milburn MV, Glass CK, Rosenfeld MG (1998) Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation. *Genes Dev* 12, 3357-3368.

Furthermore, another non-histone target for the acetyltransferase activity of CBP has been identified, further substantiating the suggestions made in the final paragraph of this paper:

Munshi N, Merika M, Yie J, Senger K, Chen G, Thanos D (1998) Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. *Mol Cell* 2, 457-467 .

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