REVIEW

The mineralocorticoid receptor and its coregulators

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Abstract

The mineralocorticoid receptor (MR) is a member of the nuclear receptor superfamily and is essential for controlling sodium transport in epithelial tissues such as the kidney and colon. Moreover, it is also present in other non-epithelial tissues and is capable of activation by both mineralocorticoids and glucocorticoids. A challenge in understanding transcriptional regulation by the MR and other nuclear receptors is to determine how tissue- and ligand-specificity is achieved. Over the past decade, it has become clear that a heterogeneous group of non-receptor proteins termed as coregulators are required to either enhance or repress nuclear receptor-mediated transactivation of target genes. A subset of these coregulators may be expected to confer specificity to MR-mediated responses by virtue of their variable tissue expression and selectivity for different ligands. Specific coregulator–MR interactions may be a suitable target in the rational design of tissue-specific MR modulators as has been described for other steroid receptors. However, the number of coregulators identified to date for the MR is very limited compared with other nuclear receptors. Understanding the full complement of MR coregulators is essential for unraveling the complexity of MR signaling pathways and will facilitate the development of selective MR modulators.

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Introduction

The mineralocorticoid receptor (MR) is a nuclear receptor (NR) that is critical for controlling sodium and potassium transport in epithelial cells, most notably in the kidney and colon (Pearce *et al.* 2003). It also plays important roles in non-epithelial tissues, such as cardiac myocytes, blood vessels, the hippocampus and adipose tissue (Lombes *et al.* 1992, 2000, Meijer 2002, Caprio *et al.* 2007). The MR is capable of binding multiple classes of steroids with high affinity, including the mineralocorticoids, aldosterone and deoxycorticosterone, the glucocorticoids (GR), cortisol (in humans) or corticosterone (in rodents), and progesterone (PR; Sutanto & de Kloet 1991). While aldosterone is considered the primary physiological MR ligand in humans (Bledsoe *et al.* 2005), in some tissues cortisol may be the primary ligand for MR, whereas PR behaves as a predominant antagonist (Fagart *et al.* 1998).

MR activation plays an important role in the pathophysiology of hypertension and cardiac fibrosis (Young & Funder 2000, Fagart *et al.* 2005). Two large clinical trials, the randomized aldactone evaluation study (Pitt *et al.* 1999), and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (Pitt *et al.* 2003), have demonstrated that MR blockade can significantly reduce morbidity and mortality in patients with heart failure. However, the underlying molecular mechanisms of these beneficial effects remain unknown. Furthermore, the use of MR antagonists is limited by the adverse renal effect of hyperkalemia (Juurlink *et al.* 2004). A tissue-selective MR antagonist that only targets heart failure while avoiding unwanted renal effects would thus be highly desirable. This is just one example of the myriad potential applications of a tissue-selective MR modulator. Others may exploit the anti-apoptotic role of MR in hippocampal degeneration (de Kloet *et al.* 2005) or seek to counteract the induction of endothelial dysfunction and vascular injury by aldosterone (Sugiyama *et al.* 2005).

For other NRs, studies have revealed numerous coregulator molecules that serve to coordinate the interaction between the NR and their target genes and in some cases define ligand- or tissue-specificity (Rowan & O'Malley 2000, Chang & McDonnell 2005, Hall & McDonnell 2005, Heemers & Tindall 2007). The search for MR-interacting coregulators is less advanced, partly due to the difficulty in purifying stable full-length MR (Clyne *et al.* 2009). This review examines the available literature on MR coregulators with a view to understand their role in the ligand- and tissue-specific activation of the MR, and their potential as therapeutic targets, as well as identifying areas for further research.

The mineralocorticoid receptor

The MR (NR3C2) is the longest member of the NR family of ligand-dependent transcription factors (Bookout *et al.* 2006). It belongs to the oxosteroid receptor subgroup that comprises of the androgen (AR), GR, and PR receptors (Bledsoe *et al.* 2005) although it is most closely related to the GR with which it shares a common ancestor (Baker *et al.* 2007, Ortlund *et al.* 2007). In the absence of ligand, MR resides in the cytosol and is rendered transcriptionally inactive by a variety of chaperone proteins including the 90-kDa heat shock protein (hsp90; Gomez-Sanchez *et al.* 2006). Ligand binding induces a conformational change in the MR resulting in its dissociation from the chaperone proteins, homodimerization, and translocation to the nucleus where it directs the transcription of target genes (Fig. 1; Lombes *et al.* 1994*a*, Hellal-Levy *et al.* 2000*a*). In addition to the classic pathway of genomic action, rapid, non-genomic action of MR signaling has also been described, in particular in terms of activation of protein kinase C and Na^+/H^+ exchange and increased intracellular calcium (Losel & Wehling 2003, Mihailidou *et al.* 2004, Funder 2005). However, this will not be discussed further as the mechanism of non-genomic action has not yet been shown to involve coregulators.

The MR contains the three principal domains characteristic of the NR superfamily: the N-terminal domain (NTD) or A/B region, the DNA-binding domain (DBD) or C region, and the C-terminal ligand binding domain (LBD) or E region (Rogerson *et al.* 2003). A flexible hinge exists between the DBD and
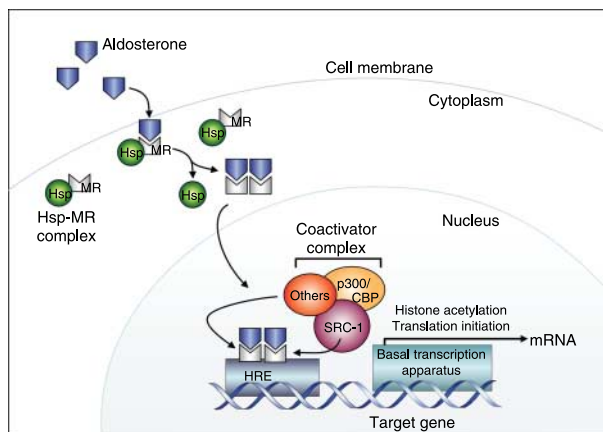

Figure 1 Schematic representation of MR signaling upon agonist ligand binding. The MR dissociates from hsp, which hold it inactive in the cytoplasm, and translocates to the nucleus where it binds to a HRE. Coactivators, such as SRC-1 and p300/CBP, are sequentially recruited to the MR to allow histone acetylation and target gene transcription to occur. MR, mineralocorticoid receptor; hsp, heat shock protein; HRE, hormone response element. The reader is referred to the text for full names of coregulators.
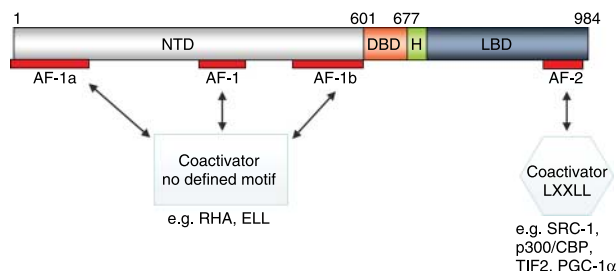

Figure 2 Schematic representation of the human MR structure displaying sites of interaction with coactivators. AF-2 is well defined and consists of parts of helices 12, 3, 4, and 5 which interact with the LXXLL motif of coactivators. AF-1 is poorly defined, and is separated into AF1a and AF1b by some groups. There is no consensus motif for coactivator binding to AF-1. MR, mineralocorticoid receptor; AF-2, activation function-2; AF-1, activation function-1. The reader is referred to the text for details on MR structure and full names of the coregulators.

LBD or D-region, but its function is poorly defined (Rogerson & Fuller 2003). For a more detailed review of MR's structure, see Pippal & Fuller (2008). As the LBD and the NTD are of particular relevance to coregulators, they will be further explored here (Fig. 2).

The MR LBD is conserved between species and has multiple functions, including ligand binding, nuclear localization, dimerization, interaction with hsps, and transcriptional coactivators and ligand-dependent transactivation via an activation function (AF), or AF-2, domain (Wurtz *et al.* 1996, Farman & Rafestin-Oblin 2001). It consists of 11 α-helices with four β-strands folded into an antiparallel three layered sandwich (Bledsoe *et al.* 2005, Fagart *et al.* 2005, Li *et al.* 2005). The crystal structure of the MR LBD reveals that aldosterone is bound in a fully enclosed pocket contacting residues in helices 3, 4, 5, 6, 7, and 11 and the β-turn (Bledsoe *et al.* 2005). Upon agonist binding, helix 12 is repositioned together with helices 3, 4, and 5 to form a hydrophobic groove on the surface of the LBD which allows binding of coactivators and subsequent MR activation (Geller *et al.* 2000, Hellal-Levy *et al.* 2000*b*, Bledsoe *et al.* 2005, Li *et al.* 2005). It is important to note that helix 12 alone is not the AF-2; rather it constitutes part of an activation surface which, together with other parts of the LBD, defines the AF-2 domain (Alnemri *et al.* 1991, Darimont *et al.* 1998).

The NTD is the least conserved domain among NRs, both in size and sequence (Warnmark *et al.* 2003). The MR has the longest NTD which shares less than 15% homology with the GR NTD (Alnemri *et al.* 1991). The MR NTD contains the constitutive AF-1 which differs from AF-2 by being ligand-independent and intrinsically disordered in its structure (Warnmark *et al.* 2003, Lavery & McEwan 2005). One study localized AF-1 to amino acids 328–382 (Govindan & Warriar 1998) while other studies separated the AF-1 into two different regions, AF-1a (amino acids 1–169) and AF-1b (amino

acids 451–600; the 147 amino acids next to the DBD), separated by an inhibitory domain (Fuse *et al.* 2000, Pascual-Le Tallec *et al.* 2003). It is postulated that the intrinsic lack of structure of the NTD allows unique secondary structure formation which permits interaction with specific coregulators (McEwan *et al.* 2007). The MR NTD also contains four consensus sumoylation motifs, with a fifth one in the LBD. These lysine acceptor sites allow post-transcriptional modification of the MR (Seeler & Dejean 2003) and transcriptional repression at certain response elements (Tallec & Lombes 2005).

Mechanisms of MR specificity

As noted, the MR can be activated by both mineralocorticoids and GRs, with distinct actions in different tissues. The mechanisms by which the MR achieves differential activation by the two corticosteroid hormones and exerts tissue-specific effects can be considered at the pre-receptor, receptor, and post-receptor levels. These three levels are well illustrated in Fig. 2 of the review by Pascual-Le Tallec *et al.* (2005).

Pre-receptor metabolism of cortisol is a key mechanism for maintaining MR selectivity in certain target tissues. Type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) converts cortisol, which circulates at concentrations 100- to 1000-fold higher than those of aldosterone (0·1–1 nM), to inactive cortisone, thereby protecting the MR from inappropriate activation by cortisol (Edwards *et al.* 1988, Funder *et al.* 1988, Bocchi *et al.* 2003, Galigniana *et al.* 2004). However, this mechanism is only present in the epithelial cells of the distal nephron, colon, sweat glands, and blood vessel walls (Alzamora *et al.* 2000, Christy *et al.* 2003, Galigniana *et al.* 2004). 11β-HSD2 expression is minimal or absent in other MR-expressing tissues including the myocardium and hippocampus.

At a receptor level, mineralocorticoid selectivity is conferred by ligand induced conformational changes that differ between cortisol and aldosterone. It has been demonstrated that aldosterone dissociates more slowly from MR and induces greater transactivation than cortisol at any given concentration (Lombes *et al.* 1994*b*, Farman & Rafestin-Oblin 2001). Moreover, the interaction between the MR NTD and the C-terminal hinge and LBD (or the N–/C–interaction), which may serve to stabilize ligand binding to the receptor (Li *et al.* 2006, Centenera *et al.* 2008), is much stronger in the presence of aldosterone than cortisol, offering another mechanism of ligand specificity at the MR (Rogerson & Fuller 2003).

Receptor isoforms are also believed to generate diversity in tissue-specific receptor response. This has been well demonstrated for the GR which has multiple potential isoforms, derived from the one gene, which are capable of producing unique biological responses to the same hormone in different tissues (Yudt & Cidlowski 2002, van der Laan & Meijer 2008). Transcription of the MR can be directed by two functional promoters, P1 and P2, to generate the isoforms hMR-α and hMR-β that differ in the 5′-untranslated region (Zennaro *et al.* 1996) and display differential tissue expressions (Zennaro *et al.* 1997). Furthermore, both promoters are found to be inducible by GRs, whereas only P2 appears sensitive to mineralocorticoids, suggesting that ligand-specificity may also be determined by the particular MR isoform generated. Another splice variant of the MR is hMRΔ5,6 (Zennaro *et al.* 2001), which will be discussed later in this review.

At the post-receptor level, an expanding library of over 300 NR coregulators has been identified over the past decade (Lonard 2007). They have been recognized to play a central role in modulating gene expression mediated by NRs and are thought to impart tissue- and ligand-specificity due to their structural and functional diversity (Lonard & O'Malley 2006).

Nuclear receptor coregulators: an overview

NR coregulators are comprised of both coactivators and corepressors and are defined as cellular factors which interact with NRs to potentiate or attenuate transactivation (McKenna & O'Malley 2000).

Steroid receptor coactivator 1 (SRC-1) was the first NR coregulator identified over a decade ago (Onate *et al.* 1995). Coactivators function as large complexes which are recruited to the target gene by NRs following ligand binding and perform many of the enzymatic reactions needed for gene expression including chromatin remodeling, histone modification, initiation of transcription, elongation of RNA chains, RNA splicing, and termination of transcriptional responses (Auboeuf *et al.* 2002, O'Malley 2007). They can also serve as docking platforms for further coregulator binding (McInerney *et al.* 1998). The ordered and cyclical recruitment of coregulator complexes have been elegantly illustrated for estrogen receptor-α (ER-α; Metivier *et al.* 2003). Many coactivators bind the AF-2 region of the LBD via a conserved NR-box that contains one or more LXXLL (where L is leucine and X is any amino acid) motifs (Heery *et al.* 1997, Darimont *et al.* 1998). Sequence conservation, however, has not been observed in AF-1 binding coactivators; they do not appear to require the LXXLL motif (Warnmark *et al.* 2003).

By contrast, corepressors interfere with transactivation by inducing histone deacetylase activity and other mechanisms to inhibit the general transcriptional machinery (Goodson *et al.* 2005). The nuclear receptor corepressor (NCoR) and silencing mediator of

retinoid and thyroid hormone receptor (SMRT) were the first corepressors discovered on the basis of their ability to bind ligand-free thyroid hormone receptor (TR) and reduce the total amount of gene product (Chen & Evans 1995, Horlein *et al.* 1995). Corepressors bind the LBD of an unliganded NR via a C-terminal 'CoRNR box' motif, defined as I/L-XX-I/V-I and recruit histone deacetylase proteins to repress gene expression (Hu & Lazar 1999). Ligand binding leads to an exchange of corepressors for coactivators, thereby allowing transcription to occur (Perissi *et al.* 2004). More recently, corepressors have been found to also interact with antagonist-bound NRs including the AR, ER, GR, and PR as well as the agonist-bound GR, suggesting that it is the ratio of coactivators to corepressors bound to a NR-ligand complex that modulates the transcriptional sensitivity of the NR (Wang *et al.* 2004*b*).

While most of the coregulators identified are widely expressed (Smith & O'Malley 2004), some display tissue-specific expression patterns and limit NR-mediated gene transcription to those tissues that express the required coactivators (Knutti *et al.* 2000, Ko *et al.* 2002). For example, the expression of SRC-1 is significantly higher in Ishikawa endometrial carcinoma cells compared with MCF-7 breast cancer cells and this can be correlated with the increased agonist activity of tamoxifen (a selective ER modulator, SERM) in Ishikawa cells (Shang & Brown 2002). Moreover, SRC-1 is specifically required for the agonist activity of tamoxifen, but not essential for other ligands such as estradiol, thereby permitting ligand-specific ER activation (Shang & Brown 2002).

Variation in the binding motif configuration of coregulators may also confer specificity. SRC-1 contains three LXXLL motifs and while a single LXXLL sequence is sufficient for activation of the ER, different combinations of two LXXLL sequences are required for the TR, retinoic acid receptor (RAR), peroxisome proliferator activated receptor (PPAR), and PR (McInerney *et al.* 1998). Distinct residues flanking the LXXLL motif have also been shown to modulate specific interactions with NRs, although it has not yet been demonstrated for the MR (Darimont *et al.* 1998, McInerney *et al.* 1998, Chang *et al.* 1999). In contrast to other NRs, the preferred binding motifs for AR are FXXLF (Chang *et al.* 2005) and WXXLF (He *et al.* 2000). Similar novel consensus sequences have not been described for the MR to date.

Coactivators of the MR

The AF-2 region of the MR binds LXXLL-containing coactivators, such as SRC-1, with high affinity, as is the case with many other NRs (Hultman *et al.* 2005). SRC-1

is a member of the p160 coactivator family of proteins (Ding *et al.* 1998) and while it only possesses weak histone acetyltransferase activity, it can sequentially recruit a chromatin remodeling complex to achieve histone acetylation and initiate transcription (Spencer *et al.* 1997, Xu & Li 2003). It is widely expressed in many tissues and cell types, and is reported to have five splice variants, with SRC1a and SRC1e being the most abundantly expressed. SRC1a has two functionally separable LXXLL motifs which bind MR with equal preference but bind other NRs with different specificities (Ding *et al.* 1998). Of note, the binding of SRC1a and SRC1e to GR and MR exhibits promoter and receptor specificity (Meijer *et al.* 2005). Coactivation by SRC1a is consistently stronger on a single response element-containing reporter, whereas SRC1e is stronger when multiple response elements are present. In terms of receptor specificity, SRC1e can enhance transcription via the AF-2 of both GR and MR, but via the NTD only in MR. The selective coactivation of MR by SRC1e in the NTD is hypothesized to be mediated via AF-1, although the exact receptor-coactivator interface has not been characterized (Meijer *et al.* 2005). The interaction between SRC-1 and MR does not appear to exhibit ligand specificity as the MR LBD interacts equally with an SRC-1 peptide in the presence of different steroid hormones (Hultman *et al.* 2005), although the result may be different if a full-length MR or SRC-1 is used.

Often complexed with SRC-1 are the global transcriptional coactivators and functional homologues p300 and CBP. p300/CBP possess strong intrinsic histone acetyltransferase activity and the ability to recruit RNA polymerase II to target gene promoters (Ogryzko *et al.* 1996). They are able to potentiate the transcriptional activity of MR via both AF-1 and AF-2. However, there is no direct interaction between p300/CBP and AF-1, raising the possibility of another intermediary nuclear factor (Fuse *et al.* 2000). Of interest, CBP has been found to facilitate hormone-induced N–/C–interaction in the AR (Ikonen *et al.* 1997), and perhaps the ability to engage both AF-1 and AF-2 in the MR contributes to the N–/C– interaction of the MR.

SRC-2 (or transcriptional intermediary factor 2, TIF2) is another member of the p160 coactivator family that can bind the MR (Voegel *et al.* 1996, Ding *et al.* 1998). It shows widespread tissue expression with the exception of the kidney, skeletal muscle, and mammary gland (Xu & Li 2003). SRC-2 has been shown to contain three partially redundant LXXLL motifs and mediate part of its action through CBP recruitment (Voegel *et al.* 1998). In the case of ER, TR, GR, AR, and retinoid X receptor (RXR), preference for one of the three NR boxes on SRC-2 has been demonstrated; however, similar receptor-specificity has not been reported for the MR (Ding *et al.* 1998, Voegel *et al.* 1998). SRC-2 can enhance

ligand-induced transactivation of both the AF-1 and AF-2 of MR (Fuse *et al.* 2000, Wang *et al.* 2004a). Similar transactivation of the MR has been demonstrated with GR interacting protein 1 (GRIP1), the mouse ortholog of human SRC-2 (Hong *et al.* 1997). Like CBP, GRIP1 has also been shown to enhance the AR N–/C–interaction, although a role in the MR N–/C–interaction has not been sought (Shen *et al.* 2005).

PPAR-γ coactivator 1 (PGC-1) is another strong coactivator of the MR (Knutti *et al.* 2000). Originally discovered as a coactivator of PPAR-γ, it has been extensively studied in the context of mitochondrial biogenesis and oxidative metabolism (Puigserver *et al.* 1998, Knutti & Kralli 2001). PGC-1α is the best studied member of the PGC-1 family and possesses powerful coactivation capacity by simultaneously recruiting a histone acetyltransferase complex including SRC-1 and CBP/p300 and facilitating the binding of the NR to the transcription initiation complex (Lin *et al.* 2005, Handschin & Spiegelman 2006). It is expressed in a tissue-specific manner with predominance in metabolically active tissues such as the heart, skeletal muscle, brown fat, kidney, brain, and liver (Knutti *et al.* 2000, Handschin & Spiegelman 2006). Tissue-specific activity has been demonstrated at different PPAR-γ target gene promoters; similar studies have not been performed for the MR (Puigserver *et al.* 1998). PGC-1α contains one NR box (LKKLL) and is able to significantly enhance hormone-dependent transcription mediated by MR via interaction with the AF-2 (Knutti *et al.* 2000). Ligand-specificity has not been demonstrated for the interaction between the MR LBD and PGC-1α (Hultman *et al.* 2005), however, the LBD alone may not accurately reflect coregulator recruitment by the full-length MR. The physiological significance of PGC-1α in MR-mediated transactivation is unclear, especially in the kidney, where PGC-1 is expressed predominantly in the proximal tubules and thick ascending loops of Henle (Portilla *et al.* 2002) while the MR is expressed in the distal convoluted tubules and cortical collecting ducts (Lombes *et al.* 1990).

It is important to note that NRs and coactivators can bind independently of the LXXLL motif, as has been demonstrated for SRC1e and TIF2/GRIP1. In the case of the MR, the AF-1 is poorly conserved and is therefore thought to recruit a unique set of non-LXXLL containing coregulators. One such coactivator is RNA helicase A (RHA; Kitagawa *et al.* 2002), which is a component of the RNA polymerase II complex that interacts with CBP/p300 to stabilize complex formation (Nakajima *et al.* 1997). RHA has been shown to bind directly to MR AF-1a and enhance transcription when the RHA/CBP complex is recruited to full-length MR in the presence of aldosterone, but not cortisol (Kitagawa *et al.* 2002). This suggests aldosterone binding

to MR may induce a conformational change that permits access by the RHA/CBP complex, while cortisol binding may induce a conformation that blocks RHA/CBP complex association, creating scope for ligand-specific actions at the MR. It may also be that the N–/C–interaction, which is uniquely aldosterone induced (Rogerson & Fuller 2003, Rogerson *et al.* 2004), is required for the RHA/CBP complex to bind the NTD.

The human elongation factor, 11–19 lysine-rich leukemia (ELL), named for its contribution to the pathogenesis of acute myeloid leukemia, is another AF-1 specific coactivator of the MR (Shilatifard *et al.* 2003, Pascual-Le Tallec *et al.* 2005). ELL is a RNA polymerase II elongation factor that increases polymerase II activity (Shilatifard 1998). ELL increases MR transcriptional activity and sensitivity to aldosterone and cortisol independently of total MR protein levels (Pascual-Le Tallec *et al.* 2005). The AF-1b region of the MR is crucial for ELL's potentiation of MR activity, while AF-1a, AF-2, and all five sumoylation sites are dispensable. By contrast, ELL markedly suppresses GR-mediated transactivation and has no effect on AR- or PR-mediated transactivation. ELL is co-expressed with the MR in the nuclei of cortical collecting duct cells of the human kidney, and is rapidly upregulated at physiological aldosterone concentrations, consistent with it being of physiological significance (Pascual-Le Tallec *et al.* 2005). Both ELL and RHA interact with RNA polymerase II, thus it may be postulated that they are recruited to different regions of the MR NTD as part of a complex that facilitates MR-mediated transactivation. Moreover, given RHA is capable of differentiating between aldosterone and cortisol and that ELL can differentiate between the MR and the GR, together, these coregulators may thus confer specificity to MR activity.

A number of other AF-1 interacting coregulators have been isolated by screening a human brain cDNA library, including death domain-associated protein (DAXX), FLICE-associated huge protein (FLASH), and Fas-associated factor 1 (FAF-1), factors implicated in cell apoptosis (Obradovic *et al.* 2004). While all three interact with both MR and GR, their effects are cell-context dependent. In a hippocampal cell line, FLASH increases the transactivation of MR and GR, FAF-1 specifically enhances MR-mediated transactivation with no effect on GR; and DAXX represses the transactivation of both MR and GR. By contrast, in a neuroblastoma cell line, the effects of FLASH and FAF-1 are attenuated while DAXX is able to differentially modulate MR- and GR-induced transactivation by potentiating or repressing their responses respectively. These proteins are expressed in the mouse hippocampus and may partly account for the different effects mediated by MR and GR in neuronal cells. However, their roles in other MR-expressing tissues are undefined.

As previously mentioned, sumoylation is a post-translational modification utilized by many NRs, including the MR (Tallec *et al.* 2003). It involves the covalent attachment of the ubiquitin-like protein SUMO-1 to its target and requires four enzymes: the SUMO protease, E1-activating enzyme, E2-conjugating enzyme, and E3 ligase (Seeler & Dejean 2003). Ubc9 is an E2-conjugating enzyme that acts as a coactivator of MR by forming a complex with the MR NTD and SRC-1, but potentiates MR-mediated transcription independently of its E2 SUMO-1-conjugating enzyme activity (Yokota *et al.* 2007). Both the full-length MR and its NTD, but not the LBD, interact strongly with Ubc9 in an aldosterone-sensitive manner. SRC-1 binds Ubc9 and synergistically potentiates MR-mediated transcription. Ubc9 is specifically recruited to the response element of the human epithelial sodium channel gene promoter together with MR in the presence of aldosterone. Furthermore, endogenous MR, Ubc9 and SRC-1 can be co-localized in the collecting duct cell nuclei of the mouse kidney, supporting a physiological role of Ubc9 in MR transactivation together with SRC-1. Ubc9 has previously been observed to interact with the GR (Gottlicher *et al.* 1996) and the AR (Poukka *et al.* 1999), although in the AR, Ubc9 interacts with the nuclear localization signal to enhance testosterone-induced transcription, also independently of its ability to conjugate SUMO-1. However, in these studies, the effect of ligand variability was not assessed, nor was the interaction of Ubc9 with other known coregulators. The spatio-temporal regulation of the dual roles of Ubc9, as SUMO E2 conjugating enzyme and NR coactivator, has yet to be determined.

As introduced earlier, splice variants of the MR may contribute to its ligand- or tissue-specific actions. hMRΔ5,6 lacks the entire receptor hinge region and the LBD (including AF-2) and has been shown to bind DNA and potentiate the transcriptional activation of wild-type hMR and hGR by recruitment of coactivators to its NTD (Zennaro *et al.* 2001). In particular, it was shown to recruit SRC-1 (both SRC1a and -1e), TIF1-α (TIF1-α) and receptor interacting protein 140 (RIP140). RIP140 increased transcriptional activation of hMRΔ5,6 despite weak binding. This is consistent with previous work demonstrating that RIP140 preferentially binds NRs via AF-2 rather than AF-1 (L'Horset *et al.* 1996). Of note, while TIF1-α interacts weakly with the MR AF-2 (Thenot *et al.* 1997), it is able to increase the transactivation of hMRΔ5,6 more than twofold and associate strongly with hMRΔ5,6 in a GST pull-down (Zennaro *et al.* 2001). hMRΔ5,6 transcripts are present at different levels in many human tissues. It is plausible that differential tissue expression of hMRΔ5,6 and its unique coactivator recruitment profile forms another mechanism of tissue-specific MR modulation.

Corepressors of the MR

NCoR and SMRT are the first corepressors identified and have been shown to attenuate MR/aldosterone-mediated transactivation (Wang *et al.* 2004a). While SMRT can induce a substantial down-regulation in aldosterone-mediated transactivation, NCoR has only a weak effect, which may reflect the experiment's choice of CV-1 cells that have high levels of endogenous NCoR. By contrast, an almost identical effect of NCoR and SMRT can be observed for the GR (Szapary *et al.* 1999).

DAXX is a transcriptional modulator that co-localizes with promyelocytic leukemia protein oncogenic domains in the nucleus, playing a role in Fas-linked cell apoptosis (Yang *et al.* 1997, Torii *et al.* 1999). As discussed earlier, it can repress MR and GR trans-activation in a dose dependent manner in mouse hippocampus-derived cells (Obradovic *et al.* 2004). However, similar repression of MR and GR activity is not observed in human neuroblastoma cells, suggesting the importance of cellular environment for transcriptional silencing. DAXX mRNA transcripts are found in the rat hippocampus and are down-regulated in the absence of endogenous aldosterone and cortisol, suggesting that DAXX may play a physiological role in regulating MR and GR activity, at least in the rat brain.

Another corepressor of the MR is the protein inhibitor of activated STAT1 (PIAS1; Pascual-Le Tallec *et al.* 2003). PIAS1 is isolated from a human kidney library using both the MR NTD and its amino acid 163–437 fragments (i.e., the inhibitory domain between AF-1a and AF-1b) as bait. It significantly inhibits aldosterone-induced transactivation but does not affect basal MR activity in the absence of ligand. This suggests that a specific ligand-dependent conformational change in the LBD is required for PIAS1 to repress MR activity. In this particular study, the repressive effect of PIAS1 appears to be MR-specific as it has no effect on dexamethasone-induced GR transactivation. The exact mechanism behind the repressive action of PIAS1 is unclear, although it plays a role in SUMO modification as an E3 ligase (Jackson 2001, Rogers *et al.* 2003). Interestingly, while PIAS1 depends on sumoylation of the MR for its repressive effect at the GR response element promoter, its repression of MR activity is sumoylation-independent in the context of a murine mammary tumor virus promoter (Pascual-Le Tallec *et al.* 2003). This suggests that the repressive effect of PIAS1 is promoter-dependent and partially relies on SUMO modification. Moreover, PIAS1 has been shown to enhance the transcriptional activity of AR and GR while repressing that of PR (Tan *et al.* 2000, Gross *et al.* 2001). PIAS1 contains two overlapping CoRNR boxes, which are the common motifs found in corepressors,

Table 1 Summary of coactivators that interact with the mineralocorticoid receptor (MR)

Coactivator	Site of interaction with the MR	Functions of the coactivator	References relevant to the MR
SRC-1	AF-2; AF-1 by SRC1e isoform	Recruits histone acetylation complex (SWI/SNF, CARM1/PRTM1, CBP) to initiate transcription; weak intrinsic histone acetyltransferase activity	Zennaro *et al.* (2001), Wang *et al.* (2004*a*) and Meijer *et al.* (2005)
SRC-2, TIF2, GRIP1 (mouse ortholog)	AF-1, AF-2	Enhances transactivation	Hong *et al.* (1997), Fuse *et al.* (2000) and Wang *et al.* (2004*a*)
p300/CBP	AF-1, AF-2	Exerts histone acetyltransferase activity; recruits RNA polymerase II to target gene promoter	Fuse *et al.* (2000)
PGC-1α	AF-2	Recruits histone acetyltransferase complex; facilitates binding of NR to transcription initiation complex	Knutti *et al.* (2000) and Hultman *et al.* (2005)
RHA	AF-1a	Component of RNA polymerase II complex; recruits CBP	Kitagawa *et al.* (2002)
ELL	AF-1b	RNA polymerase II elongation factor; prevents premature arrest and transient pausing of polymerase II	Pascual-Le Tallec *et al.* (2005)
FLASH	AF-1	Regulates cell apoptosis	Obradovic *et al.* (2004)
FAF-1	AF-1	Regulates cell apoptosis	Obradovic *et al.* (2004)
Ubc9	NTD	SUMO E2-conjugating enzyme; forms coactivation complex with SRC-1	Yokota *et al.* (2007)
TIF1-α	NTD	Transcriptional coactivator	Zennaro *et al.* (2001)
RIP140	NTD	NR coactivator	Zennaro *et al.* (2001)

however, this region is not responsible for its repression of MR activity as a PIAS1 mutant lacking the CoRNR boxes is still able to inhibit MR-mediated transactivation (Tallec *et al.* 2003).

More recently, NF-YC has been reported to be a corepressor of the MR via interaction with AF-1 (Shibata *et al.* 2008). Overexpression of NF-YC inhibits MR transcriptional activity while depletion of endogenous NF-YC increases MR transcription in an aldosterone-dependent fashion. NF-Y is a ubiquitous heterotrimeric transcription factor, composed of NF-YA, NF-YB, and NF-YC subunits, which recognizes a CCAAT box motif found in many eukaryotic promoter and enhancer elements (Nakshatri *et al.* 1996). The exact mechanism of its interaction with the MR remains to be elucidated.

Other searches for MR coregulators

The number of MR-interacting coregulators identified to date is limited to only 11 coactivators and 5 corepressors (see Tables 1 and 2). To screen for more MR coregulators, Hultman *et al.* (2005) used a mammalian two-hybrid assay to analyze 50 coregulator peptides that contain either the LXXLL or I/L-XX-I/V-I motifs, derived from 23 known coactivators and corepressors. Surprisingly, only peptides from SRC-1, activating signal cointegrator 2 (ASC2) and PGC1-α showed strong ligand-dependent interactions with the MR LBD. Full-length ASC2 is known to potentiate transactivation mediated by the RXR, RAR, TR, ER-α, and GR, but has not been previously evaluated for the MR (Lee *et al.* 1999). In a very similar study,

Table 2 Summary of corepressors that interact with the mineralocorticoid receptor (MR)

Corepressor	Sites of interaction with the MR	Functions of the corepressors	Reference relevant to the MR
SMRT	LBD	Histone deacetylase	Wang *et al.* (2004*a*)
NCoR	LBD	Histone deacetylase	Wang *et al.* (2004*a*)
DAXX	NTD	Regulates cell apoptosis; represses MR transactivation in some cell lines	Obradovic *et al.* (2004)
PIAS1	NTD $\pm$ LBD	SUMO E3 ligase; exact mechanism of repressive action unclear	Pascual-Le Tallec *et al.* (2003)
NF-YC	Unknown	Unknown	Shibata *et al.* (2008)

Li *et al.* (2005) screened 38 peptides in an α screen for competitive binding to ligand-bound MR against an LXXLL motif from SRC-2. They found that only six peptides derived from DAX1, SRC-1, SRC-2, SRC-3, PGC1-α and PGC1-β bound MR strongly. Both of these studies also demonstrated that the peptide binding profile was identical for aldosterone, cortisol, and corticosterone, although it should be noted that the MR LBD was used as bait rather than a full-length MR and only LXXLL-containing peptides were tested. It is plausible that motifs other than LXXLL may be important for ligand-specific interactions with the MR. Furthermore, the NTD of the MR neither contain an LXXLL motif, nor the F/WXXLL motif shown to be critical for the N–/C– interaction in the AR, yet it participates in an N–/C– interaction with the LBD.

Coregulators in disease and treatment

Alterations in coregulator expression have been linked to a number of pathologies, in particular hormone-responsive cancers such as breast and prostate cancer. In the case of breast cancer, a few studies have found elevated levels of steroid receptor RNA activator and amplified in breast cancer-1 (AIB1, also known as SRC-3), suggesting that raised coactivator levels may increase tumor sensitivity to estrogen and growth factors (Anzick *et al.* 1997, Leygue *et al.* 1999, Murphy *et al.* 2000, Bouras *et al.* 2001). SERMs such as tamoxifen have been effective in combating ER positive breast cancers without inducing ER antagonism in bone, in part due to their effect on tissue-specific coactivators (Hall & McDonnell 2005). In the case of prostate cancer, the loss of sirtuin 1 (SIRT 1), a histone deacetylase required for AR antagonist-mediated transcriptional repression, or the overexpression of coactivators, is postulated to play a role in the development of AR-independent disease (Dai *et al.* 2007). These coregulators are actively targeted in experimental therapy for anti-AR refractory prostate cancer (Heemers & Tindall 2007).

It is now recognized that coregulators play an important role in a range of diseases including cancer, inflammatory disease, and metabolic disorders. Out of the 102 coregulators characterized in relation to disease, the following known MR coregulators are included: ELL in leukemia, PGC-1α in the metabolic syndrome, PIAS1 in prostate cancer, SRC-1 in prostate, breast and gastric cancers, and Ubc9 in melanoma, ovarian, and lung cancers (Lonard *et al.* 2007). None of these diseases are distinctively MR driven. However, given the crucial role of coregulators in MR function, changes in coregulator expression may be implicated in pathology associated with aberrant MR activation such as cardiac fibrosis and heart failure (Costello-Boerrigter *et al.* 2007, Funder 2007, Young *et al.* 2007, Shapiro *et al.* 2008).

Conclusion and future directions

Over the last decade, it has become clear that coregulators are important in mediating the diverse and complex actions of NRs. In contrast to other NRs, especially the AR which has over 160 potential coregulators (Heemers & Tindall 2007), the MR has been demonstrated to interact with only a limited number, although it seems probable that more interacting partners exist for the MR. Future studies focused on identifying more coregulators which interact specifically with the MR will provide insights into important molecular signaling pathways for MR action. Given the observed differences in coregulator binding between full-length ER and ER LBD, it would be desirable to screen for coregulators in the context of the full-length MR bound by different ligands, using techniques such as peptide phage display, yeast two-hybrid assays or biochemical purification of MR-interacting protein complexes. Furthermore, regulation of tissue levels of coregulators in MR-mediated disease will also be informative. The discovery of tissue- or ligand-specific coregulators should facilitate the development of selective MR modulators which can confer desirable tissue-specific effects while retaining normal MR function in other tissues.

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