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MINIREVIEW

The Mineralocorticoid Receptor: A Journey Exploring Its Diversity and Specificity of Action

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The mineralocorticoid receptor (MR) integrates hormonal signaling and activates the expression of aldosterone target genes, which control various physiological processes. In recent years, evidence has been provided for an important role of MR not only in the regulation of sodium and water homeostasis but also in cardiovascular function, neuronal fate, and adipocyte differentiation. MR belongs to the steroid receptor family that displays common mechanism of action. As a result, some apparent similarities with the glucocorticoid receptor (GR) have shaded MR's own specificities. The description of its gene structure, messenger isoforms, protein variants, functional domains, and posttranslational modifications (phosphorylation, ubiquitinylation, sumoylation, acetylation) as well as a panel of interactions with coregulators, progressively depicted an original portrait of MR and shed light on its specific mechanism of action. In this review, after an overview of MR characteristics, the multiple levels of MR selectivity over other steroid receptors, in particular GR, will be described as well as the consequences for aldosterone-regulated gene expression. (*Molecular Endocrinology* 19: 2211–2221, 2005)

THE MINERALOCORTICOID receptor (MR) has long been considered as a secondary glucocorticoid receptor (GR), if not its pale copy, even though specific roles of its natural ligand, aldosterone, have been well established since the purification of electrocortin more than 50 yr ago. Aldosterone was initially restricted to the control of sodium reabsorption in the kidney, thereby being recognized as a major regulator of volume status and blood pressure (1). The cloning of a specific receptor for aldosterone (2) definitively moved MR out of the shadow of GR and opened a new era of exciting biological, biochemical, and genetic studies that have provided important insights into the complexity of MR action. MR (NR3C2) is a member of the nuclear receptor (NR) superfamily and belongs to the steroid receptor (SR) subfamily, together with GR. MR is mainly expressed in polarized epithelial tissues, such as the distal nephron or colon, where it regulates ion homeostasis through modulation of aldosterone-regulated gene expression (3). Briefly, vectorial sodium reabsorption is driven by a mechanism coupling the apical epithelial sodium channel ENaC to the basolateral Na/K-ATPase pump and is regulated by an essential kinase, the serum and glucocorticoid-regulated kinase 1 that increases functional ENaC (for more information, see Fig. S1 published as supplemental data on The Endocrine Society's Journals Online web site at http://mend.endojournals.org). Interestingly, MR expression and function extends to nonepithelial cells such as hippocampal and hypothalamic neurons, cardiomyocytes, and adipocytes. Dysregulation of the MR-aldosterone system reveals its importance in various human pathologies such as mineralocorticoid resistance (4), disorders of the nervous system (5), hypertension (6), cardiac failure, the latter is emphasized by the therapeutic benefits of antimineralocorticoids (7). Inactivation (8, 9) or overexpression of MR (10) in mice further confirmed the crucial role played by this receptor in renal and cardiac functions. Describing the physiological consequences of MR action is beyond

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Abbreviations: AF, Activating function; AR, androgen receptor; CBP, cAMP response element binding protein-binding protein; DAXX, death-associated protein; DBD, DNA binding domain; ELL, eleven-nineteen lysine-rich leukemia; ER, estrogen receptor; GR, glucocorticoid receptor; GRE, glucocorticoid response element; h, human; HDAC, histone deacetylase; 11HSD2, type 2 11β-hydroxysteroid dehydrogenase; LBD, ligand binding domain; MR, mineralocorticoid receptor; NCoR, nuclear receptor corepressor; NLS, nuclear localization signal; NR, nuclear receptor; NTD, N-terminal domain; PIC, preinitiation complex; PIAS, protein inhibitor of activated signal transducer and activator of transcription-1; PTM, posttranslational modification; RHA, RNA/nuclear DNA helicase II; SR, steroid receptor; SRC, steroid receptor coactivator 1; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; SUMO, small ubiquitin-related modifier; TIF, transcriptional intermediary factor.

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the scope of this review. It focuses on recent findings of MR diversity from its gene to proteins and their associated posttranslational modifications (PTM), as well as on the multiple molecular determinants and partners that ensure its specificity of action.

THE MR: ONE GENE, SEVERAL MESSENGER ISOFORMS, AND PROTEIN VARIANTS

Genome sequence projects have identified a fewer than expected number of genes for vertebrates. It thus appears that diverse mechanisms should exist to create the differences between organisms, given this limited repertoire of approximately 25,000 genes. It is now clear that the MR gene does not encode for one unique expressed protein, but rather for several proteins, in a manner analogous to that reported for the GR (for review see Ref. 11). Several mechanisms are involved to generate distinct MR isoforms and protein variants. Such mechanisms would appear essential to create diversity around one single gene to allow tissue- and temporal-dependent combinatorial patterns of protein expression that largely remain to be identified.

The human MR (hMR) gene, localized to chromosome 4 in the q31.1 region (12), spans over approximately 450 kb and is composed of ten exons (13) (Fig. 1). The two first exons 1, referred to as 1α and 1β, are untranslated, whereas the eight other exons encode the entire protein. Interestingly, the genomic structure of rat MR is somehow different because isolation of its cDNA (14) identified three 5′-untranslated sequences corresponding to three different exons referred to as exon 1α, exon 1β, and exon 1γ of the same gene (15). A similar genomic organization is also found for the mouse MR (Pascual-Le Tallec, L., or S. Szatl-Mazer and M. Lombès, unpublished results). Alternative transcription of these 5′-untranslated exons generates different mRNA isoforms, hMRα and hMRβ, expressed in various human aldosterone target tissues (16); however, the exact function of these first exons, which is presumably related to transcript stability and/or trans-

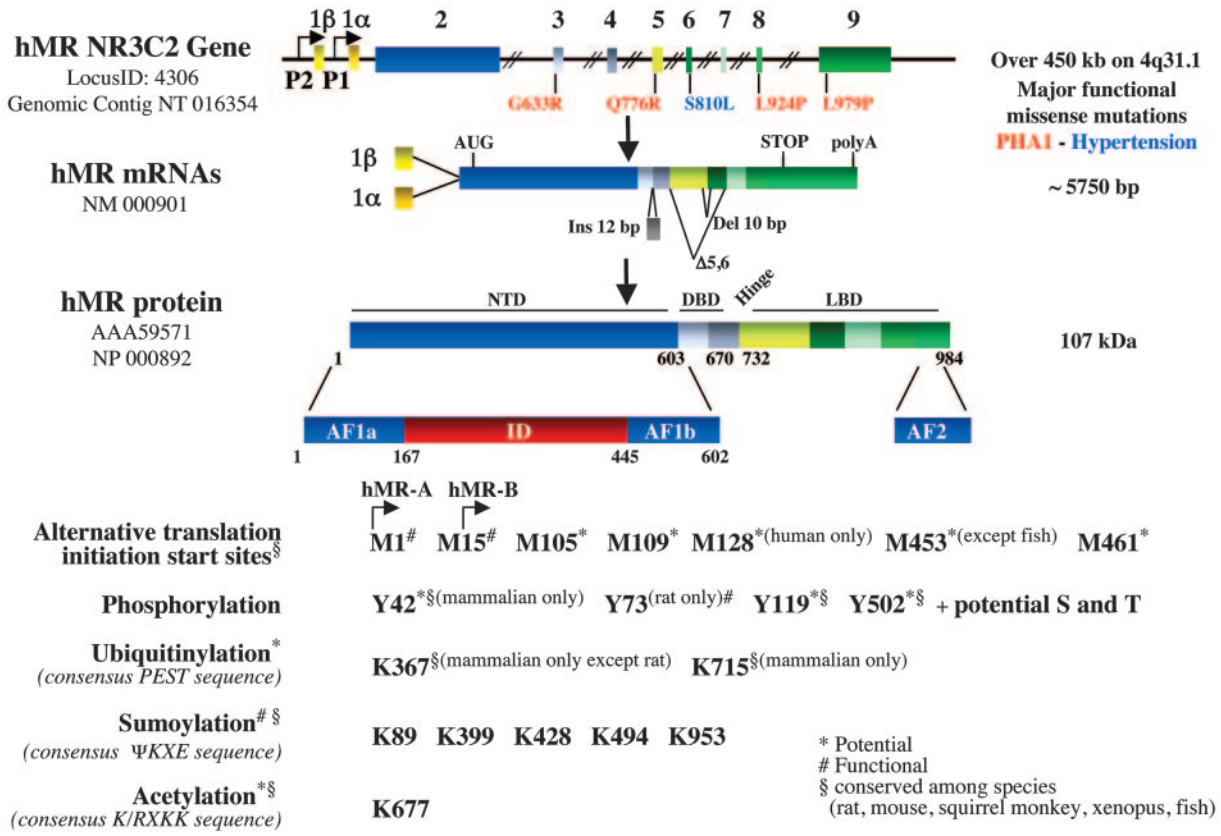

Fig. 1. The hMR Gene, mRNA Isoforms, Protein Functional Domains, and Associated PTM

The schematic representation of the gene and the naturally occurring missense mutations, responsible for either gain (in *blue*) or loss (in *red*) of MR function identified in hypertension or pseudohypoaldosteronism type 1 (PHA1), respectively, are depicted. Multiple mRNA isoforms generated by alternative transcription (1α and 1β) driven by P1 and P2 promoters or splicing events (ins12 bp, del10 bp, and Δ5,6) are translated into protein variants. The receptor harbors distinct functional domains able to activate (activating function AF1a, AF1b, and AF2) or inhibit (inhibitory domain, ID) transactivation. Alternative translation initiation sites and the positioning of residues targeted for major PTM are indicated. Amino acids numbering is based on the hMR sequence.

lational efficiency, remains to be determined. Given that MR's translation initiation site is located 2 bp downstream from the beginning of exon 2, all of these isoforms give rise to the same 107-kDa translation product. Exon 2 encodes for the N-terminal domain (NTD), small exons 3 and 4 for each of the two zinc fingers of the DNA binding domain (DBD), and the last five exons for the ligand binding domain (LBD) of the receptor. However, the existence of other MR splice variants, displaying functional roles, has been reported and seems to add diversity and to play a major role in modulation of overall receptor functions. A 12-bp insertion resulting from the use of a cryptic splice site at the exon 3/ intron C splice junction leads to an in frame insertion of four residues between the two zinc fingers of the DBD (17). This variant has no difference in terms of transcriptional activity as compared with wild-type MR (18). However, it is expressed at a slightly different abundance within various regions of the human brain (19), raising the possibility of tissue-specific alternative splicing of MR mRNAs. Another variant is the 10-bp deletion in the rat MR (20), leading to truncation in the LBD at residue 807 and unresponsiveness to aldosterone, is expressed at low levels in various rat tissues and in human kidney, but does not seem to interfere with wild-type MR activity. Finally, alternative splicing events skipping exons 5 and/or 6 lead to coexpression with the wild-type mRNA of the Δ5 or the Δ5,6 hMR mRNA isoforms (21). These transcripts code for protein variants exhibiting drastic functional alterations. For instance Δ5,6 hMR remains able to bind DNA but as the result of the lack of an LBD, due to a premature termination codon, acts in a ligand-independent manner, modulating MR and GR transcriptional capacities (21). Molecular mechanisms regulating MR transcription and translation remain to be established because tissue-specific aldosterone action is likely to be correlated with the nature as well as the relative ratio of these different isoforms.

hMR gene expression is controlled by two alternative promoters referred to as P1 and P2, which correspond to the 5′-flanking region of exon 1α and exon 1β, respectively (22). A series of hMR promoter truncations revealed that these regulatory sequences differ in their basal activities—the proximal P1 promoter possesses a stronger transcriptional activity than the distal P2 promoter—as well as in their hormonal regulation. Experiments in transgenic mice further demonstrated a distinct tissue-specific utilization of these two hMR regulatory regions *in vivo*. P1 promoter is a relatively strong promoter, active in all aldosterone-target tissues (*i.e.* distal nephron, colon, heart, brain, lung, reproductive tract, adipose tissue, and liver), whereas P2 promoter has a weaker activity with a restricted pattern of expression, presumably used during specific developmental stages or physiological situations (23).

To date, MR protein sequences are known for various mammalians, amphibians and fish (see GenBank). Studies in fish reveal the emergence of a functional MR before the appearance of aldosterone in evolution, with 11-deoxycorticosterone and glucocorticoids as natural ligands (24). These phylogenic studies reveal a similar mechanism of MR action underpinned by highly conserved sequences for all of the functional domains of the receptor. The MR displays the common nuclear receptor modular structure, composed of four distinct domains possessing specific functions (Fig. 1). MR's LBD is composed of 251 residues (~60% homology between the other SR and more than 85% homology across species) organized in 12 α-helixes and one β-sheet forming three antiparallel layers. Its three-dimensional structure was deduced by analogy to other SR LBD crystal structures (25, 26). In absence of ligand, the LBD displays multiple contact sites with chaperone proteins heat shock protein 90 (27), heat shock protein 70, and different immunophilins (28), which are released upon ligand binding thus unmasking the nuclear localization signal (NLS) 2 responsible for nuclear translocation of activated receptor (29, 30). MR's LBD also encompasses interacting surfaces responsible for heterodimerization with the GR (31). MR possesses an activating function AF2, formed and activated in a ligand-dependent manner, after agonist binding into the hydrophobic pocket of the LBD, constituted by helixes H3, H4, H5, and H12. Initially described for GR, the tau2 minimal domain of the AF2 is composed of 30 residues sufficient to activate transcription (32), *i.e.* to recruit the general transcriptional machinery. This functional domain, which is highly conserved for all SR, is also located in H12 of MR. The correct positioning of aldosterone is ensured by the hydrophobic residues L938, F941, F946, and F956 of MR helixes H11–12 (33), and stabilized by the interactions of aldosterone 3-ketone, 20-ketone, 21-hydroxyl, and 18-hydroxyl groups to LBD's polar residues Q776 and R817, C942, and N770 respectively (25, 33, 34). The consequence is the rotation of H12 occluding the pocket, the rearrangement of helixes H3 H5, and H11, together expose outside of the LBD a hydrophobic groove interacting with NR box of different coactivators defined by the LXXLL motif (where L is a leucine and X any residue) (35). Naturally occurring missense mutations of the LBD—L924P (36), Q776R, and L979P (37)—have been identified in type I pseudohypoaldosteronism patients. These mutations impair aldosterone binding, confirming the functional role of these residues for MR transactivation.

The hinge region, located between residues 671–732, encompasses a proline stretch, which permits a twist of the DBD relative to the LBD, correctly positioning the receptor to contact the general transcription machinery (38). It possesses a weak ligand-independent NLS1 responsible for receptor subcellular localization. Additionally, this region was proposed to serve as a potential link responsible for homodimerization, but not heterodimerization, of the receptor as reported for GR (31) but remains to be clearly established for MR.

The highly conserved DBD (90% homology among SR) recognizes the hormone response elements of DNA by its two zinc finger structures. It contains a P box defined as the interacting surface with the half site of the inverse repeat of the glucocorticoid response element (GRE) and a D box responsible for weak dimerization (39). A nuclear export signal has also been identified

between the two zinc fingers (40) near the NSL1 located next to the C-terminal site of the DBD. Interestingly a natural mutant of the nuclear export signal—G633R (37)—displays abnormal subcellular trafficking, predominantly nuclear retention associated with altered interaction dynamics with DNA. Given the high degree of DBD's structural and functional similarities between MR and GR, other mechanisms must be invoked to account for their distinct transcriptional activities.

The NTD of MR, which is the longest domain among all SR (602 amino acids), is highly variable between SR demonstrating less than 15% homology but is highly evolutionary conserved (more than 50% homology), strongly suggesting crucial functional importance. Indeed, the NTD possesses several functional domains responsible for ligand-independent transactivation or transrepression schematized in Fig. 1. The initial description of hMR's AF1 reported a unique central domain (residues 328–382) in the NTD (41) similar to the AF1-tau1 domain of GR (32). Rather, two distinct AF1, named AF1a (residues 1–167) and AF1b (residues 445–602) were subsequently demonstrated in both rat (42) and hMR (43), a functional organization similar to those previously reported for human androgen receptor (AR) (44) and hPR-B (45). Data have also suggested the presence of a central inhibitory domain (residues 163–437), which is sufficient to attenuate the overall transactivation strength of the NTD when fused either to AF1a or AF1b (42, 43). As a whole, the relative contribution of AF1 *vs.* AF2 in MR transcriptional activity is highly dependent on cellular and promoter contexts and appears to account for approximately 40–50% of total transactivation (21, 42, 46). These last years, the NTD had gained functional importance and could now be considered as the key determinant for MR specificity.

Finally, as reported for GR (47), MR is also expressed as at least two different protein variant forms (Fig. 1), named MRA and MRB (48), resulting from strong Kozak sequences initiating alternative translation. These variants are still hypothetical *in vivo* but clearly display distinct transactivation capacities *in vitro*. They could account for a fine-tuning in MR transcriptional activities and subsequent gene expression and provide additional support for MR diversity.

Thus, it appears that MR expression results from a complex cascade of regulatory events that involves both transcriptional and posttranscriptional mechanisms. Further studies should be devoted to identify and characterize transcription factors and signal transduction pathways regulating tissue-specific MR transcripts and thereby allowing modulation of aldosterone action most notably in the distal nephron, the cardiovascular system, and in the central nervous system.

MR IS SUBJECTED TO MULTIPLE POSTTRANSLATIONAL MODIFICATIONS

Early studies showed that MR is a phosphoprotein (49), and examination of its primary sequence reveals numerous potential phosphorylated residues (Fig. 1). Of major interest, one of the potential tyrosine phosphorylation sites, at position 73 in NTD of Fisher 344 rat MR, was found to be substituted for a cysteine in Brown Norway rat. This polymorphism perfectly correlates with the apparent insensitivity to adrenalectomy observed in this species. Indeed, a strong genetic linkage in a F2 hybrid population was detected between the MR genotype and responsiveness to corticosteroids. The Y73C substitution leads to a robust gain of function for MR with greater transactivation by aldosterone, and surprisingly by progesterone, which profoundly affects rat physiology (50, 51). Currently, direct phosphorylation on Y73 and the kinases responsible remain to be elucidated; however, these studies provided the first direct genotype/phenotype relationship in the MR signaling pathway, which is likely to be of importance in humans. Another recent study has demonstrated a rapid (within minutes of aldosterone exposure) MR phosphorylation on serine and threonine (but not tyrosine) residues, in part via protein kinase C α activation (52), providing direct evidence for a link between MR phosphorylation and ion transport. It has also been reported that MR function was enhanced in an NTD-dependent manner by PKA activation, which presumably involves phosphorylation of an associated coregulator rather than a direct effect (53). Collectively, the exact consequences of MR phosphorylation are not known but are likely to affect both its transcriptional activity as well as modulate its interaction with multiple molecular partners, and also presumably its turnover and subcellular trafficking, as demonstrated for GR (reviewed in Ref. 54).

To date, there are no consistent reports of MR ubiquitinylation. Nevertheless, on the basis of our current understanding of this PTM obtained with other SR and particularly GR, one can postulate that this modification may occur on MR. Ligand-induced GR down-regulation due to ubiquitinylation could be abrogated by proteasome inhibitor treatment or mutation of the ubiquitin-targeted lysine K426 that markedly increases GR transactivation combined with nuclear retention (55, 56). Such a mechanism is highly probable for MR because analysis by the PESTfind algorithm points out two strong potential ubiquitin acceptor lysines, at K367 in the NTD and K715 in the hinge region (Fig. 1). These PEST sequences are conserved among mammalian MR (except the NTD of the rat MR), suggesting an important role in the turnover and proteosomal degradation of the receptor. Ubiquitinylation may be considered as a potential mechanism for proteasome routing of MR and would merit further investigation.

Although MR ubiquitinylation remains hypothetical, a similar enzymatic, but functionally distinct mechanism has been extensively studied: sumoylation [for modification by SUMO (small ubiquitin-related modifier)], a PTM extremely important in the regulation of transcription factor function and in particular for SR (for recent review see Ref. 57). All SR are sumoylated (43, 58–61), except estrogen receptors (ER) α and β

(but not ER-related receptors). MR possesses four sumoylation consensus motifs in the NTD at positions K89, K399, K428, K494, and one in the LBD at position K953 (Fig. 1). The consensus site, also named synergy control motif, is defined by the peptide sequence ΨKXE, where Ψ is an aliphatic residue and X any residue. It is of interest that these lysine acceptor sites do not appear to be targeted by other PTM, thereby precluding competition by different regulatory pathways at the same residue. Importantly, these sites are highly conserved in the MR through evolution being present from xenopus and fish to mammals (Fig. 1), arguing for an essential functional role for sumoylation. Indeed, it has been shown that lysine acceptor site mutations led to a striking linear and proportional increase of MR transcriptional properties as a direct function of mutation number (43). This potentiation effect has first been reported for GR (59), then progesterone receptor (PR) (62), AR (58, 63), and many other, if not most, transcription factors (57). Thus, sumoylation could be considered as a general repressive mechanism for transcription factor action. Interestingly, the impact of sumoylation on MR is dependent on the nature of the responsive element bound by the receptor as already described for GR (59). MR transcription is repressed by sumoylation only on synergistic or natural (1) GRE, but not on hemi-sites, degenerated GRE or mouse mammary tumor virus promoter sites (43) as elegantly described for GR (59). These results indicate that dimers of MR or cooperative dimers are highly sensitive to sumoylation. Indeed, it is conceivable that SUMO or poly-SUMO groups may modify the conformation of MR monomers impairing dimerization of synergistic active MR dimers. The sumoylation process has also been implicated in the nuclear import of some proteins (57); thus, one can postulate an impact this may have on the mechanism of SR nucleocytoplamic shuttling. However, MR sumoylation mutants did not exhibit any modification in their subcellular distribution (Pascual-Le Tallec, L., or S. Szatl-Mazer and M. Lombès, unpublished results). Three distinct pathways of sumoylation implicating different classes of SUMO E3-ligase, the target-selective key enzyme, have been described (57). To date, SR seems to be only modified by the PIAS (protein inhibitor of activated signal transducer and activator of transcription-1) protein family and not by any other class of SUMO E3 ligases. The modulatory effects of PIAS proteins on MR will be described below. Next should be mentioned another analogous PTM emerging these last years: neddylation, the conjugation of the ubiquitin-like protein Nedd8 to target lysine. With respect to SR, Uba3, and Ubc12, *i.e.* the E1-activating and E2-conjugating Nedd8 enzymes, respectively, were shown to inhibit transcriptional activity of ERα, ERβ, AR, and PR (64). However, neddylation is still largely unexplored, particularly for MR.

Finally, SR acetylation mediated by the p300 and p/CAF proteins, has been mainly studied for AR and ERα (65, 66). This PTM was shown to inhibit ligand-dependent AR nuclear translocation consistent with the fact that the KXKK/RXKK acetylation motif of the hinge region exactly matches with the NLS1 of most SR, particularly the GR (67) and MR. Indeed, the AR acetylation site mutant misfolds with chaperone proteins and aggregates in the cytoplasm as a result of the inhibition of proteasomal degradation (68). The investigation of the properties of the potentially acetylated K677 of MR NLS1 may shed light on the role of acetylation in MR function, not only in terms of nuclear import and intracellular shuttling but also in terms of the balance between homo *vs.* heterodimerization with GR (31).

Taken together, lysines make their marks as "le" crucial targeted amino acid regulating, through a panel of PTM, MR, and other transcription factor functions (for review see Ref. 69). With the exception of competition between sumoylation and ubiquitinylation—for example, lysine K21 of IκBα (70)—it appears clearly that each modification occurs on specific residues. The next challenge will be to sort out the order, dynamics, and equilibrium of those multiple PTM events to decipher and connect a comprehensive network of transcriptional regulations at the level of the transactivator.

THE THREE LEVELS OF MR SELECTIVITY

The concept and the mechanisms of mineralocorticoid and MR selectivity continue to be debated. How can a receptor be selective when is equally sensitive to glucocorticoids and aldosterone and where cortisol/corticosterone plasma concentrations are much higher than those of aldosterone? How can coexpression of MR and GR in the same target cell allow activation of specific pathways and distinct physiological responses? And how can genes responsive to mineralo- and glucocorticoids be differentially activated by both corticosteroid receptors? Three cornerstones to mineralocorticoid selectivity are discussed below, presented according to the particular level of selectivity we would consider (Fig. 2).

The Prereceptor Level

The tissue distribution and expression level of MR and its specific coregulators constitute obvious selective elements that determine MR-mediated aldosterone responsiveness for a given cell type (see Fig. S1 in supplemental data). *In vivo*, MR binds both aldosterone and natural glucocorticoids with approximately the same affinity. Because cortisol/corticosterone has a 1000-fold higher concentration in plasma than that of aldosterone, the mineralocorticoid selectivity mainly depends, at a cellular level, on the enzymatic activity of type 2 11β-hydroxysteroid dehydrogenase (11HSD2), which converts cortisol into inactive cortisone resulting in the preferential aldosterone-dependent MR activity (reviewed in Ref. 34). Defects or mutations in 11HSD2 are responsible for severe hypertension in patients with apparent mineralocorticoid excess (71). However, the mineralocorticoid specificity is

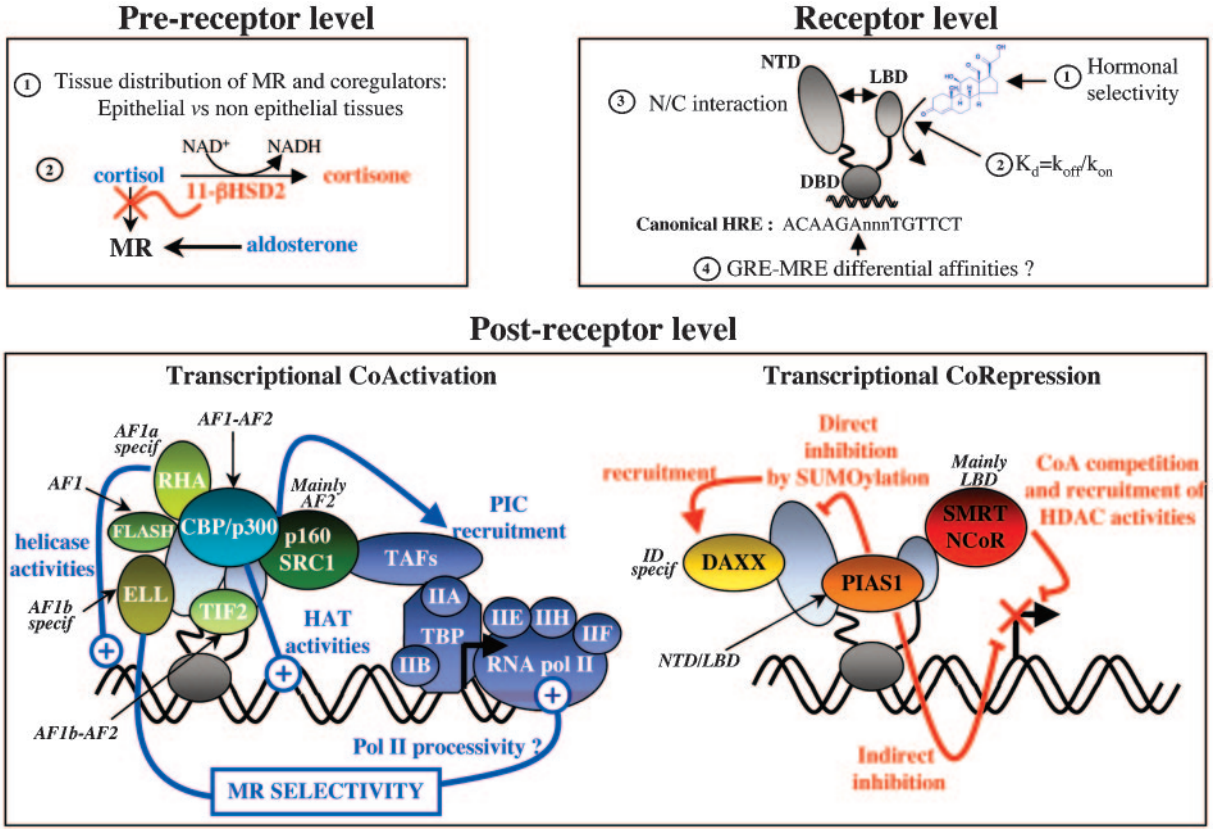

Fig. 2. The Three Levels of MR Selectivity

The tissue distribution of MR and its associated coregulators (1) together with 11HSD2 catalytic activity (2) constitute key determinants of the mineralocorticoid selectivity at the prereceptor level. Intrinsic MR properties as hormonal recognition (1), ligand binding dynamics (2), intramolecular interactions (3), and receptor-DNA binding (4) are responsible for selectivity at the receptor level. Finally, MR recruits through its functional domains (AFs or ID) distinct coactivator or corepressor complexes (and their associated properties) to ensure, at the postreceptor level, transcriptional selectivity. FLASH, FLICE-associated huge; NAD, nicotinamide adenine dinucleotide (oxidized form); NADH, NAD reduced form; TBP, TATA binding protein; TAF, TBP-associated factor; HRE, hormone response elements.

not ensured exclusively by 11HSD2 because its expression is restricted to sodium-transporting epithelial cells with very low or no activity in other aldosterone-sensitive tissues such as the heart or the central nervous system. This is a strong indication that other molecular mechanisms are important determinants of receptor specificity and action.

The Receptor Level

Mineralocorticoid selectivity is conferred at the receptor level by ligand-induced conformational changes, which differ between gluco- and mineralocorticoids leading to differential transactivation capabilities (72). Even though aldosterone and cortisol bind to MR with an affinity of the same order of magnitude—the dissociation constants Kd corresponding to the off to on value ratio are in the nanomolar range—their dissociation kinetics are quite different (72, 73). It has been shown that aldosterone dissociates more slowly from the receptor than does cortisol, indicating that aldosterone-receptor complexes are more stable and more efficient at stabilizing the helix H12 active position (33). This intrinsic property of MR to discriminate between aldosterone and glucocorticoids (72, 74) constitutes an additional molecular mechanism that ensures selectivity of aldosterone action in a kinetic point of view. This is exemplified by the S810L mutation found in patients presenting with exacerbated hypertension during pregnancy in which MR becomes activated by progesterone (75) and cortisone (76). Another important step toward mineralocorticoid specificity is the characterization of N-/C-terminal interactions that are stronger in the presence of aldosterone than cortisol (77). It implies that direct intramolecular contacts may favor ligand-dependent transconformation leading to specific recruitment of coregulators (78). Thus, disruption of N-/C-terminal interactions by the antagonist spironolactone may lead to MR inhibition (77).

The Postreceptor Level

During the last 10 yr, a battery of transcriptional coregulators have been described, some of them being

general modulators, pleiotropic in their action and cellular expression, whereas others seemed to be highly restricted to specific SR or with limited tissue distribution. As described above, SR's NTD are highly specific, not redundant in their sequences but probably conserved in their structure and functions, with composite activating or repressing domains, as also demonstrated by pioneer works using MR-GR chimeras (79, 80). These observations led different groups to postulate that the NTD was a key element if not the sole determinant of receptor singularity conferring specific interactions with particular coregulators.

Coactivators of MR

Steroid receptor coactivator 1 (SRC-1) was the first member of a large coactivator family, which includes three distinct subgroups, SRC-1, 2, and 3 (81). All SR bound to DNA interact with SRC-1 to initiate transcription by sequential recruitment of SWI/SNF chromatin remodeling complexes, histone-methyltransferase CARM1/PRTM1 proteins, and histone acetylase cAMP response element binding protein-binding protein (CBP)/p300-pCAF proteins. This results in formation of a preinitiation complex (PIC) to achieve ordered, cyclical, and combinatorial promoter gene activation as beautifully described for ERα (82). This presentation of a general and deliberately simplified mechanism of SR-mediated gene activation could be applied to MR even though all steps have not been individually reported (Fig. 2). To date, MR has been shown to interact with and be potentiated by different SRC-1 protein variants, mainly through interactions involving the AF2 domain of the LBD (33, 42, 83, 84). SRC-2/transcriptional intermediary factor (TIF) 2 and CBP/p300 are also able to increase MR AF2 function (42, 78). But other general coactivators such as TIF1α (21) or CBP/p300 (42, 78) are active on the AF1a and TIF2 and CBP/p300 (42, 78) through the AF1b domains. In addition, the NTD seems to functionally interact with receptor-interacting protein 140 (21), a member of the estrogen receptor-associated protein/receptorinteracting protein coregulator family mostly devoted to ER. Another very strong MR coactivator is peroxisome proliferator-activated receptor γ coactivator 1 (85), whose role has been underinvestigated especially with regard to its very high expression in brown adipocytes, which coincides with the novel action of MR in this tissue (86). Finally, it may be evoked that MR could alternatively interact with CRSP/MED and ARC/MED (Pascual-Le Tallec, L., or S. Szatl-Mazer and M. Lombès, unpublished results) mediator complexes as described for other SRs, particularly GR (87).

Thus far, all of the coactivators described above could be considered as general coregulators, without evident distinctive properties for individual SR. With respect to MR, a functional interaction and coactivation of an RNA helicase, RHA (RNA/nuclear DNA helicase II) recruiting CBP/p300 exclusively through the AF1a domain of MR, has been demonstrated (78). RHA can interact on double-strained DNA with topoisomerase IIα and interestingly Ubc9, forming a complex responsible for DNA relaxation (88) and also potentially regulating chromatin structure in part by CBP/p300 (Fig. 2). However, its role on other SR, and particularly GR, deserves further investigation. Fas-associated factor 1 is another example of a partially selective coregulator but seems to be a relatively modest coactivator with MR, without effect on GR, and with its action restricted to one neuronal cell line (89). The same study reported coactivation function for FLASH [FLICE (Fas-associated death domain-like IL-1β-converting enzyme)-associated huge] on both MR and GR to the same extent (89), thereby excluding a role in mineralocorticoid selectivity. The strongest example of a selective coactivator for MR is the elongation factor ELL (eleven-nineteen lysine-rich leukemia), which was originally shown to increase RNA polymerase II processivity and elongation rate by suppressing termination of mRNA synthesis and resuming transient pausing (90, 91). In addition to its elongation properties, ELL is a potent and highly selective MR coactivator on consensus and natural GRE (46). ELL directly interacts only with the NTD of MR and exerts an exclusive AF1b-dependent coactivation, as a functionally different but complementary counterpart of RHA on AF1a (Fig. 2). Of major importance, ELL behaves as a transcriptional selector because it strongly represses GR transactivation and has no effect on AR and PR activities (46). The dual role of ELL as positive or negative modulator of RNA pol II activity could be explained at least by the stage of ELL's entry into the dynamic formation of the PIC (92) before it becomes a transcriptional elongation complex (for recent review see Ref. 91). ELL constitutes the original example of selective coregulator that directly links initial recruitment of the PIC by the transactivator to subsequent properties of the transcriptional elongation complex. Could the transactivator itself dictate the composition and properties of active transcriptional complexes and thus modulate subsequent mRNA synthesis as recently suggested for splicing events (93)? Taken together, ELL represents a remarkable MR-positive, GR-negative, and SR-discriminative cofactor, which may control various physiological processes and contribute to certain pathophysiological situations. For instance, ELL may actually exert a crucial role in determining MR- *vs.* GR-mediated effects in epithelial cells (differential regulation of common target genes of ion transport) or in the brain (neuronal cell fate determination, salt appetite and volemic regulation) where an intriguing pattern of coexpression of both receptors is observed (5). ELL and MR may also be directly implicated in the pathogenesis of leukemia or other apoptotic-defective diseases triggered by glucocorticoids acting via GR (94, 95).

Corepressors of MR

Nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor

(SMRT) are paralogs of one another and function in a similar manner as corepressors of NR, constitutively tethered to their response elements on target promoters. The mechanism of action is strikingly reminiscent of coactivator recruitment, *i.e.* docking of repressors by their multiple CoRNR motifs (defined as I/LXXI/VI) to hydrophobic grooves of unliganded LBD (96). This leads to subsequent recruitment of histone deacetylase proteins (HDAC) and their HDAC activities to maintain a repressed state of gene expression until agonist binding and dissociation of the repressive complex (reviewed in Ref. 97). An evident difference for MR, and to the same extent GR, is their quasi-exclusive cytoplasmic localization in the absence of ligand (29, 30) as a result of which the unliganded receptor does not require basal repression. However, this mechanism becomes effective upon binding of antagonists, like RU486 on GR (98) or antimineralocorticoids on MR (99), which results in nuclear translocation where NCoR and SMRT are then recruited and repressive.

Another specific repressive mechanism should exist to extinguish MR activated-transcription. Recently, the death-associated protein (DAXX) was shown to be a corepressor of MR and also GR (89). This finding needs to be interpreted in the light of a study demonstrating that DAXX inhibits AR only after a prerequisite sumoylation step (100), which could be potentially achieved by PIAS proteins (see below and Fig. 2). Pioneering studies have established the physical interaction of AR with the PIAS family of proteins that modulates its transcriptional properties (101, 102). Later, this finding was extended to most SR (103). The NTD of hMR was subsequently shown to contact PIAS1, PIASxβ, and Ubc9 (43). Furthermore, PIAS1, PIASxβ but not PIAS3 behave as SUMO-E3 ligases able to sumoylate MR both *in vitro* and *in vivo* thus indicating important discriminative properties among PIAS proteins on MR (43). Importantly, PIAS1 is a specific corepressor of MR but has no effect on GR transactivation. Thus, MR's transcriptional selectivity appears not only to occur at the activation level as exemplified with ELL but also at the repression level with PIAS1. Of interest, PIAS1-mediated repression is both dependent and independent on the MR's sumoylation status as a function of the promoter context as already demonstrated for AR (63). Indeed, PIAS1 directly represses nonsumoylable MR mutants on nonsynergistic GRE (43), presumably by competing with other coregulators (104). This latter observation is reminiscent of the PIASy-mediated repression of AR, which is independent of its ability to sumoylate the receptor but thought to be related to its ability to recruit HDAC proteins (105). Thus, it is proposed that a combinatorial association between a given SR and its specific PIAS-interacting proteins leads to transcriptional repression. Searching for the precise PIAS protein repertoire associated *in vivo* with MR (*vs.* GR for example) represents an interesting task especially in the context of different aldosterone-responsive tissues (106).

CONCLUSIONS

It now appears that MR is not a simple supporting actor in the NR family but a SR that adopts diverse and yet specific attitudes. Indeed, in addition to the potential diversity of MR signaling created by a variety of receptor proteins and their interrelated PTM, additive and/or complementary molecular mechanisms lead to specific responses upon aldosterone exposure in a variety of responsive cells. The most notable feature of MR is based on its NTD that supports differential recruitment of particular coregulators responsible for major selectivity at the transcriptional level. The next exciting step will be to define the multiplicity of MR actions in the context of each promoter of target genes. MR functions now extend well beyond transcription initiation, to active transcription complexes turnover and mRNA processing in cell-specific environments. Elucidation of the basic mechanisms of MR signaling will facilitate the development of novel compounds able to modulate MR expression and to function as selective MR modulators. These will represent major steps toward the comprehension of the physiological—and pathophysiological—effects of aldosterone with consequences for improved therapeutic management in human diseases.

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