MNAR functionally interacts with both NH2- and COOH-terminal GR domains to modulate transactivation

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Faculty of Medical and Human Sciences, 1Centre for Molecular Medicine and 2Department of Cardiovascular Sciences, School of Clinical and Laboratory Sciences, University of Manchester, Manchester, United Kingdom; and 3Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas

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Kayahara M, Ohanian J, Ohanian V, Berry A, Vadlamudi R, Ray DW. MNAR functionally interacts with both NH2- and COOH-terminal GR domains to modulate transactivation. Am J Physiol Endocrinol Metab 295: E1047–E1055, 2008. —Glucocorticoids are potent anti-inflammatory agents, acting through the glucocorticoid receptor (GR) to regulate target gene transcription. However, GR may also exert acute effects, including activation of signaling kinases such as c-Src and protein kinase B, possibly via the scaffold protein, modulator of nongenomic action of the estrogen receptor (MNAR). MNAR inhibited GR transactivation in A549 cells, but in HEK293 cells there was a ligand concentration-dependent biphasic effect. Transactivation driven by low ligand concentrations was inhibited by MNAR expression, whereas higher ligand concentrations were potentiating. Further analysis revealed that MNAR inhibited transactivation by the ligand-independent activation function (AF1) but potentiated the COOH-terminal AF2 domain. The effect of MNAR was independent of c-Src activity, demonstrated by inhibitors and c-Src knockdown studies. In support of the role of MNAR in modulating GR transactivation, coimmunoprecipitation studies showed interaction between MNAR and GR in the nucleus but not the cytoplasm. Furthermore, MNAR and c-Src were also found to physically interact in the nucleus. Immunofluorescence studies showed MNAR to be predominantly a nuclear protein, with significant colocalization with GR. Deletion studies revealed that MNAR 884-1130 was communoprecipitated with GR, and furthermore this fragment inhibited GR transactivation function when overexpressed. In addition, MNAR 1-400, which contains multiple LxxLL motifs, also inhibited GR transactivation. Taken together, MNAR interacts with GR in the nucleus but not cytoplasm and regulates GR transactivation in a complex manner depending on cell type. MNAR is capable of regulating both AF1 and AF2 functions of the GR independently. MNAR expression is likely to mediate important cell variation in glucocorticoid responsiveness, in a c-Src-independent mechanism.

Glucocorticoid receptor; modulator of nongenomic action of the estrogen receptor; c-Src; glucocorticoid

Glucocorticoids (Gc) are the most potent anti-inflammatory agents known. Their effects are mediated by the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily. It is well established that on ligand binding the cytosolic GR translocates to the nucleus and exerts its effects on target genes, either by directly binding to DNA and acting as a transcription factor or by interacting with other transcription factors, including nuclear factor NF-kB (18). In addition to this well-charac-
signaling cascades (1). In addition to a cytoplasmic role, MNAR has also been reported to be localized in the nucleus and to have effects on gene transcription.

The current study explored the functional interactions between MNAR and the GR. We found interactions between the GR and MNAR in the nucleus only and found that overexpression of MNAR in A549 cells leads to impaired GR transactivation. However, further delineation of this effect in HEK cells found that this repressive effect was mediated through the NH2-terminal domain of the GR. Surprisingly, loss of the GR NH2 terminus showed transactivational enhancement by MNAR overexpression. Further studies of the MNAR molecule identified two regions that were responsible for inhibiting transactivation of the GR, namely the 400 amino acids in the NH2 terminus but also the COOH-terminal proline-rich region.

MATERIALS AND METHODS

Reagents. Dexamethasone, RU-486, radicicol, normal rabbit serum, BSA, mouse IgG, and Protein G agarose were obtained from Sigma. Protein G Dynabeads were obtained from Invitrogen. Herbsimycin A, PP2, and hygromycin solution were all obtained from Merck. Rabbit anti-MNAR was obtained from Bethyl Laboratories. Mouse anti-GR (clone 41) was obtained from BD Biosciences. Rabbit anti-phospho-Akt (Ser473) were obtained from Cell Signaling Technology. Rabbit anti-phospho-GR (Ser211), rabbit anti-Akt, and rabbit anti-phospho-GR (Ser211) were obtained from Cell Signaling Technology. Mouse anti-c-Src (clone GD11) and mouse anti-lamin A/C (clone 14) were obtained from Millipore. Horseradish peroxidase (HRP)-linked goat anti-rabbit and HRP-linked goat anti-mouse secondary antibodies were obtained from GE Healthcare. TrueBlot HRP-conjugated anti-rabbit IgG and TrueBlot HRP-conjugated anti-mouse IgG were obtained from eBioscience.

Cell culture. A549 and HEK293 cells were obtained from ECACC (Salisbury, UK) and were cultured in DMEM with Glutamax (Invitrogen) supplemented with 10% heat-inactivated FCS (Invitrogen). A549 cells were washed three times with Hank’s balanced salt solution (Sigma) and incubated in DMEM only for 48 h before experiment.

Plasmids and transfection. The FLAG-MNAR plasmid and the p133 vector were gifts from Dr. Boris Cheskis (Wyeth Research) (1). The MNAR plasmid in pcDNA3.1, pEBG vector, and GST-tagged MNAR has also been reported to be localized in the nucleus (1). In addition to a cytoplasmic role, MNAR has also been reported to be localized in the nucleus and to have effects on gene transcription.

Immunoblotting. Whole cell lysates were prepared using a Triton lysis buffer (20 mM Tris (pH 7.4), 137 mM NaCl, 2 mM EDTA (pH 7.4), 1% vol/vol Triton X-100, 25 mM β-glycerocephosphate, 10% vol/vol glycerol, 10 mM NaF, 1 mM Na3Vas, 1 mM phenylmethyl-sulfonyl fluoride (PMSF), 1 μg/ml aprotinin, and 1 μg/ml leupeptin). Protein concentration was estimated using the Bio-Rad protein assay reagent.

Cytoplasmic and nuclear fractions were prepared as before (19), briefly as follows. Cells were collected and resuspended in ice-cold buffer A [10 mM HEPES (pH 8), 1.5 mM MgCl2, 10 mM KCl, 0.5% Nonidet P-40 (NP-40), 10 mM NaF, 1 mM Na3Vas, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin] for 1 min and then spun at 3,000 g for 3 min. The supernatant was collected as the cytoplasmic fraction. The pellet was resuspended in ice-cold buffer C [20 mM HEPES (pH 8), 20% vol/vol glycerol, 0.42 M NaCl, 1 mM Na3Vas, vol/vol glycerol, 10 mM NaF, 1 mM Na3Vas, 1 mM phenylmethyl-sulfonyl fluoride (PMSF), 1 μg/ml aprotinin, and 1 μg/ml leupeptin]. Protein concentration was estimated using the Bio-Rad protein assay reagent.

Fig. 1. Modulator of nongenomic action of the estrogen receptor (MNAR) represses glucocorticoid receptor (GR) transactivation activity in A549 cells. A: FLAG-MNAR was transfected into A549 cells along with a tyrosine aminotransferase (TAT3) reporter plasmid. Open bars, transfections with the empty vector (p133); filled bars, transfections using the FLAG-MNAR plasmid and the p133 vector were gifts from Dr. Boris Cheskis (Wyeth Research) (1). The MNAR plasmid in pcDNA3.1, pEBG vector, and GST-tagged deletion constructs were described earlier (17).

The tyrosine aminotransferase (TAT) 3-Luc reporter plasmid, consisting of three copies of the glucocorticoid response element (GRE) from the TAT gene upstream of a minimal promoter linked to luciferase, was a gift from Prof. Keith Yamamoto (University of California San Francisco) (11). The human wild-type GRα in a pcDNA3 vector has been described previously (24). Two deletion constructs were described earlier (17).

Transfections were carried out using FuGene6 (Roche) as recommended by the manufacturer or by using the Nucleofector method (Amaxa Biosystems, Cologne, Germany). All reagents for nucleofection were obtained from Amaxa Biosystems.

Reporter gene assay. Cells were transfected with TAT3 reporter gene, pGL4 Renilla luciferase, and plasmids of interest by using FuGene 6 (Roche), as recommended by the manufacturer. Reporter assays were carried out using the Dual-Luciferase Reporter assay system (Promega). All experiments were performed in triplicate, on at least three separate occasions. The luciferase results were normalized to Renilla as previously described (14).

Kinase assay. The Src kinase assay was carried out using the Src assay kit (Millipore) and as instructed by the manufacturer.

Immunoblotting. Whole cell lysates were prepared using a Triton lysis buffer [20 mM Tris (pH 7.4), 137 mM NaCl, 2 mM EDTA (pH 7.4), 1% vol/vol Triton X-100, 25 mM β-glycerocephosphate, 10% vol/vol glycerol, 10 mM NaF, 1 mM Na3Vas, 1 mM phenylmethyl-sulfonyl fluoride (PMSF), 1 μg/ml aprotinin, and 1 μg/ml leupeptin]. Protein concentration was estimated using the Bio-Rad protein assay reagent.

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1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin] and incubated for 30 min on ice. The mixture was spun at 15,000 g for 15 min, after which the supernatant was collected as the nuclear fraction. Both fractions were precleared by cold centrifugation at 16,000 g for 45 min.

**Immunoprecipitation.** Protein G-Dynabeads were incubated with primary antibody overnight, as recommended by the manufacturer. After being washed, the bead-antibody complex was incubated with precleared cell fractions for 3 h at 4°C. Beads were then washed three times with NETN buffer [120 mM NaCl, 50 mM Tris (pH 8), 1 mM EDTA (pH 8), 0.5% NP-40, 10 mM NaF, 1 mM Na3VO4, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin], and then 20 μl of 6X Laemmli loading buffer were added. Before being loaded on a 10% PAGE, the complex was boiled for 10 min to ensure disaggregation of the protein complex.

Protein (100 μg) was resolved using a 10% polyacrylamide gel and wet-transferred onto a polyvinylidene difluoride membrane (Bio-Rad) overnight. The membrane was blocked with 3% nonfat dry milk or 3% BSA in Tris-buffered saline with 0.1% Tween 20 (TBST) and probed using the following primary antibodies: MNAR (1:2,000), GR (1:2,000), phospho-GR (1:1,000), c-Src (1:2,000), Akt (1:1,000), phospho-Akt (1:500), and lamin A/C (1:1,000). The membrane was washed in TBST and further probed with either HRP-linked anti-rabbit or HRP-linked anti-mouse secondary antibodies.

**Cellular immunofluorescence.** A549 cells were seeded at 1 × 10⁵ cells/ml in 24-well plates containing sterilized cover slips. After overnight incubation, cells were washed and starved from serum. A further 48 h later, cells were treated and then fixed using 4% paraformaldehyde in PBS. Fixed cells were washed and then blocked with 3% nonfat dry milk or 3% BSA in Tris-buffered saline with 0.1% Tween 20 (TBST) and probed using the following primary antibodies: MNAR (1:2,000), GR (1:2,000), phospho-GR (1:1,000), c-Src (1:2,000), Akt (1:1,000), phospho-Akt (1:500), and lamin A/C (1:1,000). The membrane was washed in TBST and further probed with either HRP-linked anti-rabbit or HRP-linked anti-mouse secondary antibodies.

**Statistical analysis.** Comparison between MNAR and control transfection data was made using an independent sample t-test run through SPSS.

**RESULTS**

**Functional interactions between MNAR and GR.** In A549 cells, MNAR overexpression inhibited GR transactivation by >50% at each concentration of dexamethasone used (Fig. 1A). This inhibitory effect was greater with increased MNAR expression (Fig. 1B), as noted previously (5).

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![Diagram](image_url)
To determine the primary target for the regulatory effect of MNAR, deletion studies of the two GR transactivation domains were performed in the HEK cell line (Fig. 2A). Although A549 cells express endogenous GR, HEK cells only express the GR at very low level. However, we observed activation of our reporter gene at higher concentrations of dexamethasone (>1 nM), and so all studies using the GR deletants employed concentrations of dexamethasone shown to have no effect on transactivation in the absence of cotransfected GR (pcDNA3 study; Fig. 2B).

In the HEK cells, MNAR exerted a repressive effect on the transcriptional activity of full-length GR at low concentrations of dexamethasone, but paradoxically, potentiated transactivation at higher concentrations of ligand (Fig. 2B). This effect was robust and reproducible.

Analysis of the isolated NH2-terminal transactivation domain (N500) showed that MNAR exerted a consistent repressive activity, and, as expected, there was no effect on activity from ligand binding. In contrast, deletion of the NH2-terminal AF1 domain (∆AF1) resulted in a construct that activated the reporter gene in a ligand-dependent fashion, but whose effect was also potentiated at all concentrations of Gc by MNAR expression (Fig. 2B). Thus MNAR can act either to inhibit transactivation through the NH2 terminus of GR or to potentiate transactivation through the COOH terminal of GR. Its actions on full-length GR are critically dependent on the

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Fig. 3. Dexamethasone activation of GR phosphorylation and both protein kinase B (Akt) and c-Src activation. A549 cells were treated with dexamethasone (100 nM) for indicated times and then lysed. Cell lysates were probed by immunoblotting using antibodies against GR or phospho-Ser211 GR (A) or Akt or phospho-Ser473 Akt (B). C: lysates from A549 cells treated with dexamethasone for indicated times were immunoprecipitated using the c-Src antibody. The kinase activity of c-Src was then determined by using the Src kinase assay kit as described by the manufacturer. D: A549 cells were treated with dexamethasone (100 nM) for 30 min and then lysed in RIPA buffer. Src was immunoprecipitated using an anti-c-Src antibody and then the following inhibitors were added to the immunoprecipitated kinase to a final concentration as follows: PP2 (0.3 µM), SU-6656 (0.6 µM), or herbimycin A (Herb A, 1 µM). Src kinase assay was carried out as described in MATERIALS AND METHODS. E: A549 cells were transfected with MNAR, TAT3-Luc reporter, and Renilla luciferase and then split into 24-well plates. Cells were pretreated for 30 min with PP2 (0.3 µM), SU-6656 (0.6 µM), or herbimycin A (0.1 µM) before treatment with dexamethasone (10 nM) for 20 h. Luciferase activity was normalized to the Renilla activity. Error bars denote the SD, and each sample point was done in triplicate.
concentration of ligand, with higher concentrations allowing the potentiation effect to predominate (Fig. 2).

The role of c-Src in MNAR function. Previous studies have suggested at the action of MNAR as a scaffolding protein, whereby it acts as a mediator in bringing nuclear hormone receptors and c-Src into close proximity (1).

To explore the role of c-Src in mediating the effects of MNAR on GR function, c-Src activation was measured in parallel with activation of Akt and Ser211 phosphorylation of GR (Fig. 3). The kinetics for these events are similar, with a rise in c-Src kinase activity that was apparent within minutes, and persisted for at least 60 min (Fig. 3C). Of the Src family of kinases, c-Src was the only member to be detectable in A549 cells by immunoblot analysis, by comparison with control A431 cells (data not shown).

Initial studies to reduce the protein level of c-Src used transfection of small-interfering RNA oligonucleotides. Although c-Src expression was reduced by 50%, there was no change in the repression observed with MNAR expression (data not shown). To ensure that the duration of knockdown was optimal for the observed effect, a separate experiment utilizing pharmacological inhibition was performed, using PP2 and SU-6656 at concentrations shown to inhibit kinase activity of c-Src (Fig. 3D). Again, there was no impact on the repressive effect of MNAR expression on reporter gene activity MNAR (Fig. 3E). Finally, a series of stable cell lines expressing a short-hairpin RNA directed against c-Src were generated (Fig. 4, A and B). We compared one clone with wild-type c-Src expression (clone 5.18), a clone generated using the control vector that also had wild-type c-Src expression (pSUPER), and a matched knockdown clone (clone 5.19). Again, there was no effect of reducing c-Src protein levels on the MNAR inhibition of GR transactivation.

Physical interactions between GR and MNAR. To explore interactions between the GR and MNAR within the cell, a series of immunoprecipitations was performed. Because the intracellular location of MNAR remains unclear with reports of both cytoplasmic and nuclear localization, we performed the interaction studies in fractionated cell lysates. The purity of each fraction was routinely determined by immunoblotting for α-tubulin and histone H3, markers for cytoplasm and nucleus respectively (Fig. 5A).

Immunoprecipitation with antibodies against MNAR specifically coimmunoprecipitated GR, but only in the nuclear fraction and in a ligand-dependent manner. Interestingly, the MNAR antibody also brought down c-Src but, again, only in the nuclear fraction (Fig. 5B). In contrast, there were clearly abundant GR and c-Src present in the cytoplasmic fraction, suggesting that the interaction between the proteins was only occurring in the nucleus.

A similar pattern of interaction was seen when the cell fractions were immunoprecipitated for GR (Fig. 5C). Again, there was evidence for interaction between GR and MNAR selectively in the nuclear fraction, although this did not appear to be altered by ligand treatment. There was no convincing, nor consistent, direct interaction observed between the GR and c-Src in the nuclear or the cytoplasmic fraction (Fig. 5C, compare control immunoprecipitate of nuclear fraction against GR immunoprecipitate).

Because subcellular fractionation may allow postlysis partition of proteins, we also examined the intracellular localization of MNAR and GR using immunofluorescence (Fig. 6). MNAR was predominantly localized within the nucleus and was unaffected by Gc treatment (Fig. 6, A and B). The GR was observed to be both cytoplasmic and nuclear in the absence of ligand, but, following Gc treatment, a typical intense nucleus stain was seen with characteristic nucleolar exclusion, as previously reported (Fig. 6A). Immunofluorescence studies revealed significant colocalization of GR and MNAR in the nucleus, both in treated and untreated cells, but with a greater degree of colocalization observed after dexamethasone treatment (Fig. 6A).

MNAR functional domain analysis. A number of functional domains within MNAR have been identified (1, 25). To determine which of these domains is important for mediating the transcriptional repressive effect, a series of deletion mutants was tested (Fig. 7A).

GST pull-down assays revealed that the MNAR 884-1130 fragment efficiently interacted with the GR, despite all MNAR constructs being expressed to a similar degree in HEK cells (Fig. 7B).

To identify a functional correlation for this interaction, the MNAR fragments were expressed in A549 cells where their
effect on the transactivation function of endogenous GR was assessed. There was an interaction between MNAR 884-1130 and GR, with inhibition of transactivation. In addition, there was inhibition of transactivation observed with MNAR 1-400, the region containing LxxLL motifs (Fig. 7, A and C).

**DISCUSSION**

Interactions between steroid receptors and c-Src have received much attention in recent times (3, 4, 21). In particular, the interactions between the PR or the ER with c-Src have been well studied. PR has an SH3 domain located at its extreme NH2 terminus that is capable of directly interacting with c-Src (2). This domain is not conserved among other steroid receptors, but ER has been found to functionally interact with c-Src via MNAR (1, 26), and the transactivation activity of GR has been reported to be opposed by MNAR expression (5). In this study, the functional interactions between MNAR and GR transactivation were studied.

There was a marked inhibition of GR transactivation observed when MNAR was overexpressed in A549 cells. In previous studies, MNAR has variously been reported to either inhibit or to potentiate steroid receptor-driven gene transactivation (5, 25). This may reflect differences between cell types studied, as well as the potential presence of other steroid receptor comodulator proteins. Therefore, to explore this MNAR-dependent inhibition of transactivation, a series of studies was performed using isolated GR transactivation domains, using the GR-deficient HEK293 cell line. Initial experiments showed an unexpectedly heterogeneous pattern of MNAR effect on the transfected wild-type GR. At low concentrations of ligand, MNAR inhibited transactivation, but, at higher concentrations of dexamethasone, MNAR expression potentiated transactivation. Although previous studies had suggested that MNAR was capable of both potentiating and inhibiting transactivation, this is the first report suggesting that it is capable of subserving both actions in the same cells.
Higher concentrations of dexamethasone were not used in HEK cells with the TAT3 reporter gene, since they resulted in activation of low levels of endogenous, full-length, wild-type GR, thereby risking confounding the results (11, 22, 23).

Studies on the constitutively active N500 GR mutant showed that MNAR robustly inhibited transactivation by >50%. However, studies on the mutant with isolated COOH-terminal transactivation domain, ΔAF1, showed a consistent potentiation of transactivation by MNAR, increasing with rising ligand concentration. This is reminiscent of the higher ligand concentrations causing MNAR potentiation on the full-length, wild-type GR. Taken together, these data suggest that the ligand-independent NH2-terminal transactivation domain is inhibited by MNAR, whereas the COOH-terminal AF2 transactivation domain can be potentiated. The biphasic effect of MNAR expression on wild-type GR is likely to result from the differing contributions of the two transactivation domains seen at different concentrations of ligand, with the modulator of non-genomic action of the estrogen receptor terminal transactivation domain predominating at low concentrations of ligand.

The ligand-activated GR is predominantly nuclear in location, but previous reports noted interactions between nuclear hormone receptors and MNAR occurring in the cytoplasm; therefore, the compartment of GR-MNAR interaction was explored. The intracellular localization of MNAR remains unclear, with reports in the literature suggesting both a cytoplasmic site of action as well as expression and action in the nucleus (9, 17, 25). Accordingly, we prepared cytoplasmic and nuclear extracts from cells and conducted a series of immunoprecipitations. There was clear evidence of interaction between GR and MNAR in the nucleus but no evidence of an interaction in the cytoplasm. We were also able to detect interactions between MNAR and c-Src, again predominately in the nuclear extracts. This suggests that, although GR and MNAR are capable of interaction, the interaction occurs predominately in the nucleus rather than in the cytoplasm. Although there is
evidence for interaction between MNAR and c-Src, this also appears to be mainly in the nucleus. There is a weak interaction between GR and c-Src that was only seen in response to ligand and exclusively in the cytoplasm. Therefore, there appears to be a hierarchy of interactions, with GR-MNAR interactions predominantly occurring in the nucleus, whereas GR and c-Src appear to interact only weakly in the cytoplasm. Taken together, this suggests that the interaction between c-Src and GR is independent of MNAR scaffolding. It further suggests that the pool of MNAR molecules binding GR is different from that binding c-Src.

The predominant nuclear colocalization of GR and MNAR is well seen in immunofluorescence studies that show very clear overlap of intracellular distribution of MNAR and GR in the nucleus, following dexamethasone administration. In the absence of ligand treatment, the GR distribution is seen across the cell; however, there is still very little overlap in distribution between GR and MNAR in the cytoplasm. In fact, commounifluorescence for MNAR and tubulin also shows very little of the total cellular MNAR to be present in the cytoplasm.

Despite applying two different genetic approaches and also pharmacological inhibition of c-Src, we were unable to show a role for c-Src in mediating the effects of MNAR on GR function. Thus the actions of MNAR on the GR in the nucleus appear to be independent of c-Src, a functional outcome entirely compatible with the failure to identify physical interactions between all three proteins in the cell nucleus.

The MNAR protein contains a number of discrete domains, the functions of which have been explored previously (1, 25, 26). We exploited these observations to identify MNAR domains capable of physically interacting with the GR. Predictions based on the MNAR sequence would have suggested that the NH2 terminus of MNAR, which harbors 10 sequential LxxLL motifs, would be capable of physically interacting with GR, whereas the COOH terminus, with proline- and glutamic acid-rich regions, may not. However, studies using cotransfection followed by GST pulldown identified that the MNAR fragment from amino acids 884 to 1130 coprecipitated significantly more GR than any of the other fragments expressed. This suggests that the 884-1130 fragment is capable of physically interacting with full-length GR.

Nevertheless, the failure to identify an interaction between GR and MNAR fragment 1-400, as predicted, does not necessarily exclude the presence of an unstable, or weak, interaction. Therefore, we also conducted a series of functional studies. Two fragments clearly and significantly inhibited transactiva-

![Fig. 7. GR is able to bind to the NH2-terminal truncated form of MNAR.](image-url)
tion. The first of these was the fragment 884-1130, the same fragment that we found to be capable of physically interacting and coprecipitating the GR in earlier analysis. The second fragment capable of replicating the inhibition was fragment 1-400, which contains the majority of the LxxLL motifs (17).

MNAR appears capable of interacting independently with both NH2 and COOH termini of GR to mediate its effects. There are precedents for transcriptional co- and coregulators acting both to potentiate GR transactivation and also acting to inhibit transactivation; for example GR interacting protein-1 potentiates GR-dependent transactivation through a consensus GRE motif but mediates the transcriptional inhibitory activity of GR when tethered to NF-kB (20). This change of role has been ascribed to the allosteric effect transmitted by the DNA-binding domain of GR. We now show that MNAR is similarly capable of modulating GR transactivation in a positive and negative manner, partly dependent on cell line, context, and on ambient Gc concentration. Understanding the functional interaction between MNAR and GR has major implications for understanding Gc action and, furthermore, the variation in Gc sensitivity seen in conditions of altered physiological status, for example, in inflammation (15).

GRANTS
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