Reconstitution of the N-terminal Transcription Activation Function of Human Mineralocorticoid Receptor in a Defective Human Glucocorticoid Receptor*

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N-terminal sequences involved in transcription activation by the human mineralocorticoid receptor (hMR) have yet to be defined. We have addressed this issue and generated overlapping internal deletion mutants hMR39–162, hMR59–247, hMR59–328, hMR162–247, hMR247–328, hMR247–382, and hMR328–382 with intact DNA-binding and hormone-binding domains. A second set of mutant receptors with unique BglII sites was generated to facilitate the isolations of fragments. Immunodetection with anti-hMR peptide antibodies and hormone-binding assays showed that the mutations did not affect the expression of the receptors or ability to bind aldosterone. Distribution of aldosterone binding activity of wild type and deletion mutants expressed in HeLa cells was predominantly nuclear. Furthermore, deletion of sequences between 59 and 390 did not affect DNA binding activity. Transfection studies with HeLa cells revealed a region around residue 247 that was crucial for normal receptor function. Deletion of amino acids 59–162 did not affect the transcriptional activity of the hMR. However, deletion of sequences 247–382 and 328–382 markedly decreased the transcription activation function. The induction of the reporter gene by the chimaera hGR71–247/hMR328–382 was 2-fold higher than with the wild type hGR, but 200-fold when compared with hGR71–262, indicating that the AF-1 domain is located between positions 328 and 382 in the hMR.

In humans, the biologically active mineralocorticoid aldosterone maintains the homeostasis of ion balance principally in the kidney, but also in the gut, salivary, and sweat glands. The evolution of the modular structure of nuclear receptors into distinct domains is largely the consequence of complex requirements that arose during cellular growth and development. The structures of the human mineralocorticoid receptor (hMR)1 and the human glucocorticoid receptor (hGR) are very similar. The N-terminal region in this family of nuclear receptors is of variable length and contains a transactivation function, the AF-1 (1). In the absence of a hormone-binding domain (HBD), this region is constitutively active. The N-terminal regions of hGR and hMR have only 15% homology, and it is this domain that is responsible for differences in target gene specificity.

In the hGR, the N-terminal activation domain is 185 amino acids long and a 58-amino acid peptide that is almost as active as the intact region has been identified (2). The experiments with hGR were, however, performed with constructs containing segments of the hGR expressed in yeast and not in mammalian cells, and the cooperative function of the hGR HBD was not taken into consideration in these analyses. Such regions in the hMR remain to be defined. Our experiments were performed in animal cells in culture, and the intact DNA-binding domain (DBD) and HBD of the hMR or hGR were used in transfection studies.

Although 94% of the 68 amino acids in the centrally located DBD of hMR and hGR are identical, the HBD has only 57% homology. The hMR contains an additional 24 amino acids including a sequence of 4 glutamines and 8 prolines encoded by repetitive nucleotide elements (1). The C terminus of the HBD also contains a hormone-dependent transcription activation function (AF-2).

In this paper, we have identified hMR sequences crucial for the transactivation function by first generating receptor mutants with unique BglII sites. In order to identify the hMR sequences capable of conferring activity upon a hGR that lacks transcriptional activity, we have constructed a series of hGR-hMR chimeras. To map the exact domain responsible for transactivation function of hMR, the chimeras were introduced into receptor-deficient CV-1 cells and sequences in the hMR (amino acids 328–382) were identified.

MATERIALS AND METHODS

DNA Constructs—Cloning was performed by standard procedure (3). MMTV-CAT and the eukaryotic expression plasmids for various steroid receptors were constructed as described previously (4, 5). The hMRmut328 was created after digesting the cDNA with ScaI-S05I. Subsequent religation generated an uninterrupted 715-amino acid sequence in an open reading frame. Similarly, digestion of hMR cDNA with BalI (amino acids 148/149 and 390/391) and religation generated a 742-amino acid hMR in an open reading frame. The wild type and mutant receptor cDNAs were cloned at the HindIII- EcoRV sites of pcDNA1. The plasmids were purified by CsCl gradient centrifugation.

Substitution Mutagenesis to introduce Unique Restriction Site in the AF-1 Domain—Unique restriction sites were introduced at the AF-1 domain of the hMR by oligonucleotide-directed mutagenesis. The 5’ region from position 1 to 351 and the 3’ region from position 352 to 984 extended to the 3’ noncoding region of the cDNA, were cloned in M13mp18 and M13mp19. Templates for single-stranded mutagenesis were prepared as described previously (6, 7).

The newly introduced restriction site is AGATCT (codons for Arg-Ser). To introduce BglII sites, the following oligonucleotides were synthesized by Life Technologies, Inc.: a, 5’-CAACAGATCTCAAGG-3’ (hMR S59R/T60S, BglII site between residues 59/60); b, 5’-CCCTT-
Deletion Mutants and Transcription Activation

Comparison of hMRwt, hMRS59RT60S, hMR162 BglII, hMR247 BglII, hMRS328RT7329S, hMRS382AR/A383S, hMR393/382 BglII, hMR162/382 BglII, hMR247/382 BglII, and hMR247/382 BglII

n = number of independent determinations. Cotransfection was with 5 μg of MMTV-CAT, 5 μg of pcDNA MR expression vector, and 5 μg of pHCl10. CAT activity was measured in extracts after normalizing to β-galactosidase activity.

| Receptor       | Binding (Aldosterone) n | Transcription activation of MMTV-CAT   |
|----------------|-------------------------|---------------------------------------|
|                | Kd (aldosterone) | % CAT activity | 1/2max [ALDO] |
| hMRwt          | 0.654 ± 0.25      | 8          | 100        |
| hMR393/162     | 0.761 ± 0.23      | 5          | 98         |
| hMR162 BglII   | 0.692 ± 0.32      | 5          | 105        |
| hMR247 BglII   | 0.643 ± 0.27      | 6          | 100        |
| hMRS328RT7329S | 0.661 ± 0.31      | 4          | 85         |
| hMRS382AR/A383S| 0.741 ± 0.25      | 4          | 129        |
| hMR393/382 BglII| 0.598 ± 0.32     | 4          | 123        |
| hMR393/162     | 0.718 ± 0.42      | 5          | 128        |
| hMR162/382     | 0.597 ± 0.29      | 4          | 122        |
| hMR247/382     | 0.672 ± 0.41      | 4          | 262        |

n = number of independent determinations. Cotransfection was with 15 μg of pcDNA MR expression vector & 5 μg of pHCl10.

| Receptor       | Binding (Aldosterone) n | n |
|----------------|-------------------------|---|
| hMRwt          | 0.654 ± 0.25      | 5 |
| hMR393/162     | 0.711 ± 0.33      | 3 |
| hMR393/247     | 0.632 ± 0.22      | 3 |
| hMR393/328     | 0.545 ± 0.37      | 3 |
| hMR393/382     | 0.661 ± 0.41      | 3 |
| hMR162/382     | 0.747 ± 0.35      | 3 |
| hMR247/382     | 0.561 ± 0.21      | 3 |
| hMR247/382     | 0.623 ± 0.52      | 3 |
| hMR393/328     | 0.764 ± 0.39      | 3 |
| hMR393/382     | 0.539 ± 0.51      | 3 |

n = number of independent determinations. Cotransfection was with 15 μg of pcDNA MR expression vector & 5 μg of pHCl10.

2 M. V. Govindan, unpublished data.
diethiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride) with or without 1 μM aldosterone for 45 min on ice and then vortexed for 10 s. The samples were centrifuged for 10 s, and the supernatants were discarded. The pellets were resuspended in 100 μl of ice-cold extraction buffer B (20 mM Hepes-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) and incubated on ice for 20 min for high salt extraction. Cellular debris was removed by centrifugation for 5 min at 4 °C, and the supernatant fractions containing the DNA-binding proteins were stored in aliquots at −70 °C. The yield was 300–350 μg of protein/5 × 10⁶ cells. Extracts containing 1 unit of β-galactosidase (~5 μg of total protein) were incubated with poly(dI·dC) on ice for 15 min in a 20-μl reaction mixture of 12 mM Hepes, pH 7.9, 80 mM KCl, 4 mM dithiothreitol, 0.2 mM EDTA (DNA-binding buffer), and 10% glycerol. Radiolabeled synthetic double-stranded MRE (5,000 cpm) was added and incubated at room temperature. The hMR-DNA complexes and free probe were then separated on a 4% polyacrylamide gel, run for 100 min and autoradiographed using intensifying screens.

**RESULTS**

**Deletions in the N-terminal Region or the Introduction of BglII Sites in the hMR Did Not Affect Aldosterone Binding**—We first measured the aldosterone binding activity of each receptor. Transfected HeLa cells exhibited aldosterone-specific binding capacity of 2985 fmol/mg of cytosolic protein (data not shown). The specific bound radioactivity varied between 86–88%, and was determined in parallel incubations containing 200-fold molar excess of non-radioactive ligand. All the hMRs have intact hormone-binding domains, hence affinity for aldosterone was unaffected (Tables I and II).

**Deletions or Substitutions in the N-terminal Region of hMR Did Not Affect Receptor Expression in HeLa Cells**—To identify functional regions within the N-terminal domain of the hMR, we have generated internal deletion mutants (Fig. 1). Previous analyses have shown that sequences between residues 328 and 390 in the hMR were crucial for transcriptional activity. A comparison of sequence shows serine-threonine at positions 59–60 and 328–329 and serine-alanine at 382–383. At positions 162 and 247, BglII sites (Arg-Ser) were created by substituting one base in the cDNAs. This did not, however, change the codon. This procedure has the following advantages. 1) It introduces a unique restriction site in the cDNA, allowing the isolation of DNA fragments for construction of fusion-protein expression vectors in bacteria and yeast; 2) it produces receptor cDNA segments for the construction of hGR-hMR chimeras that can be used to identify sequences implicated in the transcription activation function.

To rule out the possibility that variations in transcription activation were due to differential expression of the receptor, the level of hMR in HeLa cells was determined by immunoprecipitation using polyclonal antibodies raised against a synthetic hMR polypeptide. Extracts from HeLa cells transiently cotransfected with wild type hMR expression vector showed the expected 108-kDa labeled band (Fig. 2A–C). They also demonstrate that the mutations did not generate any frameshift or affect the expression of the receptors negatively. Fig. 2B and C shows that receptor mutants were expressed at similar levels. Extracts from hMR162–247 transfected cells showed a slower migrating band with an apparent molecular mass of 110 kDa. The reason for the larger protein band is not presently known. In control extracts from hMR expression vector transfected HeLa cells, no such high molecular weight protein bands were recognized by the preimmune serum.

**hMR328–382 Is Required for Normal Receptor Activity**—Table I shows the ability of the hMR BglII substitution mutants to

![Image](schematic_representation_of_wild_type_hMR_and_N-terminal_deletion_mutation_and_comparison_with_hGR.png)
induce MMTV-CAT. Although double mutation S328R/T329S did not affect affinity for aldosterone (\(K_d = 0.661\) nM), transcriptional activity was lower than that of the intact receptor. CAT activity mediated by S382R/A383S was elevated to 129% in the presence of 10 nM aldosterone. This enhancing effect was evident in other mutant receptors containing S382R/A383S. Induction of CAT was 223% by hMR S59R/T60S/S382R/A383S (MR 59/382 BglII) (Table I). Similarly, the aldosterone-stimulated activities of mutant receptors 162/382 BglII and 247/382 BglII also reached higher levels than with the intact receptor. CAT activity of hMR S328R/T329S/S382R/A383S was enhanced up to 262% when compared with wild type hMR in the presence of aldosterone, even though affinity for hormone was not affected.

**Fig. 2. Immunoprecipitation of hMR mutants.** An aliquot of total proteins equivalent to 50 units of β-galactosidase activity was extracted from HeLa cells transiently transfected with hMR expression vector(s) and labeled with \([\text{35S}]\text{methionine. Extracts were incubated with 5 µl of pre-immune or 5 µl of anti-MR antipeptide antiserum as described under “Materials and Methods.” The marker proteins were stained with Coomassie Blue, and their relative positions are indicated in kilodaltons. A, expression of hMR substitution mutants in HeLa cells. B and C, expression of hMR deletion mutants.**
Sequences around Residue 247 in the hMR Are Crucial for Transcriptional Activity—

To identify functional regions in the N-terminal AF-1 domain of the hMR, we generated a set of internal deletion mutant receptors (Fig. 3). Deletion of N-terminal sequences 59–247 generated a receptor with a 2-fold higher capacity to induce MMTV-CAT (Fig. 3, lanes 5 and 6) when compared with the wild type hMR (lanes 1 and 2). In the absence of aldosterone, transcriptional activity of hMRD59–247 (lane 5) was 50% of the level induced by the wild type hMR (lane 2). This suggests that amino acids between 59 and 247 have an inhibitory role in hMR function, since their deletion increases transcriptional activity. Subsequent deletion of amino acids 59–162 and 162–247 did not affect the transactivation potential of hMR (lanes 3, 4, 9, 10). When sequences C-terminal to 247 were deleted, receptors with reduced transactivation function were generated (lanes 11–16). hMRΔ247–328, hMRΔ247–382, and hMRΔ328–382 showed a dramatic decrease in the capacity to induce CAT. Among these, only hMRΔ247–328 showed some response in the presence of aldosterone (lane 12). This suggests that sequences between 247–382 could be responsible for normal receptor activity. To verify this possibility, we inserted sequence 247–382 in the inactive deletion mutant hMRΔ328–382 and observed that the transactivation potential was restored (lanes 17 and 18). Although the sequence between 247–328 responsible for normal CAT activity is repeated in the construct hMRΔ328–382/247–382, we did not observe a 2-fold increase in transcriptional activity. The complete loss in CAT activity caused by the deletion of residues 247–328, 247–382, and 328–382 and enhanced activity caused by deletion of residues 59–247 suggests that there is a suppressive domain adjacent to the AF-1 function that maintains normal receptor function.

The AF-1 Domain in the hMR Is Located between Residues 328 and 382—

Since the existing BglII sites at 76 and 261 in the hGR are not in frame with the hMR BglII cDNA segments, we generated a hGR mutant by substituting residues at 71/72 and 262/263. When residues 71–262 in the hGR were deleted, the resulting protein showed a loss in transcription activation function in the presence of ligand (Fig. 4, lanes 15 and 16). To identify the hMR sequences that had the capacity to confer enhancing function on the defective hGR, we constructed chimeras hGRΔ71–262/hMRΔ59–162, hGRΔ71–262/hMRΔ59–247, hGRΔ71–262/hMRΔ62–382, hGRΔ71–262/hMRΔ247–382, hGRΔ71–262/hMRΔ328–382, and hGRΔ71–262/hMRΔ162–328. If hMRΔ328–382 has an activating function, then introduction of these sequences into the defective hGR should restore transcriptional activity in the presence of dexamethasone. Additionally, this region could be interacting with similar factors as the hGR and may thus be

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**Fig. 3.** Analysis of transcription activation by wild type hMR and hMR deletion mutants. HeLa cells were transiently cotransfected with receptor expression vectors, MMTV-CAT and CH110, collected, and treated with 1 μM aldosterone as described under “Materials and Methods.” CAT activity was normalized with β-galactosidase, and data are expressed in units of β-galactosidase. The acetylated chloramphenicol derivatives were visualized by autoradiography, and the radioactivity was determined by scintillation counting. The values are the means and standard errors of triplicate experiments.
Deletion Mutants and Transcription Activation

interchangeable. These experiments were performed in CV-1 cells that do not express endogenous receptors and not in HeLa cells, as the presence of functional hGR in HeLa cells has been demonstrated (12). When sequences between 59 and 162 were deleted, transcriptional activity was not affected, suggesting that these sequences have no ligand-independent transactivation function (Fig. 3, lanes 3 and 4). The chimeric receptor hGRΔ71–262/hMR59–162 failed to induce MMTV-CAT confirming this hypothesis (lanes 3 and 4). The hGRΔ71–262/hMR328–382 and, to a lesser extent, hGRΔ71–262/hMR247–382, restored transcription activation function in the presence of dexamethasone (Fig. 4, lanes 9 and 10). Lanes 9 and 10 show that CAT induction by the hGRΔ71–262/hMR247–382 chimera reached almost the level of wild type hGR. However, induction with hGRΔ71–262/hMR328–382 was 200-fold higher than with hGRΔ71–262, localizing the AF-1 domain to sequences between residues 328 and 382 in the hMR. Hence, sequences between 328 and 382 are involved in transactivation function of hMR. Furthermore, this activating domain can be translocated to hGR and still retain the capacity to induce transcription by the chimera in the presence of dexamethasone. Thus, the N-terminal AF-1 domain in the hMR can be limited to residues 328–382.

**Transcriptional Activation by hMR328–382 Is Not Due to the Level of Receptor Expressed**—To ascertain that the transcriptional activity observed with hGRΔ71–262/hMR328–382 chimera was not due to a variation in the level of receptor expression, we conducted immunoprecipitation analyses with transiently transfected CV-1 cells. We used rabbit polyclonal antibodies raised against purified glutathione S-transferase-hMR247–382 (Fig. 5). All chimeric receptor constructs were expressed at similar levels, with variations too insignificant to account for minor differences in transcriptional activity.

**Subcellular Distribution of the Mutant hMRS**—To investigate whether the variations in transactivation capacities were due to an alteration in the cellular distribution of the receptor, we prepared cytosol and nucleosol extracts from transfected cells with 3H-labeled aldosterone and determined the specific binding (Fig. 6). In the presence of aldosterone, the cellular...
distribution of wild type and hMR deletion mutants was predominantly nuclear.

Interaction of Wild Type, hMRΔ59–328 and hMRΔ148–390 with Labeled MRE—To investigate the differences observed in transcription activation mediated by wild type and mutant hMRs, we measured their ability to bind to 32P-labeled MRE by gel mobility shift assay (Fig. 7, top panel). We prepared cell extracts from HeLa cells transfected with the receptor expression vectors to overexpress the encoded proteins. Following incubation with [3H]aldosterone, a specific gel-shifted band was observed with wild type hMR (lane 4) extract. The specificity of the interaction was assayed using hMR wild type extract prepared in the absence of aldosterone (lanes 2 and 3) or in the presence of 100-fold molar excess of non-labeled competitor MRE in the incubates (lanes 3 and 5). Similar analysis with hMRΔ59–328 (lanes 6–9) and hMRΔ148–390 (lanes 10–13) demonstrated that they had essentially wild type affinity for MRE in the presence of aldosterone (lanes 8 and 12, respectively). The specificity of the interaction was further demonstrated in parallel incubations in the presence of 100-fold molar excess competitor DNA (lanes 3 and 5, 7 and 9, and 11 and 13, respectively). The immunoblot shows (Fig. 7, bottom panel) the level of expression of hMRα and deletion mutants hMRΔ59–328 and hMRΔ148–390 used in DNA-protein interaction assay. The difference in hormonal response detected in transactivation assays did not arise from differential expression of these mutants. HeLa cells not transfected with the receptor expression vector did not react with the antibody.

**DISCUSSION**

This paper describes the functional mapping of the N-terminal AF-1 domain in the hMR. Using deletion studies, we have identified a 54-amino acid sequence in the hMR that is essential for enhancing transactivation. Activation domains with variable chemical and structural properties influence the capacity of transcription factors to mediate cell specific responses from promoters of various responsive genes (13, 14). The AF-1 region in the hER (15) is distinct from the AF-2 situated in the HBD. In the hPR, the 91-amino acid N-terminal transcription domain is rich in proline residues (16, 17); in the hGR, the 41 amino acid region contains clusters of acidic residues (2). The AF-1 domain in the hPR also interacts with an intermediary transcription factor (18), while the hGR AF-1 interacts directly with a component of the basal transcription machinery (19, 20). This reflects a further functional distinction between the various domains within the same family of nuclear receptors.

The structurally similar hGR and hMR both complex with heat shock protein (hsp) 90 (21–25). Thus, despite the 94% similarity between the DBD of the hGR and hMR and the 57% homology of HBD, the N-terminal accounts for specific cellular response to glucocorticoids and mineralocorticoids. When amino acids between 76 and 261 of hGR are deleted, there is a dramatic decline in AF-1 function and a chimera containing repeats of this domain (hGR76–261) activates transcription 3–4-fold (26). The chicken progesterone receptor PRα (78 kDa) induces the progesterone responsive ovalbumin-CAT chimeric gene, while PRβ (108 kDa) failed to induce the model gene in transient transfection assays (27). This indicates the presence of a tissue-specific signal located in the N terminus. This domain of PR can be replaced with the AF-1 domain of hGR (4). These experiments show that the AF-1 modular function can be translocated to a defective receptor. Furthermore, the modular function of sequences between residues 1 and 131 of hGR, is similar to the function of mutant hGRΔ2 (A115–130) (2). Deletion of the acidic region in the hGR referred to as transcription activation unit tau-1, reduces the transcriptional activation capacity (22, 27).

We have generated chimeras in order to examine the function of sequences involved in transcriptional activity. When fused to deletion mutant hGRΔ71–262 with intact hormone and DNA-binding capabilities, hMRΔ235–392 and hMRΔ247–392 confer transactivation capacity upon hGRΔ71–262 in the presence of dexamethasone. Sequences located N-terminal to residue 247 in the hMR are not particularly efficient in conferring activity, while C-terminal sequences confer activity upon the defective hGRΔ76–261. Our previous analyses have shown that the hMR sequences between amino acids 148 and 390 were involved in transcription regulation. When the BglII site at position 383 in the hMR, which replaces an alanine with a serine, is introduced, transcriptional activity increases. It appears as though the Ala-383 is required for normal transcriptional activity by the hMR. One reason could be the interaction with other cellular factors that act as coactivators/corepressors. Three important aspects are evident from these studies. (i) when compared with hGR wild type, sequences situated N-terminal to 247 in the hMR are inhibitory, but show no remarkable function when compared with defective hGRΔ76–261; (ii) the activity of sequences located C-terminal to 247 is similar to the wild type, or 20-fold higher when compared with hGRΔ76–261; and (iii) hMR residues between 328 and 382 are much more efficient in inducing CAT activity than hGR sequences between residues 76 and 261.

The hMRΔ59–247 increases the transcription of MMTV-CAT 2.6-fold (Fig. 2, lane 6). In fact, this mutant is more active than wild type hMR, and the constitutive level is 50% that of wild type hMR with aldosterone. We propose that this activity is modulated by negative response elements or by factors that inhibit transcription activation and name it the hMR repres-
sive function (hMR RF-1). Elimination of polyglutamine stretches between amino acids 168 and 221 located upstream of the activation domain (28, 29) in the rat AR (30) results in a receptor with increased activity. Super-receptor activity is also observed with the hGR HBD substitution mutant C638S (7).

Further deletions in the hMR show a region around amino acid 247 that is pivotal to transcriptional activity and also delineates two regions: a stimulatory function between residues 247 and 382 and an inhibitory function between residues 59 and 247. A negative regulator of the hER, when mutated,
increases transcriptional activity (31). Chimera analysis shows that the region between amino acids 247 and 382 in the hMR itself consists of more than one distinct domain. Sequences between 247 and 382 are distinct from 328–382 since hGRΔ247–382 is less efficient than wild type hGR in transcriptional activation, while the chimeric receptor hGRΔ71–262/hMRΔ298–382 induces MMTV-CAT 2-fold. The AF-1 domain in the hGR is phosphorylated at four major sites: Thr-171, Ser-224, Ser-232, and Ser-246. Ser-246 is phosphorylated by a c-Jun N-terminal kinase, and this phosphorylation inhibits hGR transcriptional activation (32). Although the hMR is also a phosphoprotein (33), it is not known which residues are implicated. The predominant accumulation of the specific aldosterone binding activity of wild type and deletion mutants of hMR in the nucleus showed that nuclear localization and specific interaction with aldosterone were not affected by these deletions. DNA binding experiments demonstrated that the abilities to bind DNA were also not affected by the deletion of sequences between 59 and 390.

The hER has two independent nonacidic transcriptional activation functions whose activities are cell type-dependent (18). It remains to be seen whether a similar cell-specific function can be attributed to the hMR sequences 59–247, 247–382, and 212–382. A third autonomous activation domain within the hER has been identified between amino acids 282 and 351, which are active in both yeast and mammalian cells (34). In the hPR, the third AF-3, which is ligand-independent, is located in the DBD (35). The steroidogenic receptor cofactor-1 (SRC-1) enhances hormone-stimulated estradiol receptor transcriptional activity (36–38). The mutation of a lysine residue in the HBD of the hER reduces the ability of the receptor to bind SRC-1 but has no effect on RIP140 protein (39). The interaction of cloned factors such as SRC-1 and RIP140 with the hMR remains to be established. Our preliminary results indicate that interactions of hormone and hormone receptor complexes with DNA, and the AF-2 domain with coactivators such as SRC-1 and GRIP, are not sufficient to induce transcription by AF-1-defective mutants.3

In conclusion, we have identified a 54-amino acid region in the hMR that has higher activity than the intact receptor and is able to confer activity upon a hGR deletion mutant that has no activity. Studies are under way in our laboratory to determine associated factors using the yeast two-hybrid system.

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3 M. V. Govindan and N. Warrier, manuscript in preparation.