Reports

Glucocorticoid Receptor Mutants That Define a Small Region Sufficient for Enhancer Activation

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Transcriptional enhancement is a general mechanism for regulation of gene expression in which particular proteins bound to specific DNA sequences stimulate the efficiency of initiation from linked promoters. One such protein, the glucocorticoid receptor, mediates enhancement in a glucocorticoid hormone–dependent manner. In this study, a region of the 795–amino acid rat glucocorticoid receptor that is active in transcriptional enhancement was identified. The active region was defined by expressing various receptor deletion mutants in stably and transiently transfected cells and examining the regulated transcription of hormone-responsive genes. Mutant receptors lacking as many as 439 amino-terminal amino acids retained activity, as did those with as many as 270 carboxyl-terminal amino acids deleted. This suggests that the 86–amino acid segment between the most extensive terminal deletions, which also includes sequences required for specific DNA binding in vitro, is sufficient for enhancer activation. In fact, a 150–amino acid receptor fragment that encompasses this segment mediates constitutive enhancement.

IN THE PRESENCE OF HORMONE, THE glucocorticoid receptor protein selectively regulates transcription by binding to specific DNA sequences, termed glucocorticoid response elements (GREs), near hormone-responsive promoters (*1, 2*). GREs are transcriptional enhancer elements that are active only in the presence of the bound receptor (*2, 3*). Glucocorticoid receptors have been characterized in some detail; the ~87-kD hormone-binding protein has been purified to near homogeneity (*4*), monoclonal antibodies have been produced (*5, 6*), and complementary DNAs (cDNAs) have been isolated and sequenced from the rat, human, and mouse (*7–9*). Biochemical studies implied that the receptor carries separate functional domains for hormone and DNA binding (*10*); this separation was confirmed and extended by in vitro expression and functional assays of a series of mutant rat receptor derivatives, which localized the specific DNA-binding domain and assessed quantitatively the effects of mutations in the hormone-binding region (*11, 12*).

Although it is generally agreed that enhancer elements serve as DNA-binding sites for specific enhancer-activating proteins, little is known about the mechanisms by which the glucocorticoid receptor, or any other enhancer activation factor, actually stimulates promoter activity. We describe here the expression of intact and mutant glucocorticoid receptors in transfected cells, with the goal of delineating a discrete region of the receptor that is sufficient for GRE enhancer activation. Two elements of our strategy differed from a similar study reported recently (*13*). First, we constructed a number of stably transfected cell lines to determine the apparent specific activities of wild-type and mutant receptor derivatives. Second, we defined directly a receptor subregion that retains enhancer activation activity, rather than inferring the positions of such regions from insertion mutations that abolish activity.

We showed previously that expression of intact receptor cDNA stably transfected into a receptor-defective cell line fully restores receptor activity (*8*); the receptor-defective line, 6.10.2, was selected (*8, 14*) from M1.19, a mouse mammary tumor virus (MTV)–infected rat hepatoma (HTC) cell line that produces functional receptors (*15*). We began the present study by establishing a series of transfected 6.10.2 cell lines, each with the potential to express a particular receptor fragment; any such receptor derivatives that are stably expressed could then be tested directly for GRE enhancer activation function. On the basis of the locations of the DNA and hormone-binding domains determined in vitro (*11, 12*; see Fig. 1), and on inferences from somatic cell genetic data (*16*), we focused first on deletions that remove various portions of the amino terminal half of the receptor.

Three deletion mutants were constructed and cotransfected into 6.10.2, together with a plasmid encoding neomycin resistance to allow isolation of stable transfectants (Fig. 1). Clonal isolates were identified that contain integrated sequences encoding each of the deletion derivatives, as well as the full-length receptor. Figure 2A shows that each expressed the predicted receptor messenger RNAs (mRNAs). In addition, translation products from these transcripts were detected (Fig. 2B) by labeling cell extract proteins with [^{3}H]dexamethasone-mesylate, which associates covalently with receptor, and then immunoprecipitating with receptor-specific monoclonal antibodies (*6*). Apparent full-length receptor mRNA and protein remained detectable in 6.10.2 at reduced levels; however, little or no residual enhancement activity was observed (see Fig. 3), which suggested that the endogenous receptor produced in 6.10.2 is nonfunctional. Notably, the apparent molecular weight of each mutant species in the transfected lines is consistent with the faithful translation of the introduced transcription units, and the apparent steady-state levels, as assessed by immunoprecipitation and by [^{3}H]dexamethasone binding (*8*), demonstrate that the mutant products accumulate to levels comparable to those seen for a wild-type receptor.

We next tested the capacity of the mutant receptors to enhance MTV transcription,

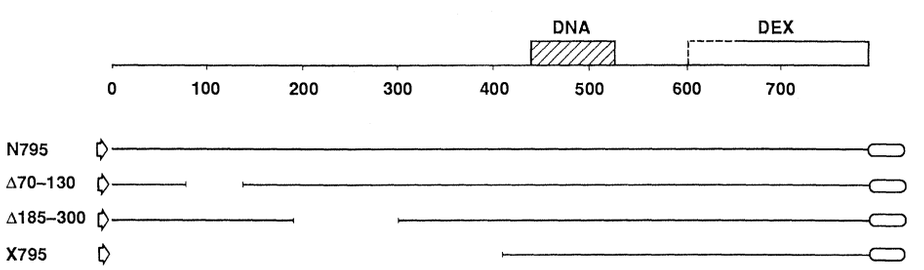

Fig. 1. Structure of receptor deletion mutants. For orientation, positions of functional domains for specific DNA binding and hormone binding, defined in vitro (*11, 12*), are given. Each receptor expression plasmid contains an enhancer/promoter combination (arrowhead) and the SV40 polyadenylation signal (oval); the solid line depicts the receptor segment present in each derivative. Translation of N795, Δ70–130, and Δ185–300 initiates from the normal receptor start site; X795 contains receptor sequences beginning at amino acid 407 and is initiated from HSV thymidine kinase leader sequences as described (*8, 12*). Receptor mutant nomenclature is as described in (*30*).

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which is hormone inducible in M1.19 but not in 6.10.2 (*14, 15*). Northern blot analyses revealed hormone-dependent accumulation of MTV RNA in each of the transfected cell lines (Fig. 3A); in particular, the three deletion mutants stimulated MTV transcription 6- to 14-fold relative to actin mRNA as an internal standard, whereas the full-length wild-type receptor yielded a 30-fold induction (see Table 1). Thus, deletion of as many as 406 NH_2-terminal amino acids produces receptor fragments that remain competent to activate MTV GRE enhancers, indicating that receptor sequences between amino acids 407 and 795 are sufficient for activity.

It seemed conceivable that the NH_2-terminal half of the receptor, which is highly conserved between rat and human (*8*), might be essential for the induction of a different subset of glucocorticoid-regulable genes in these hepatoma cells. For example, dexamethasone stimulation of α-1-acid glycoprotein (AGP) transcription (*17*) is termed a secondary response since it is blocked by cyclohexamide; in contrast, induction of MTV transcription is a primary response, which is unaffected by inhibitors of protein synthesis (*18*). One interpretation is that increased AGP transcription requires the prior transcription and translation of an additional hormone-induced gene product. In any case, Fig. 3B reveals that, as observed for MTV induction, each of the stably transfected cell lines accumulates the mature 0.9-kb AGP mRNA in a dexamethasone-dependent manner. Thus, each of the altered receptors retains the capacity to mediate hormonal regulation of two distinct classes of responsive genes.

Vanderbilt *et al.* (*19*) established that the magnitude of MTV and AGP RNA induction is proportional to receptor content in M1.19 and its receptor-transfected derivatives, indicating that intracellular receptor concentration limits the extent of these hormonal responses. Therefore, the relative specific activities of the mutant receptors can be calculated from results with the stably transfected cell lines. The receptor fragments display between 6 and 72% of the specific activity of the intact receptor with respect to both classes of hormonal response (Table 1); as expected, transfectants expressing higher levels of the mutant receptors yielded roughly proportional increases in the induction of MTV and AGP transcripts. Perhaps not surprisingly, the receptor species bearing the largest deletion displays the lowest specific activities, although the significance of this observation is unclear (see below).

Table 1. Relative enhancer activation in stable cell lines producing mutant receptors. Data were obtained by densitometry of autoradiographs in Figs. 2 and 3; RNA levels were normalized to actin. Receptor-specific activities, calculated relative to that of the intact species, N795, were determined as induced RNA equivalents per receptor protein equivalents [in the presence (+) or absence (−) of 0.1 μ*M* dexamethasone]; in no case was basal expression of MTV or AGP RNA affected by receptor transfection. The relatively high level of receptor protein expression for X795 probably reflects efficient translation initiation from the fused tk leader sequence utilized for this construction (*31*).

Receptor derivative	Receptor RNA (%)	Receptor protein (%)	Induction ratio (+/− DEX)		Relative specific activity	
			MTV RNA	AGP RNA	MTV	AGP
N795	100	100	30	33	100	100
Δ70–130	79	66	10	5	44	20
Δ185–300	68	77	14	19	57	72
X795	66	240	6	5	9	6

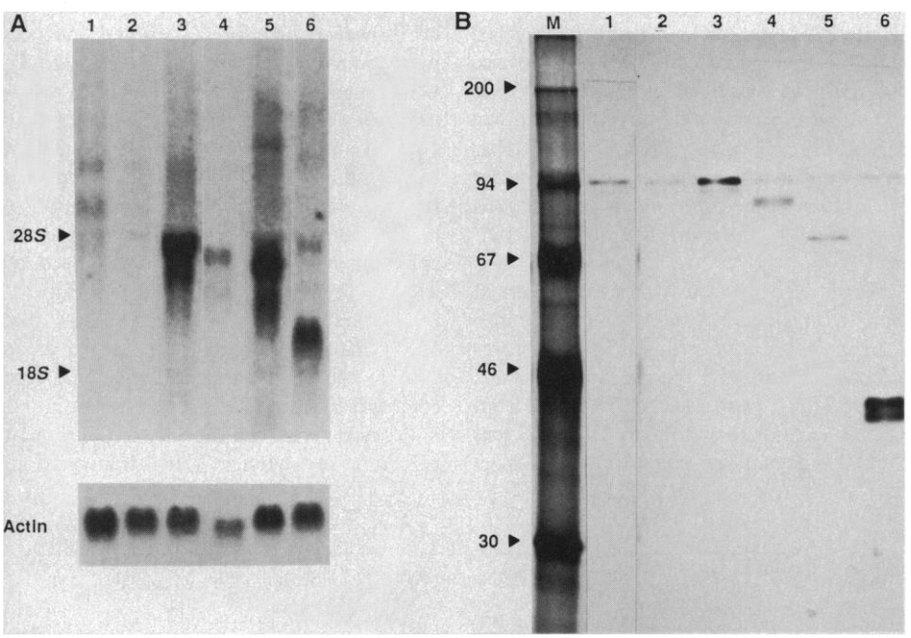

Fig. 2. Expression of receptor deletion derivatives in stably transfected cell lines. (**A**) Northern blot of total RNA (10 μg) from the following: lane 1, wild type (M1.19); lane 2, receptor-defective (6.10.2); clonal isolates of 6.10.2 stably transfected with (lane 3) N795, (lane 4) Δ70–130, (lane 5) Δ185–300, or (lane 6) X795, probed with receptor sequences. The filter was stripped and rehybridized with an actin probe as an internal control. Relative to the migration of 28*S* and 18*S* ribosomal RNA markers shown, the two full-length receptor transcripts are 6.5 and 4.8 kb (see the two bands above the 28*S* marker), whereas the cDNA transcripts in lanes 3 through 6 are 3.5, 3.2, 3.0, and 2.0 kb, respectively. (**B**) Immunoprecipitates of affinity-labeled ([^{3}H]dexamethasone-mesylate) cell extracts were subjected to electrophoresis on an SDS gel and fluorographed. Lanes 1 through 6, labeled receptor in the respective cell lines described in (A); lane M, ^{14}C-labeled marker proteins. Strong signals in lane 6 (X795 transfected cell line) probably reflect the relatively high translation initiation efficiency from the fused HSV tk leader (*31*).

We next carried out transient cotransfections to screen efficiently a larger series of receptor deletion mutants. In these experiments, an expression plasmid carrying specific receptor sequences was introduced together with a reporter plasmid harboring a hormone-responsive chloramphenicol acetyl transferase (CAT) coding region. Although 6.10.2 is a functional recipient cell line for such assays (*8*), here we used COS-7 and CV-1 cells, which also lack endogenous receptor activity and seem to display higher transfection efficiencies than 6.10.2. We first tested the three deletion mutants that had been characterized in stable cell lines, together with an additional deletion derivative, Δ273–418 (Fig. 4, rows B through E). All evoke GRE-mediated enhancement in a hormone-dependent fashion. Little can be concluded from the reduced magnitudes of the responses observed with the mutant receptors, since the efficiencies of transfection, expression, protein folding, and turnover, as well as enhancement activity per se, comprise multiple independent variables in such assays. Nevertheless, consistent results were clearly obtained with the three mutant receptors assayed in stable and transient transfections in three different cell lines.

Godowski *et al.* (*11*) demonstrated that a COOH-terminal deletion derivative lacking sequences downstream of amino acid 556

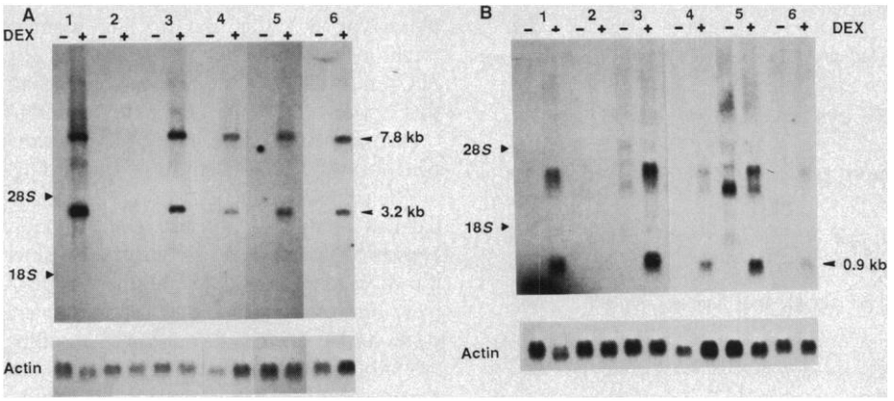

Fig. 3. Hormonal regulation of MTV and AGP transcription in receptor transfectants. Northern blots of total RNA (10 μg) from the following: lane 1, M1.19; lane 2, 6.10.2; lane 3, N795 transfectant; lane 4, Δ70–130 transfectant; lane 5, Δ185–300 transfectant; lane 6, X795 transfectant. Cultures were grown for 24 hours in the presence (+) or absence (−) of 0.1 μ*M* dexamethasone (DEX) (*32*). Filters were hybridized with MTV (**A**), AGP (**B**), or actin (A and B) probes; migration of 28*S* and 18*S* ribosomal RNA is indicated. Mature AGP mRNA is 0.9 kb; the 3.0- and 2.7-kb transcripts detected with the AGP probe have not been characterized further but may be AGP precursor RNAs whose processing may also be influenced by hormone treatment.

displays constitutive enhancer activation activity. We confirmed that observation (Fig. 4, row F) and introduced by in vitro recombination double mutations in the receptor, which yielded derivatives with both the COOH-terminal truncation and one of the four lesions in the NH_2-terminal half of the protein that are described above (Fig. 4, rows G through J). Remarkably, all of these species also retain activity, including one peptide of only 150 amino acids (Fig. 4, row J). These results thus indicate that receptor amino acids 407 through 556 are sufficient to trigger GRE-mediated enhancement.

To define the active region in greater detail, we constructed a series of NH_2- and COOH-terminal deletion mutants affecting specifically that segment of the receptor (Fig. 5). The results suggest that a region bordered by amino acids 440 and 525 is competent for enhancer activation. Notably, this same segment encompasses the specific DNA-binding domain defined in vitro (*12*) and may contain two potential "zinc finger" structural motifs (see Fig. 5) reminiscent of those proposed by Miller *et al.* (*20*) to correspond to the nucleic acid–binding domain of transcription factor TFIIIA. Three mutations that affect that region (465C, N464, and N508) displayed no detectable activity in COS-7 or CV-1 cells (Fig. 5). Therefore, the simplest interpretation of our results is that receptor activities for nuclear translocation, DNA binding, and enhancement are all contained within a small segment of the receptor between amino acids 440 and 525.

Recently, Giguère *et al.* (*13*) described the use of linker-insertion mutagenesis to analyze the human glucocorticoid receptor. Their results and ours appear to conflict, despite striking sequence conservation between the rat and human proteins (*8*). First, Giguère *et al.* described five insertion mutations between amino acids 422 and 490 that essentially abolish enhancer activation activity; they claimed that three of the mutations reside within the putative DNA-binding domain of the human receptor and that the other two define a distinct domain that is independently required for transcriptional activation. However, the five mutations define a region that corresponds to amino acids 441 through 509 of the rat receptor, a segment demonstrated to be essential for specific DNA binding in vitro (*12*). The DNA-binding domain of the human glucocorticoid receptor was not investigated, but the data from our laboratory suggest that all five mutations probably disrupt the receptor:DNA interaction. Second, Giguère *et al.* described three additional mutations, at sites corresponding to rat receptor amino acids 141, 224, and 235, that severely reduce enhancer activation activity. In contrast, we found that enhancer activation activity is retained by receptor derivatives lacking this region; for example, the Δ185–300 mutant displays nearly wild-type (60 to 70%) activity. A possible explanation for this discrepancy is that certain insertion mutations might

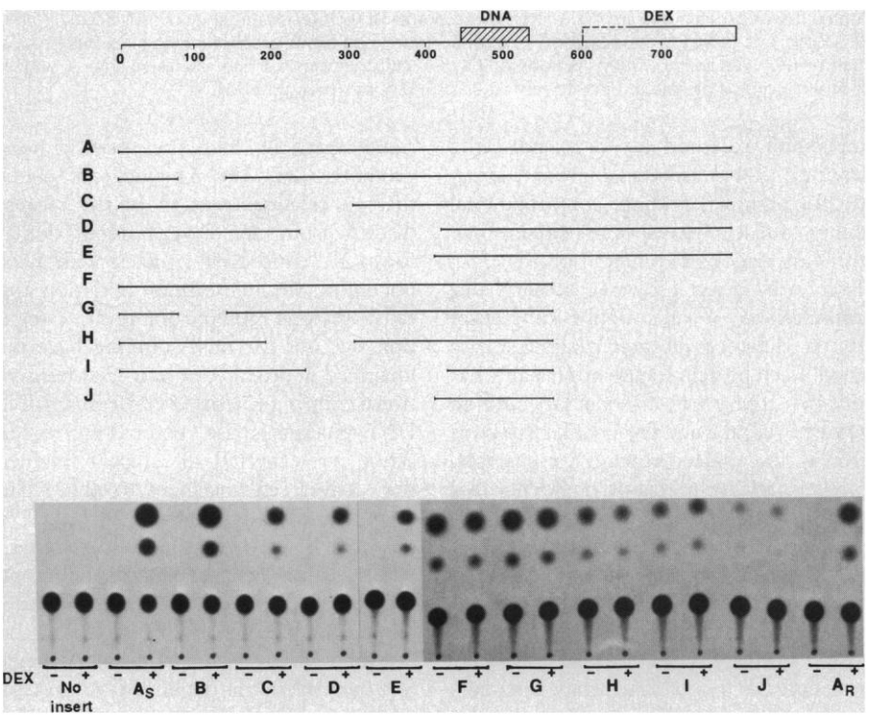

Fig. 4. GRE enhancer activation by receptor deletion mutants in transiently transfected cells. Receptor mutants and their structures relative to the wild-type protein are diagramed: row A, intact receptor (N795); row B, Δ70–130; row C, Δ185–300; row D, Δ273–418; row E, X795; rows F through J as in rows A through E, except with COOH-terminal truncation at amino acid 556. CAT assays correspond to transient cotransfections with each expression plasmid together with receptor-dependent CAT reporter plasmid, GMCS. A_S and A_R refer to the intact receptor expressed from the SV40 or RSV promoter/enhancer combinations, respectively. Transfections A_S through E were into COS-7 cells, F through A_R into CV-1 cells; duplicate transfected cultures were incubated for 48 hours with (+) or without (−) 0.1 μ*M* dexamethasone (DEX). Extracts were prepared and CAT activities determined by the standard thin-layer chromatography assay (*33*); reaction products are seen as the two upper spots in the appropriate lanes.

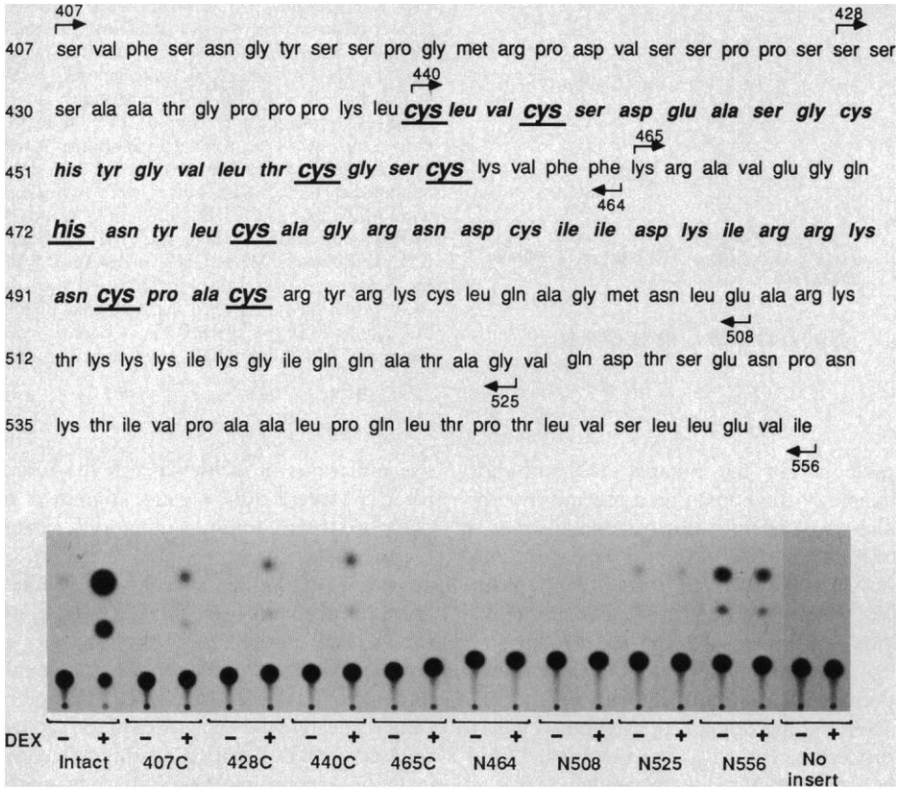

Fig. 5. Deletion mapping of the enhancer activation region. Amino acid sequence of the functional receptor derivative X556 (see Fig. 4, row J); rightward arrows, receptor starting points of NH_2-terminal deletions; leftward arrows, receptor end points of COOH-terminal deletions. Boldface amino acids depict two putative "zinc fingers" (see text); underlined residues are landmarks for this proposed structure (*20*). Transient cotransfections of COS-7 cultures were carried out as in Fig. 4 with the respective receptor plasmids together with the CAT reporter plasmid GMCS.

compromise receptor function indirectly, disrupting global aspects of protein structure (for example, folding, conformational stability, nuclear transport), rather than identifying regions of function per se.

Even with direct assays of activity, our interpretations of quantitative differences between different receptor fragments are limited. Each protein fragment probably has a distinct conformational stability and in vivo turnover rate, and few are likely to be as active as the intact protein; for example, *Escherichia coli lac* repressor fragments that include its complete DNA-binding domain nevertheless associate with the cognate sequence with only 1% of the affinity of the intact protein (*21*). Thus, in the simplest view, the reduced specific enhancer activation function of certain receptor fragments may reflect the loss of structural constraints provided by sequences outside of the domain itself. Alternatively, other regions of the receptor may also participate directly, albeit in a nonessential manner, in enhancer activation.

The receptor and other enhancer-activating proteins may function by interacting specifically with particular transcription initiation factors, perhaps by simply increasing the local concentration of a factor that is rate-limiting for initiation from a nearby promoter (*22*, *23*). This scheme predicts that an essential portion of the receptor, distinct from the DNA-binding domain, forms protein:protein contacts with a component of the transcription initiation apparatus; regions required for positive regulation but not for DNA-binding have been identified in prokaryotic and yeast transcriptional control proteins (*24*). Because specific DNA-binding is also required for receptor action, we expected all receptor fragments that activate enhancement to also include the DNA-binding domain. However, we did not expect the two activities to reside within such a small segment of the receptor protein; in fact, we have not yet identified mutations that appear to distinguish these domains. It may prove useful in this regard to characterize in detail the nt^i receptor mutants isolated from mouse lymphoma lines (*16*), which bind hormone and DNA but fail to function, apparently reflecting a defect in enhancer activation. Transcript mapping (*7*, *25*) and cDNA cloning studies (*26*) reveal that nt^i mRNAs lack coding information for the NH_2-terminal 403 amino acids of the mouse receptor (equivalent to amino acids 1 through 415 of the rat). Indeed, an initial interpretation of the nt^i phenotype was that the missing segment might include the activation domain (*8*, *16*), but our present results appear to exclude that notion. Thus, analysis of the nt^i cDNA sequence may reveal alterations that uncouple enhancer activation from DNA binding.

The primary sequence of the NH_2-terminal half of the receptor has been strongly conserved during the 70 million years since the divergence of rat and human progenitors. Interestingly, a single large exon encodes amino acids 1 through 415, residing >30 kb upstream of the multiple exons and introns that specify the COOH-terminal half of the receptor (*27*); this genomic organization may indicate that the NH_2-terminal segment was acquired relatively recently, perhaps implying an independent function. In any case, sequence conservation alone suggests that the NH_2-terminal half of the receptor is biologically important and that failure to discern its activity probably reflects only the limitations of our present assays. A number of potential activities could be tested. For example, the NH_2-terminal domain may be a structural determinant (alone or in combination with other factors) that defines essential kinetic or hormone sensitivity parameters; in this report we examined only steady-state induction at saturating hormone concentrations. A second possibility is that the NH_2-terminal sequences may be essential for receptor-mediated repression at promoters for genes such as prolactin or pro-opiomelanocortin, which are inhibited by glucocorticoids (*28*); here we examined only positive transcriptional regulation by the receptor. Third, the NH_2-terminal region might mediate combinatorial regulation, interacting with other regulatory factors that operate together to effect specific patterns of gene expression (*22*, *29*); in this study we tested only regulation by glucocorticoids alone.

REFERENCES AND NOTES

1. F. Payvar *et al.*, *Cell* **35**, 381 (1983); C. Scheidereit *et al.*, *Nature* (*London*) **304**, 749 (1983).
2. V. L. Chandler, B. A. Maler, K. R. Yamamoto, *Cell* **33**, 489 (1983).
3. K. S. Zaret and K. R. Yamamoto, *ibid.* **38**, 29 (1984); H. Ponta, N. Kennedy, P. Skroch, N. E. Hynes, B. Groner, *Proc. Natl. Acad. Sci. U.S.A* **82**, 1020 (1985).
4. Ö. Wrange, J. Carlstedt-Duke, J.-Å. Gustafsson, *J. Biol. Chem.* **254**, 9284 (1979); Ö. Wrange, S. Okret, M. Radojcic, J. Carlstedt-Duke, J.-Å. Gustafsson, *ibid.* **259**, 4534 (1984).
5. H. M. Westphal, G. Moldenhauer, M. Beato, *EMBO J.* **1**, 1467 (1982); S. Okret, A.-C. Wikström, Ö. Wrange, B. Andersson, J.-Å. Gustafsson, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1609 (1984).
6. L. P. Eisen *et al.*, *J. Biol. Chem.* **260**, 11805 (1985).
7. R. Miesfeld *et al.*, *Nature* (*London*) **312**, 779 (1984).
8. R. Miesfeld *et al.*, *Cell* **46**, 389 (1986).
9. S. M. Hollenberg *et al.*, *Nature* (*London*) **318**, 635 (1985); M. Danielsen, J. P. Northrop, G. M. Ringold, *EMBO J.* **5**, 2513 (1986).
10. J. Carlstedt-Duke, S. Okret, Ö. Wrange, J.-Å. Gustafsson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4260 (1982).

11. P. J. Godowski, S. Rusconi, R. Miesfeld, K. R. Yamamoto, *Nature (London)* **325**, 365 (1987).
12. S. Rusconi and K. R. Yamamoto, *EMBO J.*, in press.
13. V. Giguère, S. M. Hollenberg, M. G. Rosenfeld, R. M. Evans, *Cell* **46**, 645 (1986).
14. J. R. Grove, B. S. Dieckmann, T. A. Schroer, G. M. Ringold, *ibid.* **21**, 47 (1980).
15. G. M. Ringold *et al.*, *Proc. Natl. Acad. Sci. U. S.A.* 76, 665 (1979).
16. K. R. Yamamoto, U. Gehring, M. R. Stampfer, C. H. Sibley, *Recent Prog. Horm. Res.* **32**, 3 (1976).
17. H. Baumann *et al.*, *J. Biol. Chem.* **258**, 563 (1983); R. Reinke and P. Feigelson, *ibid.* **260**, 4397 (1985).
18. G. M. Ringold, K. R. Yamamoto, G. M. Tomkins, J. M. Bishop, H. E. Varmus, *Cell* **6**, 299 (1975).
19. J. N. Vanderbilt, R. Miesfeld, B. A. Maler, K. R. Yamamoto, *Mol. Endocrinol.* **1**, 68 (1987).
20. J. Miller, A. D. MacLachlan, A. Klug, *EMBO J.* **4**, 1609 (1985).
21. R. T. Ogata and W. Gilbert, *J. Mol. Biol.* **132**, 709 (1979).
22. K. R. Yamamoto, *Annu. Rev. Genet.* **19**, 209 (1985).
23. M. Ptashne, *Nature (London)* **322**, 697 (1986).
24. A. Hochschild *et al.*, *Cell* **32**, 319 (1983); I. A. Hope and K. Struhl, *ibid.* **46**, 885 (1986); J. Ma and M. Ptashne, *ibid.* **48**, 847 (1987).
25. R. Miesfeld *et al.*, in *Sequence Specificity in Transcription and Translation*, vol. 30 of UCLA Symposium on Molecular and Cellular Biology, New Series, R. Calender and L. Gold, Eds. (Liss, New York, 1985), pp. 535–545; J. P. Northrop, B. Gametchu, R. W. Harrison, G. M. Ringold, *J. Biol. Chem.* **260**, 6398 (1985).
26. R. Miesfeld *et al.*, in *Steroid Hormone Action*, UCLA Symposium on Molecular and Cellular Biology, G. Ringold, Ed. (Liss, New York, in press).
27. M. Jacobson, R. Miesfeld, S. Rusconi, unpublished data.
28. S. A. Camper, Y. A. S. Yao, F. M. Rottman, *J. Biol. Chem.* **260**, 12246 (1985); J. H. Eberwine and J. L. Roberts, *ibid.* **259**, 2166 (1984).
29. K. R. Yamamoto, in *Molecular Developmental Biology: Expressing Foreign Genes*, 43rd Symposium of the Society for Developmental Biology, L. Bogorad and G. Adelman, Eds. (Liss, New York, 1985), pp. 131–148.
30. Receptor deletion mutants are denoted according to the following nomenclature: derivatives named with N or C contain receptor amino acids extending from the NH_2-terminus or COOH-terminus, respectively, through the given amino acid number (N795 is the intact receptor); derivatives denoted with an X begin at amino acid 407 and extend through the numbered amino acid; derivatives denoted by two hyphenated numbers include the receptor segment delineated by the given amino acid numbers; derivatives denoted with a Δ lack the receptor segment delineated by the numbered amino acids. Plasmids containing the intact receptor coding sequence, RBal117, RSVGR, and SVGR1, and the SV40 expression vector SV7d, have been described previously (*8*). Internal deletion derivatives Δ70–130 and Δ185–300 were constructed by cleavage of RBal117 DNA with Nco I and Bgl II, respectively, resection with exonuclease BAL31, attachment of Xho I linkers, and recircularization. In-frame fusions were identified by translation in vitro of transcripts produced with SP6 RNA polymerase; end points were estimated (±3 amino acids) by high-resolution gel electrophoresis. The mutant coding sequences were inserted into pRSVTG, the Rous sarcoma virus expression vector [H.-P. Moore and R. B. Kelly, *J. Cell. Biol.* **101**, 1773 (1985)] used to construct RSVGR. Internal deletion derivative Δ273–418 was constructed as an in-frame fusion of the Bgl II site to a Bam HI linker of a BAL31 terminal deletion, followed by insertion into SV7d. NH_2-terminal deletion derivatives were constructed by ligating Bam HI–linked BAL31 deletions to a BAM HI–linked herpes simplex virus (HSV) thymidine kinase leader sequence (*8*); in-frame fusions were identified by in vitro translation and characterized by DNA sequencing. All mutants were inserted into SV7d, except X795, which is expressed from a human α globin promoter/SV40 enhancer combination [S. Paabo, F. Weber, T. Nilsson, W. Schaffner, P. A. Peterson, *EMBO J.* **5**, 1921 (1986)]. Carboxyl-terminal BAL31 deletions were characterized by DNA sequencing and inserted into SV7d upstream of its triple termination codons, as described elsewhere (*8, 12*). Double deletion derivatives (see Fig. 4) were constructed by fusing fragments from the respective mutant inserts upstream and downstream of the receptor Sph I site, followed by insertion into SV7d.
31. S. Rusconi and K. R. Yamamoto, unpublished data.
32. The MTV-infected rat hepatoma HTC cell derivatives M1.19 and 6.10.2 have been described (*8, 14, 15*); these lines, as well as the monkey COS-7 and CV-1 lines, were propagated in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Hyclone). The increased basal expression of MTV RNA observed in cell lines containing transfected receptor sequences (*8*) is eliminated by using fetal bovine serum rather than the defined calf serum used previously. Stable receptor transfectants were obtained by cotransfecting receptor sequences with a neomycin resistance marker, followed by G418 selection, isolation of cell clones, and characterization as described (*8*). Transient transfection of COS-7 and CV-1 cells was achieved by calcium phosphate precipitation of 5 μg of receptor expression plasmid together with 1 μg of the receptor-dependent CAT reporter plasmid GMCS (*8*); cultures were incubated in the presence or absence of 0.1 μ*M* dexamethasone for 48 hours and cell extracts were assayed for CAT activity (*33*).
33. C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982).
34. We thank S. Rusconi for several of the mutant receptor inserts and for discussions. We thank R. Harrison for receptor-specific monoclonal antibodies; R. Grosschedl, D. Sakai, and P. Sternberg for comments; and K. Rañeses for manuscript preparation. This work was supported by grants from the National Science Foundation and the National Institutes of Health; postdoctoral fellowship support was from the Leukemia Society of America (R.M.) and NIH (P.J.G.); K.R.Y. is recipient of a teacher-scholar award from the Henry and Camille Dreyfus Foundation.

27 January 1987; accepted 19 March 1987

Ice-Edge Eddies in the Fram Strait Marginal Ice Zone

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Five prominent ice-edge eddies in Fram Strait on the scale of 30 to 40 kilometers were observed over deep water within 77°N to 79°N and 5°W to 3°E. The use of remote sensing, a satellite-tracked buoy, and in situ oceanographic measurements showed the presence of eddies with orbital speeds of 30 to 40 centimeters per second and lifetimes of at least 20 days. Ice ablation measurements made within one of these ice-ocean eddies indicated that melting, which proceeded at rates of 20 to 40 centimeters per day, is an important process in determining the ice-edge position. These studies give new insight on the formation, propagation, and dissipation of ice-edge eddies.

ONE OBJECTIVE OF THE MARGINAL Ice Zone Experiment (MIZEX-84) program is to better understand the physics of mesoscale eddies along an ice edge and the role that eddies play in the processes of mass and heat exchange and in controlling the position of the ice edge. Previous studies in the Fram Strait marginal ice zone (MIZ) have established the existence of mesoscale eddies at the ice edge with scales that range from 5 to 15 km north of Svalbard (*1*) to 50 to 60 km in the western parts of the Fram Strait (*2*). Barotropic and baroclinic instability mechanisms have been suggested as eddy-generating mechanisms. Since the topography of the central part of the Fram Strait is complex (with depressions of 4000 to 5500 m and seamounts up to 1400 m below the surface), topographic generation and trapping of eddies have also been suggested (*3*). A two-dimensional model (*4*) proposed an eddy generation mechanism that included differential wind-induced ice and ocean circulation. This report describes a dedicated eddy investigation during the summer of 1984 between 77°N and 79°N along the ice edge of Fram Strait. The study used remote sensing; conductivity, temperature, and depth (CTD) observations; and ice-drifting satellite-tracked buoys that were suspended with current meters.

Remote-sensing observations were used in a near real-time mode for locating eddies and for guiding the research vessels into the eddy region. For example, the high-resolution synthetic aperture radar (SAR) mosaic on 5 July (Fig. 1) clearly shows detailed surface structure of an elliptically shaped eddy E1 on the scale of ~30 km. Since the wind was light, the floe-size distribution of 50 to 500 m reflected the upper ocean circulation. The orbital motion was cyclonic, while the spiral motion of ice toward the center indicated frictionally driven inward radial motion. The ice concentration was more than 80% at the center of the eddy (see Fig. 1, A and B). This implied that there was convergence, and that ageostrophic effects are important and must be included in realistic models of these eddies. A second eddy

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