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Mammalian Glucocorticoid Receptor Derivatives Enhance Transcription in Yeast

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In mammalian cells, the glucocorticoid receptor binds specifically to glucocorticoid response element (GRE) DNA sequences and enhances transcription from linked promoters. It is shown here that derivatives of the glucocorticoid receptor also enhance transcription when expressed in yeast. Receptor-mediated enhancement in yeast was observed in fusions of GRE sequences to the yeast cytochrome c_1 (*CYC1*) promoter; the *CYC1* upstream activator sequences were not essential, since enhancement was observed in fusions of GREs to mutant *CYC1* promoters retaining only the TATA region and transcription startpoints. It is concluded that the receptor operates by a common, highly conserved mechanism in yeast and mammalian cells.

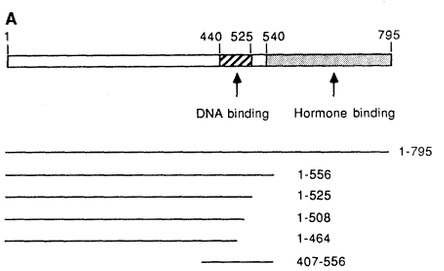
THE GLUCOCORTICOID RECEPTOR selectively regulates gene transcription in animal cells by binding in a steroid-dependent manner to specific DNA sequences termed GREs (*1, 2*). Those GREs associated with the mouse mammary tumor virus (MTV) promoter and with other genes that are regulated positively by glucocorticoids are transcriptional enhancer elements that function only in the presence of bound receptor (*2–4*). Discrete segments of the 795–amino acid rat glucocorticoid receptor have been defined that mediate nuclear translocation, hormone binding, GRE recognition, and transcriptional regulation (*5–9*). These studies also revealed that receptor derivatives lacking the hormone binding domain confer constitutive GRE-dependent enhancement (*7*); moreover, the DNA binding domain is sufficient for enhancer activation, although its apparent specific activity is low relative to the intact receptor (*8, 9*).

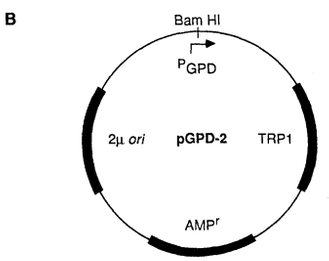
The phenomenon of transcriptional enhancement has now been documented in organisms ranging from bacteria to mammals (*10*). This widespread distribution indicates that enhancement may operate by a common mechanism and that its molecular determinants may have been conserved during evolution. Given the relatively simple physiology and genetic manipulability of *Saccharomyces cerevisiae*, we tested whether the rat glucocorticoid receptor expressed in yeast could enhance transcription from yeast promoters linked to GREs.

We first expressed in yeast a series of receptor derivatives bearing a deletion of the hormone binding domain, amino acids 557 to 795. Studies in tissue culture cells showed that receptor derivatives lacking this region confer high-level constitutive GRE-mediated enhancement. The receptor derivatives were expressed from the yeast glycerol-3-phosphate dehydrogenase promoter in parent plasmid pGPD-2 (*11*) (Fig. 1). All species except X556b, which contains receptor amino acids 407 to 556, are translated from the normal receptor initiation codon, and each terminates translation in downstream linker or vector sequences, resulting in addition of 4 to 13 nonreceptor amino acids. Comparison of receptor derivatives differing only in these COOH-terminal amino acids (for example, see N556a and N556b in Fig. 1) revealed no systematic effects of the short nonreceptor "tails."

Expression and integrity of the various receptor derivatives were assessed by immunoblotting of extracts from strains transformed stably with the receptor expression plasmids. For example, Fig. 2A (lanes 1 and 2) shows accumulation of the predicted 18- and 65-kD proteins, X556b and N556a, respectively, to steady-state levels of about 2500 molecules per cell; these intracellular concentrations are comparable to those in mammalian cells. Similar results were obtained with the other receptor constructs shown in Fig. 1.

As an initial test of DNA binding by receptor derivatives in vivo in yeast, we inserted GRE sequences between functional elements of the yeast cytochrome c_1 (*CYC1*) promoter. As shown in Fig. 3A, pLGΔ312S is a plasmid containing the intact *CYC1* promoter fused to the *Escherichia coli* β-galactosidase (*lacZ*) coding sequences (*12*); two different GRE-containing fragments were inserted at position −178 between the UAS and TATA elements of the promoter (see legend to Fig. 3A). Others have shown that insertions of nonspecific DNA fragments as large as 350 bp at this site have





Plasmid	Receptor	Receptor amino acids	Nonreceptor COOH-terminal amino acids
pGPD-2	None	None	None
pGPD-795	N795	1-795	None
pGPD-556a	N556a	1-556	GELEFPGLEDPST
pGPD-556b	N556b	1-556	GANSRV
pGPD-525	N525	1-525	AGRL
pGPD-508	N508	1-508	AGRL
pGPD-464	N464	1-464	GSRGSVDLDK
pGPD-X556b	X556b	407-556	GANSRV

Fig. 1. Yeast plasmids containing rat glucocorticoid receptor sequences. (**A**) The 795–amino acid rat glucocorticoid receptor, denoting the DNA binding domain (amino acids 440 to 525) and the hormone binding domain (amino acids 540 to 795) (*5, 8*). Receptor segments cloned into yeast plasmids are indicated below the diagram. (**B**) Receptor sequences were inserted as Bam HI fragments (*5, 7*) into the unique Bam HI site 15 bp downstream of the glycerol-3-phosphate dehydrogenase transcription start site in plasmid pGPD-2 (*11*). The resultant expression plasmid and receptor derivatives are indicated, together with the precise receptor amino acids contained in each derivative and the COOH-terminal amino acids contributed by the polylinker (see text). In each case, translation initiates at the normal receptor NH_2 terminus, except for X556b, which uses a seven–amino acid leader sequence (Met-Ala-Ser-Trp-Gly-Ser-Pro) from herpes simplex virus thymidine kinase (*23*). The pGPD-2 vector contains the replication origin and ampicillin resistance gene of pBR322, and the TRP1 selectable marker and 2μ replication origin from yeast.

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only modest effects on promoter function (*12*), but that specifically bound proteins at this position could strongly inhibit transcription (*13*). Paradoxically, we discovered that insertion of either of the GRE fragments reduced promoter activity by 10- to 100-fold in the absence of receptor and that expression of the N556a receptor derivative fully restored promoter activity (*14*). As expected, initiation from the *CYC1* promoter lacking a GRE was unaffected by the receptor (Fig. 3A). The striking decrease in *CYC1* promoter activity brought about by the GRE fragments is not understood, but it implies that a yeast protein may bind tightly to the inserted sequences. In any case, the recovery of activity in the presence of N556a indicates that this receptor derivative indeed interacts with GREs in yeast.

These results suggest either that N556a directly activates transcription in yeast or that it relieves inhibition of UAS activity by displacing a putative yeast protein bound to the GRE fragment without itself blocking UAS function. We examined these possibilities by testing the effects of GRE sequences inserted at position −178 in the absence of *CYC1* UAS elements (see legend to Fig. 3B). Thus, pSXG carries a 340-bp fragment of the MTV long terminal repeat (LTR) sequence containing a GRE; pSX26.1 and pSX26.2 include a 26-bp GRE fragment from rat tyrosine aminotransferase inserted in either orientation, and pXX46 contains a synthetic 46-bp oligonucleotide with strong GRE activity (*15*). Introduction of these constructs into yeast together with plasmids expressing N556a yielded β-galactosidase levels 30 to 150 times as high as controls without receptor.

Table 1. Transcriptional enhancement by various glucocorticoid receptor derivatives. Shown are β-galactosidase data from strain 1205-9B double transformants carrying receptor derivatives and either pSX26.1 (+GRE26) or the same plasmid lacking the GRE, pSS (−GRE26) (see Fig. 3B). Receptor derivatives are described in Fig. 1. Enzyme assays were performed as in Fig. 3A, except for the addition of 1 μ*M* dexamethasone to cultures expressing N795. Shown is the average of at least three independent assays, which varied by <20%.

Receptor derivative	β-Galactosidase	
	−GRE26	+GRE26
N795	0.7	1.3
N556a	0.6	392
N556b	0.7	584
N525	0.6	420
N508	0.7	64
N464	0.8	5.7
X556b	1.1	3.4

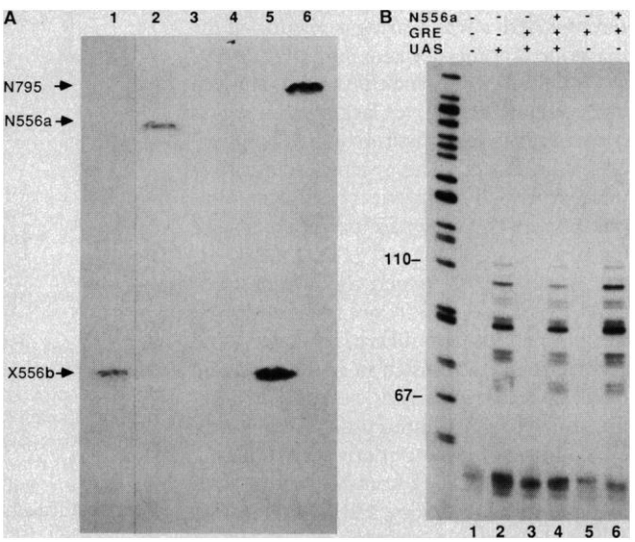

Fig. 2. Expression of glucocorticoid receptor derivatives in yeast (**A**) and primer extension analysis of *CYC1-lacZ* transcripts (**B**). (A) Shown is an immunoblot of yeast extracts fractionated by SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with a receptor-specific monoclonal antibody (*24*) and with goat antibody to mouse immunoglobulin G horseradish peroxidase–conjugated antibody (Bio-Rad). Arrows indicate the intact receptor (N795; 88 kD), and deletion derivatives N556a (65 kD) and X556b (18 kD). Yeast strains were 1205-9B (from A. Mitchell, *MATa, leu2-3, leu2-112, ura3-52, lys2, trp1, ade6*) and W303-1B (from R. Rothstein, *can1-100, his3-11, his3-15, leu2-3, leu2-112, trp1-1, ura3-1, ade2-1*). Extracts were prepared as described (*25*); each lane contained 100 μg of total protein. Lane 1, W303-1B with pGPD-X556b; lane 2, 1205-9B with pGPD-556a; lane 3, W303-1B with pGPD-2; lane 4, 1205-9B with pGPD-2; lane 5, 1205-9B with pGPD-2 plus 50 ng of pure X556b protein isolated from *E. coli* (*26*); lane 6, glucocorticoid receptor from HTC cell line 19G11.1 (*23*). (B) Total RNA was isolated from transformed yeast strains (*27*) and subjected to primer extension (*28*) with a 20-nucleotide primer (5-TCACCAGTGAGACGGGCAAC-3′) complementary to *lacZ* sense strand sequences 17 bp downstream of the Bam HI site (*29*). Extension products from 25 μg of RNA were fractionated on a sequencing gel adjacent to ^{32}P-labeled Hae III fragments from pBR322; the 67- and 110-nucleotide fragments are noted. Lane 1, untransformed host strain 1205-9B; all others are 1205-9B transformants containing: lane 2, pLGΔ312S and pGPD-2; lane 3, p312XG.2 and pGPD-2; lane 4, p312XG.2 and pGPD-556a; lane 5, pSX26.1 and pGPD-2; lane 6, pSX26.1 and pGPD-556a. Expression and reporter plasmids are described in Figs. 1 and 3; for clarity, the presence or absence of N556a, of a GRE, and of the *CYC1* UASs are indicated.

The β-galactosidase assays imply that the receptor derivatives stimulate transcription initiation from the bona fide *CYC1* promoter for all GRE-containing reporter plasmids tested (Fig. 2B). Indeed, as measured by primer extension, insertion of GREs between the *CYC1* UAS and TATA motifs drastically reduced promoter activity, and this effect was reversed upon expression of N556a (Fig. 2B, lanes 3 and 4). Similarly, N556a-dependent activation of pSX26.1, which contains a GRE but lacks UASs, reflected increased initiation at the normal start sites.

Using these same approaches, we compared the activities of various glucocorticoid receptor derivatives (see Fig. 1) in yeast. N525, which contains the first 525 amino acids of the receptor, is as active as N556a or N556b (Table 1). However, deletion of 17 additional COOH-terminal amino acids yielded N508, which retains only about 10% of the activity of N525; N464 is only about 1% active relative to N525, and X556b, which specifies receptor amino acids 407 to 556, displays similar low activity. These results parallel closely those obtained with expression of these receptor derivatives in animal cells (*7, 8*).

In contrast, expression of the full-length receptor (N795; Fig. 1) in yeast elicited no detectable stimulation of β-galactosidase activity in the presence of dexamethasone (Table 1). We have recently discovered, however, that several related corticosteroids strongly stimulate β-galactosidase expression via the intact receptor (*16*). The cause of this apparent altered ligand specificity is not understood, but these results imply the competent entry of steroids into yeast cells and that the receptor is functional for signal transduction in yeast.

Taken together, our results suggest that the glucocorticoid receptor enhances transcription in yeast and mammalian cells by a common mechanism. That is, the action of a series of receptor constructs on three distinct GREs (MTV, TAT, and a synthetic oligomer) is closely correlated in yeast and animal cells. Results similar to those obtained with GRE-*CYC1* fusions have been observed in strains containing a chromosomal GRE-*LEU2* gene (*16*). The reduced activity of the N508 receptor relative to N525 is consistent with previous findings from DNA binding studies in vitro (*5*) and from assays in transfected animal cells (*8*). Likewise, N464, which retains only one of the two "zinc fingers" of the DNA binding domain, displays low levels of activity in animal cells (*17*), as does X556, a small receptor fragment that encompasses the complete DNA binding domain (*8*). Finally, Godowski *et al.* (*18*) have defined two distinct segments of

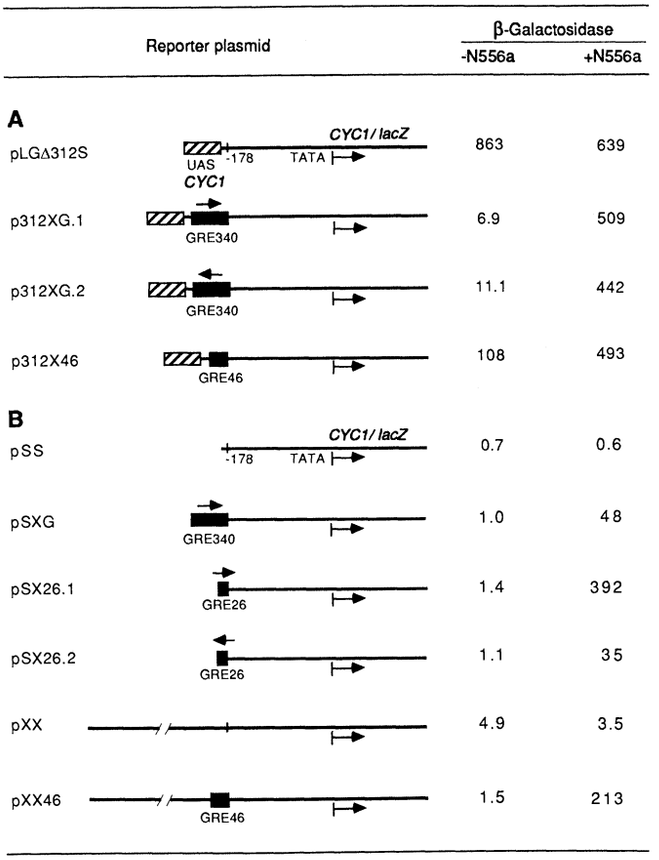

Fig. 3. Receptor-mediated enhancement of GRE-linked *CYC1* promoter constructs. (**A**) Receptor derivative N556a increases GRE-linked *CYC1* promoter activity. Cotransformants of strain 1205-9B were obtained by the method of Ito (*30*) and were selected and propagated in minimal medium deficient in uracil and tryptophan (*31*). Transformed strains expressing N556a or containing the vector alone are indicated as +N556a and −N556a, respectively. Reporter plasmids were constructed from pLGΔ312S (*12*) by inserting GREs at the XhoI site at position −178 between the UAS (hatched box) and TATA elements of the *CYC1* promoter. GRE340 is a 340-bp *Sau*3A fragment from the MTV LTR (*1*), and GRE46 is a 46-bp palindromic oligonucleotide derived from that fragment that displays strong GRE activity (*15*); arrows above GRE340 indicate orientation relative to that within the LTR. Results represent the average of at least three independent assays, which varied by less than 20%; β-galactosidase assays were performed according to Yocum *et al.* (*32*) and units are defined as 1000 times the change in optical density (OD) at 420 nm due to hydrolysis of *o*-nitrophenyl-β-D-galactoside divided by the product of the assay time (minutes) times the culture volume (milliliters) times OD at 600 nm of the culture. (**B**) Receptor-mediated enhancement of *CYC1* promoter activity is UAS-independent. Strains, plasmids, and assays are as in (A), except that the reporter plasmids carry deletions of the *CYC1* UAS: pSS was constructed by deleting a 140-bp Sal I to Sma I fragment from pLGΔ312S (*12*), plasmid pXX arose by deletion of a 390-bp Xho I to Xho I fragment from RY52 (*29*); pXX contains about 1.5 kb of upstream *CYC1* DNA not present in pSS. GRE26 is a synthetic 26-bp oligonucleotide derived from the tyrosine aminotransferase GRE (*33*).

the receptor that confer enhancement in animal cells; we find that deletions within either of these regions reduce the extent of enhancement in yeast (Table 1).

We have not detected enhancer activity in yeast from GREs residing downstream of promoters, whereas downstream activity has been observed in animal cells. This result is reminiscent of the lack of UAS activity when those elements are inserted downstream of promoters (*12*). It is interesting that UASs function competently from downstream positions in animal cells that are expressing the corresponding UAS binding protein (*19*). This implies that the capacity of regulatory elements and their cognate binding proteins to act downstream of a promoter may reflect structural or functional distinctions between yeast and animal cell transcription initiation complexes, rather than differences in the regulatory mechanisms, per se.

Our results establish that a metazoan DNA-binding transcriptional regulatory factor can function in an organism as distantly related as yeast. This finding complements reports that appeared after completion of this work showing that in cultured animal cells the yeast regulatory factor GAL4 constitutively activates animal cell promoters fused to GAL4 binding sites (*19, 20*); analogously, a derivative of the animal cell Fos protein stimulates yeast transcription when tethered adjacent to a yeast promoter through a bacterial repressor DNA binding site (*21*). These and other recent studies (*22*) may indicate strong conservation of protein-protein contacts between regulatory factors and components of the transcription initiation apparatus.

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