
The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific

M.T.Bocquel, V.Kumar, C.Stricker, P.Chambon and H.Gronemeyer

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex, France

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ABSTRACT

Normalized dose response-curves for transcriptional activation of reporter genes were obtained by co-transfecting them with increasing amounts of wild-type (wt) progesterone (PR), glucocorticoid (GR) and oestrogen (ER) expression vectors. Marked differences in both shape and magnitude of the stimulation were observed depending on whether HeLa or CV1 cells were transfected. In HeLa cells the transcriptional stimulation from a reporter gene containing the hormone responsive element (RE) present in the mouse mammary tumour virus (MMTV) long terminal repeat (LTR) increased as increasing amounts (from 0.05 to 7.5 μ g) of PR expression vector were transfected, whereas no such increase was observed in CV1 cells above 1 μ g of the same vector. In contrast, a PR mutant lacking the hormone binding domain (HBD, region E), exhibited increasing constitutive activity with increasing amounts of PR expression vector, such that in CV1 cells, but not in HeLa cells, similar activities were measured for the mutant and wt PR when 5 μ g expression vectors were transfected. Western blot analyses indicated that the differences between the two cell lines were not due to differences in the amount of receptor proteins. Using the same MMTV LTR-based reporter gene, cell-specific differences were also detected between the dose-response curves obtained for the human GR and a mutant which lacks the HBD. A PR mutant in which the N-terminal A/B region was deleted exhibited no (CV1 cells) or less than 5% (HeLa cells) of the wt-activity, whereas the corresponding GR mutant stimulated efficiently transcription in both cell lines. Identical studies with the wt human ER or a mutant truncated for the N-terminal A/B region resulted in bell-shaped dose-response curves in both HeLa and CV1 cells, whereas an ER mutant lacking the HBD was weakly active in either cell line. These data demonstrate cell- and receptor-specificity for the transcriptional activation functions present in the A/B region and the HBD of various steroid receptors and suggest that limiting factors mediate their action. The present study also emphasizes the need of establishing dose-response curves to correctly assess the relative contribution of the different regions of steroid hormone receptors in activation of transcription.

INTRODUCTION

Steroid hormone receptors, as well as receptors for thyroid hormones and retinoids, are members of a superfamily of transcriptional regulatory

proteins which, when bound to their cognate ligand, specifically recognize enhancer DNA response elements of target genes and subsequently activate transcription. Nuclear receptors are formed of discrete domains and extensive structure-function analyses have been performed to elucidate the functional significance of individual segments. Two of these regions, C and E, have been clearly defined as the domains responsible for recognition of hormone responsive elements (REs) of target genes and ligand binding, respectively (for reviews and refs see 1-5). The location of the transcription activating domain has proven to be more elusive. It is known that the N-terminal A/B regions of ER, PR and GR modulate the magnitude of the transcriptional response from certain target genes (6 - 10). The chicken PR form B, for example, activates the MMTV-LTR progesterin RE, but not that present in the ovalbumin gene promoter, whereas its naturally occurring variant, which lacks the N-terminal 127 amino acids (form A), can stimulate transcription from both target genes (11). Deletion of the human ER N-terminal A/B region diminished transcriptional activation of reporter genes containing the oestrogen REs (EREs) of either the pS2 or ovalbumin genes, whereas transcription from the vit-tk-CAT reporter gene which contains the *Xenopus* vitellogenin A2 gene ERE was not affected by this deletion (6, 12). Truncation of the C-terminal hormone binding domain (HBD) of the human ER or chicken PR generated a constitutive hormone-independent mutant that retains only 5% (ER, ref. 6) and 1% (PR, ref. 10) of the transcriptional activity of the corresponding wild-type receptor. That the HBD of the ER contains a hormone-modulated autonomous activation function was confirmed by experiments showing that chimaeric receptors, consisting of the DNA binding domain (DBD) of the yeast transcription factor GAL4 joined to the ER HBD, can activate a GAL4-responsive reporter gene in the presence of oestrogens (13).

However, some of the results obtained by others for the GR and PR have led to different conclusions. A number of mutants lacking the HBD were described that appear to exhibit constitutive activity at wild-type levels. Based on these data several groups proposed that the unoccupied HBD simply blocks a constitutive activation function localized tentatively in or close to the DBD (7-9, 14-16). Recently, however, we and others have presented evidence that both the HBD and the A/B region of GR contain autonomous transcription activation functions (13, 17, 18).

These apparent discrepancies prompted us to investigate whether they could be related to differences in the experimental conditions used by the different groups to analyze the effect of receptor mutations on their

activation function. We have studied here the effect of various parameters (amount of receptor and reporter gene transfected, nature of the recipient cell line) on this activation function. Using truncated PR, GR and ER transiently expressed in two different cell lines, we report that both the A/B region and the HBD of steroid hormone receptors contribute in a cell- and receptor-specific manner to the activation of transcription.

MATERIALS AND METHODS

Expression vectors

The human ER (hER) expression vectors HEO, HE19 and HE15, the human GR (hGR) expression vectors HG1 and HG3 and the chicken PR (cPR) expression vectors cPR1 and cPR3 have been described (6, 10). HG8 was constructed from hGR by site-directed mutagenesis such that only amino acids 368 to 777 were expressed. cPR5A is a derivative of cPR5 (10) with a stop codon following amino acid 496 and was constructed by using oligonucleotide adapters.

Transfections and CAT-assays

HeLa and CV1 (19) cells grown in 9 cm petri dishes were transfected by using the calcium phosphate co-precipitation technique, and with pCH110 (Pharmacia) as an internal control recombinant (10). β -galactosidase was determined as described (20), except that 500 μ l Z-buffer and 100 μ l ONPG (4mg/ml) were used. The dose-response curves shown in the figures correspond to mean values from at least three independent experiments (normalized for 5 units β -galactosidase) using at least two different DNA preparations of expression vectors and 5 μ g of either MMTV-CAT (in the case of PR and GR; ref 21) or vit-tk-CAT (in the case of ER; ref. 22) as reporter genes. Chloramphenicol acetylation was quantified by liquid scintillation counting of thin-layer chromatography-separated 14 C-labelled CAT-reaction products (23). Only assays in which less than 40% of the chloramphenicol was acetylated were considered.

Antibodies, immunoblots and hormone binding assays

Polyclonal antibodies against the N-terminal 275 amino acids of cPR form B were produced by immunizing rabbits with the fusion protein cPR-FP2 (24) and shown to recognize both A and B forms of the natural and recombinant cPR (our unpublished results). Cells transfected with cPR1 or cPR5A were lysed by freeze/thawing ($-80^{\circ}\text{C}/0^{\circ}\text{C}$, 3 times) in TEBGN500 (25) containing a cocktail of various protease inhibitors. Aliquots containing identical amounts of β -galactosidase (usually 20 units) were precipitated in 10% TCA, separated by 7.5% SDS-PAGE, blotted onto BA85 nitrocellulose sheets (Schleicher and

Schüll) and analyzed with anti-cPR antiserum at a dilution of 1/500 and iodinated protein A (25), using 3% nonfat milk in PBS as blocking solution. The immunoreaction to detect HG1 and HG3 was similar and used a monoclonal antibody specific for the hGR A/B region (gift of J.A. Gustafsson). The anti-HER monoclonal antibodies D75 and H226 (26) were used to quantitate HE19 and HE15, respectively, relative to HEO (see also ref. 27).

Specific tight nuclear hormone binding was measured in transfected cells after exposing them in vivo to radioactively labelled hormone [R5020 and oestradiol (5 nM) or dexamethasone (20 nM)] in the presence and absence of a 100-fold excess of non-labelled steroid. Nuclei were prepared, washed with 150 mM salt, extracted with 500 mM salt and the radioactivity was determined in a liquid scintillation counter (10).

RESULTS

1) Experimental design

Activation of transcription by wild-type (wt) and mutant cPR, hGR and hER was investigated in parallel in the two cell lines HeLa and CV1, which have been used for most of the experiments aimed at localizing the transcription activating domains of receptors [for refs see Introduction; note in this respect that COS cells are derived from CV1 cells (19)]. Dose-response curves were established for each receptor by transfecting 10 ng to 7.5 μ g of the corresponding wt or mutated recombinants. A reference recombinant, pCH110 (10), expressing the bacterial β -galactosidase, was transfected together with the receptor expression vector and the corresponding reporter gene in order to "correct" for possible variations in transfection efficiencies. CAT-assays were performed by using aliquots of whole cell extracts which contained equal amounts of β -galactosidase, and transcriptional activation was monitored by quantifying the CAT-dependent acetylation of 14 C-chloramphenicol by liquid-scintillation counting. The data were expressed as the fold-stimulation of basal reporter gene activity (measured by replacing the receptor expression vectors with the parental vector pKCR2) and plotted versus the amount of transfected expression vector (note that in the case of hGR expressed in HeLa cells, the stimulation due to endogenous hGR, as determined by transfecting the parental vector pKCR2 in the presence and absence of hormone, was subtracted). All dose-response curves were established with 5 μ g reporter recombinant. Using lower amounts of reporter gene changed the magnitude of activation, but not the shape of the curves (data not shown, see Fig. 6 for the ER). Results

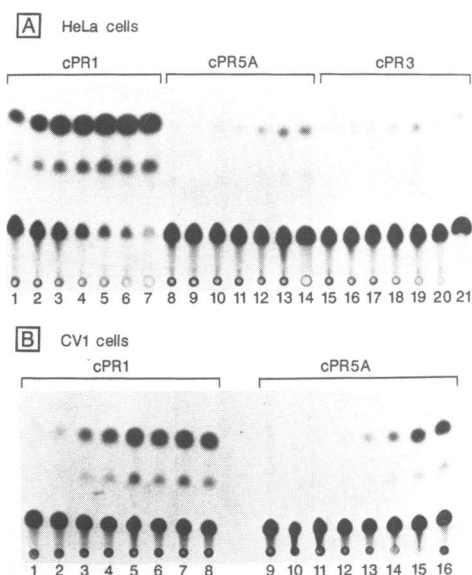


Fig. 1 - Increasing doses of transiently expressed wt and mutant cPR generate a different transcriptional response in HeLa and CV1 cells from a co-transfected cognate reporter gene. (A) CAT assays demonstrating transcriptional activation of the MMTV-CAT reporter recombinant (5 μ g) in HeLa cells by wt (cPR1) and mutant (cPR3, cPR5A; see Fig. 2) cPR. Increasing amounts of expression vectors (lanes 1-7, 8-14 and 15-21 correspond to transfections with 20, 50, 130, 320, 800, 2000 and 5000 ng) were transfected as indicated together with the reporter gene and 3 μ g pCH110 (expressing bacterial β -galactosidase) as the internal control. Note that the [3 H] is substrate exhausted at high cPR1 input (lane 7). (B) Similar analysis in CV1 cells with an identical co-transfection protocol, but using 3, 10, 30, 90, 270, 800, 2400 and 7300 ng of expression vectors in lanes 1-8 (cPR1) and 9-16 (cPR5A). No activation was observed with cPR3 in CV1 cells under our conditions (data not shown). (A) and (B) CAT-assays were performed with aliquots of whole-cell extracts containing 5 units of β -galactosidase (see text for details). Cells transfected with cPR1 or cPR3 were incubated with 100 nM progesterone.

similar to those reported here for the cPR using the reporter gene MMTV-CAT, were obtained with a PR reporter recombinant containing a synthetic PRE/GRE [PRE/GRE-tk-CAT(PTCAT), see refs. 27, 28; data not shown].

The actual amount of receptor present in the transfected cells was measured by hormone binding (Materials and Methods, data not shown) and immunoblotting analysis, as shown below for cPR (Fig. 3). In all cases a similar amount of receptor was synthesized in both HeLa and CV1 cells for a given amount of transfected expression vector (relative to either the amount

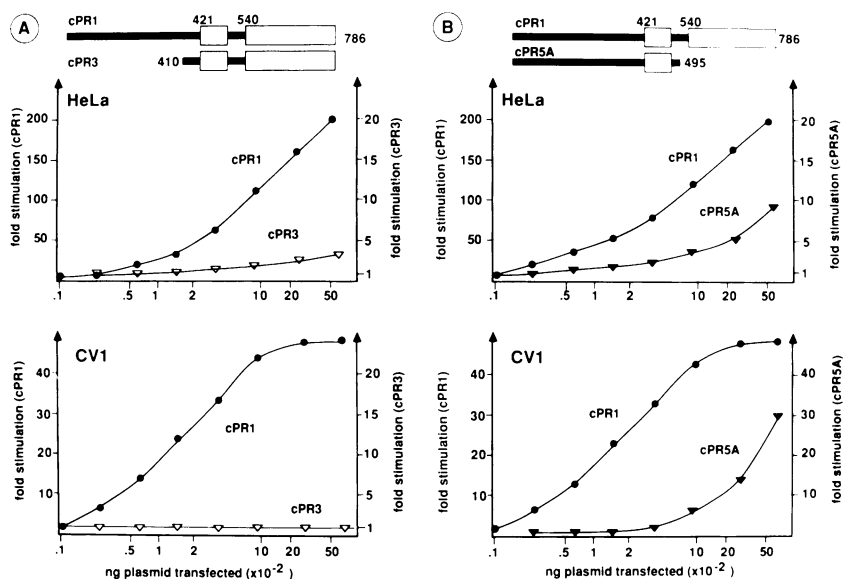


Fig. 2 - Dose-response curves for activation of transcription from 5 μ g MMTV-CAT reporter gene by (A) wild-type (cPR1, filled circles) and N-terminally (cPR3, open triangles) or (B) wild-type and C-terminally (cPR5A, filled triangles) truncated cPR expressed from increasing amounts of the corresponding vectors (as indicated), ranging from 10 ng to 7500 ng (note the log-scale) transfected into either HeLa (top panel) or CV1 (bottom panel) cells. Cells transfected with wt and N-terminally truncated receptors were exposed to 100 nM progesterone. Activation is expressed as the fold stimulation of the basal reporter gene transcription relative to that measured for a transfection in which the cPR expression vector was replaced with the parental vector pKCR2. To compensate for variations in transfection efficiencies all data were normalized to the β -galactosidase expressed from the internal reference recombinant pCH110, as described in the text. The cPR and its mutants are schematically illustrated at the very top with the two open boxes indicating DNA (left box) and hormone binding (right box) domains. Numbers refer to the first or last amino acid of a given domain or truncation. For further details on the structural organization of steroid receptors see Gronemeyer et al. (5).

of β -galactosidase expressed from the reference plasmid, or to the number of β -galactosidase stained cells).

A representative cPR experiment is presented in Fig. 1 which shows the transcriptional activation of 5 μ g MMTV-CAT when increasing amounts of cPR1, cPR3 and cPR5A, were co-transfected into HeLa and CV1 cells (the individual assays were performed with aliquots of cellular extracts containing 5 units β -galactosidase expressed from the co-transfected pCH110).

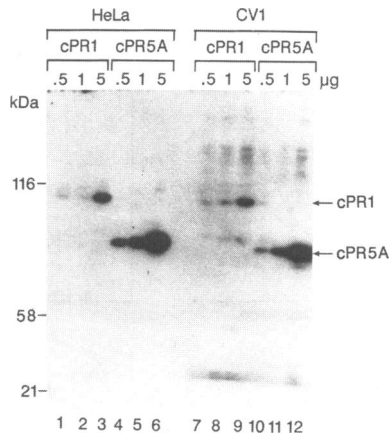


Fig. 3 - Immunoblot of wt (cPR1, lanes 1 - 3 and 7 - 9) and C-terminally truncated (cPR5A, lanes 4 - 6 and 10 - 12 ; see Fig. 2) cPR expressed in HeLa (lanes 1 - 6) or CV1 cells (lanes 7 - 12) by transfecting 0.5, 1 and 5 μ g of the corresponding expression vectors as indicated. Each lane corresponds to an aliquot of a high-salt whole-cell extract (see Materials and Methods) containing 20 units of β -galactosidase co-expressed from the internal reference recombinant pCH110. The position of marker proteins is given on the left, arrows point to the expected molecular weights of cPR1- and cPR5A-encoded receptors.

2) Transcriptional activation by the progesterone receptor

Stimulation of transcription from the MMTV-CAT reporter gene was consistently 3 to 4-fold higher in HeLa than in CV1 cells when equal amounts of the cPR expression vector (cPR1) were transfected (Figs. 1A and B, lanes 1-7 and 1-8, respectively, and Fig. 2). Notably, no further increase in the transcriptional activation was seen in CV1 cells above 1 μ g of transfected cPR1 (Fig. 1B, lanes 5-8 and Fig. 2, bottom panels). Hormone binding assays and Western blot analyses indicated that the amount of receptors synthesized in the cells increased with increasing amounts of transfected expression in the cells increased with increasing amounts of transfected expression vectors and, moreover, that a given amount of expression vector generated similar levels of receptor proteins in both cell lines (Fig. 3). When the N-terminal A/B region of cPR was deleted (cPR3), the stimulatory activity decreased by more than 95% in HeLa cells (Fig. 1A and 2A), whereas no activity could be detected in CV1 cells (Fig. 2A). Deletion of the HBD (cPR5A) resulted also in a 95% decrease of reporter gene activation in HeLa cells irrespective of the amount of transfected expression vector (Fig. 1A

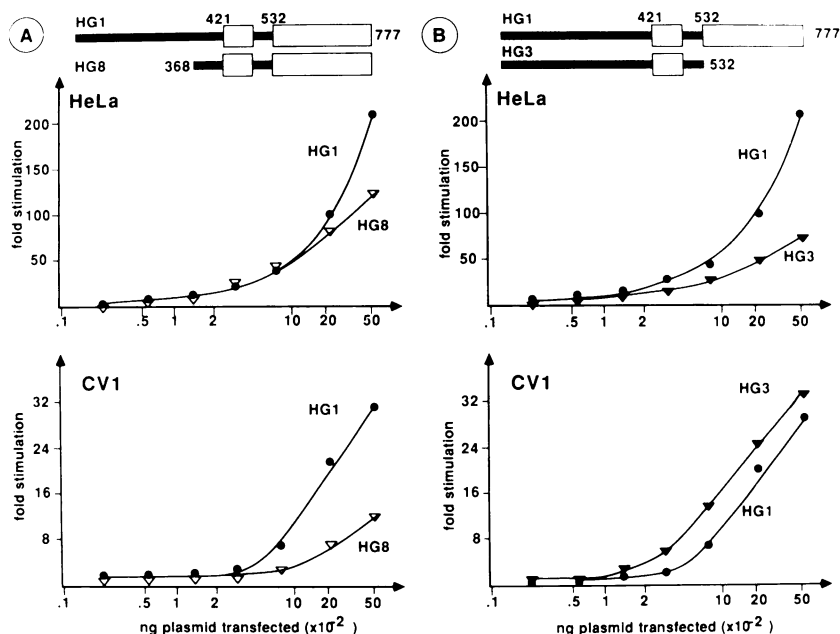


Fig. 4 - Dose-response curves of transcriptional activation of the MMTV-CAT reporter gene (5 μ g) by (A) wt (HG1, filled circles) and N-terminally (HG8, open triangles) or (B) wt and C-terminally (HG3, filled triangle) truncated hGR (illustrated at the very top) as determined by CAT-assay with normalized aliquots of whole-cell extracts of transiently transfected HeLa (top panel) or CV1 (bottom panel) cells. Cells transfected with HG1 or HG8 were treated with 10 nM triamcinolone acetonide. To generate the graphs shown for HeLa cells, the stimulation by the endogenous hGR was subtracted (approx. 10 fold; determined from HeLa cells transfected only with MMTV-CAT and treated with or without hormone). In all other aspects the figure is analogous to figure 2.

and 2B), whilst a similar decrease was observed in CV1 cells only below 1 μ g of transfected expression vector (Fig. 1B and 2B). Strikingly, high amounts (above 5 μ g) of cPR5A stimulated transcription nearly as efficiently as the wt receptor in the case of CV1, but not HeLa cells (Fig. 1 and 2). However, cPR5A was expressed in both cell lines at a level 5-times higher than cPR1 (Fig. 3), indicating that in fact even in CV1 cells the HBD-truncated mutant was less active than its wt counterpart. In contrast, cPR3 and cPR1 were expressed at similar levels as determined by hormone binding (data not shown). We conclude from these data that the A/B region (expressed in cPR5A) contains a weak autonomous transcriptional activation function which is similarly active in HeLa and CV1 cells, whereas the activation function

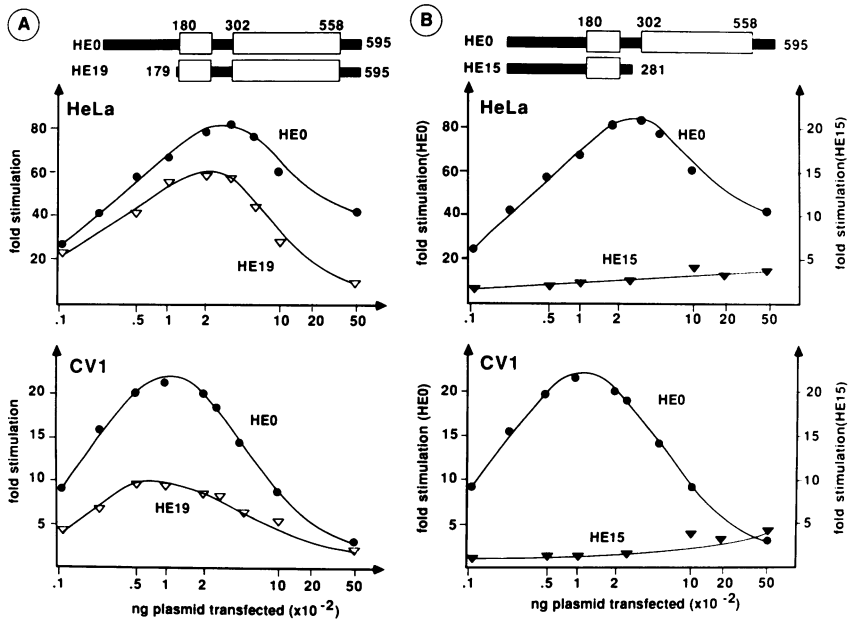


Fig. 5 - Dose-response curves of transcriptional activation of the vit-tk-CAT reporter gene (5 μ g) by (A) wt (HE0, filled circles) and N-terminally (HE19, open triangles) or (B) wt and C-terminally (HE15, filled triangles) truncated hER (illustrated at the very top) as determined by CAT-assays with normalized aliquots of whole-cell extracts of transiently transfected HeLa (top panel) or CV1 (bottom panel) cells. Cells transfected with HE0 or HE19 were incubated with 5 nM oestradiol. In all other aspects the figure is analogous to figure 2.

present in the cPR HBD, exerts very little (HeLa cells) or no (CV1 cells) autonomous activity. Its presence, however, has a clear effect in both cell lines, indicating a synergistic contribution of the two regions to transcriptional activation by wt cPR.

3) Transcriptional activation by the glucocorticoid receptor

Like cPR, hGR activated transcription from the MMTV-CAT reporter gene more efficiently (about 5-7 times) in HeLa than in CV1 cells (Fig. 4). However, in contrast to cPR, deletion of the N-terminal 367 amino acid residues (HG8) resulted in only a marginal decrease of transcriptional activation in HeLa cells and about 70% decreased activity in CV1 cells (Fig. 4A). In HeLa cells, truncation of the HBD (HG3, Fig. 4B) did not result in the large decrease in transcriptional stimulation which was characteristic for PR. Furthermore, no differences were observed between HG3 and HG1 in CV1 cells, irrespective of the amount of expression vectors transfected (Fig.

4B), although immunoblots indicated that similar amounts of HG1 and HG3 proteins were produced in the transfected cells (data not shown). Note that the contribution of the endogenous GR (resulting in an about 10-fold stimulation of MMTV-CAT transcription, as seen when the HG expression vectors were replaced by the parental pKCR2 vector) was subtracted in the case of the HeLa cells. Therefore, in these cells, no conclusions could be drawn from the portion of the curves below 1 μ g of transfected expression vector. On the other hand, at higher amounts of expression vectors, the transcriptional activation by HG1 clearly exceeded that brought about by HG8 and HG3 (top panels of Fig. 4A and B). We conclude therefore that the A/B region and the HBD of hGR have transcription activation functions which can operate autonomously and that their effects are additive in the wt receptor.

4) Transcriptional activation by the estrogen receptor

The human oestrogen receptor, expressed from HEO, stimulated transcription from the vit-tk-CAT reporter gene about 4-times more efficiently in HeLa than in CV1 cells (Fig. 5), although it was expressed in equal amounts in both cell lines (data not shown). Interestingly, the transcriptional stimulation dose-response curve exhibited an optimum for approximately 300 ng (HeLa cells) and 100 ng (CV1 cells) of transfected HEO. In both cell lines higher amounts of HEO resulted in both cell lines in a marked inhibition of transcription (Fig.5). This inhibition paralleled the increase in hER protein (as determined by immunoblotting, data not shown) and similarly bell-shaped curves were obtained when the amount of reporter gene was varied (top panel of Fig. 6). Note, however, that although the position of the optima was not significantly changed under these various conditions, the magnitude of the "auto-inhibition" was somewhat greater at low reporter gene input.

In HeLa cells, truncation of the A/B region (HE19) had little effect for low amounts of expression vector, although the maximal stimulation was lower than with HEO (Fig. 5). The HE19 "auto-inhibition" was stronger than in the case of HEO for high amounts of expression vector (Fig. 5A and Fig. 6, lower panel). When compared with HEO, HE19 was less efficient in CV1 than in HeLa cells (Fig. 5A).

Deletion of hER HBD (HE15) resulted in a drastic decrease in activation of transcription from the reporter gene in both HeLa and CV1 cells (Fig. 5B). Note, however, that at high expression vector input, due to the above described "auto-inhibition", HE15 activated transcription almost as efficiently as HEO. We conclude from these data that, using the vit-tk-CAT

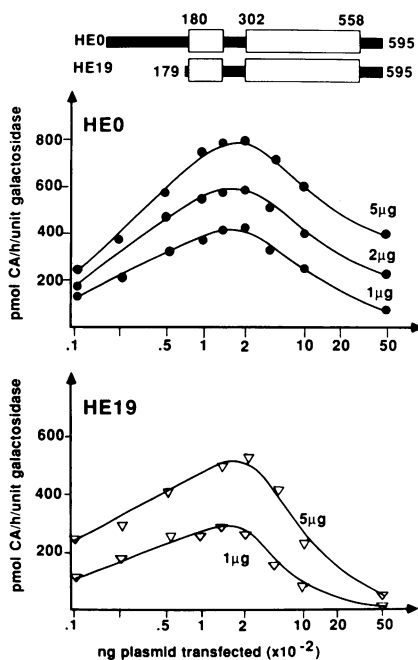


Fig. 6 - Transcriptional dose-response curves obtained by transfecting into HeLa cells the estrogen receptor expression vectors HE0 (top) or HE19 (bottom) together with 5 μ g, 2 μ g (shown only for HE0) or 1 μ g of the vit-tk-CAT reporter recombinant as indicated. Normalization of extracts for CAT-assays was relative to β -galactosidase, co-expressed from the internal reference recombinant pCH110. Transcriptional activation is expressed as pmol chloramphenicol acetylated per hour per unit of β -galactosidase contained in the corresponding aliquot of the whole-cell extract. Other aspects of this figure are as described for figure 5.

reporter gene, the activation function of hER in both HeLa and CV1 cells can be ascribed mainly to the HBD (present in HE19), whereas the A/B region (present in HE15) exerts only a minor effect. Moreover in CV1 cells these major and minor activation functions appear to act synergistically to generate the activation brought about by wt receptor.

DISCUSSION

Differential contribution of the N-terminal A/B region and the hormone binding domain (HBD) of PR, GR and ER to activation of transcription.

The systematic study in HeLa and CV1 cells of the effect of similar mutations in three steroid hormone receptors reveals striking quantitative as well as qualitative differences between the individual contributions of

either the A/B region or the HBD to the transcriptional enhancement brought about by the wt receptor. Whereas the N-terminal A/B regions of PR and ER do not contain an activating domain which functions efficiently on its own in HeLa or CV1 cells, the results obtained with the mutant HG3 suggest that the A/B region of GR can exert an autonomous transcription activation function in these cells. Data supporting this suggestion have been recently reported (17, 18). On the other hand, the region containing the ER HBD possesses a major autonomous activation function, which is efficiently exerted in the absence of the A/B region (HE19), particularly in HeLa cells. This is also the case for the region containing the HBD of the GR (HG8). These observations are in keeping with previous results from our laboratory which demonstrated that the GR and ER HBDs linked to the GAL4 DNA binding domain can efficiently activate GAL4-responsive reporter genes in a hormone-dependent fashion (13). Similar results have also been reported recently in the case of hGR (18). However, the situation appears to be different for PR, since a deletion of the N-terminal A/B region resulted in a mutant (cPR3) virtually unable to stimulate transcription. Thus, it appears that the PR does not contain any transcription activating domain which can work efficiently on its own, but that both the A/B region and the HBD have to co-operate to generate the wt receptor activation function. Such a strong synergism was not observed for the corresponding GR regions which appear to act additively, whereas the corresponding ER regions exhibit a moderate synergistic effect in CV1 cells. Thus, all of the present results suggest that the transcriptional activation functions which are present in the A/B regions and the HBDs of the three steroid hormone receptors act differently (qualitatively and/or quantitatively) on components of the transcriptional machinery to stimulate initiation of transcription.

Cell-specific differences for the transcriptional activation by PR, GR and ER mutants : an explanation of previous discrepancies.

The systematic variation of various parameters (amount of transfected expression vector and reporter gene, recipient cell), as reported here, demonstrates clearly that erraneous conclusions concerning the transcription activation potential of a transiently expressed transcription factor (such as steroid hormone receptor) can be drawn from "single-point experiments". For instance, it has been reported that truncations of the hormone binding domain of the chicken and rabbit PR, similar to that of cPR5A, generate mutants which constitutively activate transcription at wild-type levels (29, 30). However, these experiments were performed by transfecting CV1 cells with high

amounts (5 μ g or 10 μ g) of expression vectors, under conditions where the wild-type receptor dose-response curve has reached a plateau, while the mutant curve is still increasing, particularly in CV1 cells (Fig. 1 and 2B).

In the case of the glucocorticoid receptor, several groups have proposed that the HBD does not contain any transcription-activation function (see Introduction). All of these groups used CV1 cells (or the CV1-derived COS cells) as recipient cells, in which the wild type GR (HG1 vector) and the HBD-truncated mutant (HG3 vector) dose-response curves are superimposable (Fig. 4B). In contrast in HeLa cells, it is clear that HG1 stimulated transcription more efficiently than HG3, although the analysis of the GR activation function is hampered by the presence of endogenous GR (Fig. 4B). In this respect, note that the so-called "tau-2" activating domain as recently defined (18) is located within the region containing the HBD [for a description of this domain (region E) see for example ref. 5], rather than in the DNA-binding domain, as had been previously suggested (8, 16).

Evidence for the existence of limiting transcription factors mediating transcriptional stimulation by steroid hormone receptors.

What could be the underlying molecular mechanism accounting for the different transcriptional dose-response curves obtained in HeLa and CV1 cells and for the activation pattern obtained with the various truncated mutants? The lower activity of all wt receptors when tested in CV1 cells indicates a decreased efficiency of the transcriptional stimulation by steroid hormone receptors in these cells. We propose that the transcriptional activation by steroid hormone receptors is transduced through additional factor(s) which may be limiting in CV1 cells. This hypothesis would explain the curves obtained for cPR1 and cPR5A in CV1 cells: the factor mediating the effect of the HBD, but not of that mediating the effect of the A/B region would be limiting in these cells, accounting for the plateau (and lower overall activation) observed for cPR1 in CV1, but not in HeLa cells (Fig. 2). Similarly, the lower activity of GR in CV1 as compared with HeLa cells would reflect the existence in CV1 cells of a limiting factor interacting only with the GR HBD (HG3 exhibit the same activity in both cell lines, see Fig. 4; no plateau would be observed for HG1 in CV1 cells, because both regions of the GR can act independently from each other).

A prediction of the above hypothesis is that under conditions where the template is limiting (as is the case in all experiments described in this study, see above and Fig. 6), transfecting increasing amounts of receptor expression vector can generate a situation where "free" receptor molecules

will compete for such a limiting factor(s) with receptor molecules bound to the responsive element of the reporter gene. This situation should then result in an apparent "auto-inhibition". This is in fact what we have observed for ER stimulation of transcription from the vit-tk-CAT reporter gene, in both HeLa and CV1 cells. The fact that similarly shaped curves were obtained for HEO and HE19 suggests that such a factor(s) may mediate the activation function of the ER HBD. The shift in the optima for HE19 and the lower activation of transcription in CV1 cells as compared with HeLa cells (Fig. 5A) may be taken as evidence for lower expression of this factor in CV1 cells. Evidence that such a "mediating" limiting factor(s) may be shared among various steroid hormone receptors is reported elsewhere (27). The possible physiological role of a competition between these receptors for such factor(s) remains to be investigated.

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