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NEITHER THE ENDOGENOUS NOR A FUNCTIONAL STEROID HORMONE RECEPTOR BINDING SITE TRANSACTIVATE THE RIBOSOMAL RNA GENE PROMOTER *IN VITRO*

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Summary—The mammalian ribosomal RNA gene promoters exhibit a conserved sequence between positions +1 and +16 that shows a high degree of homology to the response element for glucocorticoids and progestins (GRE/PRE). These sequences bind specifically the glucocorticoid receptor and the progesterone receptor (PR) albeit with lower affinity than a canonical GRE/PRE. Because steroid hormones are known to affect expression of the ribosomal genes, we tested the influence of hormone receptors on the activity of the ribosomal RNA gene promoter in a cell-free transcription assay. Preparations of PR that induce transcription from the mouse mammary tumour virus (MMTV) promoter do not stimulate but slightly inhibit transcription from the ribosomal RNA gene promoter. This weak negative effect is not mediated through binding to the hypothetical GRE/PRE as a mutant promoter that does not bind receptor is equally repressed. Introduction of the functional MMTV GRE/PRE upstream of the basal ribosomal RNA gene promoter does not enhance its transcription in the presence of an active PR. Thus, RNA polymerase I transcription cannot be stimulated *in vitro* by cis elements and regulatory proteins that are active in RNA polymerase II transcription.

INTRODUCTION

In addition to their well established effects on the expression of protein encoding genes (for a review see Ref. [1]), steroid hormones also influence the transcription of ribosomal genes. Glucocorticoids, for instance, have been reported to inhibit ribosomal RNA synthesis in lymphoid cells [2], whereas they have an opposite effect on liver cells [3–7]. Hormonal regulation of genes transcribed by RNA polymerase II takes place by virtue of the interaction of the hormone receptors with hormone responsive elements (HRE) located in the vicinity of the regulated promoters [1]. However, the molecular mechanism of hormone action on ribosomal gene expression is unclear.

We decided to investigate the possibility that DNA sequences similar to those discovered in other hormone responsive genes may also mediate the effects of steroid hormones on the expression of ribosomal RNA. In addition, we wanted to know whether a HRE, functional in the context of a RNA polymerase II promoter, can activate RNA polymerase I-driven transcription of the ribosomal RNA gene promoter. As gene transfer experiments with ribosomal genes are difficult to interpret due to the large endogenous copy number, we chose to investigate this question *in vitro*. This approach is now possible using a recently developed cell-free transcription assay in which the purified progesterone receptor (PR) from rabbit uterus is able to efficiently induce transcription from the mouse mammary tumour virus (MMTV) promoter [8]. Here we report that the ribosomal RNA gene promoter of mammals contains a conserved binding site for glucocorticoid receptors (GRs) and PRs, exhibiting many of the properties of canonical response elements for glucocorticoids and progestins (GRE/PREs). However, binding of the PR to this hypothetical GRE/PRE does not influence its transcription by RNA polymerase I *in vitro*. Moreover, replacement of the upstream ribosomal sequences by the functional HRE of MMTV does not confer to the promoter the ability to respond to

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added PR. These results suggest that RNA polymerase I is unable to respond to DNA-bound hormone receptors that act as transactivators of RNA polymerase II-dependent transcription and that the effects of steroid hormones on ribosomal RNA transcription may be indirect or may involve mechanisms different from those reported for protein encoding genes.

EXPERIMENTAL

Plasmids

Plasmid pMribwt and the double point mutant pMrib11/12 that contain sequences from −169 to +155 (Sal I/Sma I fragment) of the mouse ribosomal RNA gene promoter cloned into the pUC9 vector were a gift of I. Grummt. pHribwt was obtained by cloning a 233 bp Ava I fragment, containing sequences from −56 to +177 of the ribosomal RNA gene promoter into the Ava I site of pGem4 (Promega Co, Madison, WI). In the plasmid pHrib12/15 positions G at +12 and C at +15 were mutated by oligonucleotide-directed mutagenesis [9] to C and G, respectively. pHribΔwt and pHribΔ12/15 were obtained by deletion of 72 bp (Sst I–Sst II fragment) at the 3′ end of the ribosomal insert of their parent plasmids pHribwt and pHrib12/15, respectively, and contain ribosomal DNA (rDNA) sequences between −56 and +116. p(MMTV–HRE)HribΔwt was constructed by subcloning a Bam HI/Hind III fragment from construct pMMTV-21H [10] containing the MMTV–HRE region between −192 and −64 into the corresponding polylinker sites of pHribΔwt located directly upstream of the ribosomal RNA gene promoter. The construct pHribwt, linearized by Pvu II, served as a template for T7 RNA polymerase transcription to generate the anti-sense RNA probe used in the RNase protection assay [11]. The MMTV–CAT templates and the riboprobe used in control experiments were described previously [8].

Receptors and DNA binding assays

GRs and PRs for the binding and transcription assays were purified as described previously [10, 12]. DNase I footprinting and methylation protection with purified GR were performed as reported previously [13] using a 341 bp Hind III/Eco RI fragment from pMribwt or pMrib11/12 containing wild-type or mutated sequences, respectively, from position −181 to +161 of the mouse ribosomal gene. Exonuclease III protection experiments were performed as described previously [12]. A 140 bp Bam HI/BstE II fragment from plasmid pHribwt, containing sequences −56/+76 of the human ribosomal gene or from the mutant pHrib12/15 was used for band shift experiments with purified receptors. To estimate the binding efficiency, gel retardation with purified receptor was also performed with a 142 bp Bam HI/Pvu II fragment from construct pGRE-1 [14] that contains the distal receptor binding site of the MMTV promoter. Gel retardation and methylation interference of the wild-type ribosomal DNA fragment were performed according to Chalepakis *et al.* [10].

Cell-free transcription

Nuclear extracts for cell-free transcription were prepared according to Dignam *et al.* [15]. The final dialysis buffer contained 12.5 mM $MgCl_2$. A mixture of two closed circular supercoiled templates (5 to 50 ng each) and the indicated amounts of nonspecific carrier [poly(dI-dC) or calf-thymus DNA] were preincubated with or without purified receptor for 20 min at room temperature in 10 mM Tris–HCl, pH 7.5, 0.1 mM EDTA and 80–100 mM NaCl. Preincubation was followed by addition of 10 to 50 μg nuclear protein and ribonucleosides triphosphates. The final transcription conditions were as described [8]. RNase protection using an anti-sense RNA probe was performed essentially as described [11].

RESULTS

Identification of a conserved binding site for GR and PR in the mammalian ribosomal RNA gene promoters

Inspection of the nucleotide sequence of the ribosomal RNA gene promoter from mouse, rat, rabbit, apes and humans reveals a conserved nucleotide sequence between positions +1 and +16 that exhibits a high degree of homology to the consensus GRE/PRE palindrome [16]. In particular, the right half of the palindrome with the hexanucleotide motif TGTYCT is present in all these species (Fig. 1) and is identical to that found in the GRE/PRE of the human metallothionein IIA gene [20] and other inducible genes [1]. In the left half of the palindrome, the

	-20 -10 +1 +11 +21
Human	TTTGGGCCGC CGGGTTATAT GCTG**ACA**CGC **TGTCCT**CTGG CGACCTGTCG
Chimp	TCTTGGGCCA CCGGGTTATT GCTG**ACA**CGC **TGTCCT**CTGG CGACCTGTCG
Rhesus	TTTTGGGCCA CCGGGTTATT GCTG**ACA**CGC **TGTCCT**TCGG CGACCTGTCG
Mouse	TATTGGACCT GGAGATAGGT ACTG**ACA**CGC **TGTCCT**TTCC CTATTAACAC
Rat	TATTGTACCT GGAGATATAT GCTG**ACA**CGC **TGTCCT**TTTG ACTTCTTTTT
Rabbit	TTTTGGACCT GCAGGTAGGT GCTG**ACA**CGC **TGTTCT**CTGG TGACTGTCGC
Consensus	T-TtGg-Cc- ---G-T---T gCTG**ACA**CGC **TGTcCT**---g ----------
Consensus GRE/PRE	**GGTACA**NNN **TGTYCT**

Fig. 1. Alignment of the sequences around the transcription initiation site of several mammalian rDNAs. The sequences of human, mouse and rat have been published [17–19]. The sequence of chimpanzee (chimp), rhesus monkey and rabbit are from P. Seperack and N. Arnheim (personal communication). The sequence homologous to the consensus sequence derived from alignment of receptor binding sites present in steroid-regulated genes [1] is written in bold characters. The GRE/PRE consensus is also given [1].

important sequence ACA is also present suggesting a potential for binding GR and PR [16]. In binding experiments with the mouse ribosomal RNA gene promoter and purified GR from rat liver, we detected a DNase I footprint over the conserved region [Fig. 2(A) lanes 1 to 3 and Fig. 2(B) lanes 1 to 3]. In addition there are weaker footprints upstream and downstream that coincide with nucleotide sequences exhibiting an homology, although weaker, to the GRE/PRE (TGTTCC at −26 in the sense strand; TGTCCT at +39 in the anti-sense strand). Methylation protection experiments [Fig. 2(C)] identified contacts between GR and the expected positions within the hypothetical GRE/PRE and over the hexanucleotide motifs [13]. A summary of these results is shown in Fig. 2(D) that also includes the results of exonuclease III footprinting experiments. The specificity of DNA binding was also supported by the results obtained with a mutant ribosomal RNA gene promoter Mrib11/12 in which positions +11 and +12 have been exchanged from TG to GA [Fig. 2(D)]. The DNase I protection in the upper strand was eliminated by this mutation [Fig. 2(A) lanes 4 to 6] and the footprint on the lower strand was weakened [Fig. 2(B) lanes 5 and 6].

Because of the complex DNase I protection pattern on the mouse ribosomal RNA gene promoter, similar experiments were performed with the human ribosomal RNA gene promoter that lacks the hexanucleotide motifs flanking the hypothetical GRE/PRE. The results of a band shift assay with purified PR from rabbit uterus are shown in Fig. 3(A). A specific complex that migrates similarly to the complex formed on the promoter distal receptor binding site of the MMTV–HRE [21] was observed with increasing concentrations of PR. This complex was not detected at low receptor concentrations with the mutant promoter Hrib12/15 in which positions +12 and +15 have been mutated from G to C and from C to G, respectively [Fig. 3(A) and (B)]. The binding specificity was further demonstrated in methylation interference experiments with the retarded complex [Fig. 3(C)]. Methylation of the expected G residues within the hypothetical GRE/PRE interfered with binding of the PR [Fig. 3(C)]. Essentially similar results were obtained with the GR (data not shown).

The experiments shown in Fig. 3(A) can also be used to determine the relative affinity of PR for the hypothetical GRE/PRE of the human ribosomal RNA gene promoter compared to the upstream receptor binding site of the MMTV–HRE [21]. A densitometric analysis of several experiments suggested that the relative affinity of the PR for the human ribosomal DNA is 8 times lower than its affinity for the MMTV oligonucleotide. A similar number was obtained with the purified GR (data not shown). Thus, these data show that both GR and PR bind selectively the hypothetical GRE/PRE in the ribosomal RNA gene promoter, albeit with an affinity considerably lower than a *bona fide* GRE/PRE.

The endogenous GR/PR binding site of the ribosomal RNA gene promoter is inactive in vitro

To investigate functional consequences of PR binding, we used a soluble nuclear extract from HeLa cells [15] that transcribes efficiently the human ribosomal RNA gene promoter. The template used for the *in vitro* transcription experiments contained human rDNA sequences between −56 and +177 (referred to as wild-type). The template includes the core promoter

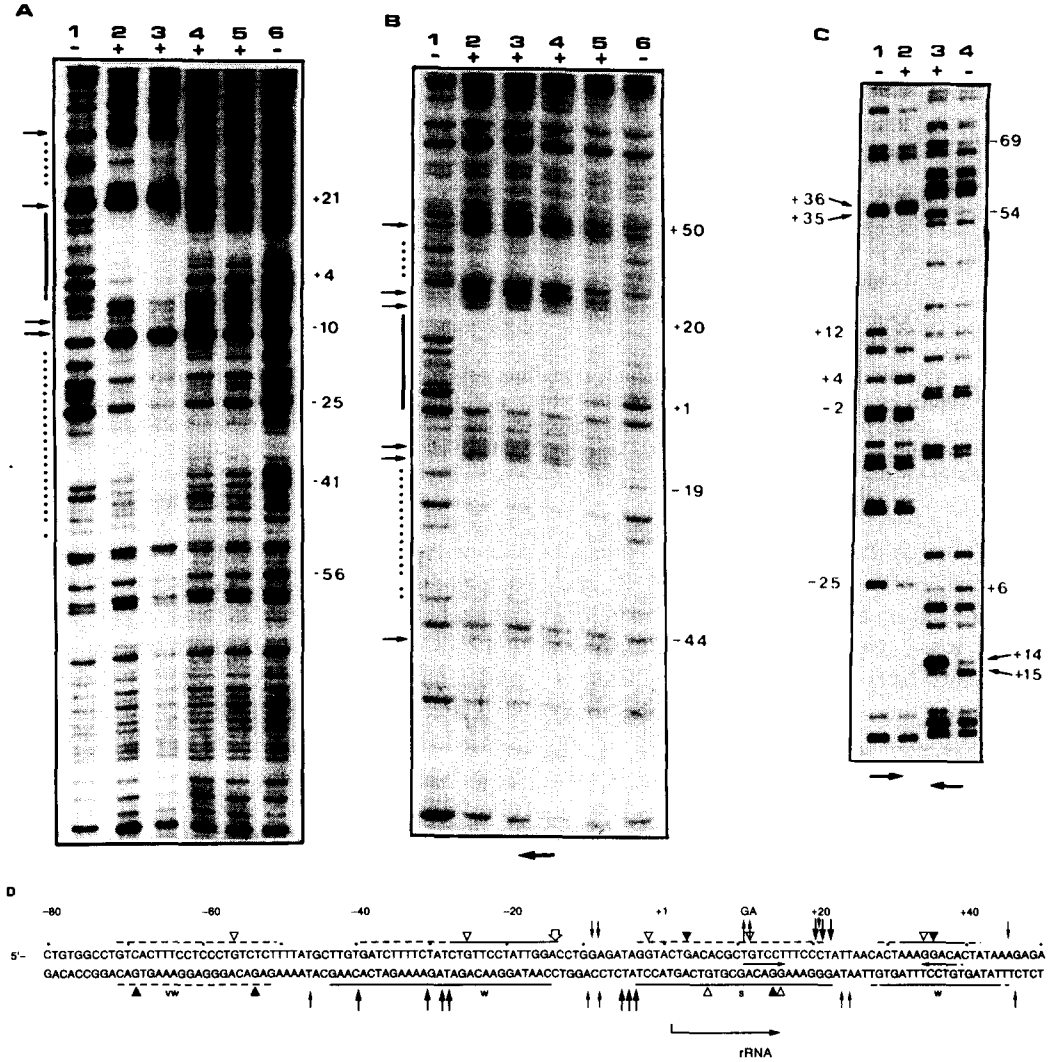

Fig. 2. DNase I footprinting and methylation protection of the mouse ribosomal DNA promoter by purified GR. (A) and (B): DNase I footprint in the noncoding (A) and coding (B) strands of the wild-type (lanes 1 to 3) and the mutant (lanes 4 to 6) promoters. Lanes 1 and 6 show control DNase I digestions in the absence of receptor. DNase I digestions of wild-type and mutant promoters after incubation with 1.6 μg (lanes 2 and 5) and 2.8 μg (lanes 3 and 4) purified rat liver GR are shown. The numbers at the right of the panels indicate the distance upstream (−) or downstream (+) of the transcription initiation site. Horizontal arrows point to receptor-dependent DNase I hypersensitive sites. The limits of the footprints are indicated on the left; dotted lines indicate weak protection. (C): Methylation protection in the noncoding (lanes 1 and 2) and in the coding strand (lanes 3 and 4). Lanes 1 and 4 represent controls without receptor, while lanes 2 and 3 show methylation protection in the presence of 1.7 μg receptor. Positions affected by receptor binding are numbered. (D): Summary of the results shown in panels A, B and C. Horizontal lines indicate the regions protected against DNase I digestion, **s**, **w** and **vw** indicate strong, weak and very weak binding sites, respectively. The hexanucleotide motifs are indicated by thin horizontal arrows. The thick vertical arrows show receptor-induced exonuclease III stops. The thin vertical arrows point to DNase I hypersensitive sites induced by receptor binding. (△) Indicate positions protected by the receptor against methylation with dimethylsulfate. (▲) Point to positions that are hypermethylated in the presence of receptor.

element, −45/+20 [22], which is essential for rDNA transcription, but lacks the upstream control element (UCE, see [23]) which increases significantly (up to a 100-fold) the efficiency of transcription initiation at the ribosomal RNA gene promoter in gene transfer experiments [23] and exhibits a much weaker effect in cell-free transcription experiments [22]. To control for specific effects of PR, we mixed the wild-type ribosomal RNA gene promoter with the HribΔ12/15 mutant that has lost the capacity to bind PR (see above). HribΔ12/15 contains an additional deletion in the 3′ end of the transcribed rDNA region that allows us to determine transcriptional efficiency of wild-type and mutant supercoiled templates in mixing exper-

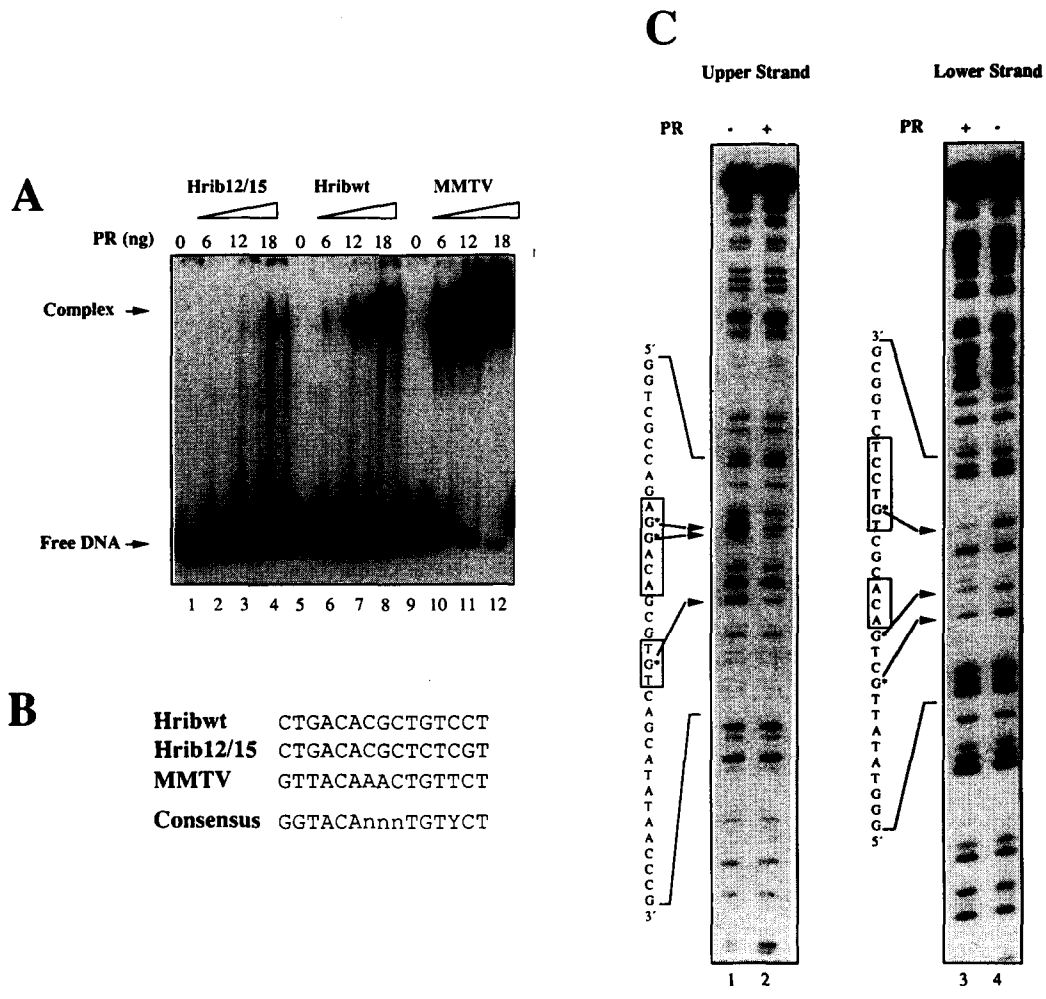

Fig. 3. Binding of purified PR to the human rDNA promoter. (A) Gel retardation of fragments containing wild-type (Hribwt: lanes 5–8) or mutated (Hrib12/15: lanes 1 to 4) rDNA sequences between positions −56 and +79 in the presence of the indicated amounts of purified PR from rabbit uterus. Lanes 9–12 represent retardation of a 142 bp fragment containing the distal receptor binding site of the MMTV promoter. (B) The sequence of the receptor binding sites in the fragments used for the gel retardation experiment shown in (A). The consensus sequence for GR and PR recognition is given [1]. (C) Methylation interference with purified PR in the noncoding (lanes 1 and 2) and coding strands (lanes 3 and 4) of the wild-type rDNA fragment used in (A). − And + refer to samples treated without or with receptor, respectively. Guanines which interfere with binding of the receptor are indicated by arrows on the left of the autoradiograms. The sequence of the human ribosomal RNA gene promoter in the bracketed region is given and the interfering guanines are labeled by asterisks. The boxed region shows the residues of the human ribosomal RNA gene promoter which are conserved among steroid receptor binding sites.

iments using the same radioactive probe [Fig. 4(C)]. Both templates were efficiently transcribed in the nuclear extract from HeLa cells [Fig. 4(A) lanes 1 and 6; and (B) lanes 1 and 5]. The lower intensity of the signal deriving from the control template HribΔ12/15 is due to a partial RNase digestion at the mismatched base pairs of hybrids between anti-sense RNA probe and HribΔ12/15 transcripts. This reduces the amount of RNase-protected fragments corresponding to the 116 nucleotide signal which is expected for correctly initiated transcripts [Signal CI mut in Fig. 4(A) and (B)]. Preincubation of a mixture of both templates with the partially purified GR or PR, at concentrations sufficient to generate a complex on the ribosomal RNA gene promoter, did not consistently alter their relative transcriptional efficiency. PR preparations showed a reproducible inhibition of the transcription of both templates which became more evident at higher receptor concentrations [Fig. 4(A) lanes 1 to 5]. Most of GR preparations had no effect on transcriptional efficiency [Fig. 4(B) lanes 5 to 8]. However, a slight stimulation of the transcription of both templates was detected using highly purified GR [Fig. 4(B) lanes 1 to 4]. A selective effect of added receptor on transcription of the wild-type

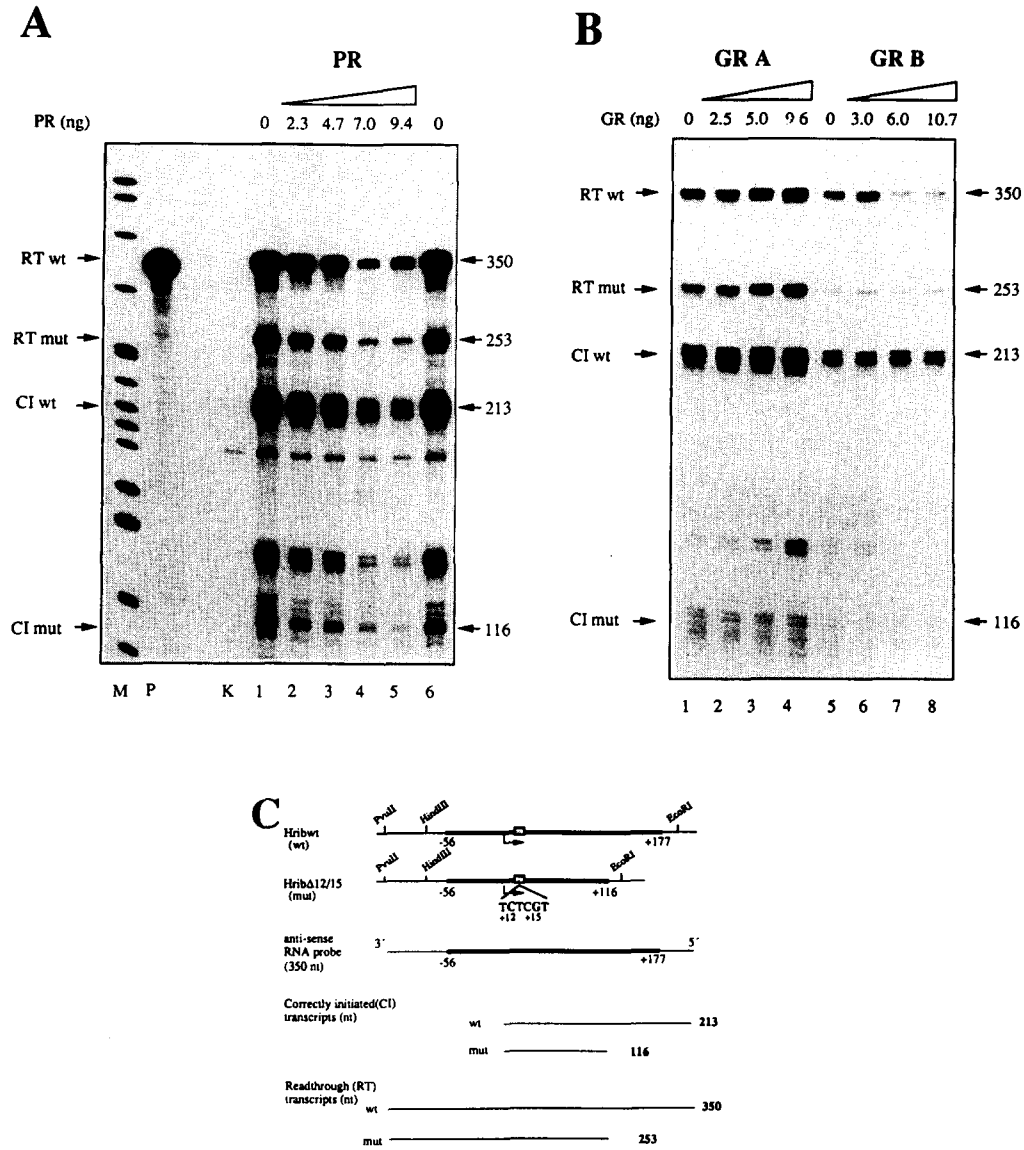

Fig. 4. Cell-free transcription from the human rRNA gene promoter in the presence of purified PR or GR. (A) A mixture of Hribwt and HribΔ12/15 (5 ng each) was preincubated with the indicated amounts of PR (lanes 2–5) and transcribed as described in Experimental. 2 μl of nuclear extract (10 μg protein), which had been preincubated with 100 ng poly(dI-dC) were used for the transcription reactions. Lanes 1 and 6 are control transcription reactions in the absence of receptor. K shows a control sample treated as 1 and 6 but in the absence of NTPs. Lanes M and P show marker fragments and the hybridization probe, respectively. Signals corresponding to correctly initiated transcripts (CI) or end-to-end transcripts (readthrough, RT) are indicated by arrows. (B) The same mixture of templates as in (A) was preincubated with the indicated amounts of purified GR (lanes 2–4 and 6–8). Two different receptor preparations were used (GR-A and GR-B). Both were prepared as described in Experimental, except that GR-A was additionally purified by fast performance liquid chromatography (FPLC) and was of a purity >70% as judged by SDS–PAGE. Transcription reaction conditions were as in (A). (C) Schematic representation of the templates used for *in vitro* transcription, the hybridization probe and the RNase-protected fragment corresponding to each transcript. Hribwt is referred to as wt and HribΔ12/15 as mut. The extent of the ribosomal RNA gene promoter in the constructs is indicated by thick lines. The boxes correspond to receptor binding sites and the mutated bps are indicated. Neighbouring restriction sites in the polylinker of the vector pGem4 are also given. The length of the hybridization probe and of the RNase-protected fragments corresponding to correctly initiated and readthrough transcripts is given below. Additional bands on the gel derive from RNase digestion at the mismatched bps (at positions +12 and +15 of the human ribosomal RNA gene promoter) in hybrids between anti-sense probe and HribΔ12/15 transcripts and are not indicated in panels (A) and (B).

template compared to the mutant HribΔ12/15 was however not detected. The same results were obtained when larger rDNA promoter constructions, containing the UCE, were used as templates in cell-free transcription (data not shown). Thus, we excluded the possibility that additional upstream factors are required for specific receptor effects on transcription.

The lack of effect of PR on the transcription of the ribosomal RNA gene promoter is not due to a defective receptor nor to the presence of an inhibitor in the nuclear extract since, as reported previously [8], addition of the same PR to a MMTV promoter template results in a clear induction of correct transcription (Fig. 5, lanes 1 to 4). We conclude that a functional PR bound to the ribosomal RNA gene promoter is not able to activate transcription by RNA polymerase I.

A bona fide HRE cannot activate RNA polymerase I transcription in vitro

We next asked whether a functional HRE derived from the MMTV promoter is able to stimulate transcription from the ribosomal RNA gene promoter *in vitro*. To this end, we cloned the MMTV sequences from −192 to −64, containing the full complement of MMTV receptor binding sites [10, 24], at position −56 of the human ribosomal RNA gene promoter. Thus, we created a human rDNA template in which the UCE was replaced by an upstream activator element, the HRE, that is functional in the context of RNA polymerase II transcription. This template was efficiently transcribed by RNA polymerase I [Fig. 5(A) lane 7], but in contrast to the MMTV promoter did not respond to preincubation with purified PR [Fig. 5(A) compare lanes 1–4 to lanes 7 and 8]. In fact, the presence of the MMTV sequence did not alter the transcriptional efficiency of the ribosomal RNA gene promoter, nor the slight inhibition of rDNA transcription observed after preincubation of the templates with PR [Fig. 5(A) compare lanes 5 and 6 to 7 and 8, respectively]. We conclude that transcription by RNA polymerase I is not affected by binding of PR to a functonal GRE/PRE located 56 bp upstream of the transcription start site.

DISCUSSION

The results presented in this paper show clearly that both GR and PR bind specifically to the promoter region of the murine and human rDNA genes. Both hormone receptors contact nucleotide sequences that exhibit considerable homology to the GRE/PRE detected in RNA polymerase II promoter and enhancer regions [1]. The selectivity of binding is demonstrated by the dramatic effect of mutations that destroy the right half of the palindromic site containing the hexanucleotide TGTYCT. The relative affinity of the purified receptors for the hypothetical GRE/PRE is about 12% of their affinity to the distal GRE/PRE of MMTV. Gel retardation experiments using oligonucleotides, in which the rDNA receptor binding site was mutated in either the first (5′) half or in the center of the palindrome to better fit the consensus [Fig. 3(B)], indicated that the most critical residues accounting for the lower affinity of PR for the Hribwt binding site relative to the MMTV distal binding site reside in the 5′ half of the palindrome, whereas the central nucleotides have only a smaller (2-fold) effect (Mathias Truss, unpublished data).

In spite of their specific binding, neither the GR nor the PR are able to activate transcription from the ribosomal RNA gene promoter *in vitro* under conditions that lead to stimulation of transcription from the MMTV promoter [8]. While the GR did not influence the transcriptional efficiency of the ribosomal RNA gene promoter in a reproducible way, a weak inhibition was consistently observed with purified PR. This negative effect was apparently independent of binding to the hypothetical GRE/PRE, as it was unaffected by mutations within the conserved half palindrome that markedly diminish receptor binding *in vitro*. The molecular basis of this weak inhibition was not investigated further.

One could argue that the lack of stimulatory effect of the purified PR on the cell-free transcription of the human ribosomal RNA gene promoter is due to the low affinity of the receptor for this binding site when compared to the distal MMTV–HRE binding site. To explore this possibility, we considered to duplicate or triplicate the number of binding sites, thereby increasing the total affinity for the receptor. However, given the position of the hypothetical GRE/PRE within the transcribed region, we decided instead to test the effect of a *bona fide* GRE/PRE located upstream of the core element of the human ribosomal RNA gene promoter. To this end, we chose the HRE of MMTV that contains four copies of the TGTYCT half palindrome and has been shown to respond to the

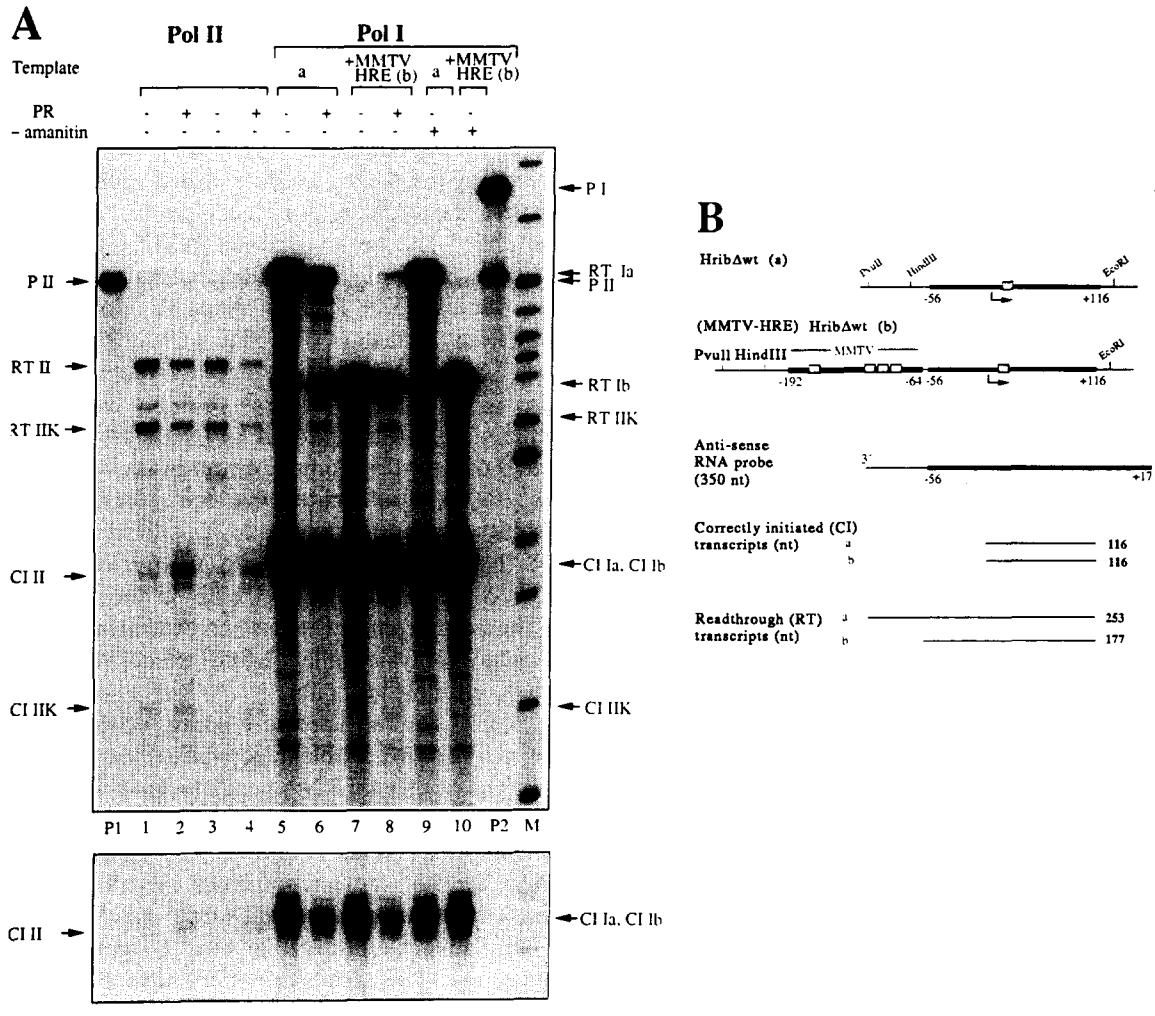

Fig. 5. Cell-free transcription of HribΔwt and of (MMTV–HRE)HribΔwt in the presence of purified PR. (A) Transcription of the following templates after preincubation in the presence of 50 ng calf thymus DNA with (+) or without (−) purified receptor protein (11 ng) as indicated. Lanes 1–4: equimolar amounts of two RNA polymerase II templates, one containing and the other lacking the HRE, 25 ng (lanes 1 and 2) or 50 ng (lanes 3 and 4) each. Lanes 5–10: equimolar amounts of a RNA polymerase I template [HribΔwt (a) or (MMTV–HRE)HribΔwt (b)] and of the RNA polymerase II control template lacking the HRE (25 ng each). Transcription followed as described by Kalff *et al.* [8]. Samples 9 and 10 were transcribed in the presence of 3 μg/ml α-amanitin. Transcripts from samples 1–4 were hybridized to a MMTV–CAT probe (P II), described previously [8] and shown in lane P1, while transcripts from samples 5–10 were hybridized to a mixture of this probe and of the ribosomal anti-sense RNA probe indicated as P I (see lane P2). RNase-protected fragments corresponding to correctly initiated or readthrough transcripts are indicated by arrows. Signals in lanes 1–4 are indicated on the left of the autoradiogram, while signals in lanes 5–10 are shown on the right side of the autoradiogram. Ia and Ib fragments correspond to HribΔwt and (MMTV–HRE)HribΔwt transcripts, respectively, and II, IIK fragments derive from transcripts of the MMTV–CAT RNA polymerase II templates containing or lacking the HRE region, respectively. The panel below shows a weaker exposure of a part of the same autoradiogram but with a better resolution of the ribosomal gene transcripts. (B) Schematic representation of the RNA polymerase I templates used for the *in vitro* transcription shown in (A) and (B). Boxes represent receptor binding sites. The boundaries of each promoter region and neighbouring restriction sites in the polylinker of pGem4 vector are indicated. RNase-protected fragments are shown as in Fig. 4(C).

addition of purified PR *in vitro* [8, 25]. The same PR preparations that reproducibly stimulated transcription from the MMTV promoter did not influence transcription from the basal ribosomal RNA gene promoter containing the MMTV–HRE at position −56. This result supports previous findings showing a lack of functional interaction between the RNA polymerase I basal transcriptional machinery and upstream elements from RNA polymerase II promoters [26]. This concept can now be extended to another family of transcriptional regulators, namely the nuclear hormone receptors. Stimulation of human rDNA transcription by the

UCE is mediated by the cooperative interaction of two factors, a DNA-binding protein, hUBF1, which also interacts with the core promoter element, and a species-specific factor, SL1 [27, 28]. This protein–protein interaction changes the footprint of hUBF1 on the ribosomal RNA gene promoter [28] and seems to determine species-specificity of rDNA expression [29]. Our and previous results [26] show the strict requirement for UCE to get upstream stimulation of RNA polymerase I transcription. In contrast to these findings, transcriptional activation of RNA polymerase II genes seems to be mediated by functional interactions between the basal transcriptional machinery and a variety of different upstream elements and activator proteins. In addition, genes transcribed by RNA polymerase III seem to share common upstream regulatory sequences with RNA polymerase II genes, such as the octamer motif [30, 31] and ATF or Sp1 elements [32]. These elements were shown to bind the same factors in both classes of promoters [31, 32]. It has also been shown that RNA polymerase III regulatory elements placed upstream of RNA polymerase II promoters increase RNA polymerase II-driven transcription from these promoters [33]. The characteristics of RNA polymerase I transcription, namely the very active transcription of only one special class of reiterated genes and the species-specificity, could partly account for the difference in regulatory mechanisms and preclude its response to upstream elements from RNA polymerase II promoters.

The effect of PR on the *in vitro* transcription of the MMTV promoter is mediated by the ubiquitous transcription factor OTF-1, also known as Oct-1 or NFIII [34]. Binding of the receptor to the HRE facilitates the interaction of OTF-1 with the MMTV promoter. The domain of the receptor involved in this synergistic interaction is not known, but our present results suggest that the same region cannot functionally interact with factors acting at the ribosomal RNA gene promoter.

An indirect effect of hormone action on ribosomal DNA synthesis via reduction of the amount or the activity of a factor required for initiation of transcription (TFIC) *in vitro* has been proposed in the case of the glucocorticoid-mediated inhibition of rRNA synthesis in murine lymphosarcoma cells [35, 36]. The down regulation of rDNA transcription occurring upon cycloheximide treatment of the cells [37, 38] or attainment of stationary phase [39] correlates with a similar reduction in TFIC activity in extracts from these cells [40]. In the latter case TFIC was defined as an activated subform of RNA polymerase I [40]. Although the stimulatory action of glucocorticoids on rDNA transcription in rat liver was reported to be exerted directly [7], later work favoured an indirect hormonal effect in this tissue via short-lived factors necessary for transcription [41]. The exact mechanisms of hormonal regulation of rDNA transcription in different tissues have still to be elucidated and the functional significance of the conserved binding site for hormone receptors in the transcribed region of the ribosomal gene remains unclear. One possibility is that it mediates regulation by other members of the receptor superfamily. As several genes have been identified encoding proteins without known ligand [1], the hypothetical DNA binding protein could act constitutively. Alternatively, if this site mediates regulation by steroid hormones, the receptors may require additional factors for efficient DNA binding and/or transcriptional regulation and these factors may be lacking in our extracts. To test this possibility one could use extracts from hepatocytes or from cells that respond to hormones with changes in ribosomal RNA synthesis. In spite of our failure to provide functional evidence, it seems unlikely that this element has been conserved without fulfilling a specific function.

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