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Dual Reporter Systems in Yeast and Mammalian Cells for Assessing Progesterone Receptor Modulators

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Abstract In the present study we describe the set-up of a new one-hybrid reporter gene assay in *Saccharomyces cerevisiae* composed of the human progesterone receptor fused to the DNA-binding domain of the yeast transcriptional activator Gal4. This assay allows the convenient estimation of receptor mediated progestogenic as well as antiprogestogenic actions of compounds. The induction of the β-galactosidase reporter gene expression correlated well with the progesterone receptor affinity and the concentration of the progestins tested. The results corresponded to those obtained from a reporter gene assay in the cancer cell line CV-1 and in vitro binding experiments using rabbit uterus cytosol. In both the yeast and CV-1 cells the activity of antiprogestins was detectable by inhibition of the progestin-induced reporter gene expression. Secondary reporter genes under the transcriptional control of receptor unrelated promoters have been introduced into yeast and mammalian test strains to distinguish between specific receptor mediated antihormone actions and nonspecific effects on cellular metabolism. J. Cell. Biochem. 73:126–136, 1999.

Key words: dual reporter systems; *Saccharomyces cerevisiae*; cancer cell lines; β-galactosidase; green fluorescent protein; firefly luciferase; *Renilla* luciferase; progesterone receptor; one-hybrid system

The progesterone receptor (PR) belongs to the family of ligand-dependent steroid hormone receptors controlling various aspects of cell growth, morphogenesis, development and homeostasis [Evans, 1988; Tsai and O'Malley, 1994]. Like other members of this family the PR is structurally and functionally characterized by distinct domains. The amino-terminal located domain possesses a ligand-independent transactivating function AF1, the central domain is responsible for DNA-binding, and the carboxy-terminal domain contains the ligand binding activity [Tsai and O'Malley, 1994]. The latter region carries also a ligand-dependent transactivation function AF2 [Danielian et al., 1992]. In the absence of a specific ligand the PR in vivo is associated to heat shock proteins and thus is transcriptionally silent. Ligand binding induces conformational changes, resulting in the release of the inhibitory proteins from the receptor followed by a transport of the active receptor/ligand complex into the nucleus. There it modulates gene expression primarily by binding as homodimer to hexameric inverted repeats spaced by three nucleotides, designated progesterone response elements (PREs) present in control regions of progesterone responsive genes [Tsai and O'Malley, 1994].

The budding yeast *Saccharomyces cerevisiae* possesses the genetic background which is necessary for the functional expression of heterologous genes [Hinnen et al., 1995]. This yeast provides a well-suited model organism to study the mechanism of the PR function independent from the complexity in mammalian cells [Imhof and McDonnell, 1996; Oñate et al., 1995; Shemshedeni et al., 1992; Xu et al., 1996]. Both, the chicken and the human PR are functionally active in yeast and induce ligand-dependent transcription through binding to PREs located in promoter regions of reporter genes [Mak et

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al., 1989; Meyer, et al., 1992; Poletti et al., 1992; Nawaz et al., 1994]. In these studies, monitoring of the PR activity was dependent on the binding of the ligand-occupied receptor to PRE sequences.

In order to study the transcription activating properties of the PR independently from the binding to its PREs, we generated a hybrid protein composed of the PR fused to the DNA-binding domain of the yeast transcription factor Gal4 (Gal4BD). This fusion protein interacted through the Gal4 moiety with a GAL1 upstream activating sequence (UAS) and induced the transcription of the lacZ reporter gene through the transactivating functions of the PR in the presence of receptor specific ligands. A similar response to PR agonists was observed by a different PRE-based transactivation assay in CV-1 cells and by in vitro binding studies using rabbit uterus cytosol.

In order to distinguish between nonspecific metabolic inhibitory effects of compounds and specific receptor mediated antiprogestogenic actions we developed secondary dual reporter systems in both yeast and CV-1 cells. These assay strains contain additional PR-independent reporter systems (the green fluorescent protein from *Aequorea victoria* and luciferase from *Renilla reniformis*, respectively) reflecting the vitality state of the cells by a simple additional fluorimetric or luminometric read-out.

MATERIALS AND METHODS

Compounds

Promegestone (R5020) was purchased from NEN Life Science Products (Boston, MA) and Mifepristone (RU486) from SIGMA (Deisenhofen, Germany). Onapristone was provided by the Jenapharm GmbH & Co. KG, Jena. Other steroids used were obtained from the steroid collection of the Hans-Knöll-Institut für Naturstoff-Forschung, Jena. All steroids were dissolved in ethanol (Uvasol 99.9%, Merck, Darmstadt, Germany).

Construction of Plasmids

Manipulation of DNA was carried out by using standard procedures [Ausubel et al., 1995]. To allow an in-frame fusion of the human PR to the yeast Gal4BD the synthetic polylinker composed of *Eco*RI, *Sal*I, *Bgl*II, *Mlu*I, *Kpn*I, *Bam*HI, and *Pst*I sites, 5′-AATTCGTCGACAGATCTACGCGTGGTACCGGATCCCTGCA-3′, and 5′-GGGATCCGGTACCACGCGTAGATCTGTCGACG-3′ was inserted between the *Eco*RI and *Pst*I sites of pGBT9 (Clontech Laboratories, Palo Alto, CA) yielding pTM152. The plasmid pRB1 was constructed by cloning the *Bam*HI released 3,7 kbp fragment of pGEM-4Z which contains the whole cDNA of the human PR B-form cDNA including parts of the 5′ and 3′ non-coding regions into the *Bam*HI site of pTM152. The plasmid pGEM-4Z was kindly provided by M. Misrahi, Strasbourg, France [Misrahi et al., 1987]. The Gal4BD and the 24th residue (Gly) of the human PR in pRB1 were spaced by Val-Asp-Arg-Ser-Thr-Arg-Gly-Thr.

The integrative vector pTY99 for expression of the green fluorescent protein (GFP) in yeast under control of the CUP1 promoter was constructed as follows: A 474 bp fragment containing the yeast CUP1 promoter was amplified by the polymerase chain reaction (PCR) using primer pairs 5′-CCGACGTCAAGCCGATCCCATTACCGAC-3′ and 5′-CGGGATCCTTCGCTGAACATTTTATGT-3′ and yeast genomic DNA as a template, digested with *Aat*II/*Bam*HI, and cloned between the respective sites of pGAD424 (Clontech Laboratories) yielding pTY35. Subsequently, the GFP-cycle 3 mutant gene (719 bp) was PCR amplified with primers 5′-GCAGATCTATGGCTAGCAAAGGAGAAG-3′ and 5′-GAAGATCTTTATTTGTAGAGCTCATCCATG-3′ from the template pBAD-GFP (Affimax, Palo Alto, CA) [Crameri et al., 1996], digested with *Bgl*II, and inserted into the *Bgl*II site of pTY35. The restriction sites within the primers are underlined. The GFP is expressed as a fusion between the first 4 residues of CUP1 followed by a spacer of two residues (Gly and Ser) and the entire GFP sequence. All products of PCR amplified DNA fragments were verified by sequencing using the LI-COR system.

The expression plasmid phPR1 used to establish the PR-reporter system in higher eukaryotic cells was a generous gift from P. Chambon and H. Gronemeyer, Strasbourg, France. It contains the coding sequence of the human PR B-form as an entire open reading frame. Transient expression in higher cells was driven from the SV40 early promoter [Kastner et al., 1990]. The pAHluc plasmid contains sequences of the mouse mammary tumor virus promoter (MMTV) taken as an 1265 bp *Ava*I to *Hpa*II fragment from the genome of the C3H strain of MMTV cut from the vector pAH as a *Bam*HI fragment and inserted into the *Bgl*II site of

pXP2 (a promoterless vector containing the luciferase cDNA from *Photinus pyralis*). The pAHluc was kindly provided by K. Nordeen [Nordeen, 1988]. As an internal control in the transient transfection assays pRL-SV40 (Promega, Madison, WI) was used which consists of the simian virus 40 promoter driving the expression of the luciferase gene from *Renilla reniformis*.

Yeast Strains and Transformation

The *S. cerevisiae* strain used throughout in this study was SFY526 (*MAT***a** *ura3–52 his3–200 ade2–101 lys2–801 trp1–901 leu2–3,-112 can*^{r} *gal4–542 gal80–538* URA3:GAL1-lacZ; Clontech Laboratories). Transformation of pRB1 into strain SFY526 was performed by the method of Klebe and co-workers which gave rise to strain BPY3 [Klebe et al., 1983]. Yeast transformants were selected and cultivated on SD synthetic medium consisting of 2% glucose, and 0.67% yeast nitrogen base without amino acids (Difco Laboratories, Detroit, MI) supplemented with the respective essential amino acids (Sigma). The GFP reporter plasmid pTY99 was linearized with *Bst*EII and integrated into the *leu2* locus of SFY526, which gave rise to strain ITH9. Subsequently, ITH9 was transformed with pRB1, resulting in test strain ITH10.

β-Galactosidase Assay in Yeast

In order to examine progesterone receptor mediated activation of transcription strain BPY3 cells were cultivated overnight in liquid selective SD medium and diluted to OD_{600} = 1.0. The pH-value was adjusted to 7.5 with 1N NaOH. 50 µl of this cell suspension were transferred into microplate wells each containing 50 µl of SD medium and the model steroids in different concentrations. By examining antagonists, R5020 was added simultaneously to a final concentration of 10^{-8} M in order to induce the reporter gene expression. The total concentration of ethanol in the cultured cells ranged between 1% (v/v) and 2% (v/v). Controls contained the same amount of solvent. After incubation for 3 h at 30°C 25 µl lysis buffer consisting of 7.5×10^{-5} M Tris-HCl, pH 7.5 (Serva, Heidelberg, Germany), 3.2×10^{-4} M β-mercaptoethanol (Sigma), 0.6% (v/v) Triton-X-100 (Serva), and 7.5 mg/ml *o*-nitrophenyl-β-D-galactopyranoside (ONPG; Sigma) were added to each well followed by a further incubation period of 90 min at 37°C. The reaction was terminated by the addition of 75 µl 1 M Na_2CO_3-solution (Serva) per well and the β-galactosidase activity was monitored spectrophotometrically by measuring the concentration of *o*-nitrophenol at 420 nm using a microplate reader (Spectramax 340, Molecular Devices, Ismaning, Germany). The ED_{50}-values of the model compounds were calculated as the concentrations which cause half of the highest progesterone effect. The IC_{50} values of the model antiprogestins were determined as the concentrations which reduce the reference value of 10^{-8} M R5020 to 50%.

Green Fluorescent Protein Assay in Yeast

The strain ITH10 was cultivated and handled as described for the β-galactosidase assay in opaque deep well plates (Sero-Wel, Bibby Sterilin Ltd, Stone, Staffs, UK) without a prior adjustment of the pH-value. Sterile aqueous $CuSO_4$-solution (Serva) was added to give a final concentration of 10^{-4} M. The basic fluorescence signal of the cells was determined at an emission wavelength of 509 nm (λ_{exc} = 395 nm) in quadruplicates using the multilabel counter Victor 1420 (Wallac-ADL-GmbH, Freiburg, Germany). After a 3-h incubation period at 30°C the measurement was repeated and the quotient of the 3-h fluorescence signal and that of the basic signal was calculated for each compound and for the reference cells. The inhibitory effect of each compound was determined by comparing these quotients.

Cell Culture and Luciferase Assay

Cells were grown at 37°C in a humidified atmosphere of air/CO_2 (95%:5%). African green monkey kidney CV-1 cells were cultured in Dulbecco's modified Eagle medium (Gibco BRL, Grand Island, NY) supplemented with 10% fetal calf serum (Serva), penicillin (100 U/ml), and streptomycin (100 µg/ml). Cells were routinely passaged 1 day before transfection (3×10^6 cells/T175, 50–80% confluent). The transient transfection assays were carried out by the calcium phosphate co-precipitation procedure in T175 flasks (Becton Dickinson, Heidelberg, Germany) with 23.3 µg indicator plasmid pAHluc and 2.33 µg receptor expression vector phPR1. Additionally, 0.23 µg pRL-SV40 was co-transfected as an internal control. Immediately after transfection and a 2 min treatment with 15% glycerol in PBS the cells were trypsi-

nated and reseeded into white opaque 96-well tissue culture plates (Falcon, 4–5 × 10^4 cells/well). The experimental medium was similar, but containing no phenol red and only 5% fetal calf serum (DCS) twice treated with dextran/charcoal. After 1–2 h hormones were added to the cells up to a final ethanol volume of 0.2%. Control cells received the same amount of vehicle. After 18–20 h incubation with the hormones the cells were washed with phosphate-buffered saline (pH 7.4). To each well 20 µl passive lysis buffer (Promega) was added. After 20 min lysis at room temperature the Promega assay solution was added and luminescence (in relative light units, RLU) was analyzed in quadruplicates using a Luminoscan Type 391 (Labsystems, Helsinki, Finland) according to the dual luciferase™ reporter assay protocol (Promega) within 30 sec.

Progesterone Receptor Binding Assay

Progesterone receptor binding affinity was measured by competitive binding of the ^{3}H-labelled tracer ORG2058 and the substances to be tested to receptors in uterus cytosol of estradiol primed juvenile rabbits [Sobek et al., 1994]. The buffer consisted of 2 × 10^{-2} M Tris-HCl (pH 7.4), 10^{-3} M EDTA, 2 × 10^{-3} M DTT, and 250 mM saccharose. The incubation was carried out for 18 h at 0–4°C. Separation of free and bound steroid was achieved by 1% charcoal/0.1% dextrane treatment. The molar IC_{50} values were evaluated for the substances and for the reference compound progesterone from various concentrations. The relative binding affinities (RBA) were calculated as quotients of the IC_{50} of progesterone and that of the substances (× 100%).

RESULTS

Development and Validation of the Progesterone Receptor Based One-Hybrid Assay in *S. cerevisiae*

For the functional expression of the human PR in yeast a one-hybrid system was constructed as described in materials and methods. The PR lacking the first 23 residues was tagged aminoterminally to the DNA-binding domain of the yeast transcription factor Gal4 (Gal4BD). The expression of this fusion protein in *S. cerevisiae* was driven from the alcohol dehydrogenase promoter present on the multicopy vector pGBT9. As a host for monitoring the Gal4BD-PR activity we used the yeast strain SFY526. This strain carries a genomic copy of a GAL1 promoter-lacZ reporter construct containing binding sites for the Gal4BD moiety of the hybrid protein. The assay principle is summarized in Figure 1.

In order to examine whether the Gal4BD-PR fusion protein is functionally active, the yeast strain BPY3 was incubated with different concentrations of progesterone, which is the natural progestin of mammalian cells, and of syn-

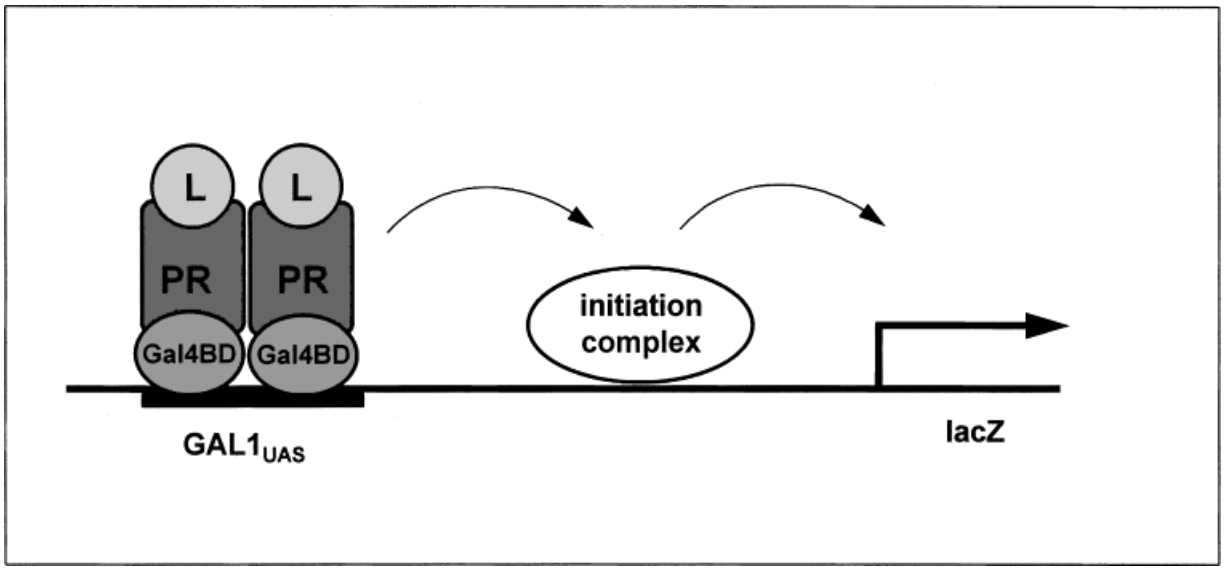

Fig. 1. Schematic illustration of the progesterone receptor one-hybrid assay in yeast. L, ligand; PR, progesterone receptor; Gal4BD, Gal4 DNA-binding domain; GAL1 $_{UAS}$, GAL1 upstream activation sequence; lacZ, gene encoding β-galactosidase.

thetic model steroids with different affinities to the progesterone receptor. The structures of the model progestins and antiprogestins used in this study are shown in Figure 2. Their relative binding affinities to the progesterone receptor in rabbit uterus cytosol are summarized in Table I. Gestodene, levonorgestrel, as well as R5020, possessed a much higher affinity to the PR compared to progesterone itself, whereas the relative binding affinity of dienogest was only about 10%. From the two antiprogestins tested RU486 showed a high, and onapristone only a weak affinity to the PR in this binding assay. These data are in good accordance with results published earlier [Cato et al, 1987, Sobek et al., 1994, Edwards et al., 1995].

Increasing concentrations of progesterone as well as of the synthetic model progestins up to 1 μM also increased the β-galactosidase activity in the yeast assay. Furthermore, compounds with a high affinity to the progesterone receptor, like gestodene ($ED_{50} < 10^{-9}$ M), levonorgestrel ($ED_{50} < 10^{-9}$ M), and promegestone ($ED_{50} = 2{,}5 \times 10^{-9}$ M), led to a higher β-galactosidase

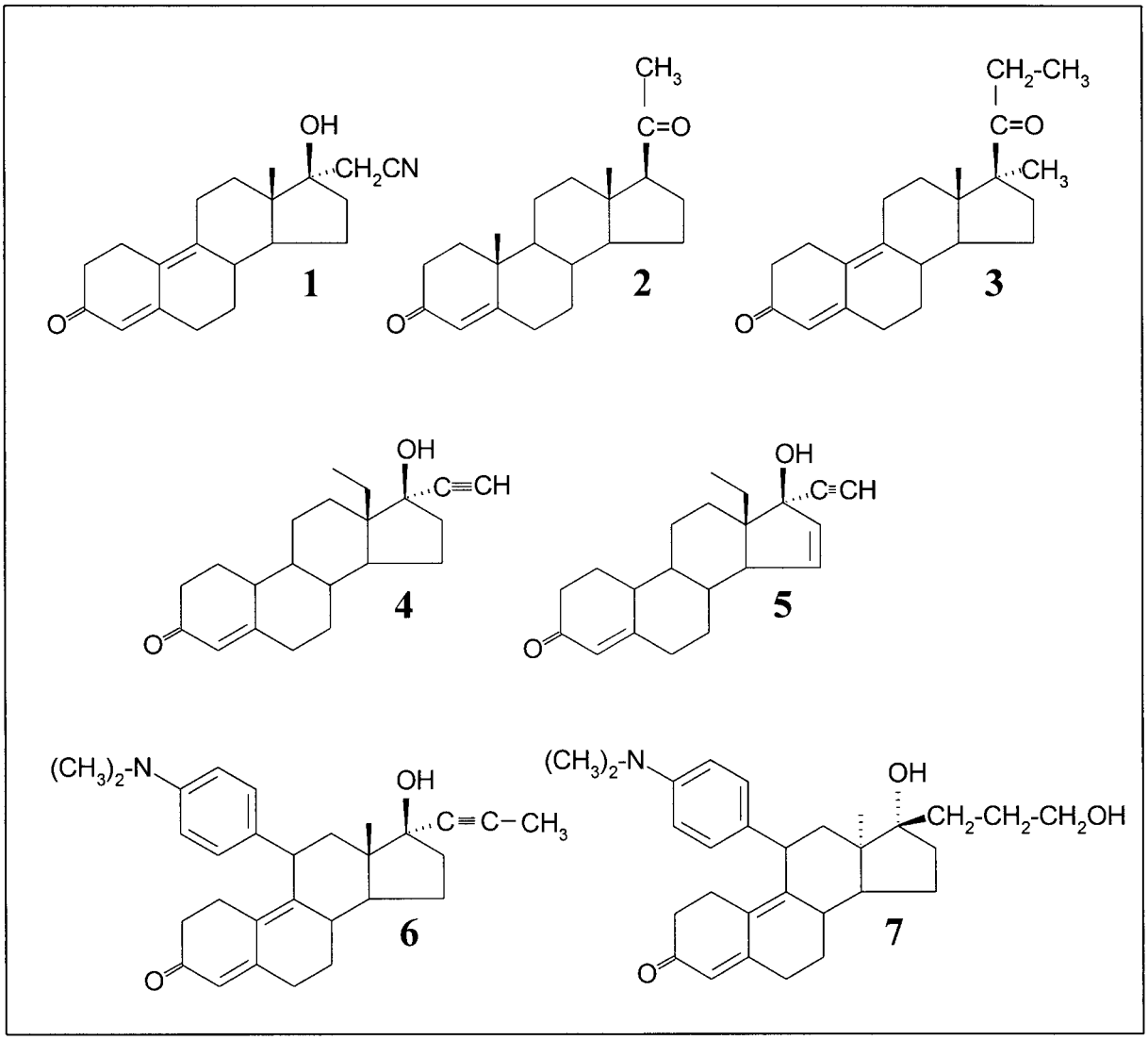

Fig. 2. Structural formulas of the progestins (1–5) and antiprogestins (6,7) used in our studies: **1:** Dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9-dien-3-one); **2:** Progesterone (4-pregnene-3,20-dione); **3:** Promegestone (R5020; 17α,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione); **4:** Levonorgestrel (18,19-dinor-13β-ethyl-17β-hydroxy-4-pregnen-20-yn-3-one); **5:** Gestodene (18,19-dinor-13β-ethyl-17β-hydroxypregna-4,15-dien-20-yn-3-one); **6:** Mifepristone (RU486, 11β-(4-dimethylaminophenyl)-17α-(1-propinyl)-17β-hydroxy-estra-4,9-dien-3-one); **7:** Onapristone (ONA, 11β-(4-dimethylaminophenyl)-17α-hydroxy-17β-(3-hydroxypropyl)-13α-methyl-gona-4,9-dien-3-one).

activity in equivalent concentrations, whereas compounds possessing a lower receptor affinity, like dienogest ($ED_{50} = 10^{-6}$ M), caused a reduced lacZ gene expression compared to that of progesterone ($ED_{50} = 3 \times 10^{-8}$ M). These results indicated that the cells responded in both a dose and affinity dependent manner to the model progestins by induction of the lacZ gene expression (see Fig. 3). Since the GAL1 promoter of the reporter construct is devoid of specific PRE sequences, DNA-binding of the hybrid protein obviously is mediated by its Gal4 DNA-binding domain. In the presence of specific ligands the transcription of the reporter gene is initiated through the activation domains AF1 and AF2 present in the PR in dependence of the ligand concentration and the ligand affinity.

In order to check whether the Gal4BD-PR fusion protein responds to compounds with antiprogestogenic activity, the yeast cells were incubated with 10^{-8} M of the progestin R5020 together with increasing amounts of either RU486 or onapristone. Treatment with RU486 resulted in a concentration dependent decrease of the β-galactosidase activity (see Fig. 4). The IC_{50} value was calculated to be about 3.5×10^{-7} M. Onapristone caused a weak inhibitory effect but only in a concentration of at least 10^{-6} M. These data corresponded well with the relative binding affinities of these compounds to the PR determined in vitro (see Table I).

TABLE I. Progesterone Receptor Binding: Relative Binding Affinities (RBA) of Model Progestins and Antiprogestins in Rabbit Uterus Cytosol

Competitor	RBA [%]	±SD	(n)
Progesterone	=100		
Dienogest	10.5	±2.5	(10)
Gestodene	880	±160	(9)
Levonorgestrel	670	±80	(6)
R5020	730	±100	(6)
RU486	500	±130	(10)
Onapristone	21	±4	(11)

Dual Reporter System for the Specification of Inhibitory Effects in Yeast

A decrease of the β-galactosidase activity in BPY3 cells after treatment with compounds may point to a specific PR antagonistic effect, but it can be caused also by a nonspecific inhibitory effect of these compounds on the yeast metabolism. To distinguish between receptor-mediated antihormonal effects and a nonspecific metabolic inhibition we generated a second PR-independent reporter system demonstrat-

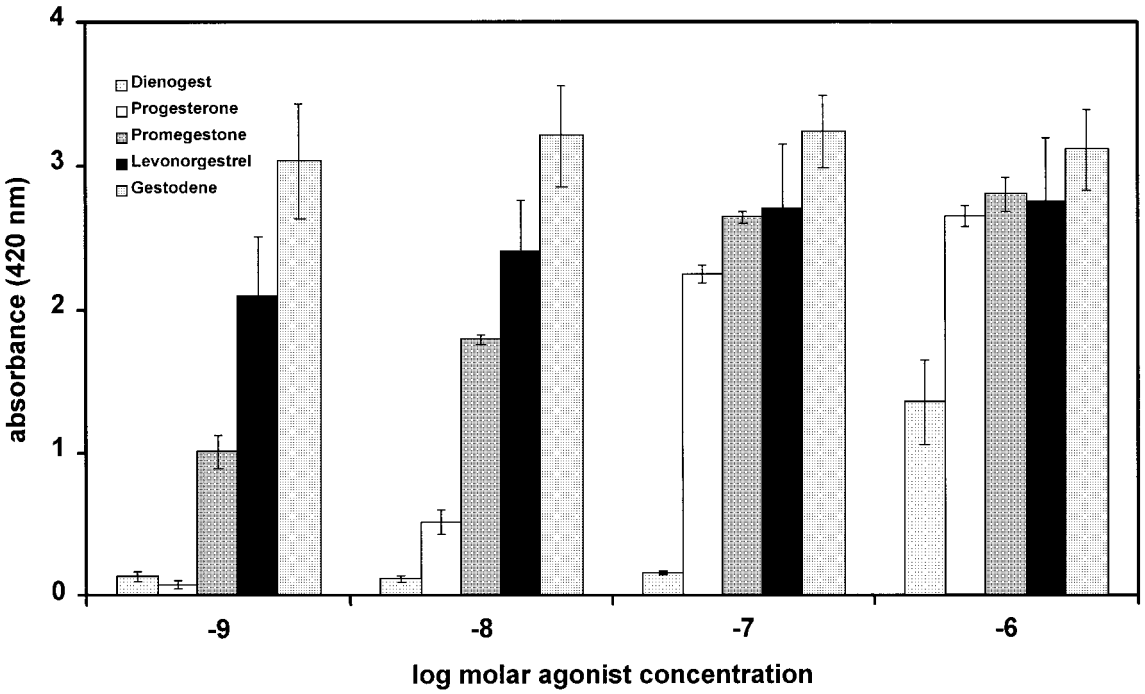

Fig. 3. Modulation of the β-galactosidase expression in yeast strain BPY3 by different progestins in a concentration- and affinity-dependent manner. Values are given in absorbance units of the product of the β-galactosidase reaction at 420 nm and are expressed in means ± S.D. of four to eight parallel measurements.

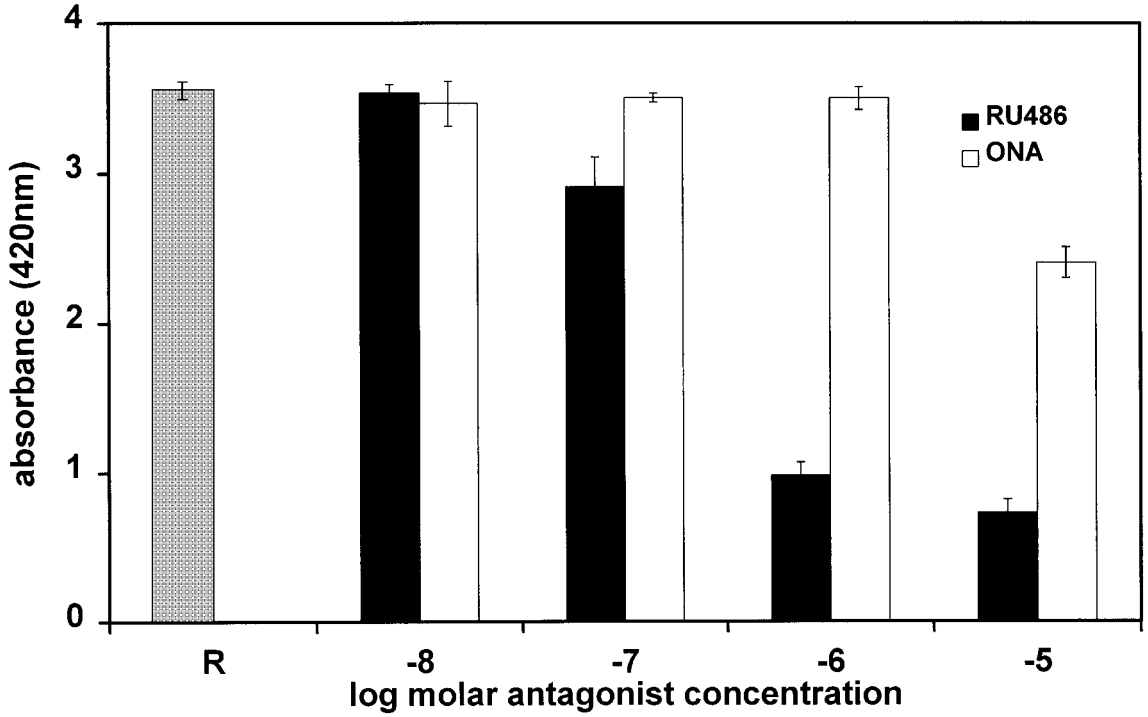

Fig. 4. Modulation of the β-galactosidase expression in *S. cerevisiae* BPY3 by different antiprogestins. The yeast cells were cultivated with 10 nmol/l of the progestin R5020 resulting in the reference value R and, in addition, with increasing amounts of either the antiprogestin RU486 or onapristone (ONA). Means and S.D. of four to eight parallel measurements are indicated.

ing the cell viability. We fused the coding sequence of the green fluorescent protein (GFP) to the yeast CUP1 promoter containing no PRE sequences. The expression of GFP in this construct was initiated by the addition of Cu^{2+}-ions. The GFP activity can be detected in intact yeast cells without cell lysis [Niedenthal et al., 1996]. The CUP1-GFP construct was integrated into the genome of strain BPY3 yielding the test strain ITH10. Cells were incubated with 10^{-8} M R5020 together with various compounds and Cu^{2+}-ions. The fluorescent signals were measured after 3 h. The common growth inhibitors amphotericin B and cycloheximide inhibited the increase of the fluorescence signal in a dose dependent manner resulting in a complete inhibition at a concentration of 10^{-6} M (data not shown). Remarkably, the PR-independent GFP expression was not significantly influenced by the antiprogestins RU486 and onapristone compared to the GFP activity of yeast cells cultivated without steroids during the incubation period (see Fig. 5). These data showed that an nonspecific inhibitory effect of the antiprogestins tested on the yeast metabolism can be excluded. For verification purposes the data were compared with those obtained by PR-dependent and PR-independent transcriptional activation assays in mammalian cells.

Dual Reporter System for the Specification of Inhibitory Effects in Mammalian Cells

In order to exclude interactions with other steroid hormone receptors the kidney cell line CV-1 from the African green monkey devoid of endogenous steroid receptors was chosen for transient transfection experiments [Fuhrmann et al., 1992]. For the detection of nonspecific metabolic effects of antihormones a dual luciferase reporter assay system was used in transient transfections. In this assay three plasmids were co-transfected into CV-1 cells containing (i) the firefly luciferase fused to the MMTV promoter containing a PRE for the progestin induction, (ii) the cDNA of the human PR gene, and (iii) the luciferase of *R. reniformis* as an internal control. The expression of the latter two genes was driven by the simian virus 40 promoter lacking PRE sequences.

R5020 induces firefly luciferase activity half maximally in the range of $5–10 \times 10^{-11}$ M (data from 6 experiments, not shown). This induction

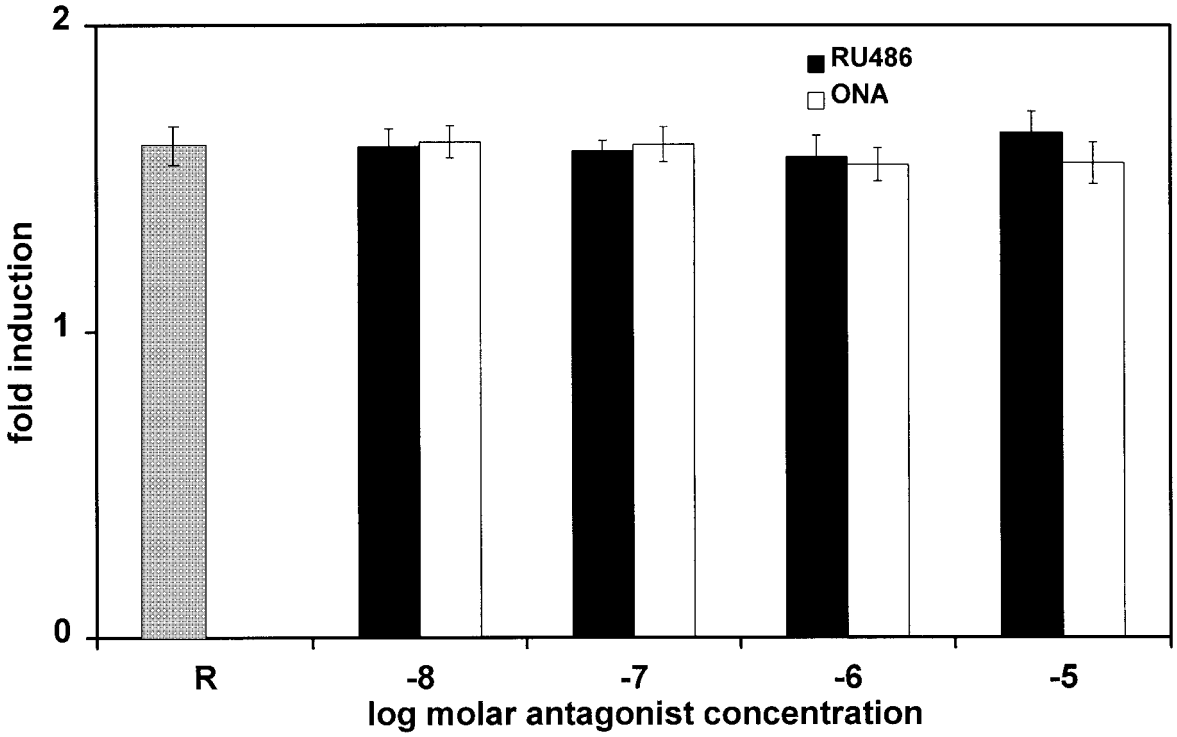

Fig. 5. Influence of antiprogestins on the copper-dependent GFP expression in the yeast reporter strain ITH10. The GFP activity is expressed as the quotient from the fluorescence signal of Cu^{2+} treated cells after the incubation time of 3 h and the basic fluorescence signal at the beginning of the incubation period. The reference value R results from the fluorescence signals obtained from cells which were incubated without antiprogestins. The means and S.D. of four to eight parallels are shown.

could be inhibited in a concentration dependent manner by the antiprogestins RU486 and onapristone. The firefly luciferase activity induced by 10^{-10} M R5020 was 314 ± 26 fold (n = 4) of the control and was more than 90% abrogated by an equimolar concentration of RU486 with an IC_{50} value of about 7×10^{-12} M (Fig. 6B). Onapristone showed such a comparable inhibition of firefly luciferase induction under the same experimental conditions only at a 100-fold higher concentration (10^{-8} M, $IC_{50} = 4 \times 10^{-10}$ M). RU486 or onapristone applied as a single compound failed to display any PR-agonistic properties at concentrations up to 10^{-6} M in this cellular system (data not shown). These data reflect the different in vitro binding affinities of RU486 and onapristone to the PR (see Table I) and correspond to the results obtained with the PR-based yeast one-hybrid assay. In analogy to the yeast system, the tested antiprogestins did not influence the PRE-independent expression of the second luciferase reporter from *R. reniformis* (Fig. 6A), whereas amphotericin B and cycloheximide induced a significant reduction of the luminescent signal (data not shown).

DISCUSSION

In this study we describe the construction and validation of assays for assessing progesterone receptor modulators in both, yeast and mammalian cells. It is well known that *S. cerevisiae* is well suited for the establishment of whole cell assays with heterologous target proteins [Kirsch, 1993]. Both human and chicken PR have been successfully expressed in yeast [Mak et al., 1989; Poletti et al., 1992; Jin et al., 1997]. Monitoring of the receptor activity in these systems was dependent on the binding of the PR to PRE consensus sequences present in the reporter construct. These idealized sequences are additionally recognized by other members of the steroid receptor family such as the glucocorticoid and androgen receptor and therefore can be considered as non-PR specific [Tsai and O'Malley, 1994]. In order to uncouple the PR binding activity to these response elements we constructed a one-hybrid system by fusion of

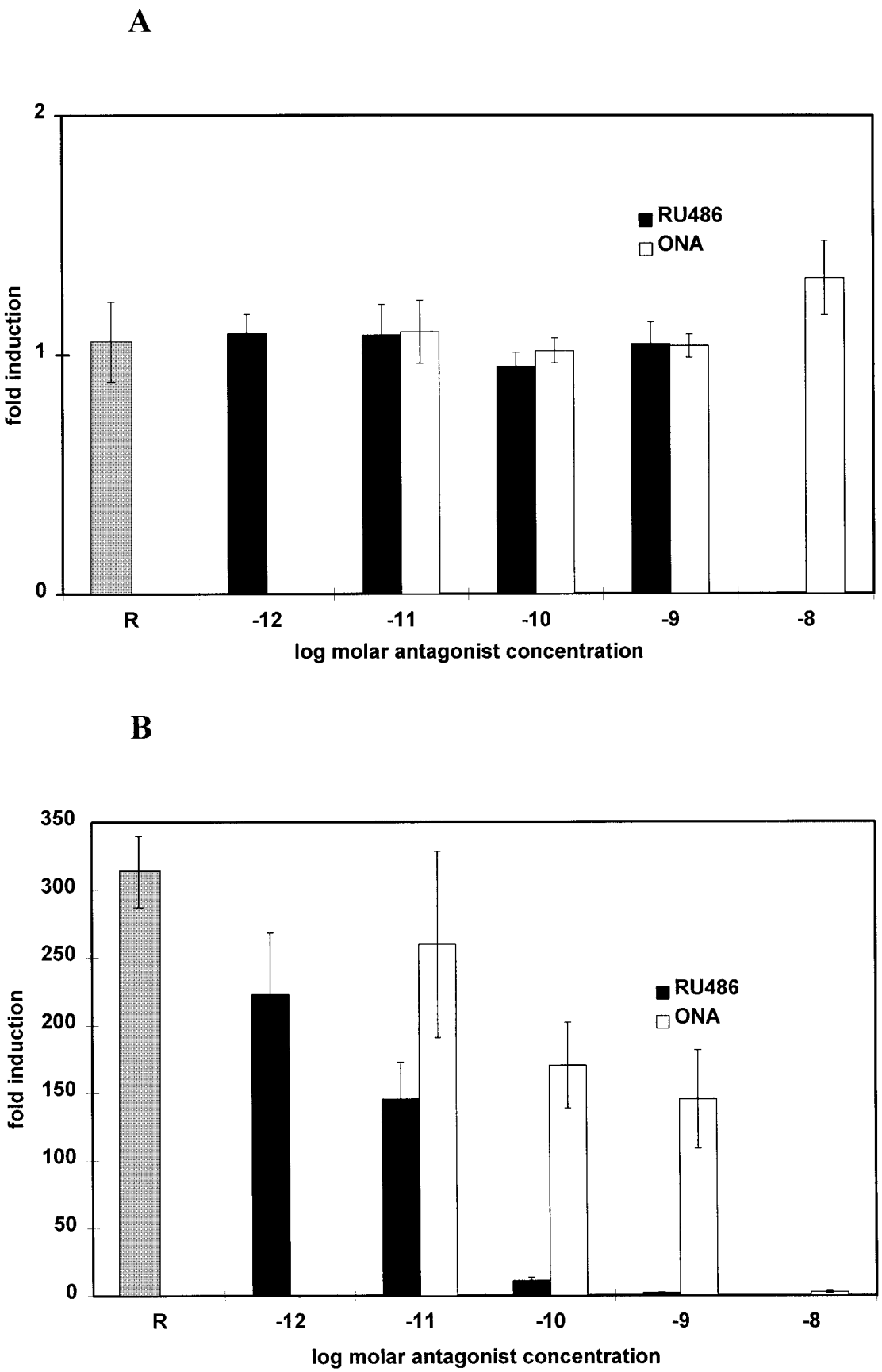

Fig. 6. Modulation of luciferase expression in CV-1 cells by progesterone antagonists; African green monkey kidney CV-1 cells, transiently transfected with the human PR, pAHluc containing the MMTV promoter and the control vector pRL-SV40, were cultivated with 100 pmol/l of the progestin R5020 together with increasing amounts of either the antiprogestin RU486 or onapristone (ONA). **A:** Luciferase activity of the luciferase reporter from *R. reniformis* (PRE independent). **B:** Normalized luciferase activity (*P. pyralis* / *R. reniformis*). Values are means ± S.D. (n = 4).

the DNA-binding domain of the yeast transcriptional activator Gal4 to the PR. The detection of the activity of the hormone stimulated PR fusion protein is obtained by the GAL1 promoter lacking PRE sequences but containing binding sites for the Gal4 moiety of the hybrid protein. Therefore, this assay allows the study of the effects of compounds on the PR activity independently from binding of the receptor to PRE sequences. The Gal4BD in our one-hybrid approach was tagged to the PR lacking the first 23 amino acids. Obviously, this amino-terminal truncation did not influence the response of the PR to the model progestins and antiprogestins.

Our quantitative analysis with various model gestagens and antigestagens showed that the Gal4BD-PR hybrid in yeast responded like the native B-form of the PR to progestogenic compounds in a concentration and affinity dependent manner. Remarkably, the Gal4BD-PR hybrid induced transcription to a more than 10-fold lower excess when tested together with a PRE-lacZ reporter construct (kindly provided by D. Picard) [Schena et al., 1991] as compared to the GAL1 promoter mediated transcription of the lacZ reporter in yeast (data not shown). Although we cannot exclude the possibility that the fusion protein did not interact properly with the PRE sequences, we propose that the PR-based one-hybrid assay is more sensitive in monitoring PR activity. Recently, an analogous study using a fusion of the estrogen receptor to Gal4BD has been described [Bush et al., 1996]. Since it is known that DNA-binding and transactivation domains of many transcription factors are functionally exchangeable [Brent and Ptashne, 1985], these systems can be considered as a general method for assessing steroid hormone receptor activity in yeast.

The assessment of inhibitory drugs to specific targets in cellular in vivo systems usually suffers from the fact that nonspecific activities on the metabolism can not be distinguished from target specific interactions. The indirect estimation of antagonistic effects by measuring the inhibition of the progestin induced reporter gene expression requires a differentiation between specific receptor mediated antihormone actions and the potential nonspecific metabolic inhibitory effects of compounds. Therefore, different secondary reporter systems (GFP and *R. reniformis* luciferase) have been introduced into yeast and CV-1 reporter strains. The use of PR-dependent and PR-independent dual reporter systems allows the discrimination of PR-specific and PR-nonspecific effects by an easy-to-handle additional fluorimetric or luminometric analysis and therefore are useful tools for assessing progesterone receptor modulators. The results with the tested model progestins and antiprogestins corresponded to the results from receptor binding studies and reported data from mammalian cells [Cato et al., 1987, Sobek et al., 1994, Edwards et al., 1995].

It is possible to cultivate yeast cells inexpensively and safely under normal laboratory conditions. The described PR one-hybrid assay is fast, convenient and reliable. Furthermore, the short assay period allows a handling of the cells under semi-sterile conditions. However, the CV-1 cell based system is more sensitive, but also more time consuming and expensive and it needs more sterile handling. Taking together, the combination of both assay systems is a useful tool for assessing PR modulators.

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