Pergamon

0960-0760(95)00095-X

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 487–495, 1995

0960-0760/95 $9.50 + 0.00

Definition of the Critical Cellular Components Which Distinguish Between Hormone and Antihormone Activated Progesterone Receptor

David L. Clemm,[1] Bryan L. Macy,[1] Dolores Santiso-Mere[1] and Donald P. McDonnell[2]*

[1] *Department of Molecular Biology, Ligand Pharmaceuticals Inc., 9393 Towne Centre Drive, San Diego, CA 92121 and* [2] *Department of Pharmacology, Box 3813, Duke University Medical School, Durham, NC 27710, U.S.A.*

The steroid hormone progesterone is a key modulator of the cellular processes associated with the maintenance and development of female reproductive function. The biological activity of this hormone is mediated by specific nuclear receptors located in target cell nuclei which upon activation are capable of modulating the transcriptional activity of promoters containing progesterone response elements. Abnormalities in the progesterone receptor (PR) signal transduction pathway are implicated in pathological states such as breast cancer, endometriosis, and uterine fibroids. As a result of the medical need to modulate PR transcriptional activity, antiprogestins, compounds which oppose the actions of progesterone and novel progesterone receptor agonists, have been developed. This review outlines our current understanding of the critical cellular components which define the pharmacology of progesterone receptor agonists and antagonists, and how this information will impact the discovery and development of additional therapeutics.

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1–6, pp. 487–495, 1995

INTRODUCTION

Intracellular receptors (IRs) constitute a super-family of related proteins which mediate the nuclear effects of steroid hormones, thyroid hormone and the non-nutritional vitamins A and D [1]. The presence of a specific intracellular receptor defines that cell as a target for the cognate hormone. The mechanisms of action of IRs are related in that they remain latent in the cytoplasm or nuclei of target cells until they bind their cognate ligand [2, 3]. Interaction with hormone then induces a cascade of molecular events leading ultimately to an association of the activated receptor with specific regulatory elements within target genes. The resulting positive or negative effects of the bound receptor on the regulation of gene transcription are determined by the cell-type and promoter-context.

The number of diseases associated with inappropriate cellular responses to steroid hormones highlights the medical and biological importance of these effectors. The reproductive steroids estrogen, testosterone, and progesterone are implicated in a variety of hormone-dependent cancers of the breast [4], ovary [5], endometrium [6] and prostate [7]. In addition, the onset of post-menopausal osteoporosis is related to a decrease in production of estrogen [8]. Consequently, the regulation of IR function is of extreme pharmaceutical importance. This is clearly illustrated by the number of IR based drugs currently on the market or in development. Tamoxifen, a potent anti-estrogen, is widely used as adjuvant therapy in the treatment of breast cancer [9]. Currently, clinical trials are underway to study this drug's effectiveness as a prophylactic agent for breast cancer [10]. In addition, RU486 (Mifepristone), a potent anti-progestin, has found applications in the treatment of meningiomas [11], endometriosis [12], and as a postcoital contraceptive [13]. The anti-androgens cyproterone acetate and flutamide remain front-line treatments in the management of androgen-dependent prostatic cancer [14, 15].

In this review, we consider the latest information on the molecular mechanism of action of the human progesterone receptor (hPR) and how this information

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.
*Correspondence to D. P. McDonnell.

will impact the discovery and development of novel hPR modulators.

TWO DISTINCT ISOFORMS OF THE hPR EXIST IN TARGET CELLS

The human PR is unique among the steroid hormone receptors in that it occurs in target tissues as two distinct subtypes, hPR-A and hPR-B, of 94 and 114 kDa, respectively [16, 17]. The PR-B isoform contains an N-terminal fragment of 164 amino acids (B164) which is absent in the hPR-A isoform. It is likely that both forms can arise as a result of either alternative initiation of translation from the same mRNA or by transcription from different promoters within the same gene [18, 19]. Interestingly, Kastner *et al.* have identified two distinct promoters in the hPR gene. These promoters which regulate the synthesis of specific transcripts corresponding to hPR-A and hPR-B are regulated independently [19]. Two forms of PR, corresponding to hPR-A and hPR-B, have been identified in most species examined, the exception being the rabbit where PR may exist as a single unique B-subtype [20]. The specific roles for each of these two PR subtypes are unclear. However, the existence of elaborate mechanisms regulating their production and the observation that the ratio of these effectors varies among target tissues suggests that differential expression of hPR-A and hPR-B in target cells may be critical for the appropriate cellular response to progesterone [21–24]. Variations in the relative expression of hPR-A and hPR-B have been observed in the endometrium where hPR-A appears to be expressed constitutively whereas hPR-B is expressed during the mid-luteal phase only [23]. In addition, Brandon *et al.* have shown that the expression level of both PR isoforms is elevated in human uterine leiomyomas relative to adjacent myometrium [24], and that a high percentage of these benign tumors contain elevated levels of hPR-A relative to hPR-B. Thus, it is clear that both the absolute level of PR expression and the relative expression of the PR isoforms are differentially regulated in target tissues. The functional significance of these events remains to be determined.

hPR-A IS A CELL AND PROMOTER SPECIFIC REPRESSOR OF STEROID HORMONE RECEPTOR FUNCTION

The biochemical properties of the PR isoforms have been analyzed extensively *in vitro*. Both forms display similar DNA and hormone binding affinities [25, 26]. Yet, the precise role of hPR-A and -B in mediating cellular responsiveness to progesterone agonists and antagonists is unknown. In order to further define the functional differences exhibited by the PR isoforms, we created mammalian expression vectors which produced exclusively hPR-B or hPR-A [27]. The transcriptional activity of these receptors was assayed in several heterologous cell lines using different progesterone responsive promoters [27]. The details of this study have been published elsewhere; however, in summary, we have determined that hPR-B functioned as a hormone dependent positive regulator of all the progesterone responsive genes examined, whereas the transcriptional activity of hPR-A was very context restricted. Interestingly, we demonstrated that hPR-A functioned as a transdominant inhibitor of hPR-B function in contexts where it had no independent positive transcriptional activity. In addition, we observed that the transcriptional activity of GR, MR, AR and ER was regulated in transfected mammalian cells by the co-expression of hPR-A [27–29]. This activity of hPR-A was induced by both agonists and antagonists of PR. The modulatory activity of hPR-A was restricted to steroid hormone receptor activated transcription as it did not affect the ability of the vitamin D receptor to regulate its target genes nor did it affect the transcriptional activity of the SV40 or RSV promoters. The ability of hPR-A to modulate hER action is particularly interesting in view of the fact that ER and PR are frequently coexpressed in reproductive target tissues. Consequently, this novel action of hPR-A enables the antiprogestins RU486, ZK112993 or ZK98299 (which do not interact directly with ER), to function as potent antiestrogens in cells where hPR-A is expressed [28]. Thus, hPR-A may have a central role in regulating the transcriptional activity of ER and other steroid hormone receptors and undoubtedly plays a major role in determining the pharmacological actions of progestins and antiprogestins.

TRANSCRIPTIONAL ACTIVATION BY STEROID HORMONE RECEPTORS AND REPRESSION BY hPR-A MAY OCCUR THROUGH DISTINCT REGULATORY PATHWAYS WITHIN THE CELL

Recently, it has been observed that the antiprogestin RU486 demonstrates non-competitive antiestrogenic activity in the primate uterus [30, 31]. Specifically, it has been shown that RU486 can oppose the actions of estrogen in the uterus, but not the oviduct, of spayed monkeys in the absence of endogenous progesterone [31]. The mechanism of this activity is unknown, however, it raises the possibility that in addition to its role as an antiprogestin, some of the biological actions of RU486 may be related to its ability to function as an antiestrogen in some cell types. There is considerable interest in understanding the molecular mechanism of action of RU486 and how this relates to its effectiveness as a therapeutic agent for breast cancer, endometriosis and family planning [11–13, 32, 33]. As a consequence, we have focused on a definition of the role of RU486 and hPR-A in regulating hER action. It is anticipated that the information obtained in these studies will be enlightening with regard to the actions of hPR-A in

modulating other steroid receptors and will reveal additional modulatory activities of RU486 and other related antiprogestins.

We have performed a series of experiments to probe the mechanism by which hPR-A modulates hER function. To date, our results indicate that hPR-A; (1) does not heterodimerize with hER; (2) has no effect on the ability of hER to interact with DNA; (3) does not effect binding of estradiol to ER; and (4) does not alter the cellular expression of hER in transfected mammalian cells ([27]; and our unpublished results). Therefore, we postulate that inhibition of hER transcriptional activity may result as a consequence of hPR-A's ability to interfere with a distal step in the hER signal transduction pathway, possibly as a result of competition of the two receptors for a common cellular target protein.

We considered that if inhibition of hER function by hPR-A was due to competition for a common target protein then repression should be overcome by increasing the expression level of hER. Conversely, if inhibition was independent of hER expression level it would suggest that these two proteins mediate their biological effects through distinct target proteins. To address this issue, we assayed the transcriptional activity of different concentrations of expressed hER in the absence or presence of a constant amount of expressed hPR-A (a concentration which we had determined to be saturating for hPR-A mediated inhibition) (Fig. 1). In the absence of co-expressed hPR-A, progesterone [Fig. 1(A)], RU486 [Fig. 1(B)] or norethindrone [Fig. 1(C)] do not effect estradiol stimulated gene transcription, suggesting that these compounds do not antagonize ER function directly. When hPR-A was co-expressed in the target cells all three PR ligands functioned as ER antagonists. Increasing the level of expressed hER over a 10-fold range had little effect on hPR-A mediated inhibition of transcription. Therefore, the absolute level of hPR-A expressed within the cell is more important for hPR-A inhibitory activity than the ratio of expressed hPR-A and hER. This suggests that the action of hPR-A was not "competitive" where both receptors were competing for the same target but rather was "non-competitive". Thus, we feel that hPR-A and hER interact with distinct cellular targets, or alternatively, that they contact distinct sites on a common target.

Accessing currently available information, we have developed a working model to explain how hPR-A can act as a transcriptional repressor of hER (Fig. 2). Although alternative mechanisms are possible, this approach has facilitated our experimental design. Conceptually, we consider that the target proteins for hPR-A and the steroid receptors could be steroid receptor-specific transcription factors or adaptor proteins. Alternatively, they may be factors which are part of the general transcription machinery. Interestingly, intracellular hormone receptors have been shown *in vitro* to interact with the basal transcription factor TFIIB [34], although the functional significance of this interaction is unknown. In addition, several laboratories have shown that enhancer binding proteins may communicate with the general transcription machinery through interactions with TFIID. It has been shown also that TFIID is a multiprotein complex comprising the TATA box binding protein (TBP) and TBP associated proteins (TAFs) [35]. To date, at least eight

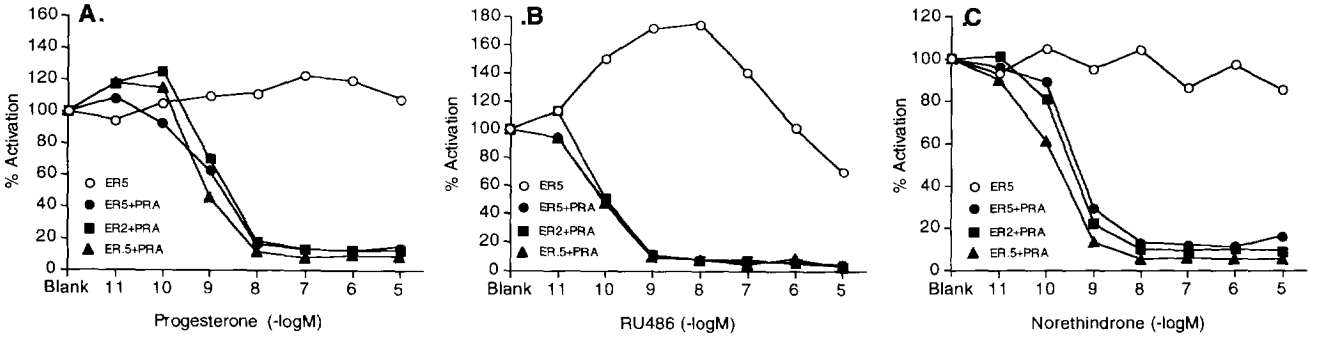

Fig. 1. Inhibition of hER transcriptional activity by hPR-A is independent of hER expression level. Monkey kidney CV-1 cells were transiently transfected with increasing concentrations of an hER expression plasmid (as indicated) alone or in the presence of a vector expressing hPR-A. The concentration of hPR-A expression vector (0.5 μg/ml) was shown previously to be maximal for hPR-A mediated repression of hER activity. Each transfection condition included an MMTV-ERE-LUC reporter plasmid (10 μg/ml) and the pCH110 (expressing b-galactosidase) plasmid as an internal control (5 μg/ml). The transriptional activity in these set-ups was measured following the addition of 10^{-7} M 17β-estradiol alone or estradiol in the presence of increasing concentrations of progesterone (A), RU486 (B), or norethindrone (C), as indicated. Following incubation, the cells were harvested, and luciferase and β-galactosidase activities were measured. The data are presented as % activation, where the 100% value represents the activity of hER in each condition in the absence of any added PR ligand. Each data point shown represents the average of triplicate determinations of the transcriptional activity under a given experimental condition and are representative of several individual experiments. The average coefficient of variation at each hormone concentration was <15% in this experiment.

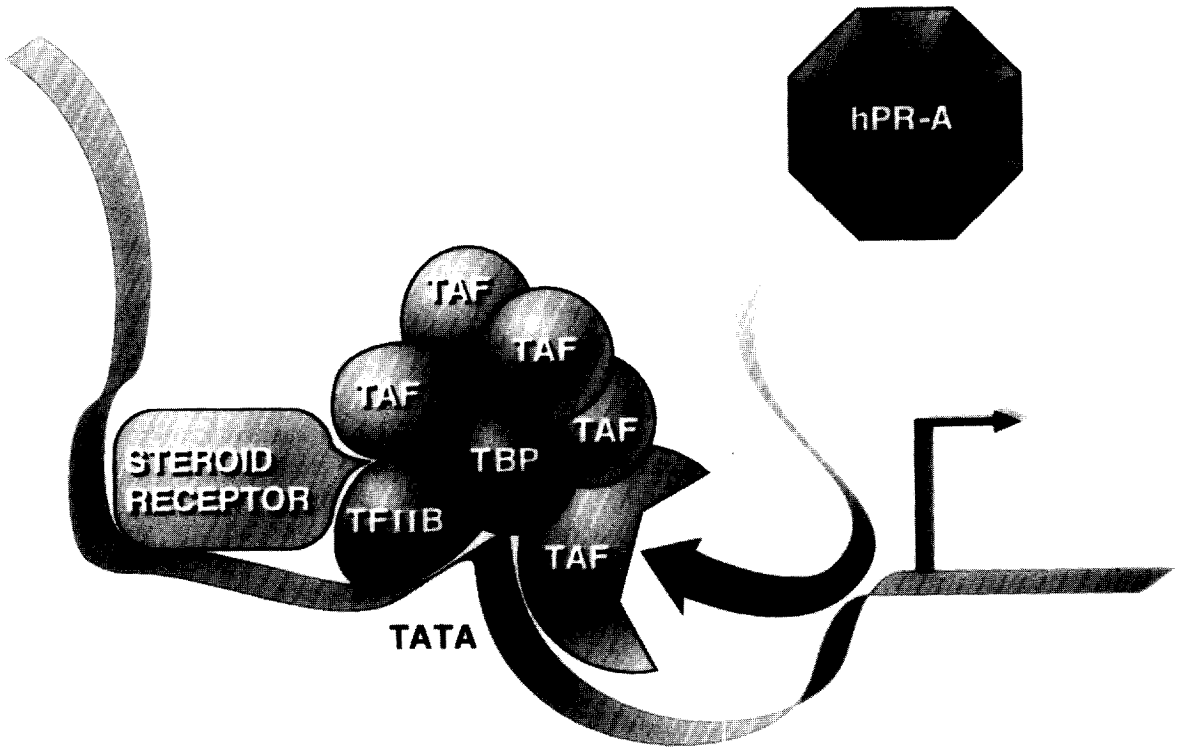

Fig. 2. The hPR A-form functions as a transdominant inhibitor of hER function. This schematic diagram outlines a hypothesis which would explain how hPR-A exerts an inhibitory effect on the transriptional activity of the steroid hormone receptors. Upon interaction with its cognate ligand, the steroid hormone receptor interacts with its specific response element within the promoters of target genes. The DNA bound receptor then interacts directly and/or indirectly with the general transcriptional machinery to facilitate the stabilization of the transcription pre-initiation complex and the enhancement of RNA polymerase activity [50]. A direct association of PR with the transcription factor TFIIB has been reported, supporting this hypothesis [34]. Recently, it has been shown that different TATA box protein associated factors (TAFs) mediate the interaction of the VP-16, Sp-1 and the hER protein with the transcription machinery [35, 36]. Similarly, it is possible that the different steroid receptors may contact the transcriptional machinery in different ways through these TAFs. Our model would predict that the interaction of hPR-A isoform of the hPR induces a conformatinal change in this protein such that it interacts with a target protein distinct from that required for the other steroid receptors. The consequence of this interaction is to block the transcriptional acitivity of the hormone activated steroid receptors. Conclusive proof of this model awaits direct biochemical evidence indicating that the cellular targets for hPR-A and the other steroid hormone receptors are distinct.

drosophila TAFs (250, 150, 110, 80, 60, 40, 30α and 30β) and a human TAF have been cloned and characterized [35, 36]. When assayed *in vitro*, it has been shown that $dTAF_{II}110$ permits a functional interaction of Sp1 with the general transcription machinery, $dTAF_{II}150$ permits transcriptional regulation by NTF-1 and $dTAF_{II}40$ contacts VP16 [35, 37, 38]. In view of the fact that steroid receptors contact the basal transcription apparatus *in vitro* [34], we consider that the transcriptional enhancement activities of the steroid hormone receptors and the inhibitory activity of hPR-A could possibly be mediated through interactions with different TAF proteins in the TFIID complex as detailed in our working hypothesis as outlined in Fig. 2.

ANTAGONISM OF PR FUNCTION

Several antiprogestins (compounds which oppose the actions of progesterone) have been developed and are currently being used for the treatment of a variety of endocrine-related disorders. Additionally, these compounds are useful tools with which to dissect the PR signal transduction pathway. All of the currently available antiprogestins interact directly with the hormone binding domain of PR and competitively inhibit progesterone binding. This event alone is insufficient, however, to completely block receptor activation as both agonists and antagonists of PR promote displacement of heat shock proteins, permit dimerization and facilitate association of the receptor with DNA [39]. Thus, it appears that antiprogestins must block PR mediated transcriptional activity at some step downstream of DNA binding. All but one antiprogestin, ZK98299 (Onapristone), appear to function in this way [40, 41]. The mechanism of action of ZK98299 is distinct in that it binds to receptor but does not promote the formation of a high affinity PR–DNA complex when assayed *in vitro*. One possible interpretation of this result is that ZK98299 prevents receptor

dimerization, a requisite step for DNA binding. This distinction has been challenged by recent data from Milgrom's laboratory which demonstrate that *in vitro*, at concentrations of ligand which saturate the receptor, ZK98299 is functionally identical to other antiprogestins [42]. Irrespective of these results, however, the current basis for classifying PR antagonists is whether they prevent (type I) or promote (type II) the association of receptor with DNA *in vitro* (using the nomenclature of Klein-Hitpass) [40].

In support of this *in vitro* classification, several groups have shown that in some cell and promoter contexts the pharmacology of PR antagonists can be altered by co-addition of cAMP analogs [43–45]. In particular, it has been shown that type II antiprogestins (but not type I) can function as PR agonists in the presence of 8-Br-cAMP (a cAMP analog) [43]. Additionally, it has been shown that DNA binding activity is required for these responses to cAMP. Thus, it appears that antagonist activated PR, associated with its DNA response element, is a target for cAMP stimulated processes which act on the receptor allowing it to activate transcription. It will be interesting to determine whether observed mechanistic differences displayed by antiprogestins *in vitro* are reflected by distinct biological activities *in vitro* [40].

AGONISTS AND ANTAGONISTS INDUCE DISTINCT ALTERATIONS IN PR STRUCTURE

An important clue to understanding how the cellular transcriptional machinery distinguishes between PR agonists and antagonists was provided by the elegant studies of Allan *et al.* [46]. By performing limited protease digestion of *in vitro* synthesized PR in the absence or presence of ligands, it was demonstrated that progesterone and RU486 induce distinct conformational changes within the receptor protein. Using specific monoclonal antibodies, this conformational change was shown to occur at the extreme carboxyl terminus of the receptor [27, 46]. Thus, the ability of the transcriptional machinery to distinguish between these agonist and antagonist induced structures may be critical determinants of the biological activity of these compounds. Using a similar approach, we have extended these analyses to examine other PR agonists and antagonists. The results are shown in Fig. 3. Using *in vitro* synthesized hPR-A, we confirmed that the unliganded receptor was extremely sensitive to treatment with trypsin (Fig. 3, lanes 2–4) whereas a specific 30 kDa fragment was protected when the synthetic PR agonist, R5020, was included in the incubations (Fig. 3, lanes 5–7). Digestion of the RU486–PR complex gave rise to a distinct 28 kDa protected fragment (Fig. 3, lanes 8–10). Interestingly, incubation with the antiprogestin ZK98299, afforded equal protection of both 30 and 28 kDa fragments. This latter digestion pattern, representing an "intermediate" between that obtained with either R5020 or RU486, may indicate a true mechanistic difference in the way ZK98299 interacts with PR. Alternatively, it may result as a consequence of this compound's low affinity for PR [42] (D. Mais, Ligand Pharmaceuticals Inc., unpublished results).

All of the antiprogestins tested thus far using the protease digestion assay are derived from 19-nortestosterone. Consequently, it was important to determine whether the distinct digestion patterns observed above reflect an activity of all antiprogestins, or whether they are related to the chemical class from which the antiprogestins were derived. Therefore, the protease digestion assay was performed on PR in the presence of norethynodrel and norethindrone, agonists which are derived from 19-nortestosterone. For comparative purposes, we analyzed the PR agonists 17α-hydroxyprogesterone and medroxyprogesterone acetate (derived from 17α-hydroxyprogesterone) in the same way. The results of this analysis are shown in Fig. 4. Interestingly, regardless of their chemical derivation, all 4 agonists afford protection from trypsin treatment of the identical 30 kDa receptor fragment, suggesting that the structure of PR in the presence of chemically distinct agonists is similar. This indicates that the protease digestion assay can be used to distinguish PR agonists from antagonists and, importantly, it functions in this manner independently of the chemical derivation of the

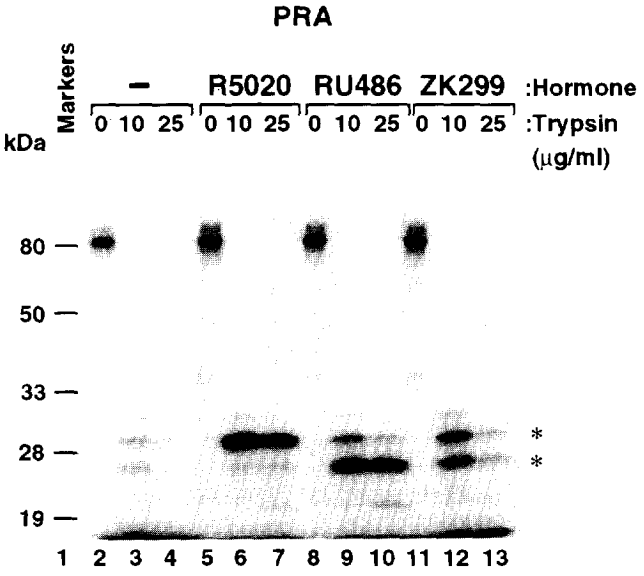

Fig. 3. PR agonists and antagonists induce distinct alterations in PR structure. Radiolabeled hPR-A was synthesized *in vitro* using a coupled rabbit-reticulocyte transcription–translation and [^{35}S]methionine. Subsequently, this receptor preparation was incubated with ethanol as a control (lanes 2–4), 1μ M R5020 (lanes 5–7), RU486 (lanes 8–10), or ZK98299 (lanes 11–13) for 20 min at room temperature. The complexes which formed were then subjected to digestion by trypsin for 10 min as indicated. The digestion products were analyzed by denaturing PAGE and visualized by autoradiography. The mobility of known molecular weight standards are indicated (lane 1). The predominant digestion resistant receptor fragments are indicated by asterisks.

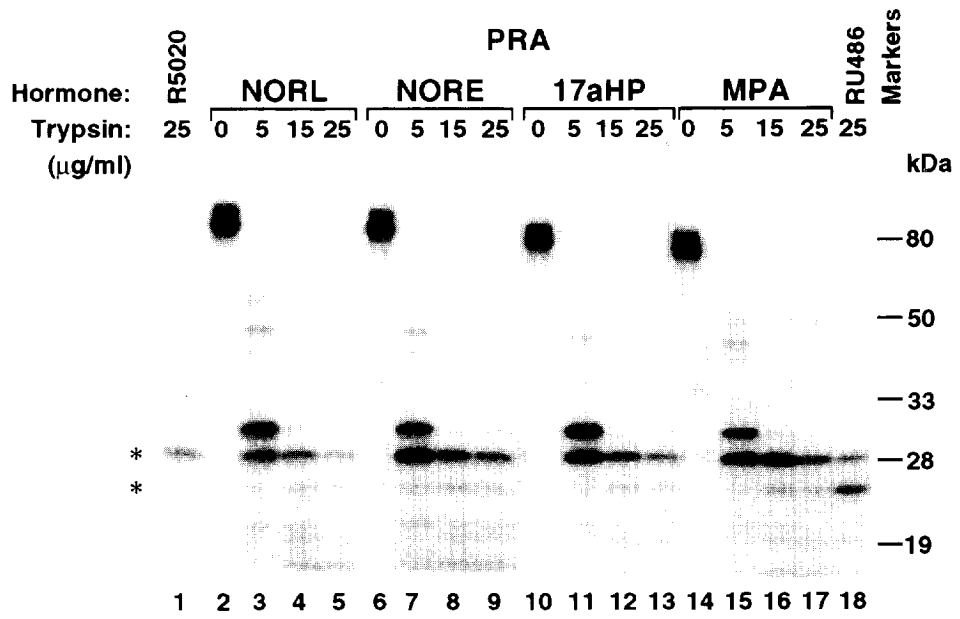

Fig. 4. Chemically distinct PR agonists induce similar alterations in PR structure. Radiolabeled hPR-A was synthesized *in vitro* using a coupled rabbit-reticulocyte transcription-translation and [^{35}S]methionine as indicated in Fig. 3. The radiolabeled receptor was incubated with either 1μM norethynodrel (NORL, lanes 2–5), norethindrone (NORE, lanes 6–9), 17αhydroxyprogesterone (17aHP, lanes 10–13), or medroxyprogesterone acetate (MPA, lanes 14–17) for 20 min at room temperature. The complexes which formed were then subjected to digestion by trypsin for 10 min as indicated. The digestion products were analyzed by denaturing PAGE and visualized by autoradiography. The products resulting from trypsin digestion of PR–R5020 and PR–RU486 complexes are included as controls. The mobility of known molecular weight standards are indicated. The predominant digestion resistant receptor fragments are indicated by asterisks.

compounds. These data firmly support the original hypothesis set forth by Allan *et al.* that receptor agonists and antagonists induce distinct structural alterations within PR [46], and suggests further that it is the ability of the cellular transcriptional machinery to recognize these distinct receptor conformations that determines agonist and antagonist efficacy.

ISOLATION OF RECEPTOR MUTANTS WHICH RESPOND TO PR ANTAGONISTS AS AGONISTS

Using a progesterone responsive transcription unit in yeast in which intact hPR-B is expressed, we sought to determine genetically the structural elements within PR which discriminate between agonist and

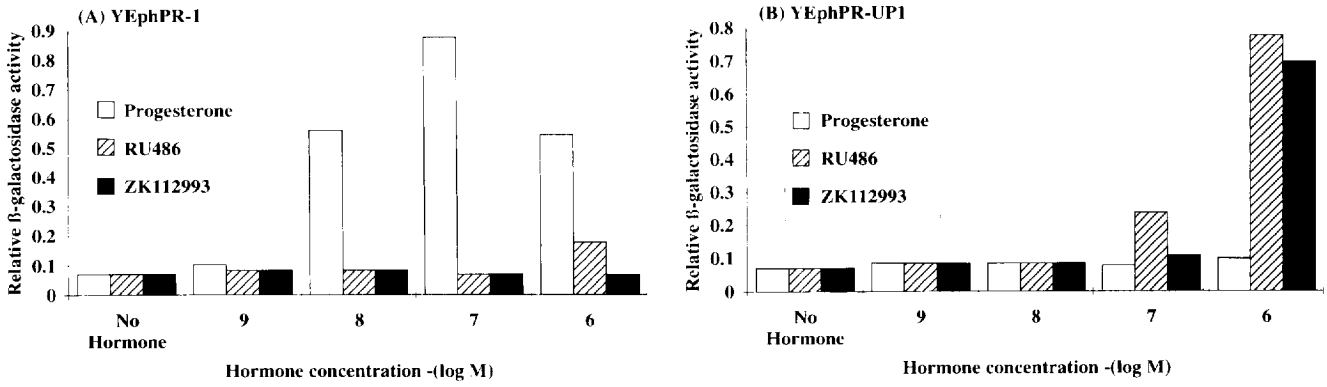

Fig. 5. Sequences within the carboxyl-tail enable PR to discriminate between agonists and antagonists. The yeast strain BJ5409 was transformed with the expression plasmids YEphPR1 or YEphPR-UP1 encoding authentic hPR-B or a mutant hPR-B in which 42 amino acids were removed by deletion [47]. This yeast strain was subsequently transformed with the YRpG2 reporter plasmid which contains 2 copies of the PRE inserted into an enhancerless CYC1 promoter fused to β-galactosidase. Transformants were grown overnight in selective media and plated out in 96-well plates at a cell density of O.D.$_{600\,nm}$ of 0.01. Compounds of interest were added to each well at final concentrations ranging from 10^{-5}–10^{-11} M as indicated. The cultures were grown for 16 h at 30°C, harvested, and β-galactosidase activity was measured.

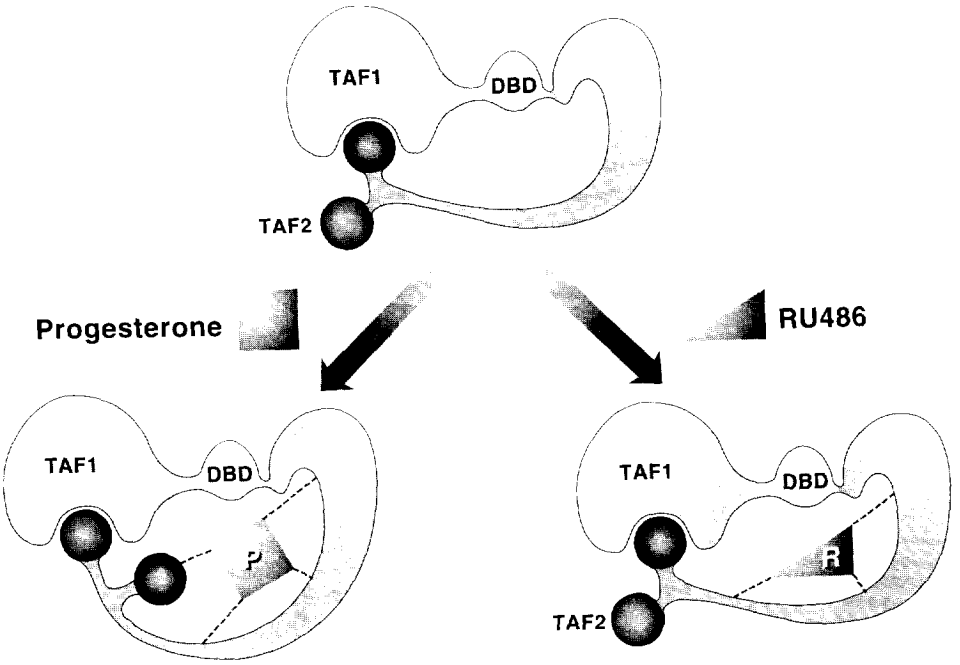

Fig. 6. PR agonists but not antagonists can overcome the inhibitory activity of the PR carboxyl tail. Accumulating evidence supports the hypothesis that the carboxyl tail of PR serves to keep the receptor in a transriptionally inactive form in the absence of hormone. Since it has been shown that PR requires at least two transactivation sequences, AF-1 and AF-2, to efficiently regulate gene transcription, we postulate that one effect of the PR tail may be to inhibit AF-1/AF-2 synergy. We and others have shown that interaction of PR with progesterone induces an alteration in receptor structure permitting its dissociation from heat shock proteins and delivery to DNA. It is considered that the agonist induced conformational changes within the receptor permit AF-1/AF-2 synergy by overcoming the inhibitory activity of the PR-tail, ultimately facilitating its productive association with the transcription apparatus. When antagonsists interact with PR, they also induce a conformational change within the receptor which is incompatible with heat shock protein interaction. We propose, however, that the specific antagonist induced structural alterations within PR do not permit the efficient interaction of AF-1 and AF-2. Thus, antagonists block PR transcriptional activity by preventing the DNA bound receptor from interacting productively with the general transcription machinery.

antagonists. The details of this study have been published and are only considered in brief below [47]. For this analysis we created libraries of random PR mutants using error prone PCR reactions to induce alterations in the receptor coding sequence. These libraries were transformed into a yeast strain containing a progesterone responsive promoter and screened for mutant receptors which demonstrate altered responsiveness to progesterone and RU486. Using this approach, we identified one clone, PR-UP-1, in which RU486 but not progesterone functioned as a receptor agonist (Fig. 5). We determined that this phenotype resulted from a truncation of 42 amino acids from the carboxyl terminus of PR [47]. Hormone binding analysis indicated that RU486 but not progesterone could interact with the mutant receptor. This information suggested that progesterone and RU486 do not interact with PR in the same manner.

When assayed in transiently transfected mammalian cells the transcriptional activity of the PR-UP-1 mutant receptor was stimulated by RU486, but not progesterone, as was observed in yeast [47]. This indicates that the regulatory process highlighted by this mutation in yeast was conserved in mammalian cells and further validates our use of yeast as a model system for steroid hormone action. This important information indicated that the protein sequences required for progesterone and RU486 binding were distinct, and that the carboxyl tail of the receptor may be part of a functional domain of PR responsible for maintaining the receptor in a transcriptionally inactive form in the absence of hormone.

Using the information gained from the biochemical and genetic experiments detailed above we have developed a working model to explain the mechanism by which PR distinguishes between agonists and antagonists (Fig. 6). It has been shown previously that PR requires two distinct sequences (activation sequences; AFs) to allow a productive association of the receptors with the transcriptional machinery [19, 48]. The AF-1 sequence located in the amino terminus of steroid hormone receptor and the AF-2 sequence located within the carboxyl terminus must cooperate to permit transcriptional activation. We propose that in the inactive state the carboxyl tail of the receptor prevents AF-1 and AF-2 from interacting. Thus, inhibition may occur as a result of an intermolecular interaction, as depicted, where the tail prevents AF-1/AF-2 synergy

directly, or alternatively (not depicted) it may require an additional cellular factor. We propose that in the presence of PR-agonists a conformational charge occurs within the receptor which disrupts the inhibitory effects of the tail region, thus facilitating interactions critical for transactivation. It is further considered that the conformational changes occurring within PR following antagonist binding, permit displacement of heat-shock proteins, but are not sufficient to overcome the effect of the inhibitory tail domain. In the case of PR-UP-1, where the inhibitory tail is removed by deletion, we propose that AF-1 and AF-2 associate and form a transcriptionally active receptor. However, since it appears that the UP-1 mutated receptor remains associated with heat shock proteins in the absence of hormone (E. Vegeto and D. P. McDonnell, unpublished results), the transcriptional activity of this mutant is manifest only when the protein is delivered to DNA. Since the agonists tested do not interact with PR-UP-1 they are unable to displace the heat shock proteins. However, antagonists such as RU486 are capable of performing this task and so appear as transcriptional activators. It is likely, therefore, that any compounds (agonists or antagonists) which interact with the PR-UP-1 protein and facilitate heat shock protein displacement will function as agonists. Interestingly, a model similar to ours has evolved from studies of mechanism of action of the VP16 acidic activator [49]. Specifically, it was demonstrated that the activity of TFIIB is inhibited by an intramolecular inhibition involving sequences in both ends of the protein. The VP16 acidic activator is capable of disrupting this interaction by inducing a distinct conformational change within TFIIB permitting its interaction with TFIIF and RNA polymerase. Whether or not the carboxyl tail of PR functions analogously remains to be determined.

CONCLUSIONS

The information obtained from these and related studies will impact our understanding of the cellular mechanisms which distinguish between hormone agonists and antagonists. In addition, it provides a series of molecular tools with which to predict the *in vivo* biological activity of novel PR modulators. The current state of the art reveals a firm understanding of how agonists and antagonists affect PR structure. The remaining frontiers are to define the mechanism by which the cellular transcription machinery distinguishes agonist from antagonist activated progesterone receptors. The genetic tools currently available and the ability to reconstitute PR activity *in vitro* will assist greatly in the resolution of this issue.

Acknowledgements—The authors would like to thank their friends and colleagues who shared their data and insights with us. Parts of the work presented in this review were supported by an NIH grant to DPM (#DK43267).

REFERENCES

1. Evans R. M.: The steroid and thyroid hormone receptor superfamily. *Science* **240** (1988) 889–895.
2. Beato M.: Gene regulation by steroid hormones. *Cell* **56** (1989) 335–344.
3. O'Malley B. W. and Tsai M-J.: Molecular pathways of steroid hormone action. *Biol. Reprod.* **46** (1992) 163–167.
4. Sunderland M. C. and Osborne C. K.: Tamoxifen in premenopausal patients with metatastic breast cancer: a review. *J. Clin. Oncol.* **9** (1991) 1283–1297.
5. Rao B. R. and Slotman B. J.: Endocrine factors in common epithelial ovarian cancer. *Endocrine Rev.* **12** (1991) 14–26.
6. Dreicer R. and Wilding G.: Steroid hormone agonists and antagonists in the treatment of cancer. *Cancer Invest.* **10** (1992) 27–41.
7. Daneshgari F. and Crawford E. D.: Endocrine therapy of advanced carcinoma of the prostate. *Cancer* **71** (1993) 1089–1097.
8. Barzel U. S.: Estrogens in the prevention and treatment of postmenopausal osteoporosis. *Am. J. Med.* **85** (1988) 847–850.
9. Jordan V. C., Fritz N. F. and Tormey D. C.: Endocrine effects of adjuvant chemotherapy and long-term tamoxifen adminstration on node-positive patients with breast cancer. *Cancer Res.* **47** (1987) 624–630.
10. Henderson B. E., Ross R. K. and Pike M. C.: Hormonal chemoprevention of cancer in women. *Science* **259** (1993) 633–638.
11. Poisson M., Pertuiset B. F., Hauw J. J., Philippon J., Buge A., Moguilewsky M. and Philibert D.: Steroid hormone receptors in human meningiomas, gliomas and brain metastases. *J. Neurol. Oncol.* **1** (1983) 179–189.
12. Kettel L. M., Murphy A. A., Mortola J. F., Liu J. H., Ulmann A. and Yen S. S.: Endocrine reponses to long-term administration of the anti-progesterone RU486 in patients with pelvic endometriosis. *Fertil. Steril.* **56** (1991) 402–407.
13. Baulieu E-E.: Contragestation and other clinical appliations of RU486, an antiprogesterone at the receptor. *Science* **245** (1989) 1351–1357.
14. De Voogt H. J.: The position of cyproterone acetate (CPA), a steroidal anti-androgen, in the treatment of prostate cancer. *Prostate* **4** (1992) 91–95.
15. Benson R. C.: A rationale for the use of non-steroidal anti-androgens in the management of prostate cancer. *Prostate* **4** (1992) 85–90.
16. Schrader W. T. and O'Malley B. W.: Progesterone binding components of chick oviduct: characterization of purified subunits. *J. Biol. Chem.* **247** (1972) 51–59.
17. Horwitz K. B. and Alexander P. S.: *In situ* photolinked nuclear progesterone receptors of human breast cancer cells: subunit molecular weights after transformation and translocation. *Endocrinology* **113** (1983) 2195–2201.
18. Conneely O. M., Maxwell B. L., Toft D. O., Schrader W. T. and O'Malley B. W.: The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. *Biochem. Biophys. Res. Commun.* **149** (1987) 493–501.
19. Kastner P., Krust A., Turcotte B., Stropp U., Tora L., Gronemeyer H. and Chambon P.: Two distinct estrogen-regulated promoters generate transcipts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J.* **9** (1990) 1603–1614.
20. Loosfelt J., Atger M., Misrahi M., Guiochon-Mantel A., Meriel C., Logeat F., Benarous R. and Milgrom E.: Cloning and sequence analysis of rabbit progesterone-receptor complementary DNA. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 9045–9049.
21. Boyd P. A. and Spelsberg T. C.: Seasonal changes in the molecular species and nuclear binding of the chicken oviduct progesterone receptor. *Biochemistry* **18** (1979) 3685–3690.
22. Shyamala G., Schneider W. and Schott D.: Developmental regulation of murine mammary progesterone receptor gene expression. *Endocrinology* **126** (1990) 2882–2889.
23. Feil P. D., Clarke C. L. and Satyaswaroop P. G.: Progestin-mediated changes in progesterone receptor forms in the normal human endometrium. *Endocrinology* **123** (1988) 2506–2513.
24. Brandon D. B., Bethea C. L., Strawn E. Y., Novy M. J., Burry K. A., Harrington B. S., Erickson T. E., Warner C., Keenan E. J. and Clinton G. M.: Progesterone receptor messenger

ribonucleic acid and protein are overexpressed in human uterine leiomyomas. *A. J. Obstet. Gynec.* **169** (1993) 78–85.
25. Lessey B. A., Alexander P. S. and Horwitz K. B.: The subunit characterization of human breast cancer progesterone receptors: characterization by chromatography and photoaffinity labelling. *Endocrinology* **112** (1983) 1267–1274.
26. Christensen K., Estes P. A., Onate S. A., Beck C. A., DeMarzo A., Altman M., Lieberman B. A., St John J., Nordeen S. K. and Edwards D. P.: Characterization and functional properties of the A and B forms of human progesterone receptors sythesized in a baculovirus system. *Molec. Endocr.* 5 (1991) 1755–1770.
27. Vegeto E., Shahbaz M. M., Wen D. X., Goldman M. E., O'Malley B. W. and McDonnell D. P.: Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Molec. Endocr.* 7 (1993) 1244–1255.
28. McDonnell D. P. and Goldman M. E.: RU486 exerts antiestrogenic activities through a novel progesterone receptor A form-mediated mechanism. *J. Biol. Chem.* **269** (1994) 11,945–11,949.
29. McDonnell D. P., Shahbaz M. S., Vegeto E. and Goldman M. E.: The human progesterone receptor A-form functions as a transcriptional modulator of mineralocorticoid receptor transcriptional activity. *J. Steroid Biochem. Molec. Biol.* **48** (1994) 425–432.
30. Wolf J. P., Hsiu J. G., Anderson T. L., Ulmann A., Baulieu E. E. and Hodgen G. D.: Noncompetitive antiestrogenic effect of RU486 in blocking the estrogen-stimulated luteinizing hormone surge and the proliferative action of estradiol on endometrium in castrate monkeys. *Fertil. Steril.* **52** (1989) 1055–1060.
31. Slayden O. D., Hirst J. J. and Brenner R. M.: Estrogen action in the reproductive tract of rhesus monkeys during antiprogestin treatment. *Endocrinology* **132** (1993) 1845–1856.
32. Horwitz K.: The molecular biology of RU486. Is there a role for antiprogestins in the treatment of breast cancer? *Endocrine Rev.* **13** (1992) 146–163.
33. Romieu G., Maudelonde T., Ulmann A., Pujol H., Grenier J., Cavalie G., Khalaf S. and Rochefort H.: The antiprogestin RU486 in advanced breast cancer: preliminary clinical trial. *Bull. Cancer.* 74 (1989) 455–461.
34. Ing N. H., Beekman J. M., Tsai S. Y., Tsai M. J. and O'Malley B. W.: Members of the steroid hormone receptor superfamily interact with TFIIB (S300-II). *J. Biol. Chem.* **267** (1992) 17,617–17,623.
35. Tjian R. and Maniatis T.: Transcriptional activation: a complex puzzle with a few easy pieces. *Cell* 77 (1994) 5–8.
36. Jacq X., Brou C., Lutz Y., Davidson I., Chambon P. and Tora L.: Human TAFII30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell* **79** (1994) 107–117.
37. Hoey T., Weinzierl R. O. J., Gill G., Chen J-L., Dynlacht B. D. and Tjian R.: Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of coactivators. *Cell* 72 (1993) 247–260.
38. Chen J-L., Attardi L. D., Verrijzer C. P., Yokomori K. and Tjian R.: Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* **79** (1994) 93–105.
39. Takimoto G., Tasset D., Eppert A. and Horwitz K.: Hormone-induced progesterone receptor phosphorylation consists of sequential DNA-independent and DNA-dependent stages: analysis with zinc finger mutants and the progesterone antagonist ZK98299. *Proc. Natn. Acad. Sci. U.S.A.* **89** (1992) 3050–3054.
40. Klein-Hitpass L., Cato A., Henderson D. and Ryffel G.: Two types of antiprogestins identified by their differential action in transcriptionally active extracts for T47D cells. *Nucl. Acids. Res.* **19** (1993) 1227–1234.
41. Bocquel M-T., Ji J., Ylikomi T., Benhamou B., Vergezac A., Chambon P. and Gronemeyer H.: Type II antagonists impair the DNA binding of steroid hormone receptors without affecting dimerization. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 205–215.
42. Delabre K., Guiochon-Mantel A. and Milgrom E.: *In vivo* evidence against the existence of antiprogestins disrupting binding to DNA. *Proc. Natn. Acad. Sci. U.S.A.* **90** (1993) 4421–4425.
43. Beck C. A., Weigel N. L., Moyer M. L., Nordeen S. K. and Edwards D. P.: The progesterone antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. *Proc. Natn. Acad. Sci. U.S.A.* **90** (1993) 4441–4445.
44. Sartorius C. A., Tung L., Takimoto G. S. and Horwitz K. B.: Antagonist-occupied human progesterone receptors bound to DNA are functionally switched to transcriptional agonists by cAMP. *J. Biol. Chem.* **268** (1993) 9262–9266.
45. Nordeen S. K., Bona B. J. and Moyer M. L.: Latent agonist activity of the steriod antagonist, RU486, is unmasked in cells treated with activators of protein kinase A. *Molec. Endocr.* 7 (1993) 731–742.
46. Allan G. F., Leng X., Tsai S-T., Weigel N. L., Edwards D. P., Tsai M-J. and O'Malley B. W.: Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. *J. Biol. Chem.* **267** (1992) 19,513–19,520.
47. Vegeto E., Allan G. F., Schrader W. T., Tsai M-J., McDonnell D. P. and O'Malley B. W.: Mechanism of RU486 antagonism is dependent on the conformation of the carboxyl-terminal tail of the human progesterone receptor. *Cell* **69** (1992) 703–713.
48. Bocquel M. T., Kumar V., Stricker C., Chambon P. and Gronemeyer H.: The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell specific. *Nucl. Acids Res.* **17** (1989) 2581–2595.
49. Roberts S. G. E. and Green M. R.: Activator-induced conformational change in general transcription factor TFIIB. *Nature* **371** (1994) 717–720.
50. Klein-Hitpass L., Weigel N. L., Allan G. F., Riley D., Rodriguez R., Schrader W. T., Tsai M-J. and O'Malley B. W.: The progesterone receptor stimulates cell-free transcription by enhancing the formation of a stable preinitiation complex. *Cell* **60** (1990) 247–257.