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# Progesterone Receptor and the Mechanism of Action of Progesterone Antagonists

**Dean P. Edwards,[1]* Magda Altmann,[1] Angelo DeMarzo,[1] Yixian Zhang,[2] Nancy L. Weigel[2] and Candace A. Beck[1]**

[1]*Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262 and* [2]*Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, U.S.A.*

**Currently available progesterone antagonists have been suggested to fall into two categories based on differences in how they interact with and inactivate the progesterone receptor (PR). The anti-progestin ZK98299 (Type I) impairs PR association with DNA, while Type II compounds (RU486, ZK112993, ZK98734) promote PR binding to DNA. Type II agents, therefore, appear to inhibit receptor activity at a step downstream of DNA binding, presumably failing to induce conformational changes in PR structure required for enhancement of transcription. This paper discusses both published and unpublished data supporting the concept of two types of progestin antagonists. Using PR-mediated induction of reporter genes in breast cancer cells as an assay for biological response, both types of anti-progestins, after correction for difference in steroid binding affinity, inhibit progestin induction substoichiometrically. However, Type II anti-progestins are more potent, inhibiting at lower ratios of antagonist to agonist than ZK98299. This suggests that in addition to behaving by classical competitive mechanisms these compounds (in particular Type II) may exhibit additional activity as transrepressors of PR in the same cell bound to hormone agonist. Transrepression may occur by the combined mechanisms of heterodimerization and competition for binding to DNA. In support of this, mixed ligand dimers form readily in solution between a PR subunit bound to agonist and another bound to either type of anti-progestin, whereas these mixed ligand dimers bind poorly, if at all, to specific progesterone response elements (PREs) *in vitro*. Additionally, when added as a single ligand, Type II agents increase PR dimerization in solution and PR affinity for PREs as compared with single ligand dimers formed by progestin agonist. This contrasts with ZK98299, when given as a single ligand, which reduces PR affinity for PREs without disrupting solution dimerization. Thus the higher affinity of PR for PREs may account for the greater biological potency of Type II compounds as compared with ZK98299. As a further distinction between types of antiprogestins, ZK98299 minimally stimulates phosphorylation of PR whereas RU486 increases site-specific phosphorylation of PR in a manner indistinguishable from that of hormone agonist. Additionally, ZK98299 is not susceptible *in vivo* to functional switching to a partial agonist by cross talk with cAMP signal transduction pathways, as occurs with Type II compounds. Thus, ZK98299 under certain conditions may be a more pure antagonist than Type II compounds.**

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## INTRODUCTION

The biological effects of sex steroid hormones on growth and development of reproductive tissues and on the growth and progression of endocrine-dependent neoplasias [1–8] are mediated by intracellular receptors that are members of a gene family of ligand-dependent transcriptional activators [9–11]. In addition to receptors for the sex steroid hormones and glucocorticoids, the gene family also includes receptors for thyroid hormone (TR), vitamin $D_3$ (VDR), retinoic acid (RAR), and a number of orphan receptors for which a ligand has not yet been identified. Intracellular receptors are modular proteins composed of separate domains for ligand binding, DNA binding and transcriptional enhancement. For the steroid hormone group of receptors, the C-terminal ligand binding domain (LBD) also harbors sequences for a second ligand-dependent transcriptional activation domain,

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*Correspondence to D. P. Edwards.

dimerization, nuclear localization and binding of heat shock proteins [9–11].

There are similarities in the general mechanism by which ligands activate steroid hormone receptors. In the absence of hormone, receptors form an inactive oligomeric complex with heat shock protein 90 (hsp90), hsp70, immunophilins and possibly other proteins of unknown identity [12–14]. In response to binding hormone, receptors dissociate from the oligomeric complex and acquire the ability to dimerize [15–18] and to bind to hormone response elements (HREs) that are usually located in the regulatory region of steroid responsive genes [19]. Receptor association with HREs leads to an increase or decrease in transcription [20] by mechanisms that are not fully understood. A central event in the activation process appears to be a ligand induced conformational change in receptor structure [21]. Major unanswered questions are the nature of ligand induced structural changes and how receptors interact with the transcriptional machinery to alter transcription.

Several synthetic ligands (both steroidal and non-steroidal) for estrogen (ER) and progesterone receptors (PR) have been developed which compete for binding with the natural hormone and are capable of inhibiting receptor activity. The anti-estrogen tamoxifen has been widely used clinically for treatment of endocrine-dependent breast cancer [reviewed in 1, 2] and the anti-progestin RU486 has been widely used as a post-coital contraceptive and for medical abortion [22, 23]. These drugs have also proven to be valuable for dissecting normal receptor activation mechanisms. Anti-progestins have been categorized as two types based on their effects on PR function *in vitro*. Type I compounds, which includes ZK98299 (Onapristone) and possibly others, have been suggested to prevent PR from associating with DNA [24, 25]. Type II anti-progestins, which include RU486 and several other similar compounds, appear to promote receptor dissociation from hsps, dimerization and interaction with DNA. Thus Type II compounds have been proposed to act at a step downstream of DNA binding [24–30]. The precise molecular mechanism responsible for non-productive association of the PR-RU486 complex with DNA is not known. Studies have suggested that progestin agonists and RU486 induce distinct conformational changes in PR structure [22, 31–35]. Thus, RU486 may fail to expose receptor surfaces required for interaction with the transcription apparatus. Currently available anti-estrogens also appear to fall into two general categories, those, such as Tamoxifen, which permit ER binding to DNA and the steroid analog ICI 164,384 which may prevent or reduce ER association with DNA [36, 37].

In human cells PR is expressed as two forms, full length PR-B and PR-A which has a 164 amino acid N-terminal truncation [38]. The two proteins are synthesized from a single gene, arising by alternate initiation of translation from a single PR mRNA, or by alternate transcription from two promoters [38, 39]. The two forms of PR have long been suspected to have different functional properties. This has now been confirmed by transfection studies, where PR-A was shown to act as a trans-dominant repressor of PR-B and other receptors in the glucocorticoid receptor (GR) subfamily under conditions where PR-A does not itself function as an activator [40]. This repressor function of PR-A appears to be cell and target gene specific. Thus PR-A appears to have the potential for dual functions depending on the context; activation or repression. Additionally, PR-B when expressed alone can mediate partial agonist-like effects of anti-progestins possibly by binding to non-PRE (progesterone response elements) sites of complex promoters [41].

This paper discusses both published and unpublished studies on the mechanism of action of progestin antagonists with regard to the biology and biochemistry of human PR. For the most part we have not distinguished between biological effects mediated by PR-A or PR-B. We do take advantage of the expression of full length and truncated PR protein to detect receptor dimers. The studies described are designed more to explore how different types of anti-progestins interact with the progesterone receptor.

## RESULTS AND DISCUSSION

### *Biological activity of anti-progestins: substoichiometric inhibition of progestin action by Type II compounds*

Currently available anti-progestins are steroid analogs with an aromatic substituent at the $11\beta$ carbon position which appears to be essential for antagonist activity [25]. The primary structural difference between antiprogestins is the 17-carbon side chain. The chemical structure of the Type I anti-progestin ZK98299 (Onapristone), and several Type II compounds are shown in Fig. 1. The biological activity of ZK98299 and Type II anti-progestins was determined by their ability to inhibit progestin (R5020) induction of a mouse mammary tumor virus (MMTV) reporter gene that we have stably introduced into PR rich T47D breast cancer [28]. In this cellular context, anti-progestins behave as pure antagonists showing little or no induction of CAT activity themselves, while they are effective inhibitors of R5020 induction [28]. Interestingly, Type II compounds suppressed reporter gene induction by $\approx 50\%$ when present at a concentration 10–20-fold lower than R5020 and induction was fully suppressed when R5020 and antagonists were at approximately equal molar concentrations. As an example, results with the Type II anti-progestin ZK112993 are shown in Fig. 2. In contrast, the Type I compound ZK98299 required an equal or slightly higher concentration than R5020 to suppress activity by 50% and maximal suppression required an approx. 20-fold higher molar excess over R5020. To determine

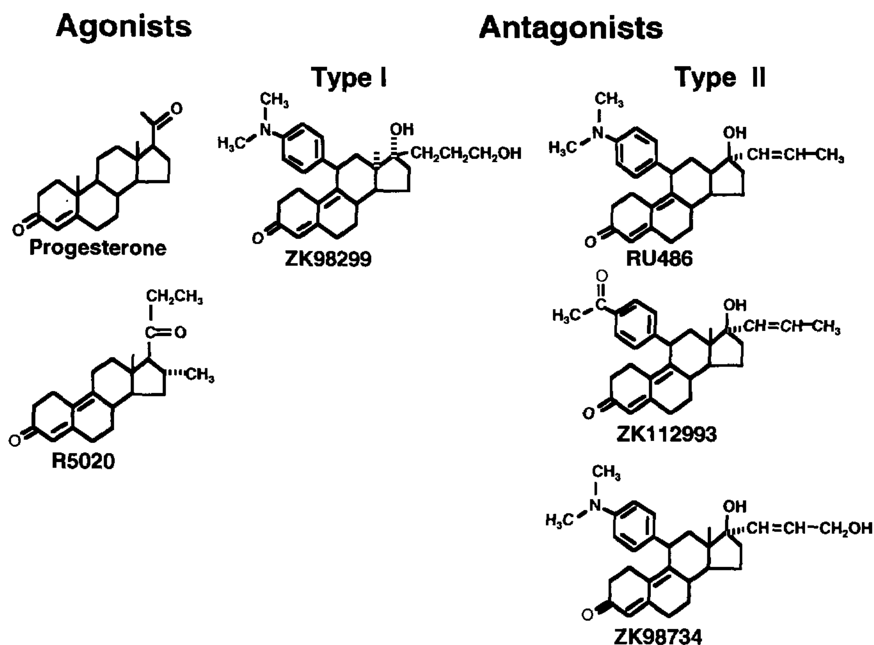


Fig. 1. Chemical structures of the natural hormone progesterone, the synthetic progestin R5020, Type I anti-progestin ZK98299 (Onapristone) and the Type II anti-progestins RU486 (Mifepristone), ZK112993 and ZK98734.

whether differences in ligand affinity might contribute to the potency of Type II anti-progestins, we have measured the relative binding affinity of various anti-progestins for PR. All Type II compounds tested (RU486, ZK112993, ZK98734) have the same binding affinity as R5020, whereas ZK98299 has an approx. 20-fold lower affinity (not shown). Since R5020 and Type II compounds bind with equal affinity to PR, and are stable in cell culture to metabolism [42], the biological potency of these anti-progestins at such low ratios of antagonist to agonist suggests that they inhibit progesterone action substoichiometrically and thus act by mechanisms in addition to that of a classical competitive antagonist. After adjusting for a 20-fold lower binding affinity, ZK98299 also appears to inhibit at a lower ratio of antagonist to agonist than expected by a simple competitive mechanism.

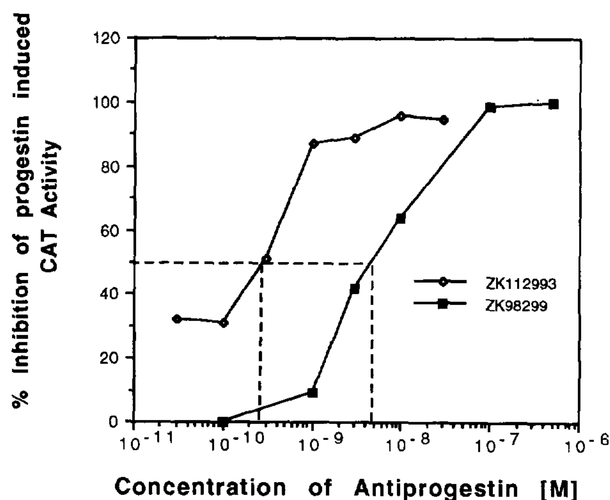


Fig. 2. Inhibition of progestin ($5 \times 10^{-9}$ R5020) induction of the MMTV-CAT reporter gene expression in T47D breast cancer cells by increasing concentrations of ZK98299 or ZK112993.

*Evidence from in vitro studies that Type II antiprogestins may act as trans-dominant repressors of PR bound to hormone agonist by the combined mechanism of heterodimeration and competition for binding to PREs*

The potency of anti-progestins (in particular Type II compounds) at such low ratios of antagonist to agonist could be explained by receptors bound to anti-progestins acting as trans-dominant repressors, in the same cell, of PR bound to hormone agonist. Several mechanisms could account for this including heterodimerization and competition for DNA binding sites. To begin to explore these possibilities we have determined the ability of PR bound to agonist to dimerize *in vitro* with PR bound to antagonist. Mixed ligand dimers were detected by expressing recombinant full-length PR-B and N-terminally truncated PR-A in a baculovirus system [43, 44]. Separately expressed PR-A was bound to RU486 and PR-B was bound to R5020, excess free steroid was removed and the two receptor forms were mixed *in vitro* under conditions that minimize ligand exchange. Receptors were then immunoprecipitated with a monoclonal antibody specific to N-terminal sequences in PR-B (B-30) and analyzed for the presence of co-precipitating PR-A as evidence of dimerization [43]. Solution dimerization between PR-A bound to RU486 and PR-B bound to R5020 was found to be unimpaired compared to that

obtained when both PR subunits were bound to the same ligand [43]. However, mixed ligand dimers that readily formed in solution exhibited an impaired ability to bind to specific progesterone response element (PRE). As shown by electrophoretic mobility shift assay (EMSA), intermediate mobility A/B dimers complexed to PREs are predominant when PR-B and PR-A are each bound to R5020, whereas the intermediate A/B heterocomplex is substantially reduced when PR-A is bound to RU486 and PR-B is bound to R5020 (Fig. 3). Similar results were obtained with other Type II anti-progestins, ZK112993 and ZK98734. Neither compound impaired mixed-ligand solution dimerization of PR, while the dimers that formed in solution bound poorly to DNA by EMSA. In fact, the mixed ligand dimers formed in the presence of ZK89734 and ZK112993 showed little or no DNA binding and thus bound even less well to DNA binding than mixed ligand dimers formed in the presence of RU486 (unpublished, DeMarzo and Edwards). Studies by Meyer *et al.* [27] similarly showed that when PR-A bound to RU486 was mixed with PR-B bound to R5020, that A/B heterocomplexes did not bind efficiently to DNA by EMSA. However, solution dimerization was not examined in this study, and it was thus concluded that PR bound to antagonist was not capable of dimerization with PR bound to agonist due to incompatibility of dimerization interfaces [27]. As an alternative explanation, we propose that mixed ligand dimers are capable of forming in solution but bind inefficiently to DNA [43]. It is tempting to speculate that mixed ligand dimers assume an asymmetric orientation incompatible with recognition of palindromic PREs. Formation of heterodimers that fail to bind DNA is a potential mechanism to inactivate at least some PR in the cell bound to hormone agonist.

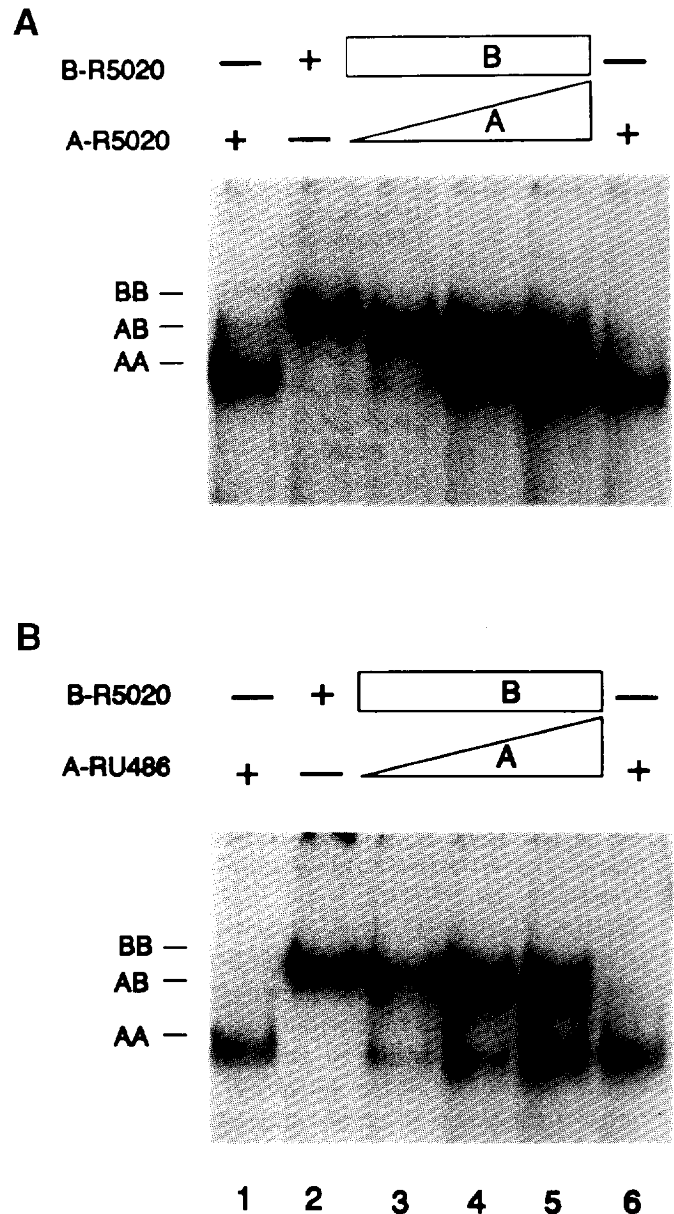


**Fig. 3. Mixed ligand R5020/RU486 PR dimers exhibit reduced DNA binding as compared to single ligand dimers. Separately expressed recombinant PR-A and PR-B from baculovirus were bound to either R5020 or RU486 as indicated and incubated with a [$^{32}$P]PRE oligonucleotide probe and receptor–DNA complexes were detected by EMSA. (A) A constant amount of PR-B (20 fmol) bound to R5020 was mixed with increasing amounts of PR-A bound to R5020 (0, 10, 20, 40 fmol). (B) A constant amount of PR-B bound to R5020 was mixed with increasing amounts of PR-A bound to RU486. The figure shows only the region of the gel containing receptor–DNA complexes and the resolved AA, AB and BB dimeric complexes are indicated. Note that the faint mixed-ligand intermediate AB heterocomplex in panel B has a different relative mobility compared with single-ligand AB heterocomplex in panel A (reproduced from ref. [43]).**

To begin to investigate whether competition for PREs might also contribute to the potent antagonist activity of Type II compounds, we have compared the effects of different anti-progestins as single ligands, in the absence of hormone agonist, on the relative binding affinity of PR for PREs *in vitro*. As shown by EMSA with nuclear extracts of T47D breast cancer cells, binding of human PR to PREs *in vitro* is dependent on ligand and induction occurs with the agonist R5020, Type II antagonists (RU486 and ZK112993) but not with the Type I antagonist ZK98299 (Fig. 4). Also shown are the three closely resolved DNA complexes demonstrated in previous studies to correspond to AA, AB and BB dimers [27, 43, 44]. It should also be noted that Type II antagonists (RU486, ZK112993) promote a higher affinity interaction of PR with DNA than the agonist R5020. When we examined the effects of the same ligands on solution dimerization of PR (single ligand dimers detected by co-immunoprecipitation of PR-A with PR-B) we observed that salt treatment to remove associated heat shock proteins was sufficient to promote dimerization. Addition of R5020 produced little further effect on solution dimerization whereas Type II compounds increased dimerization which thus correlated with increased DNA binding affinity (Fig. 4). In contrast, the Type I anti-progestin

ZK98299 neither enhanced nor impaired solution dimerization. Thus in agreement with the studies of Bocquel *et al.* [29], ZK98299 appears to impair DNA binding of PR without disrupting dimerization. This suggests that dimerization may be necessary but not sufficient for DNA binding and that additional ligand induced conformational changes are required that apparently are different for Type I and II anti-progestins. Taken together these *in vitro* receptor analyses are consistent with the idea that heterodimerization and competition for PREs may be responsible for the potent substoichiometric effects of Type II anti-progestins. In contrast, heterodimerization may contribute to the activity of the Type I compound ZK98299 but without competition for PREs as a component. Proof of this will require detection of mixed ligand dimers *in vivo*, assessment of the biological activity of mixed ligand dimers, and direct measurement of PR binding to PREs *in vivo*.

Figure 5 is a schematic of how PR bound to a Type II compound (RU486) may act as a transrepressor of PR bound to hormone agonist. A portion of cellular PR bound to agonist dimerizes with PR bound to antagonist and the resulting mixed ligand dimer has an impaired ability to bind to PREs. This effectively sequesters a portion of cellular PR bound to progesterone in an inactive form. Single ligand dimers bound to RU486 have a higher affinity for PREs than single ligand dimers bound to progesterone. Thus competition for PREs impedes binding of the activated PR–progesterone complex to its specific target DNA.

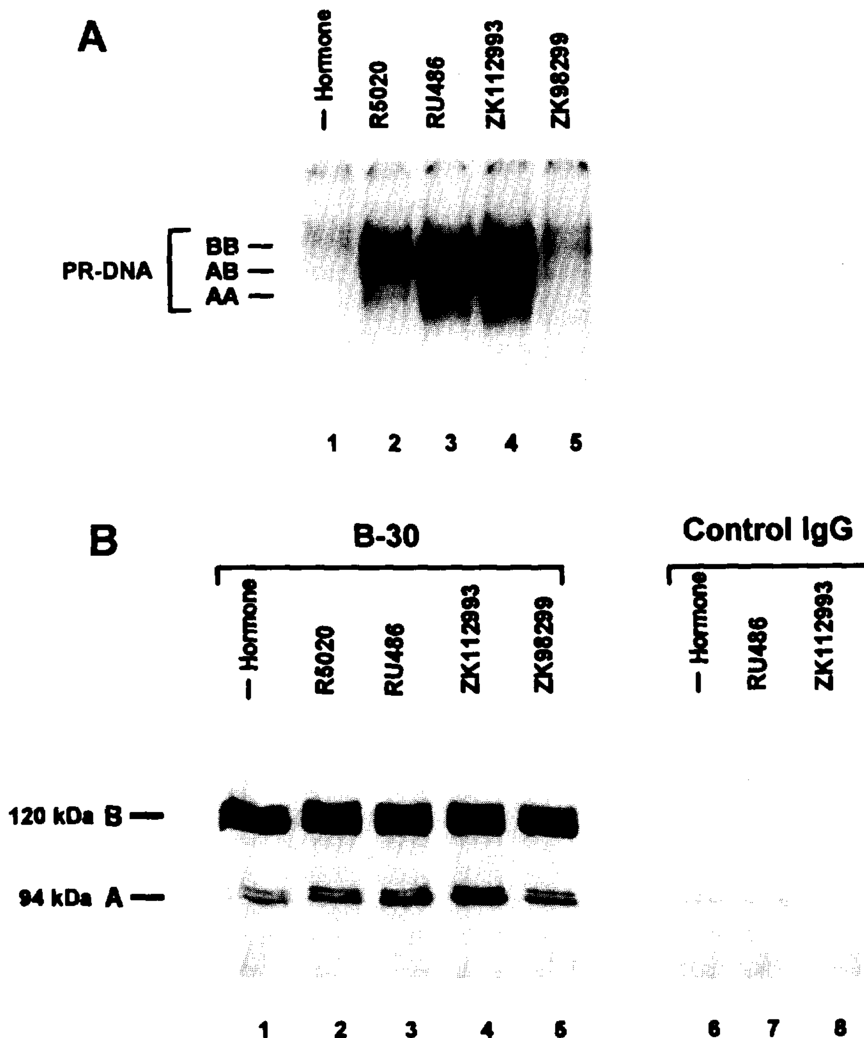


Fig. 4. (A) Type II anti-progestins *in vitro* enhance binding of PR to specific DNA as detected by EMSA and (B) solution dimerization of PR as detected by co-immunoprecipitation assay. Nuclear extracts of T47D cells containing endogenous PR-A and PR-B were bound to the ligands indicated and samples submitted to EMSA using a PRE [$^{32}$P]oligonucleotide probe or to co-immunoprecipitation assay with the PR-B specific antibody (B-30). (C) As controls for co-immunoprecipitation, samples were also immunoprecipitated with an unrelated antibody.

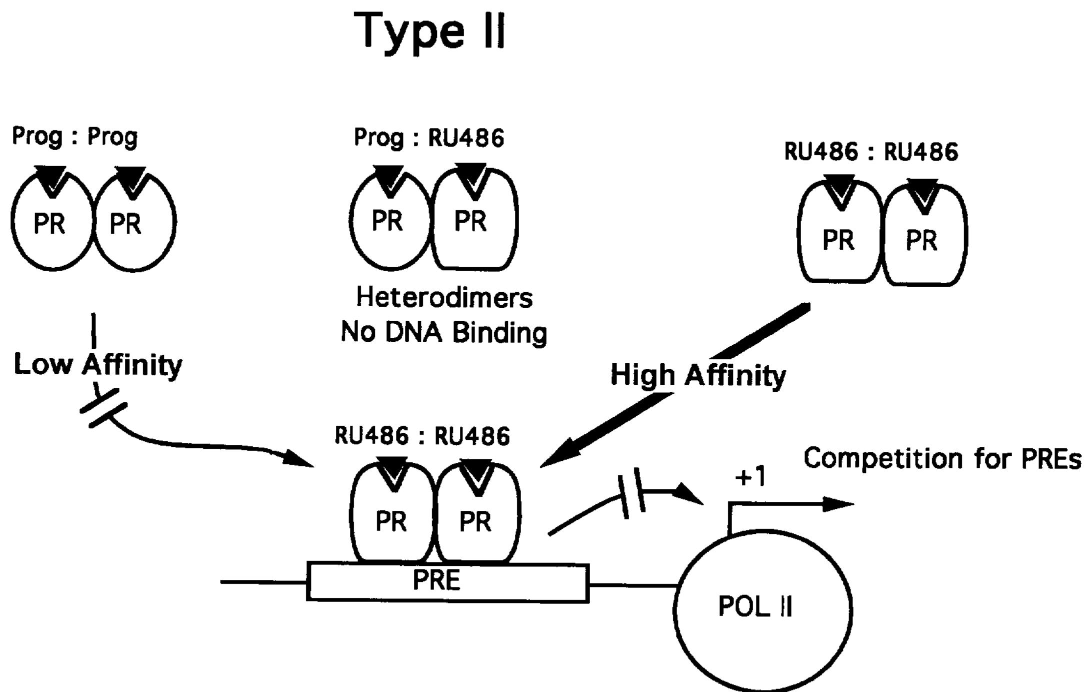


Fig. 5. Schematic of the proposed mechanism of action of Type II anti-progestins.

*Nonproductive association of PR with DNA is correlated with a distinct conformational change in receptor structure induced by RU486*

Several studies have shown that RU486 as a single ligand (in the absence of progestin agonists), induces binding of PR to PREs not only *in vitro* but also *in vivo* [26–30, 45]. Therefore, it appears that single ligand dimers formed by RU486 that bind to PREs (Fig. 5), interact nonproductively. The mechanism for nonproductive association with DNA is not known but likely involves induction or stabilization by RU486 of a PR structure that is distinct from that induced by hormone agonist, thus failing to expose receptor surfaces required for interaction with the transcriptional machinery. Several lines of evidence indicate that RU486 induces a distinct conformation in PR, both with PR in solution and when bound to DNA. First, PR–DNA complexes detected by EMSA exhibit faster mobility when receptor is bound to RU486 and other Type II compounds, as compared to receptors bound to agonist (Fig. 4) [28, 43, 44]. Secondly, a site directed monoclonal antibody (C262) against the C-terminal tail of human PR is differentially recognized by receptors bound to agonist or antagonist [31]. Thirdly, progestins and anti-progestins induce different partial proteolytic digestion patterns of PR [21, 35]. As shown in Fig. 6, the C262 antibody recognizes and supershifts the receptor DNA complex by EMSA in the presence of RU486 but does not recognize and supershift complexes when PR is bound to the progestin agonist R5020. The C262 antibody also does not bind and immunoprecipitate the PR-R5020 complex from solution but does so efficiently with the PR-RU486 complex [31]. Interestingly, C262 also competes with R5020 for binding to PR but does not compete with RU486 [31], and deletion of 41 amino acids from the C-terminal tail of PR results in loss of progestin binding while high affinity RU486 binding is retained [34]. These results taken together, indicate that progestins and RU486 do not contact the same amino acids in the ligand binding domain (LBD) and that they induce different conformational changes in the C-terminal tail of PR. The precise nature of the conformational change induced by anti-progestins is not known. Nor is it known whether differences in conformation may also be localized to other regions of PR or whether Type I and Type II compounds stabilize the same or different conformational states. Although ZK98299 and RU486 were reported to induce similar partial proteolytic digestion patterns [35], whether they have different effects on PR structure has not been rigorously examined. The fact that ZK98299 and Type II compounds have such different effects on solution dimerization and PR–DNA binding *in vitro* taken together with the differences in how the two types of compounds interact with PR *in vivo* (see below), suggests that ZK98299 and other anti-progestins may affect PR conformation differently.

*Type I (ZK98299) and Type II (RU486) anti-progestins exert different effects on PR in vivo*

Co-transfection studies reported by Delabre *et al.* [45] have shown that both ZK98299 and RU486 promote dimerization and binding of PR to PREs *in vivo*

suggesting that there may not be a mechanistic distinction between anti-progestins. PR missing nuclear localization sequences was capable of translocating to the nucleus in response to ZK98299 or RU486 addition by dimerization with co-transfected wild type PR, and wild type PR in response to addition of either ligand was able to compete with a constitutively active DNA binding mutant (truncated PR missing the LBD), for binding to PREs in the intact cell. Therefore, it was concluded that both compounds antagonize PR by similar mechanisms at a step downstream of DNA binding and that the only difference between the two was the lower binding affinity of ZK98299 for PR requiring that it be given in higher concentrations to obtain the same effect. However, studies discussed below do indicate that ZK98299, even when added at higher concentrations (20–50-fold) to compensate for its lower binding affinity, affects PR *in vivo* differently than RU486 and other Type II compounds. For example, progestin agonists and Type II antagonists (RU486, ZK98299, ZK112993) all promote efficient cytosol depletion-nuclear accumulation of PR. In contrast, ZK98299 promotes only a partial cytosol depletion–nuclear accumulation (data not shown). Thus *in vivo* binding of PR to nuclear components is reduced by ZK98299 which is consistent with the lower binding affinity of PR for PREs *in vitro* (Fig. 4).

When added to T47D cells, ZK98299 also affects the phosphorylation of PR differently than progestin agonists or RU486. We and others have shown that progestins increase phosphorylation of human PR in at least two stages, a rapid ligand-dependent phosphorylation followed by a ligand and DNA-dependent phosphorylation that is associated with reduced mobility of PR on SDS–gel electrophoresis [46–48]. RU486 stimulates a similar increase in net phosphorylation of PR and an upshift of PR mobility on SDS-gels. In contrast ZK98299 stimulates only a minimal net increase in PR phosphorylation (1.18-fold vs 2-fold for RU486) and fails to induce PR upshifts on SDS-gels (unpublished and [47]). Human PR-B has at least 9 phosphorylation sites, all on serine residues, which are located in the N-terminal A/B domain (Zhang, Beck, Edwards and Weigel, unpublished). Based on phosphopeptide mapping, the majority of sites are constitutively phosphorylated while at least 3 sites appear to be highly hormone-dependent. One of the hormone-dependent sites is responsible for the upshift of PR on SDS-gels and is most likely both a ligand and DNA-dependent site, since Takimoto *et al.* showed that

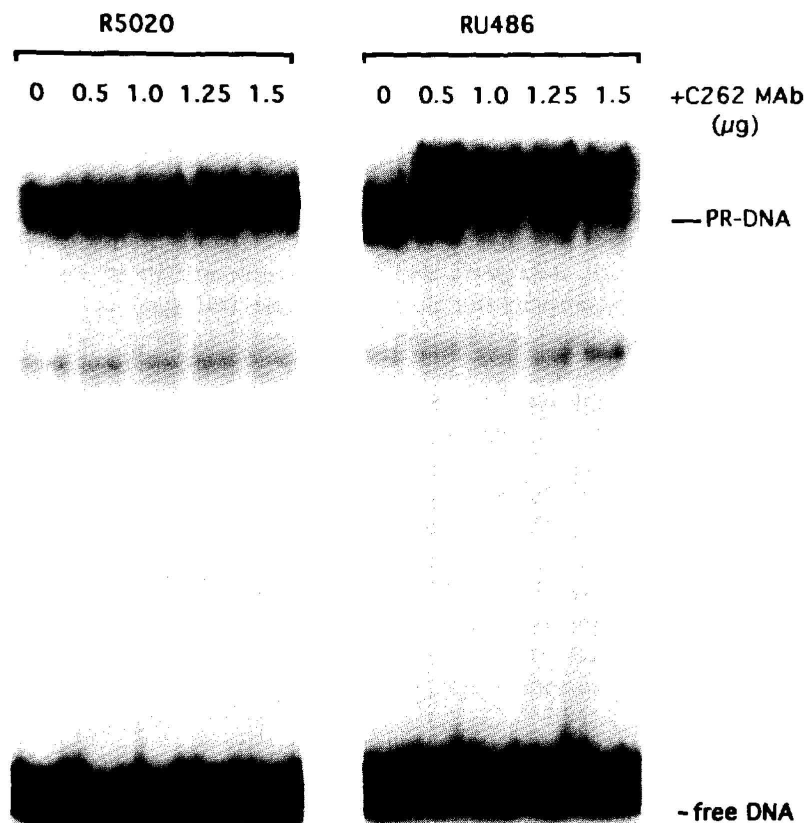


Fig. 6. Differential recognition of PR bound to RU486 and a progestin agonist (R5020) by a site-directed monoclonal antibody (C262) to the C-terminal tail of human PR. PR in nuclear extracts of T47D cells were incubated with a [$^{32}$P]oligonucleotide PRE probe and DNA complexes were detected by EMSA. PR was bound to either the progestin agonist R5020 or the antagonist RU486 in the presence or absence of varying concentrations of the C262 antibody (reproduced from ref. [31]).

PR-upshifts do not occur with a zinc finger DNA binding mutant [47]. Additionally, we showed that the upshift occurs subsequent to DNA binding [46]. In contrast to RU486 which stimulated phosphorylation of both ligand-dependent and DNA-dependent sites in a manner indistinguishable from that of R5020, ZK98299 produced only a minimal increase in the ligand-dependent sites and failed to phosphorylate the ligand and DNA dependent site (Beck, Edwards, Weigel, unpublished). Thus, at the level of individual sites, the phosphorylation state of PR bound to ZK98299 is more similar to that of unliganded PR than PR bound to agonist. Since conformational changes in PR structure and/or dissociation from hsps are likely required for hormone-dependent phosphorylation, these results also suggest that ZK98299 affects PR structure differently than other anti-progestins.

Various activators of cell signal transduction pathways can potentiate progestin-dependent PR-mediated gene transcription. Effects have been observed with activators of PKA, protein kinase C and with the protein phosphatase inhibitor okadaic acid [42, 46]. These agents enhanced the transcriptional activity of PR as opposed to altering PR synthesis or other functional properties such as steroid or DNA binding. For reasons that are not clear, we have been unable to detect substantial ligand-independent activation of human PR as has been reported for other steroid receptors such as ER and chicken PR [49–51]. Since these cell signaling pathways involve protein phosphorylation cascades, we naturally questioned whether convergence between pathways might be mediated by direct phosphorylation of PR. However, we and others have observed that these agents did not detectably alter the phosphorylation state of PR itself nor that of GR in similar studies [46, 52]. Therefore, it appears that alternate pathways do not modify PR directly but alter another factor(s) that mediates receptor interaction with the transcription apparatus.

Alternate signal transduction pathways have also been shown to be able to change the activity of PR bound to RU486 and related anti-progestins [42, 53]. As shown in Fig. 7, RU486 in T47D breast cancer cells stably transfected with the progestin inducible MMTV-CAT reporter gene, behaves as a pure antagonist, failing to induce CAT activity when added alone, while effectively inhibiting progestin induction. In cells that were co-treated with 8Br-cAMP to elevate intracellular cAMP levels, RU486 acquired substantial agonist activity capable of inducing MMTV-CAT to a level that was 60% of that induced by R5020 alone. Also shown in Fig. 7 is the potentiation of progestin-induced responses by cAMP and that RU486 fails to completely antagonize R5020 induction in cells co-treated with 8Br-cAMP. cAMP was also found to change the activity of other Type II anti-progestins such as ZK112993 but did not affect the antagonistic activity of ZK98299 [42]. As shown in Fig. 8, ZK98299

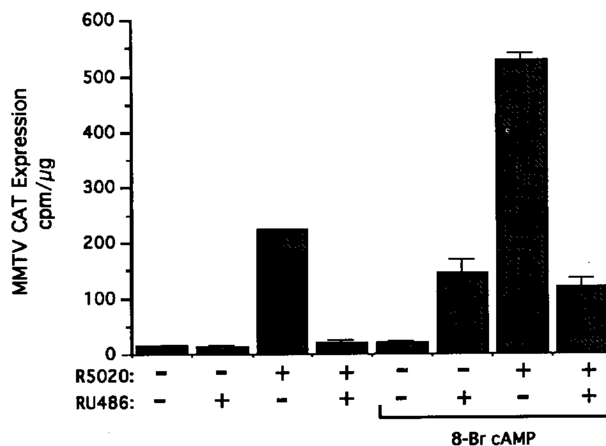


Fig. 7. Effect of 8Br-cAMP on PR-mediated induction of the MMTV-CAT reporter gene. PR-expressing T47D cells stably transfected with MMTV-CAT were treated with the hormones indicated in the Figure in the presence or absence of 8Br-cAMP. CAT activity was measured as described and expressed as cpm of [$^3$H]Ac-CoA converted per mg protein (reproduced from ref. [42]).

behaves as a pure antagonist both in the absence and presence of 8Br-cAMP. Thus, Type II anti-progestins appear to be more susceptible to functional switching by alternate signal transduction pathways than ZK98299. Additionally, ZK98299 was found to be capable of inhibiting the agonist activity of RU486 in cells co-treated with 8Br-cAMP [42]. These results suggest that ZK98299 antagonizes PR function by a different mechanism than Type II anti-progestins, and that ZK98299 may be a more pure antagonist. At present this antagonist–agonist switch for anti-progestins has been observed only by activators of cAMP signaling pathways. However, potentiation of agonist-like activity by cAMP has been shown in more than one cell type and with different target genes including the endogenous human metallothionein-II-A [54].

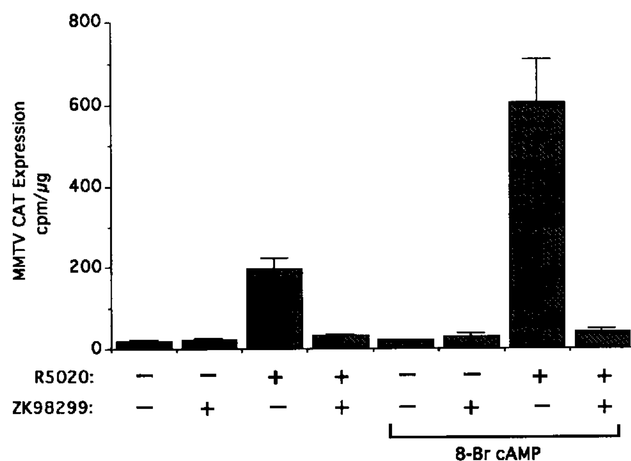


Fig. 8. The Type I anti-progestin ZK98299 is not affected by cAMP. T47D cells stably transfected with MMTV-CAT were treated as in Fig. 7 (reproduced from ref. [42]).

A similar modulation of the anti-estrogen tamoxifen by cAMP signaling pathway has also been observed. In co-transfection studies, cAMP elevating agents were observed to increase the ability of tamoxifen to induce ER-mediated gene transcription [55]. Additionally, stimulation of dopaminergic signaling pathways was observed to potentiate the agonist activity of the ER–tamoxifen complex, resulting in induction of estrogen responsive genes with a magnitude approaching that stimulated by estradiol [56].

Modulation of the nuclear receptor–antagonist complex by alternate signaling pathways is a potentially important finding that may explain in part the resistance to anti-estrogens that frequently occurs in breast cancer patients [57, 58]. Crosstalk between signal transduction pathways could be initiated during the course of tumor progression as a result of changes in growth factor production or oncogene activation. Thus cellular resistance to anti-steroids may not be resistance *per se*, but a switch in the activity of the nuclear receptor–antagonist complex. Currently, this functional agonist–antagonist switch by alternate pathways has been observed only for cAMP and dopaminergic signaling pathways. Whether there are other physiological regulators in breast cancer cells or whether growth factor signaling pathways are involved in modulation of the activity the receptor–anti-steroid complex remains to be determined. This unsuspected level of regulatory complexity highlights the importance of studying the molecular biology of receptors. A better understanding of the mechanisms of action of intracellular receptors and how anti-steroids and other factors affect receptor function are central to the discovery of the molecular basis for cellular resistance to steroid antagonists in breast cancer. Ultimately this may lead to improvements in the therapeutic efficacy of current compounds and to the development of novel steroid antagonists.

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