MINIREVIEW

Mechanism of Action of Progesterone Antagonists

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The effects of progesterone on target tissues are mediated by progesterone receptors (PRs), which belong to a family of nuclear receptors and function as ligand-activated transcription factors to regulate the expression of specific sets of target genes. Progesterone antagonists repress the biological actions of progesterone by "actively" inhibiting PR activation. This work discusses the first clinically used progesterone antagonist RU486 and closely related compounds in terms of how these compounds inhibit progesterone action through heterodimerization and competition for DNA binding and by the recruitment of corepressors to promoters of target genes to repress transcription. We discuss cellular factors that may influence the activity of these compounds, such as the availability of coactivators and corepressors and the context of specific target promoters in any given cell type. We also discuss steroidal and nonsteroidal antagonist selectivity for PR versus other steroid hormone receptors and suggest that it may be possible to develop tissue/cell specific modulators of PR. Exp Biol Med 227: 969–980, 2002

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In women, the steroid hormone PG is a key regulator of reproductive events associated with the establishment and maintenance of pregnancy, including uterine and mammary gland development and also the process of ovulation. PG also has physiological actions in nonreproductive target tissues such as the brain. The biological actions of PG are diverse and often opposing. For example, in the cycling uterus, PG inhibits estrogen-induced proliferation of glandular epithelial cells during the follicular phase, but later induces proliferation of stromal cells during the luteal phase. In the normal mammary gland, PG has proliferative effects for epithelial ductual side branching and is also required for the differentiation of lobuloaveolar structures.

During pregnancy, PG causes the uterine lining to undergo decidualization and is necessary for the implantation and maintenance of the embryo. It also promotes uterine quiescence by inhibiting contractions of the smooth muscles of the uterus. It is this physiological action of PG on the uterus, priming the lining for implantation, which became the focus of worldwide efforts to improve birth control methods. Because the interruption of PG synthesis or elimination of circulating PG is not possible in women by current methods, efforts were directed toward developing a PG antiprogestin that would decrease or suppress the effects of PG. Mifepristone (RU486) was the first PG antagonist developed that exhibited antiprogesterone activity in humans. Since the first clinical trial in 1982, RU486 has been used in many clinical studies in the gynecologic and obstetrical fields.

Numerous clinical studies have been aimed at defining the optimal dose and schedule of administration of RU486 for the purposes of pregnancy termination. Studies have also been conducted on using RU486 for expulsion after intrauterine fetal death, cervical ripening before surgical abortion, labor induction, emergency postcoital contraception, and other forms of contraception (1–3). RU486 has also been investigated for treatment of endometriosis and fibroids, meningiomas, and certain progestin responsive tumors such as leiomyosarcomas (reviewed in 2). Clinical trials have evaluated RU486 as a first-line agent as well as a second-line agent in the treatment of metastatic breast cancers. Although partial responses were reported, RU486 had no impact on prolonged disease-free survival, and only a few patients had transient remission (2).

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The effects of PG on target tissues is mediated by progesterone receptors (PRs) that function as ligand-activated transcription factors to regulate the expression of specific sets of target genes. The focus of this review is on the mechanism of action of PR antagonists. We first outline the structure and corresponding functional properties of PR and the mechanism of action of PR when bound to hormone agonist. We then summarize what is known about the mechanism of action of the first clinically used and best characterized PR antagonist, RU486, and closely related compounds. We discuss some other steroidal PR antagonists in terms of selectively for PR versus glucocorticoid receptor (GR) and the concept of "active antagonists." Finally, we describe some nonsteroidal PR antagonists and their potential usefulness.

Structure and Functional Properties of PR

PR is a ligand-activated transcription factor that belongs to a large family of nuclear receptors which include receptors for the following: (i) steroid hormones (estrogen, progesterone, glucocorticoid, androgen, and mineralocorticoid); (ii) other lipophilic hormones and ligands (thyroid hormone, retinoic acid, 9-*cis* retinoic acid, vitamin D3 and eicosanoids, fatty acids, lipids); and (iii) orphan receptors that have no known ligand (4–6).

As a member of the nuclear receptor family, PR contains three conserved functional domains, including the N-terminus, a centrally located DNA binding domain (DBD), and C-terminal ligand binding domain (LBD) (Fig. 1). Three-dimensional atomic structures of isolated DBD and LBDs have revealed common motifs for these regions. The core DBD contains two asymmetric zinc fingers, each with a zinc ion coordinated by four conserved cysteine residues. An alpha helix extends between the two zinc fingers, which make base-specific contacts in the major groove of the DNA. The PR LBD consists of 12 α-helices and four β-sheets that fold into a three-layer α helical sandwich containing a central core positioned between helix bundles on either side. This structure creates a hydrophobic wedge-shaped cavity in which the steroid hormone (ligand) is buried. By comparison, little is known about the structure of the N-terminal domain. Biophysical and biochemical data indicate that the N-terminal domain is in a nonglobular extended conformation with little secondary structure (7, 8). This is the least conserved region among family members with respect to both length and amino acid sequence. The N domain is functionally important because it is required for full transcriptional activity of steroid hormone receptors and for many cell- and target gene-specific responses.

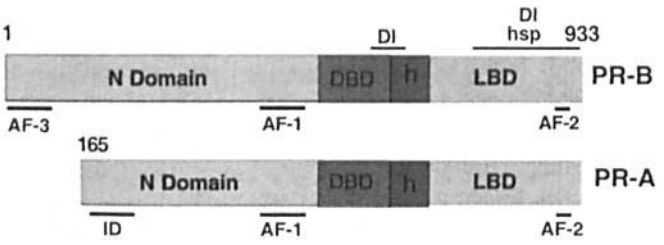


Figure 1. Domain organization of the human PR-A and -B isoforms. N domain, N-terminus; DBD, DNA binding domain; h, hinge; LBD, ligand binding domain. Transcription activation domains; AF-1, AF-2, and AF-3. Dimerization domain (DI); Inhibitor domain (ID); hsp, heat shock protein binding region.

Other functional and structural determinants have been identified within these broader three domains. In addition to binding steroid hormone, the LBD contains determinants for dimerization in the absence of DNA, binding of heat shock proteins (hsps), and for nuclear localization. The DBD contains a second nuclear localization and dimerization domain that is dependent on DNA binding. Steroid receptors contain at least two transcription activation domains (AFs), AF-1 in the N-terminal domain and highly conserved AF-2 in the C-terminal LBD. These are autonomous transferable domains required for the DNA bound receptor to transmit a transcriptional activation response and they function as specific binding sites for coactivators. AF-2 located in the LBD is hormone dependent and becomes activated as a result of the steroid hormone inducing a repositioning of the C-terminal most α helix-12 in such a way as to create a specific hydrophobic binding pocket for members of the p160 family of steroid receptor coactivators (SRCs). Little is known about AF-1 in the N-terminus. It can function independent of AF-2 in a constitutive manner or can synergize with AF-2 in a ligand dependent manner. The coactivators that bind to and mediate the activity of AF-1 are yet not well defined.

The human PR exists as two isoforms, PR-A and PR-B (94 kDa and 120 kDa, respectively; Fig. 1). The two isoforms are expressed from a single gene by alternate promoter usage. PR-A differs from PR-B by lacking 164 amino acids (aa) at the N-terminus (9). Although the two forms of PR have similar steroid hormone and DNA binding activities, they have distinct functional activities. *In vitro* cell culture experiments have shown that the transcriptional activities of the two PR isoforms vary depending on the cell type and context of the target gene promoter. PR-B in general, on classical PRE targets (progesterone response element), is a much stronger activator than PR-A. However, PR-A can be a strong activator under specific cell and target gene contexts (10, 11). The stronger activation potential of PR-B is caused in part by the existence of a third activation domain (AF-3) within the first N-terminal 164 aa that is unique to PR-B (Fig. 1; 12). Under certain cell and target promoter contexts PR-A is inactive as a transcription factor and can function as a ligand-dependent transdominant repressor of other steroid receptors including PR-B, estrogen receptor (ER), androgen receptor (AR), mineralocorticoid receptor, and GR. PR-A can act in this repressor mode in response to binding either progestin agonists or antagonists. An inhibitory domain (ID) responsible for this transrepressor function has been mapped to the first 140 N-terminal (aa 165–305) amino acids of PR-A (Fig. 1). ID is functional and transferable to other steroid receptors such as chicken PR

and human ER that do not exhibit this transrepressor activity. The fact that the sequence within ID is present in both PR isoforms but is only active in the context of PR-A suggests the PR-B–specific N-terminal segment plays a role in suppressing the ID domain. This suppression is thought to occur through the PR-B N-terminal segment exerting a long-distance effect on the conformation of the PR-A N-terminus. How PR-A can repress transcriptional activity of other steroid receptors remains unclear. Studies with PR isoform specific gene knock out mice and transgenic mice that overexpress either PR-A or PR-B have provided evidence that the two forms of PR have distinct physiological roles *in vivo* (13–15). Selective knockout of PR-A in mice has a strong phenotype in the uterus but not in the mammary gland, suggesting that the PR isoforms have tissue-specific roles (13, 14). Transcription factors that harbor both activation and repression domains or are expressed as truncated forms capable of functioning as dominant transrepressors have been identified in several different families of transcription factors. These naturally occurring transrepressors have important physiological roles in shutting off activation responses at specific times during development and differentiation or under specific physiological conditions. PR-A has been suggested to have a similar role among the steroid hormone receptors that may be particularly relevant in the uterus where progesterone is known to antagonize the growth stimulatory activity of estrogen (11, 13, 14).

PG-Induced Activation of PRs

PG and other steroid hormones are lipophilic molecules capable of readily passing from circulation across the cell membrane. Once inside a target cell, they bind to and convert their cognate receptor from inactive to active transcription factors. Receptor activation involves multiple steps, including a conformational change and dissociation from a multiprotein sequestering complex consisting of protein chaperones including hsps and immunophilins (references in 16). Receptors then dimerize and bind to specific DNA sequences within the regulatory promoter region of steroid responsive genes, referred to as hormone response elements (HREs). Consensus HREs consist of inverted repeat hexanucelotide sequences separated by three unspecified nucleotides to form a 15-base pair (bp) recognition site, each bound by a symmetric receptor homodimer. The DNA bound receptor can either increase or decrease rates of target gene transcription through additional interactions that facilitate assembly or stabilization of the preinitiation complex at the promoter (Fig. 2).

Receptor interaction with the general transcription machinery is believed to occur through recruitment of coactivator proteins. Coactivators have no DNA binding activity and associate with target genes solely through protein interaction with DNA bound receptors. The autonomy of the receptor LBD permitted the use of genetic and biochemical approaches to identify ligand-dependent AF-2–interacting coactivators. These include the p160 family of coactivators (SRC-1, TIF-2, ACTR, p/CIP, RAC3, and ABI1) as well as CBP/p300. In addition to the p160 family, numerous unrelated coactivator proteins have also been identified, including RIP140, ERAP160, TIF1, hRPF-1, and ARA70 (see reviews in 4–6). Nuclear receptor interaction domains of these coactivators have been mapped to a highly conserved motif (LXXLL), termed the NR box (6). p160 coactivators possess multiple copies of NR boxes, suggesting that mul-

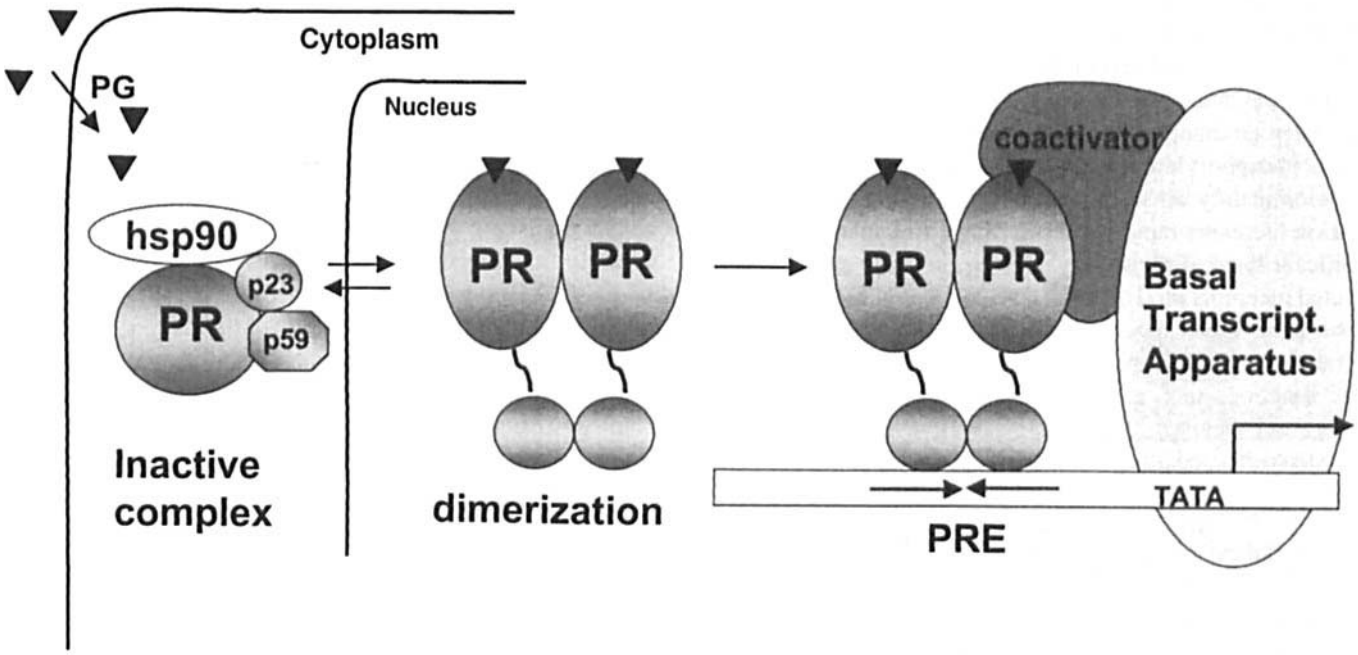


Figure 2. PG activation of progesterone receptor. Binding of PG to the inactive receptor complex induces a conformation change, which leads to immunophilin and hsp dissociation, receptor dimerization, DNA binding, and recruitment of coactivators to facilitate communication with the basal transcription apparatus. PRE, progesterone response element.

tiple regions of the coactivators interact with the receptors (17). In addition, the x-ray crystal structure of estrogen bound ER-LBD in the presence of a peptide containing a LXXLL motif shows that the NR box binds AF-2 and fits into the hydrophobic groove formed by the LBD (18). Some coactivators possess intrinsic enzyme activity for acetylation (histone acetyltransferase activity-HAT) or methylation of core histone proteins. These chemical modifications of core histones can relieve the repressive effects of chromatin on transcription by relaxing nucleosome structure and facilitating access of the general transcription machinery to the promoter. Gene-targeting studies in mice have confirmed the coactivator function of the p160s in a physiological context. Gene disruption of the mouse SRC-1 and SRC-3 resulted in the impaired growth and development of certain steroid responsive tissues, suggesting that loss of SRCs results in partial resistance to hormones (19, 20). SRC-3–null mice also exhibit a short stature phenotype, suggesting that SRC-3 is also important for regulating normal somatic growth (21). Interestingly, genetic ablation of the E6-AP steroid coactivator in mice revealed defects in reproductive functions that were different from the SRC knockout mice (22). These differences suggest that different coactivators mediate a subset of steroid hormone action and support the hypothesis that coactivators contribute to tissue-specific hormone action. Although steroid hormone receptors do not usually interact with corepressors, certain members of the nuclear hormone receptor superfamily, including retinoid and thyroid hormone receptors, can actively silence gene transcription through the recruitment of corepressors that possess histone deactylase enzyme activity (HDAC). HDACs mediate the opposite effect of histone acetyltransferases (HATs) by stimulating a condensation of nucleosome structure and impairing access of the general transcription machinery to the promoter.

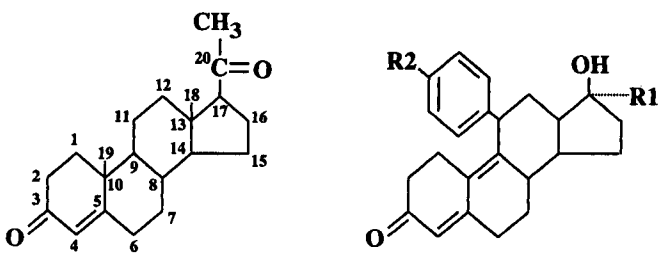
An additional event that is likely involved in PG-dependent activation of receptors is phosphorylation. Phosphopeptide-mapping studies indicate the presence of multiple phosphorylation sites in the N-terminus of human PR, predominantly on serine residues (23, 24). PR phosphorylation increases rapidly after hormone treatment, and a significant level of phosphorylation occurs only after the activated receptors bind to DNA (23, 24). The functional role of phosphorylation has not been well defined; however, several studies have implicated an influence on multiple receptor functions such as DNA binding, transcriptional activation, and receptor stability. Phosphorylation may also be involved in mediating cross-talk with other signaling pathways (23, 25).

Mechanism of Action of the Antagonist (RU486) and Related Compounds

Binding Mechanism. The simplest approach to antagonize PG is to effectively compete for binding of the physiological hormone ligand to PR with an inactive synthetic analog (steroidal or nonsteroidal). RU486 is among the most widely used progestin antagonists. The main structural characteristics of RU486 that correlate with its antagonist activity are the phenyl-aminodimethyl group at the 11-β position of the steroidal skeleton (R1) and the carbon 11 side chain (R2) (Fig. 3). Although RU486 binds with high affinity to PR (in fact, it binds PR with a slightly higher affinity than PG) and effectively competes for PG binding, it does not make the same contacts in the LBD as agonist. As evidence for this, truncation of the last 42 amino acids at the C-terminus of PR abolishes binding to progestin agonist while retaining binding to progestin antagonist (26). Also, a single amino acid substitution Gly→Cys at position 722 in the LBD of human PR abolishes RU486 binding whereas retaining high affinity binding for PG approximately equal to that of wild-type PR (27).

Dimerization and DNA Binding by PR. The mechanism by which RU486 inactivates PR is complex and remains incompletely understood. The receptor activation steps of dissociation from hsps, dimerization, and binding to PREs are not impaired. Although earlier studies suggested that RU486 might act by stabilizing the inactive PR-hsp complex and thus prevent PR from interacting with DNA (3), the preponderance of data does not support this as a mechanism. In fact, RU486 has been observed to induce a stronger dimerization of PR and tighter association of PR with DNA than hormone agonists (28, 29). Additionally, there is no evidence that RU486 causes PR to make different contacts with PREs than PG. Based on DNA footprinting assays, PR was found to make identical base specific contacts with the HRE of mouse mammary tumor virus in the presence of agonist and RU486 (30, 31).

We and others have shown that RU486 promotes PR binding to PREs not just *in vitro* but also within intact cells (*in vivo*). This has been shown by several experimental approaches, including promoter interference assay, induction of gene activation mediated by a PR-VP16 activation domain chimeric receptor, and by the ability of the PR-RU486 complex to effectively inhibit, through competition for PREs, agonist-activated GRs or a constitutively active truncated PR lacking the LBD (32, 33). Although the steroidal antagonist ZK98299 (Onapristone) was initially cat-



Progesterone RU 486

Figure 3. Chemical structure of progesterone and the progesterone antagonist RU486 (mifepristone). The positions of the carbons in the steroid ring are numbered in the progesterone structure. The structure of RU486 shows substitutions at the 11β and 17α positions that are characteristic of steroidal antiprogestins.

egorized as mechanistically distinct from RU486 by preventing PR binding to DNA, this categorization was based on *in vitro* gel shift experiments (34). Subsequent gel shift experiments showed that ZK98299 does induce PR binding to PREs *in vitro*, although the tightness of the association is lower than RU486. Additionally, ZK98299 effectively stimulated PR binding to PREs in intact cells by the approaches used to detect the influence of RU486 on PR-DNA binding *in vivo*. Thus, we conclude that RU486 and other structurally related steroid antagonists all work by a similar mechanism that does not involve inhibiting activation steps that lead to PR binding to specific target DNAs.

Altered Conformation in the LBD Induced by RU486 Inhibits AF-2 Coactivator Recruitment. The mechanism for nonproductive interaction of PR with DNA in the presence of RU486 is not completely worked out. However, studies with p160 coactivators and structure of LBDs bound to agonist versus antagonist have provided much insight. RU486 induces a conformation in the C-carboxyl terminal tail of PR that is distinct from that induced by hormone agonist. This alternate conformation inactivates AF-2 and does not permit interaction with SRCs (35). There are several lines of evidence indicating that RU486 induces an alternate conformation in the C-terminus. RU486-bound PR altered the electrophoretic mobility of PR-DNA complexes when compared with agonist-induced complexes in polyacrylamide native gels (36, 37). A monoclonal antibody to the C-terminus of PR (C262) recognized PR-bound RU486 but not agonist-bound PR, suggesting the C262 epitope is accessible in the presence of RU486 but is not available in the presence of agonist. In addition, using limited proteolytic digestion analysis, antagonist-bound PR LBD gave a digestion pattern distinct from agonist bound PR LBD (38). Modeling of the RU486-bound PR LBD crystal structure predicted that RU486 displaces helix 12 (39) and results in helix 12 disrupting the hydrophobic groove required for coactivator binding. Thus, the displacement of helix 12 induced by RU486 blocks coactivator binding to AF-2 and renders the receptor transcriptionally inactive (40).

Influence of RU486 on Amino- and Carboxyl-Terminal Domain Interaction. Under most cellular and target promoter conditions, full transcriptional activity of steroid receptors requires functional synergy between AF-1 and AF-2 (37). Studies with ER (41) and AR (42) suggest that this functional synergy involves a ligand dependent intramolecular association between the N- and C-terminal domains of receptor. Using a mammalian two-hybrid interaction system, we observed a hormone-agonist–dependent functional interaction between N-terminal domains (PR-A and PR-B) and the hinge LBD (hLBD) of human PR (43). These interactions appear to involve direct protein contacts as determined by *in vitro* protein-protein interaction assays using purified expressed domains of PR (43). RU486 failed to induce an interaction between the N-domains and the hLBD of PR *in vitro* and functionally inhibited hLBD interaction with N-domains in whole cells by mammalian two-hybrid (43). These data indicate that RU486 fails to induce, or impairs, a physical association between the N- and C-domains of PR. These data suggest that in addition to blocking AF-2 coactivator binding, the altered conformation in the LBD induced by RU486 may contribute to inactivation of receptor by interfering with physical association between the amino and carboxyl domains. N- and C-domain interaction may also be necessary for formation of a proper surface for additional coactivator interactions, possibly involving AF-1 coactivators, but this is still unclear at the present time.

Influence of Antagonists on PR-Mediated Transactivation Through Interaction With Other Transcription Factors

The activities and mechanism of action of steroid antagonists on gene transcription have been largely defined by their ability to influence receptor function through consensus HREs. However, there is increasing evidence that a number of steroid-regulated genes are not regulated by direct binding of receptor to classic HREs but through receptor interaction with other sequence-specific transcription factors (Fig. 4). Although this mode of regulation can be either positive or negative, it is more commonly a pathway for negative gene regulation by steroid receptors. As a variation of this mode of regulation are genes that contain composite response elements consisting of a less than optimal DNA binding site for the steroid receptor (often a HRE half-site) that overlaps, or is adjacent to, a binding site for another sequence specific transcription factor (Fig. 4). Examples of PR cross-talk with other transcription factors are repression of NF-kB activity (through interaction with RelA-p65 subunit), inhibition of prolactin-induced Stat5-mediated activation of the β casein gene, repression of AP-1 (*fos*/jun) activity and potentiation of c/EBPβ mediated gene activation (44–47). Because NF-kB is activated by various

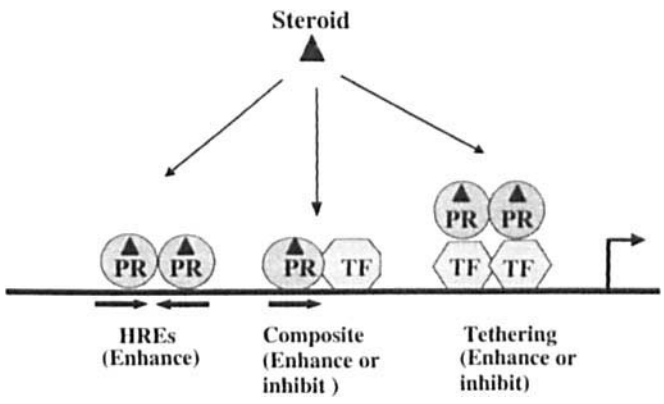


Figure 4. Different mechanisms for gene regulation by steroid receptors. Left, direct binding of PR to HREs within promoters of PG-responsive genes. Middle, composite element consisting of a weak nonconsensus HRE and a neighboring site for another sequence-specific transcription factor (TF). Right, tethering response element. PR interacts with another DNA bound transcription factor through protein-protein interaction to either enhance or inhibit transactivation.

cytokines, cross-talk with PR is thought to be involved in the immunosuppressive effects of progesterone during pregnancy. PR cross-talk with AP-1, c/EBP, and Stat5 is thought to be involved in proliferative and differentiation functions of progesterone in the mammary gland and uterus, respectively. An important question is how steroid antagonists influence this mode of PR-mediated gene regulation. RU486 and ZK98299 were shown to induce PR-mediated repression of the RelA subunit of NF-κB induction of the human ICAM-1 gene in a manner similar to that of the progestin agonist R5020 (45). We found that both RU486 and ZK98299 inhibited prolactin Stat5-mediated induction of a β-casein reporter gene, similar to the way progestin agonists (47) and PR-repression of AP-1 were reported to be induced by PG agonist and RU486 (44). Thus, it appears from these studies that PR antagonists can behave as agonists on target genes regulated through PR interaction with other transcription factors. There is increasing evidence that many natural steroid responsive genes do not contain canonical HREs. Therefore, understanding how antagonists influence PR-mediated transcription on these indirect elements is an important consideration in profiling their biological activity *in vivo*.

Active Antagonists

RU486 and related steroid antagonists are more potent than predicted by simple competition for PG binding and prevention of p160 coactivator recruitment by inactivation of AF-2. RU486 effectively antagonizes PG activation of PR at concentrations that are much less than substoichiometric with PG. Three mechanisms appear to contribute to this unusual potency of RU486 as an antagonist of PG. First, RU486 promotes a higher affinity interaction of PR with DNA than the agonist R5020 *in vitro* (48), and we and others have shown that antagonists-bound PR can effectively compete with binding of agonist-bound PR to PREs *in vivo*. This provides a mechanism for PR-RU486 to inhibit PR-agonist complexes in *trans* through competition for DNA sites. A second contributing mechanism is the ability of PR bound to antagonist to heterodimerize with PR bound to R5020. We showed by coimmunoprecipitation assay *in vitro* (28) and a mammalian two-hybrid assay with receptor ligand specificity mutants (49) that PR bound to RU486 can heterodimerize with PR bound to an R5020. Using electrophoretic mobility shift assay (EMSA), we also observed that mixed R5020/RU486 heterodimers had a significantly reduced ability to bind to PREs (28). Our results were similar to the results of Meyer *et al.* (37), who found that mixed R5020/RU486 heterodimers could not bind to PREs using EMSA. Heterodimerization could potentially sequester a portion of cellular PR bound to agonist in an inactive form, without requiring direct binding of RU486 to PR.

A third contributing factor to the potency of RU486 to antagonize PG is the ability of PR to recruit corepressors to promoters in the presence of RU486. PR in the absence of ligand, or presence of agonist, has weak affinity for corepressors. Nuclear receptor corepressor (NCoR) was identified by yeast two-hybrid assay as a factor that interacts with RU486-bound PR but not agonist-bound PR (50). Subsequent studies revealed a direct physical association between RU486-occupied PR-B and NCoR *in vitro* (51) as well as interactions with NCoR and silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) in mammalian cells (52). Evidence suggests that corepressor interaction is of functional consequence, as was shown by overexpression of NCoR and SMRT, which suppressed the partial agonist activity of RU486-bound PR (50). Also, mixed agonists, which function as weak agonists or antagonists depending on the cell and promoter context (53), induced an interaction of intermediate strength with corepressors (52) in the two-hybrid assay as compared with agonists and more pure antagonists. And antagonist-bound PR-A was shown to have a higher affinity for SMRT than antagonist-bound PR-B (54), correlating with transactivation abilities of the PR isoforms. These observations support a model in which PR has a high affinity for SMRT or NCoR only in the presence of antagonists and that the conformational change induced by agonists increases the affinity of the receptor for coactivators, an event which is incompatible with PR-corepressor interactions (52). Figure 5 depicts the different mechanisms proposed to contribute to the potency of RU486 and related compounds as PG antagonists. These include the ability of the PR-antagonist complex to inhibit PR complexed to PG in *trans* through heterodimerization and competition for binding to PREs. And the ability of PR homodimer-antagonist complexes that bind to PREs to recruit corepressors to the promoter of target genes and actively repress gene transcription. This model has lead to the concept that RU486 and other related steroidal compounds are "active PG antagonists."

Signal Transduction Cross-Talk Potentiates Partial Activity of RU486

As with most steroid antagonists, RU486 is not a pure antagonist. RU486 exhibits partial agonist/antagonist activity under certain cellular conditions. The A and B isoforms of PR respond differently to antagonists; RU486-occupied PR-B can function as a partial weak agonist under certain cellular conditions whereas RU486-occupied PR-A cannot (37, 55). The partial agonist activity of RU486 is dependent on an intact AF-1 region of the receptor (37).

Our laboratory and the Horwitz group showed that the protein kinase A activator 8-bromoadenosine 3′,5′-cyclic monophosphate (8-bromo-cAMP), strongly potentiates the agonist activity of RU486 (and other related progestin antagonists) under cellular and target promoter contexts where RU486 is a complete antagonist (56, 57). However, 8-bromo-cAMP does not affect ZK98299 activity (56). Also, RU486 in the presence of 8-bromo-cAMP is only partially effective in antagonizing R5020 action (56). It should be noted that the activity of 8-bromo-cAMP is not restricted to antagonists because it will also potentiate the

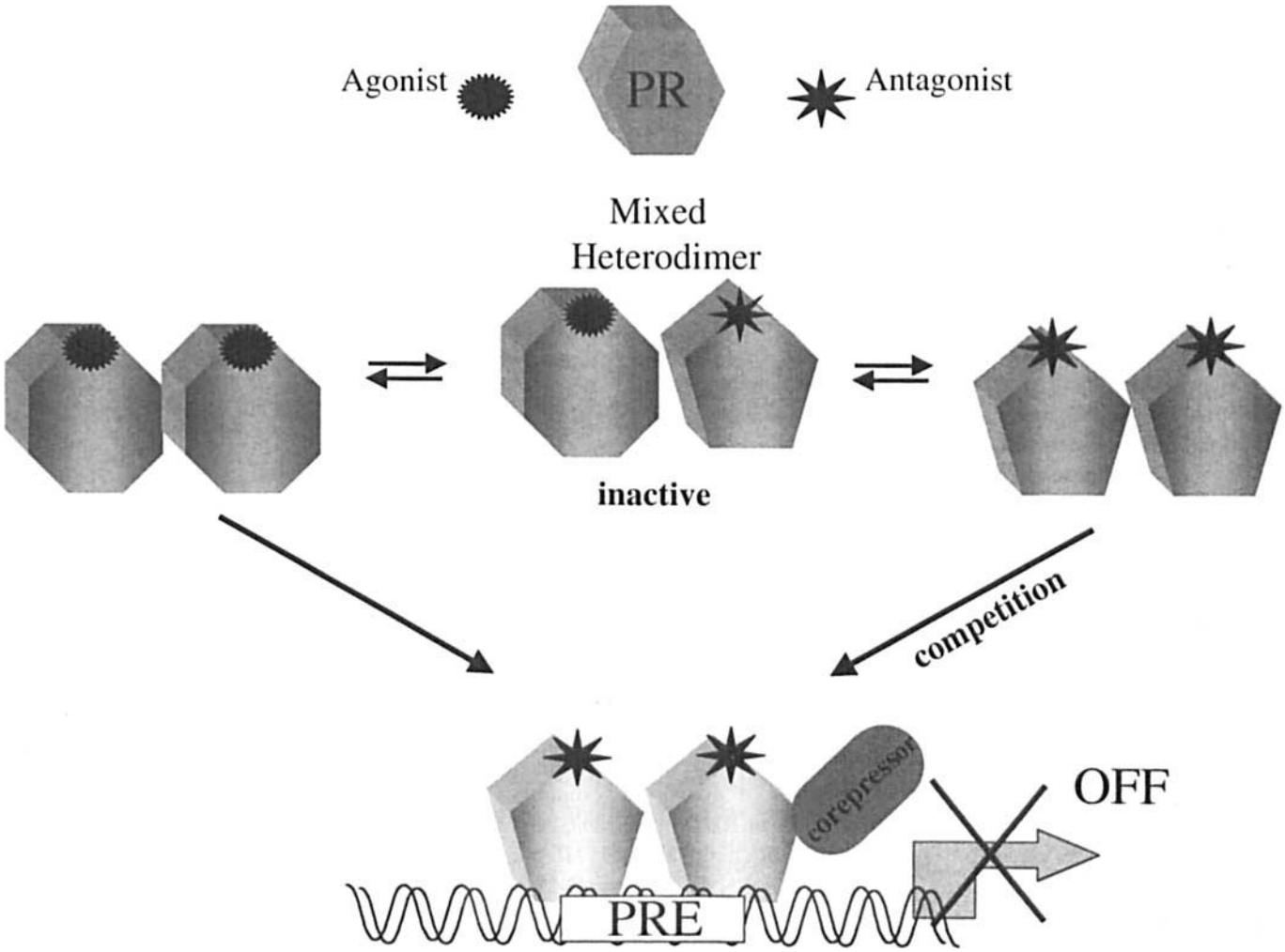


Figure 5. Mechanism of "active" PR antagonists. PG antagonists compete with agonist for binding to PR and promote the activation steps of dimerization and binding to specific PREs of target DNA. However, antagonists induce an altered conformation in PR that is transcriptionally inactive, resulting in a nonproductive interaction of receptor with DNA. Nonproductive DNA interaction is caused by PR recruitment of corepressors instead of coactivators. In addition to this mechanism, the PR antagonist complex can inhibit the PR agonist complex in *trans* by combined mechanisms of competing with the PR agonist complex for binding to PREs and by dimerization with the PR agonist complex to produce an inactive mixed ligand dimer.

activity of agonist-bound PR (56, 57). The mechanism by which 8-bromo-cAMP potentiates PR activity has not yet been determined. However, it has become increasingly evident that the target of 8-bromo-cAMP activation is not receptor phosphorylation itself but receptor-interacting proteins. One such target may be the p160 coactivator SRC-1. A recent study with chicken PR (cPR) showed that 8-bromo-cAMP induced phosphorylation of two sites in SRC-1 and that this phosphorylation of SRC-1 facilitated interaction with and activation of cPR (58). cAMP may also act by disrupting the association of PR with corepressors in the presence of RU486. It has been reported that cAMP dissociates, or inhibits, the interaction of antagonist bound PR with NCoR or SMRT in the mammalian two-hybrid assay (52). Taken together, these results suggest that cAMP induced partial agonist activity of RU486 is caused by the disruption of PR corepressor interactions accompanied by facilitation of PR-coactivator interactions.

Because the partial agonist activity of RU486 is thought to be mediated primarily by AF-1, coactivators that bind AF-1 may be responsible for this activity. We and others have shown that p160s and associated components of the coactivator complex (CBP and pCAF) can interact with the N-terminus of PR and mediate functional enhancement (17). However, these associations with the N-terminal regions are much weaker than with AF-2, suggesting that AF-1 activity may be mediated by as-yet undescribed proteins. SRA is a recently identified endogenous RNA transcript that functions as a selective coactivator for AF-1 of steroid receptors (59). We recently identified a protein, Jun dimerization protein-2 (JDP-2), which functions as a PR N-terminal domain coactivator independently of AF-2 and p160s and can strongly potentiate the partial agonist activity of RU486 (60). Although JDP-2 directly interacts with the DBD of PR, and not the N-terminus, it appears to function as a docking factor to recruit or stabilize other general coactivator interactions (such as CBP/pCAF) with AF-1 in the N-terminus that lies adjacent to the DBD.

It has become increasingly evident that the activity of steroid analogs is determined not only by the ligand and the receptor but also by the coregulatory proteins and context of specific target gene promoters available in any given cell type. Because steroid antagonists are more effective inhibitors of AF-2 than AF-1, the relative balance between corepressors and AF-1 selective coactivators, may be more important than that of corepressors and AF-2 coactivators (Fig. 6). In theory, steroid antagonists can exhibit a broad range of tissue/cell specific agonist/antagonist activities dependent on the many possible combinatorial interactions between the conformation of receptor induced by ligand, the cellular availability of coactivators and corepressors and the composition of the accessible target gene promoters. Because of this tissue/cell selective activity of steroid antagonists, they have become more appropriately termed selective steroid receptor modulators; SERMs for ER modulators and SPRMs for PR modulators (61).

Other Steroidal Antagonists-PR Versus GR Selectivity

Steroidal antiprogestins are chemically characterized by substitutions at the 11β and 17α positions of the steroid ring system of progesterone (Fig. 3). Although many of these compounds have high affinity for PR and potency as PR antagonists, they all exhibit some level of cross-reactivity with GRs. Since the original discovery of RU486, much effort has been devoted to modifications of chemical structures with the goal of generating a compound that is PR specific. Although an antiprogestin that does not bind to the GR has yet to be developed, studies with steroidal antiprogestins other than RU486 have provided insights into possible chemical modifications of the steroidal skeleton that may reduce the affinity for GR while maintaining affinity for PR.

Onapristone (ZK98299), which contains a 13α-configured retro steroid, has a lower GR binding affinity than RU486. However, this compound also has a reduced affinity for PR as well. Although PR binding was also compromised, the chemical structure of ZK98299 emphasized the importance of the stereochemical structure of the D-ring of the steroid in receptor binding and selectivity. Compounds Org. 31710 and Org. 31806 also showed reduced GR binding but unlike ZK98299, maintained RU486 levels of PR binding. Org. 31710 and Org. 31806 have a 17-spiroether group combined with a 6β-methyl group in the B-ring at position 6 and 7 respectively (62). Functional properties of these compounds revealed that small substituents in the B-ring reduce the affinity for GR. The displacement of the dimethylaminophenyl group by acetophenone groups, as found in ZK112993, also reduced binding to GR (63).

One of the most important modifications in the steroid skeleton of antiprogestins in regards to reducing binding to GR appears to be substitutions at position C-17. Several compounds contain this modification. Org. 33628 was the next generation of compounds in the same series as Org. 31710 and Org. 31806 and contains an acetophenone group at C-11 and a methylene-furan substitution at C-17. These substitutions not only result in a 25-fold lower binding to

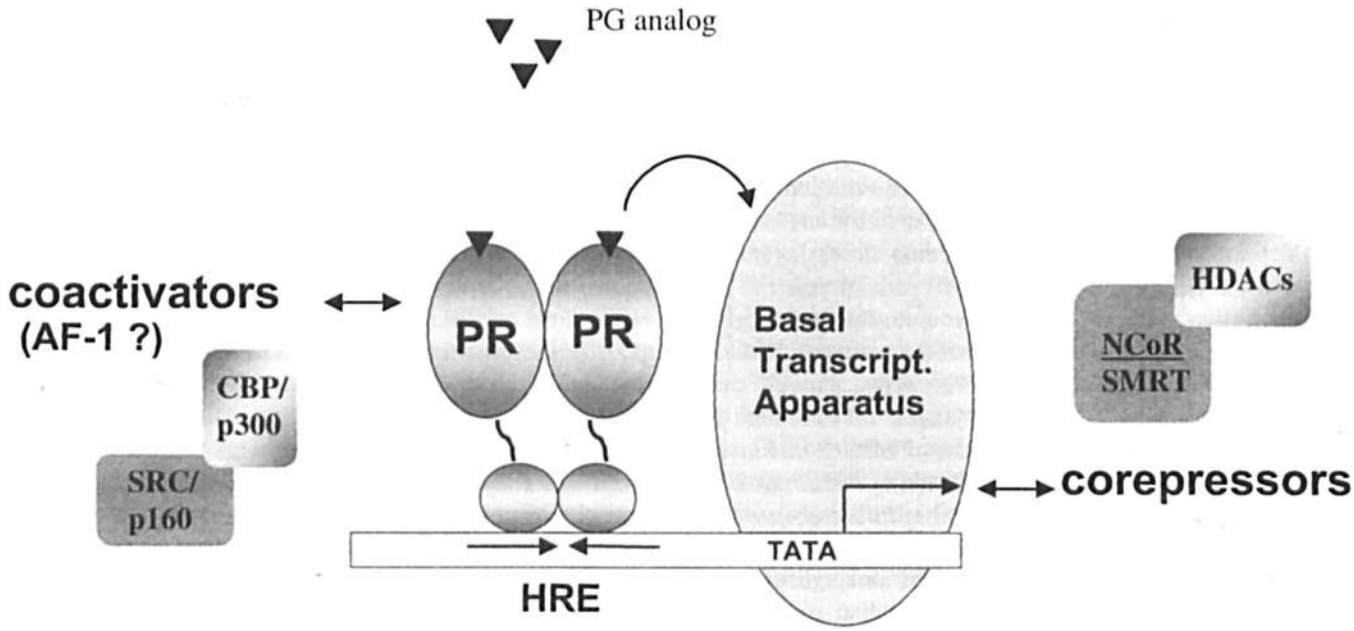


Figure 6. Cell-specific factors that determine activity of PR in response to various ligands. PG agonist or antagonist (▶) bind PR and drives receptor to bind to promoters upstream of progesterone responsive target gene. The transcriptional activity of the receptor-ligand complex is determined by several cell-specific factors. These include cellular availability of coactivators and corepressors and PR affinity for coactivators (SRC/p160 and CBP/p300) or corepressors (such as NCoR or SMRT and associated HDACs) that are determined by the specific ligand-induced conformation of the receptor. Additionally the nature of target gene promoters accessible to PR plays an important role. Shown is a consensus HRE; refer to Figure 4 for other types of elements

GR as compared with RU486 but also a 2-fold higher affinity for PR than RU486 (63). The more recently described ZK230211 has a 17α-pentafluorethyl side chain at position C-17. When antiglucocorticoid activity was assessed *in vivo* by the ability to reverse thymus involution induced by glucocorticoids, ZK230211 was found to have a markedly reduced antiglucocorticoid activity compared to RU486 (64).

Studies by Wagner *et al.* (65) have suggested an alternative mechanistic approach for development of PR selective antagonists with no GR antagonist activity that does not rely on widely different affinities of ligands for GR and PR. The compounds RTI-012 and RTI-022 have similar binding affinities for PR and GR; however, they act as "active antagonists" of PR and "competitive antagonists" of GR. Both compounds fail to promote GR translocation and binding to DNA, whereas they promote efficient interaction of PR with the target DNA. Because much smaller concentrations of "active antagonists" than "competitive antagonists" can be used to effectively inhibit steroid receptor activity, the authors show that at low concentrations of RTI-012 or RTI-022, a complete functional separation of PR and GR antagonism could be achieved. Thus, the mechanism-based approach to develop dissociated antiprogestins appears to be a new and powerful tool in developing antagonists that are functionally selective for PR. Together with traditional direct binding approaches, it may soon be possible to develop antiprogestins that are specific for PR with minimal activity on GR.

Nonsteroidal Antagonists

Currently available antiprogestins all share a common steroidal skeleton derived from a 19-nor-testosterone backbone. These compounds all exhibit some degree of cross-reactivity with other steroid hormone receptors with potential for undesirable side effects *in vivo*. To search for potent antiprogestins with reduced cross-reactivity with GR and resultant endocrine side effects, considerable efforts have been made to discover structurally novel modulators of PR.

Functional high through-put co-transfection assays have been used to screen natural products that modulate PR activity. A crude extract of the marine alga *Cymopolia barbata* was found that inhibits progesterone-stimulated reporter gene expression in cells transfected with hPR and an appropriate reporter gene construct (66). Purification of the active constituents of the extract yielded the antagonist (3R)-cyclocymopol monomethyl ether (LG100127) (66, 67). LG100127 was found to block expression of a progestin target gene, alkaline phosphatase, in the human breast cancer cell line T47D and was also able to displace [^{3}H] progesterone from binding to hPR. However, cross-reactivity with other steroid receptors limited the usefulness of LG10027. Although it did not interact with hGR and hER, the compound showed reactivity with hAR.

Because the functional cell co-transfection assay was used successfully to identify the LG100127 compound, it was also used to screen a defined chemical library, resulting in the identification of 1, 2-dihydro-2,2,4-trimethyl-6-phenylquinoline pharmacophore (LG001447) (68). Investigations into the structure-activity relationships (SAR) of the LG001447 pharmacophore using a series of 6-aryl-1,2 dihydro-2,2,4-tri-methylquinolines identified two compounds LG120753 and LG120830 which were not only able to block the effects of progesterone *in vitro*, but also function as antiprogestins *in vivo* after oral administration to rodents (68). In addition, these compounds had potencies comparable or equal to Onapristone (ZK98299). However, these compounds displayed limited cross-reactivity with hGR and hAR (68). Further SAR analysis of the 6-phenyl 1,2-dihydroquinoline analogues has been performed and yielded 6-thiophene 1,2-dihydroquinoline analogues (69). Although these compounds behaved as good antiprogestins, they also displayed antagonist activity for AR and GR.

Tetrahydropyridazines are another class of nonsteroidal compounds that have shown antiprogestin activity. Discovered in a random screening program in which compounds were evaluated for the ability to compete with radiolabeled R5020 for cytosolic PR obtained from rabbit uterus, the tetrahydropyridazines emerged as novel compounds that demonstrated only modest binding affinities to PR but showed no affinity for AR, ER, and GR at concentrations of up to 10 μM (70, 71). Because the tetrahydropyridazines showed highly selective binding for PR, they were evaluated for agonist and antagonist activity in T47D cell-based gene transcription assays and for activity on PR-DNA binding. Many of the tetrahydropyridazines had agonist activity whereas only a few were shown to have mixed agonist/antagonist activity in the T47D cell proliferation assay. Of the few compounds with antagonist activity *in vitro*, only one compound, RWJ26329, behaved as an antagonist *in vivo*. RWJ26329 was able to inhibit progesterone-stimulated uterine transformation of the estrogen-primed rabbit uterus (71). Mechanistically, little is known of how RWJ26329 functions as an antagonist. DNA binding studies with tetrahydropyridazine compounds that behave as PR agonists suggest that these compounds may not induce the optimal conformation of the PR required for dimerization and subsequent binding to DNA. This data is consistent with the fact that although tetrahydropyridazines are highly selective for PR, they show reduced potency relative to steroidal progestins in all PR-dependent functional assays tested.

A random screening of a defined chemical library has identified derivatives of PF1092C, ((4*a*R,5R,6R,7S)-6,7 dihydroxy-4α,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho [2,3-*b*]furan-2(4H)-one) with antiprogestin activity. Initially discovered as fungal metabolites, two compounds CP8400 and CP8401, have good to moderate affinity for hPR by *in vitro* binding assays and behaved as antagonists in an alkaline phosphate expression assay in T47D cells (72). Importantly, these derivatives of PF1092C possess selective affinity for hPR; with little to no affinity for rat AR, hGR and hER.

Analogs of the antiandrogen, flutamide, have been in-

vestigated for antiprogestin activity based on the observation that certain synthetic steroids (e.g., cyproterone acetate) have dual progestin/antiandrogenic properties (73). The nonsteroidal antiandrogen hydroxyflutamide was therefore considered as a starting point for medicinal chemistry aimed at antiprogestin activity. Various chemical modifications of flutamide (haloalkyl, aryl, haloalkyl/arylalkyl) were made that resulted in increased antiprogestin activity as assessed by abortifacient activity during early pregnancy in pigtailed monkeys (74). However, these compounds consistently exhibited varying degrees of androgen and antiandrogen activity. Hopefully, continuous efforts to establish structure-activity relationships for non-steroidal compounds will lead to identification of potent PR antagonists with minimal cross reactivity with GR and AR.

Summary

PG antagonists repress the biological actions of progesterone by "actively" inhibiting PR activation. PR bound to antagonist can inhibit PR bound to agonist in *trans* through heterodimerization and competition for DNA binding. Once bound to target gene promoters, the PR antagonist complex can recruit corepressors and actively repress gene transcription. Cellular specific factors are also an important determinant of the activity of PR analogs. The agonist/antagonist activity of compounds like RU486 is thought to be a reflection of the balance between expression and availability of coactivators and corepressors and the context of specific target promoters available in any given cell type. The currently available PG antagonists have provided insights into the mechanism of action of PR and suggest that it may be possible to develop tissue/cell specific modulators of PR with minimal cross-reaction with GR and other steroid hormone receptors.

1. Spitz IM, Croxatto HB, Robbins A. ANTIPROGESTINS: Mechanism of action and contraceptive potential. Annu Rev Pharmacol Toxicol **36:**47–81, 1996.
2. Cadepond F, Ulmann A, Baulieu E-E. RU486 (Mifepristone): Mechanisms of action and clinical uses. Annu Rev Med **48:**129–156, 1997.
3. Baulieu E-E. Contragestion and other clinical applications of RU486, an antiprogesterone at the receptor. Science **245:**1351–1357, 1989.
4. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev **14:**121–141, 2000.
5. Edwards DP. Coregulatory proteins in nuclear receptor action. Vitam Horm **55:**165–218, 1999.
6. McKenna NJ, O'Malley BW. Combinatorial control of gene expression by nuclear receptors and coregulators. Cell **108:**465–474, 2002.
7. Bain DL, Franden MA, McManaman JL, Takimoto GS, Horwitz KB. The N-terminal region of the human progesterone A-receptor. J Biol Chem **275:**7313–7320, 2000.
8. Bain DL, Franden MA, McManaman JL, Takimoto GS, Horwitz KB. The N-terminal region of human progesterone N-receptors. J Biol Chem **276:**23825–23831, 2001.
9. Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J **9:**1603–1614, 1990.
10. Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem **277:**5209–5218, 2002.
11. Giangrande PH, McDonnell DP. The A and B isoforms of the human progesterone receptor: Two functionally different transcription factors encoded by a single gene. Rec Prog Horm Res **54:**291–313, 1999.
12. Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS, Horwitz KB. A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol Endocrinol **8:**1347–1360, 1994.
13. Conneely OM, Lydon JP. Progesterone receptors in reproduction: Functional impact of the A and B isoforms. Steroids **65:**571–577, 2000.
14. Conneely OM, Mulac-Jericevic B, Lydon JP, DeMayo FJ. Reproductive functions of the progesterone receptor isoforms: Lessons from knock-out mice. Mol Cell Endocrinol **179:**97–103, 2001.
15. Shyamala G, Yang X, Silberstein G, Barcellos-Hoff MH, Dale E. Transgenic mice carrying an imbalance in the native ratio of A to B forms of progesterone receptor exhibit developmental abnormalities in mammary glands. Proc Natl Acad Sci USA **95:**696–701, 1998.
16. Cheung J, Smith DF. Molecular chaperone interactions with steroid receptors: An update. Mol Endocrinol **14:**939–946, 2000.
17. Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai M-J, Edwards DP, O'Malley BW. The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. J Biol Chem **273:**12101–12108, 1998.
18. Shiau A, Barstad D, Loria P, Cheng L, Kushner P, Agard D, Greene G. The structural basis of estrogen receptor/coactivator recognition and the antagonist of this alteration by tamoxifen. Cell **7:**927–937, 1999.
19. Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai M-J, O'Malley B. Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science **279:**1922–1925, 1998.
20. Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley B. The steroid receptor coactivator SRC-3 (pCIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proc Natl Acad Sci USA **97:**6379–6384, 2000.
21. Wang Z, Rose DW, Hermanson O, Liu F, Herman T, Wu W, Szeto D, Gleiberman A, Krones A, Pratt K, Rosenfeld R, Glass CK, Rosenfeld MG. Regulation of somatic growth by the p160 coactivator p/CIP. Proc Natl Acad Sci USA **97:**13549–13554, 2000.
22. Smith CL, DeVera DG, Lamb DJ, Nawaz Z, Jiang YH, Beaudet AL, O'Malley B. Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. Mol Cell Biol **2:**525–535, 2002.
23. Weigel N. Steroid hormone receptors and their regulation by phosphorylation. Biochem J **319:**657–667, 1996.
24. Knotts T, Orkiszewski R, Cook R, Edwards D, Weigel N. Identification of a phosphorylation site in the hinge region of the human progesterone receptor and additional amino-terminal phosphorylation sites. J Biol Chem **276:**8475–8483, 2001.
25. Lange CA, Shen T, Horwitz KB. Phosphorylation of human progesterone receptors at ser-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. Proc Natl Acad Sci **97:**1032–1037, 2000.
26. Vegeto E, Allan GF, Schrader WT, Tsai M-J, McDonnell DP, O'Malley BW. The mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell **69:**703–713, 1992.
27. Benhamou B, Garcia T, Lerouge T, Vergezac A, Gofflo D, Bigogne C, Chambon P, Gronemeyer H. A single amino acid that determines the sensitivity of progesterone receptors to RU486. Science **225:**206–209, 1992.
28. DeMarzo AM, Onate SA, Nordeen SK, Edwards DP. Effects of the

steroid antagonist RU486 on dimerization of the human progesterone receptor. Biochemistry **31:**10491–10501, 1992.
29. Skafar D. Differences in the binding mechanism of RU486 and progesterone to the progesterone receptor. Biochemistry **30:**10829–10832, 1991.
30. Kuhnel B, El Ashry D, Edwards DP, Nordeen SK. Mapping contacts between unpurified human progesterone receptor and the hormone response element of mouse mammary tumor virus. DNA **10:**703–713, 1989.
31. Chalepakis G, Arnemann J, Slater E, Bruller HJ, Gross B, Beato M. Differential gene activation by glucocorticoids and progestins through the hormone regulatory element of mouse mammary tumor virus. Cell **53:**371–382, 1988.
32. Delabre K, Guiochon-Mantel A, Milgorm E. *In vivo* evidence against the existence of antiprogestins disrupting receptor binding to DNA. Proc Natl Acad Sci USA **90:**4421–4425, 1993.
33. Gass EK, Leonhardt SA, Nordeen SK, Edwards DP. The antagonists RU486 and ZK98299 stimulate progesterone receptor binding to DNA *in vitro* and *in vivo* but have distinct effects on receptor conformation. Endocrinol **139:**1905–1919, 1998.
34. Klein-Hitpass L, Cato ACB, Henderson D, Ryffel G. Two types of antiprogestins identified by their differential action in transcriptionally active extracts from T47D cells. Nucl Acids Res **19:**1227–1234, 1991.
35. Onate SA, Tsai SY, Tsai M-J, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science **270:**1354–1357, 1995.
36. El-Ashry D, Oñate SO. K. NS, Edwards DP. Human progesterone receptor complexed with the antagonist RU486 binds to hormone response elements in a structurally altered form. Mol Endocrinol **3:**1545–1558, 1989.
37. Meyer M-E, Pornon A, Ji J, Bocquel M-T, Chambon P, Gronemeyer H. Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. EMBO J **9:**3923–3932, 1990.
38. Allan GF, Leng X, Tsai SF, Weigel NL, Edwards DP, Tsai M-J, O'Malley BW. Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. J Biol Chem **267:**19513–19520, 1992.
39. Williams SP, Sigler PB. Atomic structure of progesterone complexed with its receptor. Nature **393:**392–396, 1998.
40. Tanebaum D, Wang Y, Williams S, Sigler P. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. Proc Natl Acad Sci USA **95:**5998–6003, 1998.
41. Kraus WL, McInerney EM, Katzenellenbogen BS. Ligand-dependent, transcriptionally productive association of the amino- and carboxyl-terminal regions of a steroid hormone nuclear receptor. Proc Natl Acad Sci USA **92:**12314–12318, 1995.
42. He B, Kemppainen JA, Wilson EM. FXXLF and WXXLF sequences mediate the NH2-terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem **275:**22986–22994, 2000.
43. Tetel MJ, Giangrande PH, Leonhardt SA, McDonnell DP, Edwards DP. Hormone-dependent interaction between the amino- and carboxyl-terminal domains of progesterone receptor *in vitro* and *in vivo*. Mol Endocrinol **13:**910–924, 1999.
44. Bamberger A-M, Bamberger C, Gellersen B, Schulte HM. Modulation of AP-1 activity by the human progesterone receptor in endometrial adenocarcinoma cells. Proc Natl Acad Sci USA **93:**6169–6174, 1996.
45. Kalkhoven E, Wissink S, van der Saag PT, van der Burg B. Negative interaction between the RelA(p65) subunit of NF-κB and the progesterone receptor. J Biol Chem **271:**6217–6224, 1996.
46. Christian M, Pohnke Y, Kempf R, Gellersen B, Brosens JJ. Functional association of PR and CCAAT/Enhancer-binding protein β isoforms: Promoter-dependent cooperation between PR-B and liver-enriched inhibitory protein, or liver-enriched activatory protein and PR-A in human endometrial stromal cells. Mol Endocrinol **16:**141–154, 2002.
47. Edwards DP, Leonhardt SA, Gass-Handel E. Novel mechanisms of progesterone antagonists and progesterone receptor. J Soc Gynecol Invest **7:**22–24, 2000.
48. Edwards DP, Altmann M, DeMarzo A, Zhang Y, Weigel NL, Beck CA. Progesterone receptor and the mechanism of action of progesterone antagonists. J Steroid Biochem Mol Biol **53:**449–458, 1995.
49. Leonhardt SA, Altmann M, Edwards DP. Agonist and antagonists induce homodimerization and mixed ligand heterodimerization of human progesterone receptors *in vivo* by a mammalian two-hybrid assay. Mol Endocrinol **12:**1914–1930, 1998.
50. Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB. The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol **11:**693–705, 1997.
51. Zhang X, Jeyakumar M, Petukhov S, Bagchi MK. A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor. Mol Endocrinol **12:**513–524, 1998.
52. Wagner BL, Norris JD, Knotts TA, Weigel NL, McDonnell DP. The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. Mol Cell Biol **18:**1369–1378, 1998.
53. Wagner BL, Pollio G, Leonhardt SA, Wani MC, Lee DY-W, Imhof MO, Edwards DP, Cook CE, McDonnell DP. 16α-substituted analogs of the antiprogestin RU486 induce a unique conformation in the human progesterone receptor resulting in mixed agonist activity. Proc Natl Acad Sci USA **93:**8739–8744, 1996.
54. Giangrande PH, Kimbrel ER, Edwards DP, McDonnell DP. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. Mol Cell Biol **20:**3102–3115, 2000.
55. Tung L, Mohamed MK, Hoeffler JP, Takimoto GS, Horwitz KB. Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. Mol Endocrinol **7:**1256–1265, 1993.
56. Beck CA, Weigel NL, Moyer ML, Nordeen SK, Edwards DP. The progesterone antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. Proc Natl Acad Sci USA **90:**4441–4445, 1993.
57. Sartorius CA, Tung L, Takimoto GS, Horwitz KB. Antagonist-occupied human progesterone receptors bound to DNA are functionally switched to transcriptional agonists by cAMP. J Biol Chem **268:**9262–9266, 1993.
58. Rowan BG, Garrison N, Weigel N, O'Malley B. 8-Bromo-cyclic AMP induces phosphorylation of two sites in SRC-1 that facilitate ligand-independent activation of the chicken progesterone receptor and are critical for functional cooperation between SRC-1 and CREB binding protein. Mol Cell Biol **23:**8720–8730, 2000.
59. Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ, O'Malley B. The steroid coactivator, SRA, functions as a RNA and is present in an SRC-1 complex. Cell **1:**17–27, 1999.
60. Wardell SE, Boonyaratanakornkit V, Adelman JS, Aronheim A, Edwards DP. Jun dimerization protein-2 (JDP-2) functions as a progesterone receptor N-terminal domain coactivator. Mol Cell Biol **22:**5451–5466, 2002.
61. Katzenellenbogen BS, Katzenellenbogen JA. Defining the 'S' in SERMs. Science **295:**2380–2381, 2002.
62. Mizutani T, Bhakta A, Kloosterboer HJ, Moudgil VK. Novel antiprogestins ORG 31806 and 31710: Interaction with mammalian progesterone receptor and DNA binding of antisteroid receptor complexes. J Steroid Biochem Mol Biol **42:**695–704, 1992.
63. Kloosterboer HJ, Deckers GH, de Gooyer ME, Dijkema R, Orlemans EOM, Schoonen GEJ. Pharmacological properties of new selective antiprogestagen: Org 33628. Ann N Y Acad Sci **761:**192–200, 1995.
64. Fuhrmann U, Hess-Stumpp H, Cleve A, Neef G, Schwede W, Hoffman J, Fritzemeier KH, Chwalisz K. Synthesis and biological activity of a novel, highly potent progesterone receptor antagonists. J Med Chem **43:**5010–5016, 2000.
65. Wagner BL, Pollio G, Giangrande P, Webster JC, Breslin M, Mais DE, Cook CE, Vedeckis WV, Cidlowski JA, McDonnell DP. The novel

progesterone receptor antagonists RTI 3021-012 and RTI 3021-022 exhibit complex glucocorticoid receptor antagonist activities: Implications for the development of dissociated antiprogestins. Endocrinol **140:**1449–1458, 1999.

66. Pathirana C, Stein RB, Berger TS, Fenical W, Ianiro T, Mais DE, Torres A, Goldman ME. Nonsteroidal human progesterone receptor modulators from marine alga Cymopolia barbata. Mol Pharmacol **47:**630–635, 1995.
67. Hamann LG, Farmer LJ, Johnson MG, Goldman ME, Mais DE, Davtian A, Bender SL, Jones TK. Synthesis and biological activity of novel nonsteroidal progesterone receptor antagonists. Ann NY Acad Sci **761:**383–387, 1995.
68. Pooley CLF, Edwards JP, Goldman ME, Wang M-W, Marschke KB, Crombie DL, Jones TK. Discovery and preliminary SAR studies of a novel, nonsteroidal progesterone receptor antagonist pharmacophore. J Med Chem **41:**3461–3466, 1998.
69. Zhi L, Tegley CM, Pio B, West SJ, Marschke KB, Mais DE, Jones TK. Nonsteroidal progesterone receptor antagonists based on 6-thiophenehydroquinolines. Bioorgan Med Chem Lett **10:**415–418, 2000.
70. Combs DW, Reese K, Phillips A. Nonsteroidal progesterone receptor ligands. 1. 3-Aryl-1-benzoyl-1,4,5,6-tetrahydropyridazines. J Med Chem **38:**4878–4879, 1995.
71. Palmer S, Campen CA, Allan GF, Rybczynski P, Haynes-Johnson D, Hutchins A, Kraft P, Kiddoe M, Lai M-T, Lombardi E, Pedersen P, Hodgen G, Combs DW. Nonsteroidal progesterone receptor ligands with unprecedented receptor selectivity. J Steroid Biochem Mol Biol **75:**33–42, 2000.
72. Tabata Y, Iizulka J, Masuda NT, Shinei R, Kurihara K-I, Okonogi T, Hoshiko S, Kurata Y. Fungal metabolites, PF1092 compounds and their derivatives, are nonsteroidal and selective progesterone receptor modulators. Eur J Pharm **430:**159–165, 2001.
73. Neumann F, Graf K-J, Hasan SH, Schenck B, Steinbeck H. Central actions of antiandrogens. In: Martini L, Motta M, Eds. Androgens and Antiandrogens. New York: Raven Press, pp163–177, 1977.
74. Dukes M, Furr BJA, Hughes LR, Tucker H, Woodburn JR. Nonsteroidal progestins and antiprogestins related to flutamide. Steroids **65:**725–731, 2000.