All known progesterone target cells coexpress two functionally different progesterone receptor (PR) isoforms: 120-kDa B-receptors (PR-B) and N-terminally truncated, 94-kDa A-receptors (PR-A). Their ratio varies in normal and malignant tissues. In human breast cancer cells, homodimers of progesterone-occupied PR-A or PR-B regulate different gene subsets. To study PR homodimers and heterodimers, we constructed breast cancer cell lines in which isoform expression is controlled by an inducible system. PR-negative cells or cells that stably express one or the other isoform were used to construct five sets of cells: (i) PR-negative control cells (Y iNull), (ii) inducible PR-A cells (Y iA), (iii) inducible PR-B cells (Y iB), (iv) stable PR-B plus inducible PR-A cells (B iA), and (v) stable PR-A plus inducible PR-B cells (A iB). Expression levels of each isoform and/or the PR-A/PR-B ratios could be tightly controlled by the dose of inducer as demonstrated by immunoblotting and transcription studies. Induced PRs underwent normal progesterin-dependent phosphorylation and down-regulation and regulated exogenous promoters as well as endogenous gene expression. Transcription of exogenous promoters was dependent on the PR-A/PR-B ratio, whereas transcription of endogenous genes was more complex. Finally, we have described several genes that are regulated by induced PR-A even in the absence of ligand.

Progesterone exerts its effects through progesterone receptors (PRs), which are ligand-dependent members of the nuclear receptor family of transcription factors. Two PR isoforms exist in progesterone target tissues: the 120-kDa B-isoform (PR-B) and the N-terminally truncated, 94-kDa A-isoform (PR-A). In transient transfection systems, the two receptors have markedly different transcriptional effects (1–3). Antiprogestins have partial agonist effects only on PR-B, whereas PR-A functions as repressors (1, 3, 4). Differential regulation by the two PR isoforms occurs on endogenous genes as well. Microarray analyses demonstrate that the two PRs up- and down-regulate different subsets of genes in breast cancer cells (5). For example, although the genes encoding the cell cycle regulatory proteins p21 and cyclin D1 are equally well up-regulated by both receptors (6), the anti-apoptotic gene bcl-xL is uniquely up-regulated by PR-A, whereas PR-B uniquely up-regulates C/EBPβ, STAT5α, integrin α6 (ITGA6), and tissue factor F3 (5), all genes important for mammary gland growth, differentiation, and/or breast cancer.

An additional complexity arises from the fact that the two isoforms are coexpressed in the same cell. Therefore, the function of PR-A/PR-B heterodimers may differ from that of the homodimers. Because of this, the ratio of PR-A to PR-B in a tissue is likely to control its response to progesterone. In the uterus, PR-A/PR-B ratios vary extensively during the menstrual cycle (7, 8), leading to variable progesterone responsiveness. PR knockout mice and transgenic mice that overexpress one PR isoform demonstrate the importance of a balanced isoform ratio. Mice that express only PR-B exhibit normal mammary gland development, but have severe reproductive tract anomalies (9), indicating that isoform expression defects are tissue-specific. Transgenic mice that overexpress PR-A exhibit abnormal mammary gland development, including ductal hyperplasia, extensive ductal branching, and decreased cell-cell adhesion, all features associated with neoplasia (10). In contrast, overexpression of PR-B reduces ductal branching and alveolar development (11). Taken together, the data suggest that PR-A and PR-B have physiologically different tissue-specific functions and that maintenance of appropriate isoform ratios is required for normal progesterone responses.

The two PRs are expressed at equimolar levels in the normal human breast during the menstrual cycle (12). Whether the PR ratio fluctuates during development or pregnancy is unknown. In human breast cancers, measurement of total PR levels is an important guide to disease prognosis and response to hormone therapies (13, 14). However, the role of each isoform in clinical decision-making is unknown, but three studies have addressed this (12, 15, 16). Immunoblot analyses of 202 PR-positive breast cancers show that in ~50% of tumors, one isoform exceeds the other (12, 16). Among such invasive tumors, PR-A predominates in ~80% of cases (12). Tumors that overexpress PR-A are less differentiated than tumors that overexpress PR-B (15). Interestingly, in culture, human breast cancer cells that overexpress PR-A detach from the monolayer in response to progesterone (17), a phenotype associated with high-grade malignancies.

In PR-positive T47Dco human breast cancer cells, the two isoforms are constitutively expressed at equimolar levels (18, 19). These cells are ideal models to study progesterone action...
because PR induction does not require estrogen pretreatment. In previous studies, a PR-negative subline (T47D-Y cells) was isolated from T47Deco cells and used to stably reintroduce constitutively expressed PR-A (T47D-YA) or PR-B (T47D-YB cells) (20). Using the edcsyne-inducible system (21), we have now used these cells to engineer five new cell lines. T47D-Y cells were used to create cells that inducibly express (i) no PR (Y iNull cells), (ii) PR-A (Y iA cells), or (iii) PR-B (Y iB cells). Additionally, to manipulate isoform ratios, (iv) T47D-YA cells were modified to inducibly express PR-B (A iB cells), and (v) T47D-YB cells were modified to inducibly express PR-A (B iA cells). The five cell lines all have the same parental cell background. Four express pnonasterone A (pnonA)-inducible PRs in a tightly regulated manner; and in the cells that express one isoform constitutively, the PR-A/P-R-B ratio can be controlled by induction of the other isoform. We demonstrate that liganded, induced receptors undergo progesterone-dependent phosphorylation and down-regulation and control exogenous progesterone-responsive promoters and endogenous gene transcription. A novel finding is that upon receptor induction, subsets of genes defined by microarrays are regulated even in the absence of ligand. One of these genes encodes prolactin receptors (PRLRs).

EXPERIMENTAL PROCEDURES

Cell Lines and Culture—The PR-positive T47Deco breast cancer cell line, isolation of its PR-negative clonal derivative T47D-Y, and construction of PR-positive T47D-YA and T47D-YB cells have been described (20, 22). Cells were routinely cultured in 75-cm² plastic flasks and incubated in 5% CO₂ at 37°C in a humidified environment. The stock medium consisted of minimum essential medium (MEM) with Earle’s salts containing 1-glutamine (292 μg/liter) buffered with sodium bicarbonate (2.2 μl/mg), insulin (6 μg/ml), and 5% fetal calf serum (Hyclone Laboratories, Logan, UT). The T47D-YA and T47D-YB cells were grown in 200 μg/ml G418 (Sigma). The Y iNull, Y iA, Y iB, A iB, and B iA cells were maintained in medium as described above with 300 μg/ml Zeocin (Invitrogen) and 145 units/ml hygromycin B (Calbiochem). A iB and B iA cells were also maintained in 200 μg/ml G418.

Plasmid Construction—The edcsyne-inducible mammalian expression plasmids, and the pVgRXR, pLHDhygro, and pLHDLacZ plasmids were constructed as described (5). RNA samples were prepared from cells independently isolated from T47Dco cells and used to stably reintroduce constitutively the hygromycin B resistance gene were digested with EcoRV and dephosphorylated. The PR-B cDNA (human PR1, gift of P. Chambon) (23) was released with EcoRI, and its ends were filled with Klenow and ligated to the EcoRV-digested pLHDhygro vector to generate pLHDhygro. pLHDhygro was created by BarnHI digestion of pLHD/hygro to excise the 5'-PR-B cDNA region (B-upstream segment), followed by religation. DNA was sequenced for orientation and content.

T47D Cell Transfection and Selection of Stable Cell Lines—Approximately 3 million T47D-Y, T47D-YA, or T47D-YB cells were transfected with 15 μg of the pVgRXR plasmid. After 48 h, the cells were placed in medium containing 500 μg/ml Zeocin to kill untransfected cells. Positive clones were expanded and tested for VgRXR expression and function by β-galactosidase expression from the transcriptionally active LacZ vector containing five VgRXR-binding sites (edcsyne/glucocorticoid response element) upstream of the LacZ reporter. Cells were induced for 24–48 h with 10 μg pDNA (Invitrogen) and lysed in 1X lysis buffer (Pharmingen, San Diego, CA), and β-galactosidase assays were performed as described (6). Three clones with the highest induction (T47D-TV, T47D-YAV, and T47D-YBV) were selected and used to generate secondary stable cells. T47D-TV cells were transfected by electroporation with 15 μg of the pLHD/abygro, pLHD/abygro, or pLHDLNull/hygro construct; T47D-YAV cells were transfected with pLHD/abygro; and T47D-YBV cells were transfected with pLHD/abygro. After 48 h, cells were placed in medium containing hygromycin B (195 units/ml). Surviving clones were expanded and assayed for PR expression by immunoblotting after 24 or 48 h of induction with 10 μg pDNA. pLHDLNull/hygro (Y iNull) cells were screened for an intact inducible promoter region by PCR (data not shown).

Transcription Assays—Cells were harvested, washed with phosphate-buffered saline, and resuspended in medium containing 6% charcoal-stripped serum (CSS). Four million cells, 12 μg of pMMTV-Luc reporter (gift of S. Nordeen) (24) or pRRE/TATAα,Luc, and 1 μg of pCMV-β-gal as an internal control were electroporated to 220 V and 950 microfarads. Replicate sets were plated in four 35-mm dishes in MEM containing CSS, induced with 10 μg pDNA or Me₂SO for 24 h, and then treated with EtOH or 10 nm R5020 for 24 h. Cells were harvested, washed with phosphate-buffered saline, and lysed with 1X lysis buffer. β-Lactamase and β-galactosidase assays were performed as described (6).

RESULTS

Construction and Description of Cells—The edcsyne-inducible mammalian expression system (21) was used to construct cells that inducibly express one or the other PR isoform in the background of PR-negative T47D-Y cells (20). The cells were stably transfected with the VgRXR plasmid, which encodes both the modified edcsyne receptor (Vg) and retinoid X receptor (RXR) regulatory proteins; selected in antibiotic; and expanded. Cells were transiently transfected with the inducible LacZ-positive control plasmid and induced with pDNA, and the clone with the highest induction of β-galactosidase (termed T47D-TV) was stably transfected with plasmids containing five VgRXR-binding sites upstream of the PR-A (pLHD/abygro) or PR-B (pLHD/abygro) cDNA or no cDNA (pLHD/hygro). Transfected cells were selected in antibiotic; expanded; induced with pDNA; and screened by immunoblotting or RT-PCR to select clones that inducibly express PR-A (Y iA cells), PR-B (Y iB cells), or no PR (Y iNull cells). Fig. 1 shows an immunoblot from the three cell lines treated either with vehicle or with pDNA. PR regulation was tightly controlled, and PRs were undetectable in the uninduced cells and the control Y iNull cells. Multiple independent clones were characterized, and no significant dif-
ferences were observed among them. Therefore, one representative clone for each inducible cell type is shown.

T47D-YA and T47D-YB cells, which constitutively express PR-A and PR-B, respectively, were transfected with the VgRXR plasmid; selected in antibiotic; expanded; and screened with the inducible LacZ construct. The highest expressors, called T47D-YAV and T47D-YBV, were used to create the secondary cells. YAV cells received the IndB construct; YBV cells received the IndA construct. Positive clones were selected in hygromycin B, expanded, and screened by immunoblotting (Fig. 1). In the absence of inducer, only the constitutive isoform was expressed. Upon addition of ponA, the second isoform appeared. Expression of the inducible isoform did not affect expression of the constitutive isoform, and receptor levels were similar to those found in T47Dco cells, which express both PR-A and PR-B naturally (Fig. 1).

**PR Induction Is ponA Dose-dependent**—Because heterodimerization of the VgRXR regulatory protein is dependent on binding of ponA, inducer concentration determines the amount of protein produced by the target gene. To demonstrate this, cells were treated with increasing concentrations of ponA (Fig. 2). PR induction was detectable with 1–3 μM ponA in Y iA and Y iB cells (Fig. 2A). In A iB cells, the PR-A/PR-B ratio ranged from 2.3 to 0.9 depending on the ponA dose (Fig. 2B). In B iA cells, the PR-A/PR-B ratio ranged from 0.3 (at 3 μM ponA) to 2.5 (at 10 μM ponA) (Fig. 2B). For the studies described below, a 10 μM ponA dose was used.

**PR Induction Is Time-dependent**—Cells were treated with vehicle or ponA for 3 to 72 h (Fig. 3). PR induction was readily observed at 12 h and peaked at 24 h (Fig. 3, A and B). Without addition of fresh ponA, PRs stayed at high levels for varying periods of time: 72 h in Y iB and Y iA cells (Fig. 3A) and 34–48 h in B iA and A iB cells (Fig. 3B). For the studies described here, a 24-h induction time was used. These time course and ponA concentration data are similar to those reported for in vivo experiments (27).

**PROgesterone-dependent Down-regulation of PRs Despite Continuous ponA**—Wild-type PRs undergo ligand-dependent down-regulation coincident with strong transcriptional activation (27). To determine whether the inducible PRs exhibit this physiologically important response, Y iB cells were treated with or without ponA for 24 h and then with or without the progestin R5020 for 72 h while ponA was continued (Fig. 4). In the absence of R5020, PR-B were detectable for at least 72 h (Fig. 4A, left panel). In the presence of R5020, PR-B were ~70% down-regulated by 12 h and 95% down-regulated by 24 h (Fig. 4A, right panel). This time course of down-regulation was identical to that observed for PRs expressed by their endogenous promoters in T47D cells and for PR expression driven by the exogenous SV40 promoter in the T47D-YA and T47D-YB cells. It occurred despite the continuous presence of ponA and indicates that down-regulation is a post-transcriptional phenomenon. The molecular mass upshift of R5020-occupied PR-B in the right panel is indicative of ligand-dependent phosphorylation (28–30).

**Receptor Turnover in the Absence of ponA**—To define the time course of PR disappearance after ponA withdrawal, Y iB cells were induced with ponA for 24 h. ponA was then washed out; cells were treated with or without R5020; and PR-B levels were measured for 0–72 h thereafter (Fig. 4B). In the absence of R5020, PR-B declined by 39% at 12 h and by 93% at 24 h following ponA removal (Fig. 4B, left panel). The PR loss in the absence of R5020 was due to a halt in transcription coupled with protein turnover. Ligand-dependent down-regulation due to R5020 treatment (Fig. 4B, right panel) accelerated this process, with 98% loss of PR-B by 12 h.

**PR-A/PR-B Heterodimers in Transient Transcription Assays**—To test whether the induced receptors are functional as homo- or heterodimers, cells were transiently transfected with MMTV (24) or PRE2-TATAtk promoter-luciferase reporters and treated for 24 h with 1) vehicle, 2) R5020, 3) ponA, or 4) ponA and R5020 (Fig. 5). In Y iA cells, no transcription above basal levels was observed except in set 4, which received ponA and R5020. Transcription induced by PR-A homodimers was lower from the simple PRE2-TATAa promoter (2–3-fold) than from the complex MMTV promoter (10-fold) (Fig. 5, upper left panel). The pattern in Y iB cells was similar, except for the typically much higher levels of transcription observed with PR-B homodimers from both PRE2-TATAa (50-fold) and MMTV (60-fold) due to activation function 3 of PR-B (Fig. 5, upper right panel). Note the 10-fold difference in the scales of the two panels. No stimulation was observed in Y iNull cells transfected with PRE2-TATAa-Luc and treated with ponA and progesterone, demonstrating that VgRXR is not activated by progesterone and is not functional on a PRE (data not shown).

To study the influence of heterodimers, A iB and B iA cells...
were treated with or without ponA for 24 h, followed by vehicle or R5020 for 24 h (Fig. 5, lower panels). A iB cells express PR-A constitutively, so in the absence of ponA, R5020 induced the PRE2 reporter by 3-fold and the MMTV reporter by 20-fold (set 2), as expected for PR-A homodimers. The contribution of PR-B resulting from ponA induction led to marked rises in transcription levels to 15- and 80-fold, respectively (set 4). However, maximum levels in A iB cells (∼45,000 luciferase units) did not approach the levels seen in Y iB cells (∼200,000 units). At equimolar levels of PR expression, binomial distribution analysis predicts that ∼50% of PRs are heterodimers and that 25% are PR-A or PR-B homodimers (31). The repressor contribution of PR-A as the homodimer and/or heterodimer requires further study. Similarly, in the B iA cells, the strong transcription observed with constitutively expressed PR-B homodimers (120–130-fold induction over baseline levels from both reporters) (set 2) was reduced by ∼75% (PRE2TATAT) and 50% (MMTV) upon induction of PR-A (set 4). Thus, paradoxically, although the induced cells contain higher total PR levels, their transcription levels are lower. These experiments document the inhibitory actions of PR-A on PR-B-mediated transcription in transient transfection assays (1, 3), using models in which the mechanisms can be addressed.

Model Cells to Study PR-A Versus PR-B Regulation of Endogenous Genes—In exogenous expression systems, coexpression of the two PRs leads to transcription levels that differ from those seen with each receptor alone (Fig. 5). This has led to the long-held generalization that PR-A are repressors of PR-B activity. Without appropriate experimental models, however, this has not been analyzable on endogenous genes. The new cell lines were designed to address this deficiency since our long-term goal is to analyze this issue in a global manner. In Fig. 6, we show preliminary results using two endogenous genes: bcl-xL, which we previously showed to be up-regulated specifically by PR-A; and tissue factor, which we previously showed to be up-regulated specifically by PR-B (5). We now investigated what happens to the endogenous regulation of each gene when the other PR isoform is added, using the four PR-expressing cell lines. Each transcript was measured by RT-PCR after treatment with 1) vehicle, 2) progesterone, 3) ponA, or 4) ponA plus progesterone. Immunoblotting was performed to quantify PR induction (data not shown, but see Fig. 3).

Fig. 6A shows the data for bcl-xL. The transcript was induced 2-fold above basal levels in Y iA cells (which express PR-A and RNAs).
were treated with progesterone) only in set 4. In A iB cells, the transcript was again weakly up-regulated by PR-A (1.8-fold; set 2). Expression of approximately equimolar PR-B increased transcription (2.4-fold; set 4). This suggests the interesting possibility that the PR-A/PR-B heterodimer may also regulate \textit{bcl-xL} transcription. Furthermore, \textit{bcl-xL} was also up-regulated by progesterone in T47Dco cells, which coexpress PR-A and PR-B (5). In B iA cells, \textit{bcl-xL} transcription was not regulated by PR-B (set 2) and was up-regulated when PR-A was induced and progesterone was added (set 4).

What about tissue factor, which is specifically regulated by PR-B? Fig. 6B shows 4.5-fold up-regulation of tissue factor in Y iB cells induced with 10 \( \mu \text{M} \) ponA for 24 h; then ponA was removed, and no R5020 was added (left panel), or ponA was removed and R5020 was added (right panel). Cells were harvested at the specified times, and immunoblotting was performed as described under “Experimental Procedures.”

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**Model Cells to Study Ligand-independent Effects of PRs**—These new cells are also uniquely suited to study ligand-independent gene regulation by PRs. This is demonstrated in Fig. 7 by microarray methods using Y iA cells; Y iNull cells served as controls for VgRXR activity. Cells were treated with 1) vehicle, 2) progesterone (6 h), 3) ponA, or 4) progesterone (6 h) plus ponA. RNA was extracted, and polyA\(^+\) RNA was prepared, derivatized, and hybridized to Affymetrix chips (5) displaying \(-12,000\) human genes. Data for three (Y iA) or two (Y iNull) time-separated experiments were generated and analyzed statistically (5). Results for four genes are described (Fig. 7);
asterisks denote statistically significant ($p < 0.01$) induction by unliganded PR-A (set 3) over PR-negative states (sets 1 and 2) in Y iA cells. None of the genes were regulated in Y iNull cells. The four genes shown are the cell cycle inhibitor p21 (Fig. 7A), ENC1 (ectodermal-neural cortex 1, Fig. 7B), the cell adhesion molecule (PCDH1) (Fig. 7C), and PRLR (Fig. 7D). The effect of unliganded PR-A on the PRLR gene was subtle (1.7-fold), but reproducible and statistically significant ($p < 0.01$). Because PRLRs are of interest in the breast, we confirmed the results by RT-PCR (Fig. 7E). PRLR transcript levels increased 1.8-fold after ponA induction of PR-A (set 4) in three independent experiments. The PR requirement was confirmed by the ability of RU486 to suppress the ponA component (set 5) without affecting basal levels (set 2).

**DISCUSSION**

The **Models**—The ecdysone-inducible system produces tight, ponA dose-dependent regulation of PRs. These models allow us to isolate the effects of each PR isoform and to vary isoform ratios while controlling for confounding factors such as differences in the genetic backgrounds of cells. The receptors retain wild-type biochemical properties as monitored by their ligand-dependent down-regulation, ability to be phosphorylated, and transcriptional regulation of endogenous genes and exogenous promoters. Control cells that contain VgRXR and an inducible construct lacking the PR cDNA insert (Y iNull) assess pleiotropic effects, if any, of the VgRXR regulatory heterodimer. With regard to other control issues, wild-type ecdysone receptors utilize the same chaperone and some co-regulator proteins as steroid receptors (32, 33), and this could theoretically impact PR expression and/or function. However, PRs are produced at high levels in these cells, even when VgRXR activation is prolonged by ponA treatment (Fig. 3). Thus, it is unlikely that chaperones are limiting. Similarly, although it is unknown whether the modified ecdysone receptors and PRs utilize common co-regulatory proteins, the very high transcription levels obtained with progestins and the excellent repression obtained with the antiprogestin RU486 (data not shown) suggest that co-regulators are also not limiting. In addition to controlling expression of each isoform independently, two of the cell lines allowed us to overexpress one isoform over the other by ~2-fold (Fig. 2B). We anticipate generating even larger excursions in this ratio by adding a rexinoid to the ponA (26). Other uses of these cells are to study the PR dependence of non-genomic effects of progesterone (34, 35) and to study ligand-independent effects (see below).

**Ligand-independent Effects of PRs**—The Y iA cells demonstrated ligand-independent regulation by human PR-A of a subset of endogenous genes, four of which are shown in Fig. 7 for illustrative purposes. In preliminary experiments, we found a much larger number of genes uniquely regulated by unliganded PR-A than by unliganded PR-B. This is surprising because the opposite is the case for regulation by ligand (5). Ligand-independent effects of nuclear receptors are unusual, and the mechanisms are unclear. Chicken PR-A are activated in a ligand-independent manner by cAMP and epidermal growth factor (36, 37). Despite attempts to do so, this phenomenon has not been reliably demonstrated for human PRs (38). In addition to chicken PRs, several other nuclear receptors can be activated by dopamine through D1 receptors (39), and dopamine placed directly into the third ventricle of the brain increases female rat sexual behavior in a progesterone-independent, but PR-dependent manner (40). Interestingly, two genes regulated by PR-A in a ligand-independent manner, ENC1 and the cell adhesion molecule DSCAM (data not shown), are expressed at high levels in neuronal cells and may be additional brain targets of unliganded PRs. We can only speculate about the mechanisms for ligand-independent gene regulation by PRs. Treatment with 8-bromo-cAMP does not alter chicken PR (41) or human PR (42) phosphorylation, suggesting that the direct target for phosphorylation by this signaling pathway may be one or more transcriptional co-regulatory proteins. For example, the co-regulatory protein SRC-1 is phosphorylated following cAMP treatment and increases the transcriptional activity of PRs (43).

PRLRs are known to be progesterone-regulated (44). Their expression is complex and involves multiple tissue specific promoters. The rat PRLR gene contains tissue-specific promoters that are regulated by several transcription factors, including SP1, STAT5, and C/EBPβ (45, 46). Two promoters, PIII and Pν, drive PRLR expression in human cells (47). The human Pν promoter contains a putative nuclear receptor-binding site, but neither promoter contains a consensus PRE. However, there are putative SP1-binding sites in both PRLR promoters (47). These SP1 sites are of interest because we have previously shown that progesterone regulation of the promoter for the cell cycle inhibitor p21 is indirect, through tethering of PRs to SP1 (48) rather than binding to PREs. These studies also demonstrated that unliganded PR-A, but not PR-B, interact directly with SP1 (48), perhaps explaining the greater ligand-independent transcriptional efficacy of PR-A that we observed in the present study (Fig. 6B). Note that p21 is also regulated by PR-A in a ligand-independent manner (Fig. 7A). The physiological relevance of ligand-independent gene regulation is unknown. Because PRLRs and PRs are coexpressed in immature mouse mammary epithelial cells (49) and both genes are expressed in...
normal breasts of postmenopausal women (50, 51), it is conceivable that ligand-independent mechanisms are engaged during such progesterone-deficient states.

PR-A/PR-B Ratios—At the present time, total PR levels are routinely measured in breast cancers as a guide to therapy. However, given the important functional differences between PR-A and PR-B, summing the levels of the two receptors to arrive at this total is uninformative. In fact, we show here, using exogenous promoters, that as the contribution of PR-A to total PR levels increases, transcription levels can paradoxically decrease (Fig. 5). However, regulation of endogenous genes may be more complex, depending, among other things, on the maturity of the receptors and the treatment time. Because PRs are post-translationally modified by sumoylation\(^2\) and by phosphorylation in a time-dependent manner after protein synthesis (52), this could provide an explanation for differences in function between nascent and mature receptors. These new cell lines provide ideal models to study post-translational modifications and their effects on receptor maturation and biological activity.

Our studies suggest that the dominant-negative effect of PR-A on PR-B may be promoter-specific and may differ on exogenous promoters versus endogenous genes. Interestingly, in progesterone-treated mammary carcinoma cells, the stably integrated MMTV promoter is activated by constitutively expressed PRs, whereas transiently expressed PRs fail to activate transcription (53). Endogenous progesterone-responsive genes may also be differentially regulated depending on whether PR expression is transient or constitutive. Our inducible cells provide a unique model system to examine this question, as PR-A or PR-B can be constitutively or transiently expressed depend-

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\(2\) Abdel-Hafiz, H., Takimoto, G. S., Tung, L., and Horwitz, K. B., submitted to JBC.
ing on whether ponA treatment is continuous or temporary. We are also in a position to study how the ratio of the two isoforms influences transcription of endogenous genes in human breast cancer cells. Several studies indicate that an imbalance in the PR-A/PR-B ratio is physiologically damaging. Breast cancers with an excess of PR-A are less differentiated than tumors with balanced levels of the two isoforms (15). PR-A and PR-B are both present in the normal endometrium. However, only PR-A is detectable in endometriosis (54), whereas overexpression of PR-B is associated with highly malignant forms of endometrial, cervical, and ovarian cancers (55, 56). Equimolar levels of the two PR isoforms have been detected in normal human brain cells, but human chordomas express an excess of PR-B, which is associated with abnormal cell growth (57). Thus, an imbalance in the PR-A/PR-B ratio appears to alter cell growth and other cellular responses to progesterone, but little is known about the underlying mechanisms. The cell lines described here will allow us to investigate these mechanisms.

Acknowledgments—We are grateful to Steve Nordeen for the MMTV-Luc placidum, Pierre Chambon for PR expression vectors, and J. Danny Graham for helpful discussions and advice. We also acknowledge the University of Colorado Cancer Center Gene Expression Core and Sequencing Core Laboratories.

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