Activation of the Human Androgen Receptor through a Protein Kinase A Signaling Pathway*

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Androgens, acting via their receptors, regulate the development and maintenance of the differentiated function of the male reproductive system (1–3). The androgen receptor (AR) is a member of a large family of transcription factors known as the steroid/thyroid receptor superfamily. This superfamily includes receptors for steroid and thyroid hormones and the retinoids, as well as orphan receptors for which the ligands are not known. The receptors are ligand-dependent transcription factors and have distinct functional domains involved in transcriptional activation and repression, DNA binding, and steroid binding. In the presence of ligand (steroid hormone), the receptor is activated, resulting in stimulation or repression of transcription of genes that are under steroid hormone control (4). Hormone antagonists (either natural or synthetic molecules) compete with the agonist for binding to the receptor and block transcriptional activation by the receptor (5, 6).

The activity of many transcription factors, including steroid receptors, is regulated by their phosphorylation status. In the case of these steroid receptors, there is an enhanced phosphorylation of the receptor and/or associated proteins with a concomitant increase in transcriptional activity of the receptor upon binding of the hormone to the receptor (7). This occurs predominantly at serine and threonine residues of the receptor and has been well documented for the progesterone receptor (PR) (8), the glucocorticoid receptor (9), and the AR (10, 11). Phosphorylation of the estrogen receptor (12) and the retinoic acid receptor β (13) also occurs on tyrosine residues. Three phosphorylation sites have been identified for the human AR; two in the amino-terminal region (serine 81 and serine 94) and one in the hinge region between the DNA- and ligand-binding domains (serine 650). Site-directed mutagenesis of these residues to alanine residues resulted in a reduction in the transcriptional activity of the receptor by 30% for the 650 site but had no effect in the case of the 81 and 94 sites (10).

These results suggest that receptor phosphorylation can potentially modulate gene activation. Several steroid receptors, including the chicken progesterone receptor (cPR) and human estrogen receptor, can be activated in the absence of steroid by growth factors, neurotransmitters, and other agents that directly or indirectly increase intracellular kinase activity or decrease phosphatase activity (14–19). Examples of these compounds are 8-bromo-cyclic AMP (an analog of cyclic AMP that acts via the protein kinase A pathway), forskolin (an activator of adenylate cyclase), okadaic acid (an inhibitor of phosphatases 1 and 2A), vanadate (an inhibitor of protein phosphatase 2A), growth factors, and the neurotransmitter dopamine. On the other hand, other members of the superfamily, like the human progesterone receptor (hPR) and the glucocorticoid receptor, cannot be activated in the presence of kinase modulators alone but can be activated in a synergistic fashion by steroid and kinase modulators (20, 21). Co-treatment with steroid hormone antagonists and kinase modulators can be either inhibitory or synergistic, depending on whether the antagonist blocks receptor-DNA interaction or not, and on the receptor and promoter context. Culig et al. (22) have reported that the AR can be activated in the absence of steroid by the growth factors, keratinocyte growth factor (KGF), insulin-like growth factor-1 (IGF-1), and epidermal growth factor (EGF) and that the anti-androgen, casodex, can block this activation. In the case of the rat AR, there is a synergism between the protein kinase A and C pathways and the steroid pathway to stimulate transcriptional activation rather than a steroid-independent activation of the AR (23). De Ruiter et al. (24) also have reported similar cross-talk between the protein kinase C (but not

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1 The abbreviations used are: AR, androgen receptor; ARE, androgen response element; PR, progesterone receptor; cPR, chicken PR; hPR, human PR; EGF, epidermal growth factor; KGF, keratinocyte growth factor; IGF-1, insulin-like growth factor-1; PKI, protein kinase A inhibitor; CAT, chloramphenicol acetyltransferase; PAGE, polyacrylamide gel electrophoresis.

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the protein kinase A pathway) and the steroid pathway for the human AR.

Androgens play an important role in the growth and differentiation of the prostate. In prostate cancer, there is an unchecked proliferation of the prostate epithelial cells. Treatment for prostate cancer includes castration or removal of testicular androgens as well as treatment with anti-androgens, estrogens, or luteinizing hormone-releasing hormone. These therapies are usually beneficial in the earlier stages of cancer but are not effective in more advanced or recurring androgen-insensitive tumors (2, 25). The therapies described above do not eliminate the AR itself (26); if indeed the AR can be activated by alternate signaling pathways that may be activated in rapidly proliferating cancer cells (27), the tumor may progress at an even faster rate.

In this study, we address the issue of whether the human AR can be activated in the absence of androgens by agents that directly modulate protein kinase A activity. We also test the efficacy of two potent anti-androgens, casodex and flutamide, in blocking AR-mediated transcriptional activation.

EXPERIMENTAL PROCEDURES

Materials—All cell culture reagents and the protein kinase A inhibitor peptide (PKI (6-22) amide) were purchased from Life Technologies, Inc. Forskolin was obtained from Sigma, and actinomycin D was purchased from Sigma. Casodex (ICI 176,334) was a gift from Zeneca Pharmaceuticals (Macclesfield, United Kingdom). R1881 (methyltrienolone) and [3H]cholamiphencol were purchased from DuPont NEN, [α-32P]dGTP and [32P]-protein A from ICN (Irvine, CA), and poly-L-lysine from Sigma. All other chemicals were reagent grade.

Plasmids—The reporter construct GRE_E1bCAT was provided by Dr. John Cidlowski (28). It has two glucocorticoid or androgen response elements (AREs) from the tyrosine aminotransferase promoter, followed by the TATA box of the adenosine E1b gene inserted upstream of the choromalcin hydroxysterol transferase (CAT) gene. The -286PCAT plasmid was a gift from Dr. Robert Matsukis and has a 286-base pair sequence of the rat probasin promoter (which has two AREs as well as potential binding sites for other transcription factors) linked to the CAT gene (29). The mutant and wild-type human AR constructs were provided by Drs. Marco Marcelli and Michael McPhaul (30, 31). They are expression vectors driven by the cytomegalovirus promoter. Plasmid DNA was prepared using the pZ2S3 column purification procedure (5 Prime — 3 Prime, Inc., Boulder, CO), and the amount was quantitated at 260 nm.

Cell Culture—Monkey kidney CV1 and COS-1 cells and human prostate epithelial PC-3 cells (obtained from the American Type Culture Collection, Rockville, MD) were grown and maintained in early to mid-log phase in Dulbecco’s modified Eagle’s medium and Dulbecco’s modified Eagle’s medium/F-12 medium, respectively, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin in incubators with humidified air and 5% carbon dioxide at 37°C. Twenty-four h prior to transfection, cells were washed with Hanks’ buffered saline solution, trypsinized, and plated at a density of 2 × 105 cells/well in six-well plates in medium supplemented with 10% charcoal-stripped serum. For the electrophoretic mobility shift assays, COS-1 cells were plated at 105 cells/100 × 20-mm² plates. Immediately prior to transfection, the medium was changed to Dulbecco’s modified Eagle’s medium or Dulbecco’s modified Eagle’s medium/F-12 devoid of serum.

Transfection—CV1, COS-1, and PC-3 cells were transfected by the nonconcurrent adenoviral-mediated DNA transfer technique (32). In brief, the replication-deficient adenovirus, di312, was purified from infected human 293 embryonic kidney cells (stably transfected with adenovirus type 5) by a series of cesium chloride density gradient ultracentrifugation steps. The virus was then coupled covalently to poly-L-lysine. For each well of a six-well plate containing 2 × 10⁶ cells/well, the indicated amount of reporter DNA was mixed with 0.5 µg of reporter DNA in Hepes buffered saline, pH 7.2, and then incubated with 1.5 × 10⁷ virus particles. Thirty min later, additional poly-L-lysine (1.3 µg poly-L-lysine/µg DNA) was added to shrink the DNA on the surface of the virus. The virus/DNA complex mixture was added to cells and allowed to incubate for 2 h in medium lacking serum, following which the medium was supplemented with charcoal-stripped serum to a final concentration of 5%, and the infection was allowed to continue.

Treatments—Twenty-four h following infection, cells were treated with ethanol vehicle or 10⁻¹⁰ M R1881 or as indicated, 1–4 × 10⁻³ M forskolin, 5 × 10⁻⁷ M casodex, or 5 × 10⁻⁶ M flutamide for 24 h. All of the agents were dissolved in ethanol vehicle and added to cells to a concentration not exceeding 0.2% ethanol, which we deemed to be nontoxic to cells by trypan blue exclusion.

CAT Assays—After 24 h of treatment with different agents, cells were harvested by scraping in 1% sodium deoxycholate, 1% EDTA, and 150 mM NaCl (pH 8.0) and collected by centrifugation. Cells were resuspended in 0.25 M Tris, pH 7.5, and lysed by three to four rounds of freeze-thawing. Protein concentrations were determined by a Bio-Rad microtiter plate assay, which is a modification of the Bradford assay (33). The CAT activity was determined by incubating 5–10 µg of total protein with [3H]cholamiphencol (20 µCi/µg) and butyryl CoA as described previously (16). Acylated cholamiphencol was extracted using a mixture of tetramethyl pentadecane/xylenes (2:1) and counted in a liquid scintillation counter. Reagent blanks were subtracted from the sample counts.

Immunoblot Analysis—After 24 h of treatment with different agents, cells were harvested and lysed as described earlier, except that cell extracts were prepared in 0.4 M NaCl homogenization buffer (50 mM potassium phosphate, pH 7.4, 30 mM sodium fluoride, 1 mM EDTA, 1 mM EGTA, and 12 mM 3-mercaptopropionic acid). Equal amounts of protein samples were separated by electrophoresis on a 6.5% SDS-polyacrylamide (SDS-PAGE) gel and transferred to nitrocellulose by a liquid transfer method. A polyclonal antibody to the human AR generously provided to us by Dr. Michael McPhaul was used to detect the expressed AR protein using 125I-labeled protein A (specific activity, >30 µCi/µg) (30).

Nuclear Fractionation of the AR—After 24 h of treatment with different agents, cells were harvested and resuspended in a lysis buffer (0.1% Triton X-100, 10 mM Tris, pH 7.5, 1 mM EDTA, 12 mM 3-mercaptopropionic acid, and protease inhibitors). The cytoplasmic fraction was separated by centrifuging at 3000 rpm in an Eppendorf 5415 microfuge for 5 min and retaining the supernatant. The nuclear pellet was washed with 10 mM Tris, pH 7.5, and then resuspended in 0.4 M NaCl homogenization buffer. This was incubated on ice for 1 h, then centrifuged at 12,000 rpm, and the supernatant was used as the nuclear fraction. Nuclear fractions were electrophoresed on a 6.5% SDS-PAGE gel, transferred to nitrocellulose, and detected with AR antibody as described before (30).

Electrophoretic Mobility Shift Assay—ARE oligo DNA described by Tsai et al. (34) was labeled with α-[32P]dGTP (3000 Ci/mmol) using Klenow polymerase. The probe was purified on a Quick-Spin Sephadex G-25 column (Boehringer Mannheim). After treatment of COS-1 cells for 24 h with the different agents, cells were harvested in 0.4 M NaCl TEBG (10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM dithiothretol, and 10% glycerol) with protease inhibitors. Cell extracts were prepared as before, and protein levels were determined. Twenty µg of cell extract were incubated with a pre-mix buffer of poly (dA-dT) and gelatin, then 20,000 cpm of radiolabeled ARE probe (specific activity = 5 × 10⁶ cpm/µg DNA) was added and incubated for 1 h on ice. The reaction mixtures were loaded on a 4% polyacrylamide gel and run at 20 mA for 3 h at 4°C, as described previously (20). The gel was dried and exposed to x-ray film (Eastman Kodak Co.).

RESULTS

The results of transfections of AR-negative CV1, COS-1, and PC-3 cells with AR expression vectors and inducible reporter constructs are presented below. From DNA titration curves, 62.5 ng of AR DNA and from steroid concentration curves, 10⁻¹⁰ M R1881 were chosen as optimal for the transfection and treatment of cells.

Activation of AR and cPR but not hPR by Forskolin in CV1 Cells—In Fig. 1A, the steroid-inducible reporter GRE_E1bCAT alone or in conjunction with the AR expression vector was transfected into CV1 cells. The cells were treated with ethanol vehicle, 10⁻¹⁰ M R1881, or 2 × 10⁻⁵ M forskolin. The reporter alone was not activated by androgen or forskolin. However, transfected AR induced CAT activity in cells treated with R1881 or forskolin. The extent of activation with forskolin was 73% of that seen with R1881. Therefore, both androgens as well as alternate pathways involving protein kinase A inducers can activate AR-mediated gene expression independent of androgen.
Previous studies of the cPR and hPR using conventional transient transfection techniques had shown that the cPR, but not the hPR, was activated by ligand-independent pathways. Moreover, Ikonen et al. (23) failed to detect ligand-independent activation of the rat AR using conventional transient transfection procedures. To determine whether our ability to elicit ligand-independent activation of the human AR was a function of the adenovirus-mediated infection procedure, we compared the activity of the AR with the cPR and hPR under identical conditions. These results are shown in Fig. 1, B and C. The cPR (A form) and hPR (B form) were co-transfected with the GRE_E1bCAT reporter and subsequently treated with 10^{-8} M progesterone (for the cPR) or 10^{-8} M R5020 (for the hPR) or with 2 \times 10^{-5} M forskolin. As shown in Fig. 1B, the cPR can be
activated by $10^{-8}$ M progesterone as well as by forskolin analogous to the AR activation by $10^{-10}$ M R1881 and forskolin. The hPR, shown in Fig. 1C, can be activated by $10^{-8}$ M R5020 but not by forskolin, whereas the AR can be activated by R1881 as well as forskolin. In summary, the human AR, like the cPR but not the hPR, exhibits ligand-independent activation.

Expression of AR in CV1 Cells—In Fig. 2, the same amounts of AR expression plasmid used as for CAT activity determination (62.5 ng) were transfected into CV1 cells, and the total protein (extracted in a high salt homogenization buffer) was electrophoresed on a 6.5% SDS-PAGE gel. The AR is expressed as a doublet with a molecular mass of approximately 112 and 110 kDa when visualized by a polyclonal antibody to the AR.

In Fig. 3, CV1 cells were transfected either with reporter alone (GRE2E1bCAT) or together with wild-type AR or AR mutants having amino acid substitutions in the DNA binding domain. The two mutant AR DNAs had substitutions at positions 574 (cysteine to arginine) for the first mutant and at 595 (serine to glycine) for the second mutant (31). The wild-type AR was activated to an equal extent by treatment with $10^{-10}$ M R1881 and $2 \times 10^{-5}$ M forskolin. However, neither of the mutant receptors (which have greatly reduced or no DNA binding ability, as determined previously) could be activated with R1881 or forskolin. Western analysis confirmed that the wild-type and mutant receptors were expressed at similar levels (data not shown). Therefore, it appears that binding of the AR to the ARE is critical for the ligand-independent and ligand-dependent activity of the AR.

Forskolin Increases Nuclear AR Levels, Whereas Casodex and Flutamide Inhibit This Effect in CV1 Cells—Previous studies have shown that in the absence of hormone, steroid receptors are predominantly found in the cytoplasmic fraction of cells, but that subsequent to hormone treatment, high salt is required to extract the receptors (35). To determine the effects of R1881, forskolin, and the anti-androgens on tight nuclear binding of the AR, CV1 cells were transfected with the AR expression vector and treated with ethanol, $10^{-10}$ M R1881, $2 \times 10^{-5}$ M forskolin, or a combination of R1881 or the anti-androgens with forskolin. Nuclear fractions were isolated as described under “Experimental Procedures” and run on a 6.5% SDS-PAGE gel. Fig. 7 shows that the AR nuclear steady-state levels are increased over control (Fig. 7, lane 2) slightly in the forskolin-treated (Fig. 7, lane 3) and substantially in the R1881-treated samples (Fig. 7, lane 1), and that casodex and flutamide block the control and forskolin-mediated increase in nuclear AR levels (Fig. 7, lanes 4 and 6-8 versus lanes 2 and 3).

Forskolin Enhances Whereas Casodex and Flutamide Inhibit AR Binding to AREs in COS-1 Cells—To determine whether the DNA binding ability of the AR was affected by the treatments described below, we prepared whole-cell extracts and measured DNA binding using an electrophoretic mobility shift assay. COS-1 cells were transfected with no DNA (mock trans-
fection) or 5 μg of AR expression vector and treated with ethanol vehicle, 10^{-10} M R1881, or 2 \times 10^{-5} M forskolin, with or without PKI at 10 μg/ml. Bars, S.D. In B, CV1 cells were transfected with the constitutive CAT construct pSV\_CAT and then grown in the presence or absence of 10 μg/ml PKI as above. Bars, S.D. In C, high salt cell extracts were prepared, and 5 μg of protein were loaded per lane on a 6.5% SDS-PAGE gel as before. The AR doublet protein band can be visualized at 112 and 110 kDa.

**DISCUSSION**

Steroid receptors are hormone-regulated transcription factors that control the expression of target genes (4). Recently, there has been a great deal of evidence for alternate regulatory mechanisms for a number of members of this superfamily. This regulation can either be in the form of ligand-independent activation (where ligand equates to steroid), synergism between steroid and signaling pathways, or an alteration of a steroid antagonist behavior to that of an agonist in the presence of modulators of phosphorylation (14, 15, 20, 21). In this report, we demonstrate that the human AR can be activated in the absence of androgens through an alternate signaling pathway involving forskolin, a stimulator of adenylyl cyclase. This ligand-independent activation of the AR was observed in both monkey kidney CV1 cells as well as in human prostate PC-3 cells transiently transfected with the AR expression vector and with two separate androgen-inducible reporter constructs (one an artificial promoter and the other, a natural context promoter). The cyclic AMP analog, 8-bromo-cyclic AMP, can also activate the AR in a ligand-independent manner (data not shown), albeit to a lower extent than forskolin (for 8-bromo-cyclic AMP, activation seen is between 20 and 25% of that with R1881). We also demonstrate that the cPR, but not the human counterpart, can also be activated by forskolin in CV1 cells. This is in agreement with previous reports showing that the cPR, but not the hPR, can be activated by other agents such as 8-bromo-cyclic AMP, phorbol esters, okadaic acid, and EGF (16, 20).

Alternate activation of the human AR in the absence of steroid has also been demonstrated by Cülig et al. (22) in DU-145 and LNCaP prostate tumor cell lines in response to IGF-1, KGF, and EGF. The magnitude of the ligand-independent activation of the AR by forskolin that we observe can range from experiment to experiment from 25% to as much as 100% as that seen with R1881. The data shown in Figs. 1 and 5 are representative of activation levels normally seen in CV1 and PC-3 cells, respectively. Neither R1881 nor forskolin increased AR steady-state protein levels over control levels under these conditions. We have not, however, characterized AR protein turnover. The activation of the AR by forskolin and R1881 can be attributed to enhanced AR activity per se rather than an increase in basal activity due to increased protein expression. Treatment with PKI reduced ligand-independent activation, and to a somewhat lower extent, ligand-dependent activation without altering receptor expression levels. PKIs also inhibit the ligand-depend-
ent activity of the cPR (8) and of the hPR (36), suggesting that this pathway normally plays a role directly or indirectly in hormone-dependent steroid receptor function.

Analysis of the effects of forskolin on tight nuclear binding and the ability of receptor to bind to DNA (Figs. 7 and 8) indicate that both phenomena are enhanced but are less than that seen with R1881. This suggests that an additional aspect of receptor function, such as interactions with other transcription factors or co-activators, is also being affected by forskolin. Previous studies have shown that casodex and flutamide inhibit R1881-mediated nuclear localization and DNA binding (35, 37–40). In this study, we show that casodex and flutamide can also inhibit forskolin-mediated translocation as well as DNA binding in a similar manner to the block of the ligand-dependent activation, indicating that the activation by forskolin is an AR-mediated event. The ability of antagonists to block ligand-independent activation or to prevent stimulation of activity by protein kinase activators is by no means universal. In some cases, the anti-estrogen, tamoxifen, stimulates estrogen receptor activation in the presence of dopamine or protein kinase modulators (18, 41). Moreover, treatment of cells with 8-bromo-cyclic AMP can cause the progestin/glucocorticoid antagonist, RU486, to act as an agonist (20, 21). Interestingly, antagonists that apparently block DNA binding, such as the progestin antagonist ZK98299 or the estrogen antagonist ICI 164,384, typically block ligand-independent activation and do not act as agonists in the presence of modulators of kinase activity (18, 20). The fact that pure anti-androgens (which are devoid of steroid activity and/or inhibit AR binding to DNA) block ligand-independent activation suggests that treatment with these anti-androgens may be more effective in blocking receptor activity in prostate cancer rather than solely attempt-

Fig. 4. Inhibition of AR-mediated transcription by anti-androgens in CV1 cells. Following infection of CV1 cells with AR and GRE_E1bCAT reporter, the cells were treated with ethanol, 10^{-10} M R1881, or 2 \times 10^{-5} M forskolin alone or in combination with the two potent anti-androgens, casodex (5 \times 10^{-7} M) or flutamide (5 \times 10^{-6} M). The anti-androgen treatment alone is shown in the first group of columns. The second group of columns correspond to combinations of steroid with/without the two anti-androgens. The third group of columns depicts forskolin alone and in combination with anti-androgens. These results are representative of three independent experiments; bars, S.D.

Fig. 5. Ligand-independent activation of the AR by forskolin in human prostate PC-3 cells. PC-3 cells were transfected with the -286PCAT alone (first group of columns) or in combination with AR expression vector at 62.5 ng of DNA (second group of columns) per 2 \times 10^5 cells. Cells were treated with ethanol, 10^{-10} M R1881, or 1 \times 10^{-5} M forskolin. Bars, S.D.

Fig. 6. An intact DNA binding domain of the AR is critical for ligand-independent activation of the AR in CV1 cells. CV1 cells were transfected with reporter alone or in combination with wild-type AR and two AR mutants having amino acid substitutions in the DNA binding domain. The first group of columns correspond to the reporter-alone transfectants, whereas the second group indicates wild-type receptor, and the third and fourth groups correspond to mutant 574* and the double point mutant 595* and 615*, respectively. The cells were treated with ethanol, R1881, or forskolin as before. This graph is representative of two independent experiments; bars, S.D.
be species- and tissue-specific) and steroid receptors can regulate gene expression. Our experiments were performed in CV1 cells and PC-3 cells with two reporter constructs, GRE-E1bCAT (an artificial promoter-reporter) and -286PB-CAT (a natural context promoter-based reporter), both of which were activated in a ligand-independent manner. Factors such as species specificity of steroid hormone receptors, cell lineage, and promoter context of androgen-inducible reporter constructs may determine whether the AR can interact with critical cellular transcription factors to regulate gene expression in the absence of androgen. In fact, Culig et al. (22) have shown that the context of the promoter in the reporter gene (the number of ARES) also governs ligand-independent activation of the AR in response to growth factors. In the presence of a two ARE-reporter, IGF-1, EGF, and KGF activated the AR in the absence of steroid, but the ligand-independent activation with EGF and KGF was not evident when a reporter with a single ARE was used. Our data indicate that the AR can be activated by other pathways besides growth factor receptor-mediated events in the absence of androgen.

The implications of these findings for alternate activation of the AR may be important in understanding the progression of prostate cancer. We propose that activation of the AR may occur (by signaling pathways that modulate phosphorylation) even after androgen withdrawal therapy in individuals with prostate cancer, resulting in the androgen-independent growth of these cells. Our data suggest that these ligand-independent pathways may be blocked efficiently in vitro with the anti-androgens, casodex and flutamide.

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REFERENCES

1. Sadi, M. V., and Barrack, E. R. (1993) Cancer 71, 2574–2580
2. Yan, G., Fukabori, Y., Nikdaropoulos, S., Wang, F., and McKeehan, W. L. (1992) Mol. Endocrinol. 6, 2123–2128
3. Yuan, S., Frachtenberg, J., Mills, G. M., Brown, T. J., Xu, F., and Keating, A. (1993) Cancer Res. 53, 1304–1311
4. Beato, M. (1989) Cell 56, 335–344
5. Horwitz, K. B. (1992) Endocr. Rev. 13, 146–163
6. Kallio, P. J., Janne, O. A., and Palvimo, J. J. (1994) Endocrinology 134, 998–1001
7. Orti, E., Bodwell, J. E., and Munck, A. (1992) Endocr. Rev. 13, 105–128
8. Denner, L. A., Schrader, W. T., O’Malley, B. W., and Weigel, N. L. (1990) J. Biol. Chem. 265, 16548–16555
9. Hoek, W., and Groner, B. (1990) J. Biol. Chem. 265, 5403–5408
10. Zhou, Z. Y., Kempainen, J. A., and Wilson, E. M. (1995) Mol. Endocrinol. 9, 605–615
11. van Laar, J. H., Berrevoets, C. A., Trapman, J., Zegers, N. D., and Brinkmann, A. O. (1991) J. Biol. Chem. 266, 3734–3738
12. Migliaccio, A., DiDomenico, M., Green, D., de Alcón, A., Kajtić, E. L., Blasi, F., Chambon, P., and Auricchio, F. (1989) Mol. Endocrinol. 3, 1061–1069
13. Rochette-Egly, C., Gaub, M., Lutz, Y., Ali, S., Scheuer, I., and Chambon, P. (1992) Mol. Endocrinol. 6, 2197–2209
14. Denner, L. A., Weigel, N. L., Maxwell, B. L., Schrader, W. T., and O’Malley, B. W. (1990) Science 250, 1740–1743
15. Power, R. F., Mani, S. K., Codina, J., Connelly, O. M., and O’Malley, B. W. (1991) Science 254, 1636–1639
16. Zhang, Y., Bai, W., Allgood, V. E., and Weigel, N. L. (1994) Mol. Endocrinol. 8, 577–584
17. Ignar-Trowbridge, D. M., Nelson, K. G., Bidwell, M. C., Curtis, S. W., Washburn, T. F., Madlachlan, J. A., and Korach, K. S. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6458–6462
18. Smith, C. L., Connelly, O. M., and O’Malley, B. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6120–6124
19. Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotô, Y., Nishida, E., Kawashima, H., Metzger, D., and Chambon, P. (1995) Science 270, 1491–1494
20. Beck, C. A., Weigel, N. L., Moyer, M. L., Nordeen, S. K., and Edwards, D. P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4441–4445
21. Moyer, M. L., Bond, K. C., Boná, B., DaFranco, D. B., and Nordeen, S. K. (1993) J. Biol. Chem. 268, 22933–22940
22. Culig, Z., Hubsch, A., Cronauer, M. V., Radmayr, C., Trapman, J., Hittmair,
23. Ikonom, T., Palumbo, J. J., Kalil, P. J., Reinikainen, P., and Janne, O. A. (1994) Endocrinology 135, 1359–1366
24. de Ruiter, P. E., Teuwen, R., Trapman, J., Dijkema, R., and Brinkmann, A. O. (1995) Mol. Cell. Endocrinol. 110, R1–R6
25. Carter, H. B., and Coffey, D. S. (1995) Mol. Cell. Endocrinol. 110, R1–R6
26. Van Der Kwast, T. H., Schalken, J., Ruizeveld de Winter, J. A., van Vroothen, C. C. J., Mulder, E., Boersma, W., and Trapman, J. (1991) Int. J. Cancer 48, 189–193
27. Voeller, H. J., Wilding, G., and Gelman, E. P. (1991) Mol. Endocrinol. 5, 209–216
28. Allgood, V. E., Oakley, R. H., and Cidlowski, J. A. (1993) J. Biol. Chem. 268, 20870–20876
29. Rennie, P. S., Bruchovsky, N., Leco, K. J., Sheppard, P. C., McQueen, S. A., Cheng, H., Snook, R., Hard, A., Bock, M. E., MacDonald, B. S., Nickel, B. E., Chang, C. S., Liao, S. S., Cattini, P. A., and Matusik, R. J. (1993) Mol. Endocrinol. 7, 23–36
30. Tilley, W. D., Wilson, C. M., Marcelli, M., and McPhaul, M. J. (1990) Cancer Res. 50, 5382–5386
31. Zopp, S., Marcelli, M., Deslypere, J. P., Griffin, J. E., Wilson, J. D., and McPhaul, M. (1992) Mol. Endocrinol. 6, 409–415
32. Allgood, V. E., Wetzel, N. L., and O'Malley, B. W. (1994) Seventy-Sixth Endocrine Society Meeting, 631 (abstr.) The Endocrine Society, Bethesda
33. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
34. Tsai, S. Y., Carlstedt-Duke, J., Weigel, N. L., Dahlman, K., Gustafsson, J.-A., Tsai, M. J., and O'Malley, B. W. (1988) Cell 55, 361–369
35. Veldscholte, J., Berrevoets, C. A., Brinkman, A. O., Grootegeest, J. A., and Mulder, E. (1992) Biochemistry 31, 2393–2399
36. Beck, C. A., Weigel, N. L., and Edwards, D. P. (1993) Mol. Endocrinol. 6, 607–620
37. Wong, C., Xhou, Z., Sar, M., and Wilson, E. M. (1993) J. Biol. Chem. 268, 19004–19012
38. Verheist, J. J., Denis, L., Van Vliet, P., Van Poppel, H., Braeckman, J., Van Cangh, P., Mattelaer, D., and Mahler, C. (1993) Clin. Endocrinol. 41, 525–530
39. Mauhder, A., and von Angerer, E. (1993) J. Cancer Res. Clin. Oncol. 119, 669–674
40. Kemppainen, J. A., Lane, W. V., Sar, M., and Wilson, E. M. (1992) J. Biol. Chem. 267, 968–974
41. Fujimoto, N., and Katzenellenbogen, B. S. (1994) Mol. Endocrinol. 8, 296–304