Follicle-stimulating Hormone Inhibits All-trans-retinoic Acid-induced Retinoic Acid Receptor α Nuclear Localization and Transcriptional Activation in Mouse Sertoli Cell Lines*

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The regulation of retinoic acid receptor alpha (RARα) signal transduction has not been well characterized. In this study, we determined whether all-trans-retinoic acid (tRA) and follicle-stimulating hormone (FSH) modulate RARα receptor subcellular localization, leading to changes in its transcriptional activity and protein expression in mouse Sertoli cell lines. We found that tRA induced the nuclear localization of RARα within 30 min and that longer term exposure increased the receptor transcriptional activity and RARα protein expression. Conversely, FSH suppressed the tRA-induced nuclear localization, transcriptional transactivation, and protein expression of RARα. Treatment with two different protein kinase A-selective antagonists reversed the inhibitory actions of FSH on tRA-dependent RARα nuclear localization and transcriptional activity. These results are consistent with the involvement of protein kinase A in mediating the inhibitory effects of FSH. For the first time, we demonstrate a unique signaling convergence between the RARα and the FSH-mediated signaling pathways, which may have significant implications in the testis because both are critical regulators of testis physiology.

Vitamin A is required for various fundamental physiological processes including vertebrate development, cellular differentiation, vision, and reproduction (1, 2). The biologically active form of vitamin A is retinoic acid, which includes all-trans-retinoic acid (tRA) and 9-cis-retinoic acid (3). The biological response of retinoic acid is mediated through two families of nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors, each of which consists of three receptor subtypes, α, β, and γ (4). Retinoid receptors are transcription factors that regulate the transcription of retinoic acid-responsive genes.

Retinoid receptors belong to the larger superfamily of steroid/thyroid hormone receptors. Several members of this superfamily including the progesterone, the glucocorticoid, the estradiol, and the peroxisome proliferator-activated receptors are regulated at the level of nuclear trafficking (5–9). It was demonstrated that activation of cellular kinases increased the nuclear translocation and transcriptional activity of these receptors (8, 9). However, little is known about the regulation of RARα subcellular localization and how this may affect its subsequent transcriptional activity. Previously, Tahayato et al. (10) reported that down-regulation of protein kinase C decreased RARα nuclear localization and subsequent retinoic acid response element (RARE)-dependent transcriptional activity. In addition, although the regulation of nuclear trafficking was not examined, cAMP-dependent protein kinase (PKA) has been shown to decrease the transcriptional activity of RARα and to inhibit the tRA-induced expression of all three retinoic acid receptor mRNAs in PC12, B16 melanoma, and F9 teratocarcinoma cells (11–13). In contrast, other reports indicate that the phosphorylation of RARα on serine 369 by an overexpressed catalytic subunit of PKA increased the transcriptional activity of the receptor in CV-1, HeLa, and COS-1 cells (14, 15).

Interestingly, potential physiological effector molecules, for example, hormones and growth factors, which may stimulate PKA activity and influence RARα transcriptional activity, have not been examined. Of particular interest in the study of Sertoli cells is the potential signaling interaction of follicle-stimulating hormone (FSH) with RARα. FSH signaling in the Sertoli cell is unique because it is the only cell type in males that expresses the receptor for FSH (FSHR) (16). It is well established that FSH interaction with its membrane receptor activates a stimulatory G-protein that leads to an induction of cAMP followed by activation of PKA (17). Once activated, PKA phosphorylates various proteins, including the transcription factor, cAMP response element-binding protein, thereby potentiating subsequent changes in gene expression (16).

Furthermore, both RARα and FSH are critical regulators of testis physiology (18–20). It has been shown that the activity of RARα is essential for male reproduction. Transgenic RARα knockout male mice are sterile, exhibiting a degenerated testis morphology similar to that of vitamin A-deficient rats (18). Similarly, mice harboring targeted gene disruptions for either the FSH-β gene (19) or FSHR gene (20) have significantly decreased testis size and spermaticogenic output (75% fewer sperm) (19). In this report, we demonstrate how FSH inhibits tRA-induced RARα nuclear translocalization and how this influences receptor transcriptional activity and receptor protein expression in mouse Sertoli cell lines.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**MSC-1 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 5% fetal calf serum (FCS), supplemented with penicillin (100 IU/ml) and streptomycin (100 μg/ml) at 37 °C in a saturated atmosphere of 5% CO2. MSC-1F cells, which are MSC-1 cells stably transfected with the FSH receptor cDNA (clone 64), were maintained in culture in the same medium except for the addition of 0.25 μg/ml cAMP.
of 250 μg G418 (Life Technologies, Inc.). Cells were grown to approximately 50% confluency before being serum-starved for 48 h with 0.1% FCS in DMEM to reduce endogenous tRA.

Cells were treated with various concentrations of tRA (Sigma), dibutyryl-cAMP (dbcAMP) (Sigma), and either human pituitary FSH or ovine FSH (National Hormone and Pituitary Program, NIDDK, National Institutes of Health). Cycloheximide (Sigma) was added at a concentration of 1.5 μg/ml. Various concentrations of the PKA-selective inhibitors, H-89 and Rp-8-Br-cAMPS (Calbiochem, San Diego, CA), were added 30 min prior to treatment with activators. H-89 has been shown to be a specific inhibitor of PKA (21–23), with a Ki value of 0.048 μM. H-89 does not have an ability to inhibit other cellular protein kinases, but at much higher concentrations (i.e. PKG, Kᵣ = 48 μM; protein kinase C, Kᵣ = 31.7 μM and, casein kinase, Kᵣ = 38.3 μM). On the other hand, Rp-8-Br-cAMPS acts as a competitive analog to endogenous cAMP and prevents the dissociation of the regulatory and catalytic subunits of PKA, thus preventing PKA from phosphorylating substrates. The Ki value of Rp-8-Br-cAMPS for PKA is 5 μM.

Mouse Sertoli Cell Line (MSC-1) Stably Transfected with FSHR cDNA—Rat FSHR cDNA was cloned by reverse transcriptase-polymerase chain reaction from rat Sertoli cell mRNA into pSELECT (Promega Corp., Madison, WI) (24). The expression construct (pFSHREx) was generated by subcloning the EcoRI-XbaI fragment containing the rat FSHR cDNA into the EcoRI-XbaI sites of pcDNAs (Invitrogen, Carlsbad, CA). MSC-1 cells were transfected with 0.5 μg of supercoiled pFSHREx and neomycin-resistant colonies selected by culturing transfected cells in DMEM supplemented with 5% fetal calf serum and 600 μg/ml G418 for 14 days.

Northern Blot Analysis of Clones—Northern blot analyses were performed as described previously (24). Briefly, 10 μg of total RNA from stably transfected MSC-1F cells was separated on a 1.5% agarose gel and transferred to Immobilon-P membranes (Millipore, Bedford, MA). Membranes were blocked with 5% Blotto (Carnation, Los Angeles, CA), incubated with a polyclonal anti-RARα antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:3000 dilution, and incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody at 1:2500. Antibody-antigen complexes were detected by the Enhanced Chemiluminescence system (Amersham Pharmacia Biotech). Densitometric analysis was performed using the Molecular Dynamics SI Densitometer. Levels of RARα were normalized to levels of total protein as determined by Coomassie Blue staining of the membrane.

Transient Transfection Using Luciferase and β-galactosidase Reporters—A 2.8-kilobase RARα cDNA from the plasmid LRARαs (29) was subcloned into the multiple cloning site of the pHook expression vector (Invitrogen, San Diego, CA). MSC-1 and MSC-1F cells were grown to approximately 75% confluency in 24-well plates containing 5% FCS in DMEM before being serum-starved in 0.1% FCS in DMEM for 24 h. Cells were transfected with 75 ng of pHookRARα, 250 ng of the reporter plasmid pRARE-tk-Luc, and 75 ng of pHookLacZ (Invitrogen, San Diego, CA) using LipofectAMINE transfection method (Life Technologies, Inc.). 5 h following transfection, an equal volume of 4% FCS in DMEM was added to the cells. The following day, the medium was replaced with 2% FCS in DMEM, and cells were treated with various agents. Cells were then harvested 16 h post-treatment. Luciferase activity was analyzed using a Luciferase Assay System (Promega, Madison, WI) and a luminometer (E&G Microlumat, Berthold Systems, Aliquippa, PA). Transfection efficiency was normalized to β-galactosidase activity.

Stable Transfection of MSC-1 Cells with a Functional FSH Receptor—In 1992, Peschon et al. (30) developed an immortalized Sertoli cell line, designated MSC-1. Although MSC-1 cells share many similar characteristics with primary Sertoli cells, MSC-1 cells do not express FSHR (31). Therefore, to examine the potential effects of FSH on RARα signaling, MSC-1 cells were stably transfected with the cDNA encoding the full-length FSH receptor. Northern blot analysis was then performed to determine which transfected MSC-1 clones expressed recombinant FSHR mRNA. These analyses identified several clones that expressed appreciable levels of mRNA corresponding to the FSH receptor with approximate size of 2.6 kilobases (data not shown). Clone 64, which expressed RARα protein levels determined by Western blot analysis consisted of one-way analysis of variance followed by pairwise comparison of the means α = 0.05 (Tukey’s test, Minitab 10 Xtra; Minitab, Inc., State College, PA).

RESULTS

Stable Transfection of MSC-1 Cells with a Functional FSH Receptor—In 1992, Peschon et al. (30) developed an immortalized Sertoli cell line, designated MSC-1. Although MSC-1 cells share many similar characteristics with primary Sertoli cells, MSC-1 cells do not express FSHR (31). Therefore, to examine the potential effects of FSH on RARα signaling, MSC-1 cells were stably transfected with the cDNA encoding the full-length FSH receptor. Northern blot analysis was then performed to determine which transfected MSC-1 clones expressed recombinant FSHR mRNA. These analyses identified several clones that expressed appreciable levels of mRNA corresponding to the FSH receptor with approximate size of 2.6 kilobases (data not shown). Clone 64, which expressed FSH receptor mRNA, was selected and designated MSC-1F (Fig. 1A). To confirm that the expressed FSH receptor was functional, MSC-1F cells were treated with FSH for 1 h, and the subsequent induction of the FSH-responsive c-fos mRNA was examined. These analyses confirmed the presence of functional FSH receptors in MSC-1F cells (Fig. 1B).

Fig. 1. MSC-1F cells express functioning FSH receptor. A, 10 μg of total RNA isolated from rat primary Sertoli cells (S), untransfected MSC-1 cells (M), and MSC-1F cells (clone 64) stably transfected with FSHR cDNA (64) were subjected to Northern blot analyses using a radiolabeled cDNA for the FSH receptor as a probe. 10 μg of total RNA isolated from untreated MSC-1F cells (clone 64) (C) and MSC-1F cells treated with 25 ng/ml FSH for 1 h (1h+F) (B) were subjected to Northern blot analyses using a radiolabeled cDNA for c-fos as a probe.

FSH and dbcAMP Inhibit tRA-mediated RARα Nuclear Localization—To investigate the factors that regulate RARα nuclear translocation and thus potentially regulate the transcriptional activity of RARα, MSC-1F cells were serum-starved for 48 h and treated with tRA, FSH, dbcAMP, and the PKA-
selective antagonists, H-89 and Rp-8-Br-cAMPS. Cells were then fixed with methanol, and the subcellular localization of RARα was detected using anti-RARα antibody. Immunofluorescent confocal microscopy revealed that treatment with tRA increased RARα nuclear localization compared with cells treated with the vehicle alone (Fig. 2, compare A and B). Conversely, treatment of cells with FSH inhibited the tRA-induced nuclear localization of RARα (Fig. 2D). Likewise, treatment with dbcAMP, a cell permeable analog of cAMP, also inhibited the tRA-induced nuclear localization of RARα (Fig. 2G). However, treatment of cells with FSH or dbcAMP in the absence of tRA did not change the subcellular localization of RARα compared with untreated cells (data not shown). Furthermore, to determine whether dbcAMP and FSH suppression of RARα nuclear localization was through the activation of PKA, MSC-1F cells were pretreated with the PKA-selective inhibitors, H-89 and Rp-8-Br-cAMPS. Treatment of cells with these two PKA-selective inhibitors alleviated the inhibitory actions of dbcAMP and FSH on the nuclear localization of tRA-induced RARα (Fig. 2E, F, H, and I). Similar results were obtained for MSC-1 cells treated with dbcAMP and PKA-selective antagonists (data not shown).

Western Blot Analyses of RARα in the Nuclear Extracts of MSC-1 and MSC-1F Cells—To quantitate the changes in RARα nuclear translocation, MSC-1F and MSC-1 cells were treated for 30 min, and nuclear extracts were obtained as described under “Experimental Procedures.” Nuclear protein extracts were then subjected to SDS-polyacrylamide gel electrophoresis followed by Western blot analysis using anti-RARα antibody. Western blot analysis showed a significant increase of RARα protein in the nuclear fraction of MSC-1F and MSC-1 cells following tRA treatment compared with untreated cells (Fig. 3, A and B, respectively, compare lanes 1 and 2). This nuclear localization of RARα also occurred in the presence of cycloheximide, a protein synthesis inhibitor, indicating that increases in receptor seen in the nucleus is not a result of increased newly synthesized receptor and that no additional protein synthesis is required for nuclear translocation (data not shown). In contrast, Western blot analysis of nuclear extracts from MSC-1F cells treated with increasing concentrations of FSH revealed a dose-dependent decrease of tRA-induced RARα nuclear localization (Fig. 3, A, lanes 3–6, and E). Similarly, treatment of MSC-1 cells with dbcAMP also inhibited the nuclear translocation of tRA-induced RARα (Fig. 3B, compare lanes 2 and 3). In addition, it was also observed that pretreatment with H-89 alleviated the inhibitory effects of FSH and dbcAMP in a dose-dependent fashion, suggesting that the inhibitory effects of FSH on RARα nuclear translocation is at least partially mediated through cAMP-dependent PKA (Fig. 3, A, lanes 7–9, and B, lanes 4–8).

To determine the extent of cytoplasmic contamination in the nuclear extracts, which may influence relative levels of RARα, Western blot analyses of these same nuclear extracts and a cytoplasmic extract from MSC-1F cells (Fig. 3C) and MSC-1 cells (Fig. 3D) were performed using an antibody against actin, a cytoplasmic protein marker. A low amount of cytoplasmic contamination was evident as indicated by the presence of actin in the nuclear extracts from both cell lines. Equally important, the levels of cross-contamination in the nuclear extracts were consistent across treatments. Therefore, changes in RARα protein levels in the nuclear fractions (Fig. 3, A and B) cannot be attributed to differential cytoplasmic contamination but are because of changes in nuclear translocalization of RARα under varying experimental conditions.

Regulation of RARα Transcriptional Activity by tRA, dbcAMP, and FSH—To investigate whether increases in RARα nuclear localization are related to increases in RARE-dependant transcription by RARα, MSC-1F cells were transiently transfected with a luciferase reporter construct, designated pRARα-kLuc (29), containing three RAREs from the RARβ promoter. In addition, an expression vector containing the wild type RARα gene (pHookRARαSN) was cotransfected to observe more specifically RARα-mediated transcriptional activity. A β-galactosidase plasmid (pHookLucZ) was also cotransfected to normalize for transfection efficiency across treatments. After treatment of transfected cells with various agents, cells were
FSH Inhibits RARα Activity

Fig. 3. FSH and dbcAMP decreases tRA-induced RARα nuclear localization. MSC-1F cells (A, C, and E) were incubated with 0.5 μM tRA and 10, 50, 100, or 250 ng/ml FSH for 30 min, whereas MSC-1 cells (B, D, and F) were incubated with 0.5 μM tRA and 0.25 mM dbcAMP for 30 min. In addition, when PKA-selective inhibitors were used, MSC-1F cells were pretreated with 1, 5, or 25 μM H-89 for 30 min prior to incubation with 0.5 μM tRA and 75 ng/ml FSH (A and E), whereas MSC-1 cells were pretreated with 1, 2, 5, 10, or 20 μM H-89 for 30 min prior to incubation with 0.5 μM tRA and 0.25 mM dbcAMP (B and F). The nuclear extracts were collected, in addition to one cytoplasmic fraction (cyto), and RARα and actin proteins were detected by Western blot analysis using anti-RARα antibody (A and B) and anti-actin antibody (C and D), respectively. The levels of RARα protein in the nuclear fraction were determined by densitometric analysis from three independent experiments, and results are plotted as relative levels of RARα protein in the nuclear fraction of treated cells versus untreated control cells (means ± S.D.). (B and F). Asterisks denote a significant difference from control levels (p ≤ 0.05).

Fig. 4. Regulation of RARα transcriptional transactivation by tRA and FSH. MSC-1F cells were transiently cotransfected with 75 ng pHookRARα, 250 ng of the reporter plasmid, pRAR-E-tk-Luc, and 75 ng of pHookLacZ as described under "Experimental Procedures." After transfection, MSC-1F cells were either treated with vehicle alone as a control (lane C), treated with 50 ng/ml FSH alone (lane F), with 0.01, 0.1, 1.0, and 10 μM tRA for 24 h, or with 10, 50, and 150 ng/ml FSH in the presence of 1.0 μM tRA. In addition, MSC-1F cell were pretreated with 5, 25, 100, and 250 μM Rp-8-Br-cAMPS or with 1, 5, and 10 μM H-89 for 30 min before incubation with 50 ng/ml FSH and 1.0 μM tRA for 24 h. Data are presented as relative luciferase activity as compared with control (lane C), following normalization to β-galactosidase, and are the average of three independent assays, each conducted in triplicate (means ± S.D.).

Prior to treatment with tRA. Cycloheximide suppressed tRA-induced RARα protein expression (Fig. 5A, lanes 7–11), demonstrating that the up-regulation of RARα expression by tRA treatment is due to new protein synthesis.

To investigate whether FSH or dbcAMP had similar inhibitory effects on RARα protein expression as they did with recep-
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In experiments, and the results are plotted as relative levels of RAR and dbcAMP on tRA-induced RAR. The addition of H-89 alleviated the inhibitory effects of also pretreated for 30 min with the PKA-specific inhibitor, dbcAMP was mediated by PKA, MSC-1F and MSC-1 cells were

![Image 104x479 to 500x729](image)

**Fig. 5. Inhibition of tRA-induced RARα protein expression by FSH and dbcAMP in MSC-1 and MSC-1F cells.** MSC-1 cells were treated with 0.5 μM tRA with or without 1.5 μg/ml cycloheximide (A and C). In addition, MSC-1F cells (B and D, lanes 1–9) were incubated with 0.5 μM tRA with or without 75 ng/ml FSH, and MSC-1 cells (B and D, lanes 10–13) were incubated with 0.25 mM dbcAMP in the presence of 0.5 μM tRA. The cell lysates were collected at the time intervals indicated, and the RARα protein levels were determined by Western blot analysis using anti-RARα antibody (A and B). The levels of RARα protein in the cell lysates were determined by densitometric analysis from three independent experiments, and the results are plotted as relative levels of RARα protein in the total fraction of treated cells verses untreated control cells (means ± S.D.) (C and D). Asterisks denote a significant difference from control levels (p ≤ 0.05).

tor nuclear localization and transcriptional activity, MSC-1F cells were treated with FSH and MSC-1 cells were treated with dbcAMP, in the presence of tRA. Cells were harvested in intervals of 1, 3, 6, and 24 h and Western blot analysis conducted using anti-RARα antibody. Treatment with FSH or dbcAMP abolished the tRA-induced increase of RARα protein expression in MSC-1F and MSC-1 cells, respectively (Fig. 5B, compare lanes 2–5 with lanes 6–13). To determine whether the inhibition of tRA-induced RARα protein expression by FSH and dbcAMP was mediated by PKA, MSC-1F and MSC-1 cells were also pretreated for 30 min with the PKA-specific inhibitor, H-89. The addition of H-89 alleviated the inhibitory effects of FSH and dbcAMP on tRA-induced RARα expression, returning the time-dependent pattern of receptor protein levels to almost that of tRA treatment alone (Fig. 6).

**DISCUSSION**

The regulation of receptor subcellular localization is of great importance in understanding the transcriptional activity of receptors belonging to the steroid/thyroid hormone receptor superfamily (5–9). In this report, we demonstrate that the ligand, tRA, increases the nuclear translocation of RARα within 30 min in mouse Sertoli cell lines (MSC-1 and MSC-1F) and that this localization is independent of new protein synthesis. This regulation of nuclear localization by ligand is consistent with the previous finding in the vitamin A-deficient rat model in which the RARα subcellular localization in germ cells changed from primarily nuclear to more cytoplasmic following retinol depletion (33). These results clearly demonstrate the importance of ligand in the regulation of RARα receptor subcellular localization.

Equally important, this increase in nuclear localization is followed by a dose-dependent increase in the transcriptional activity of an RARE-containing luciferase reporter gene and the expression of the endogenous RARα gene. Specifically, RARα expression is induced in a time-dependent manner by tRA treatment, increasing significantly by 1 h, reaching a maximum of 2.5-fold within 3 h, and then declining by 24 h. This increase requires new protein synthesis and is probably due to the RARα serving as an endogenous RARE-containing reporter gene because an RARE has previously been located within the promoter region of RARα (32). The pattern of increase in the expression of RARα protein by tRA treatment in mouse Sertoli cell lines parallels the retinol-induced effect on RARα protein expression previously observed in vivo in the testis of vitamin A-deficient rats (33). Following an injection of retinol into a vitamin A-deficient rat, the RARα protein level reached a maximum level at 4 h and then decreased to a basal level within 24 h. At the mRNA level, there was nearly a 3-fold increase of RARα mRNA as early as 2 h post-injection of retinol, followed by a subsequent decrease by 12 h (33). Altogether, these results suggest a positive feedback loop for RARα operating in the Sertoli cell lines in the presence of tRA, beginning with a relatively rapid nuclear localization of RARα receptor, leading to enhanced transcriptional activity of RARα, and resulting in increased expression of endogenous RARα.

Although the ligand, tRA, acted as a positive regulator of RARα nuclear localization and transcriptional activity, treatment of MSC-1F cells with FSH or MSC-1 cells with dbcAMP in the presence of tRA inhibited RARα nuclear translocation, RARE-reporter gene transcriptional activity, and RARα protein expression. The effect of dbcAMP on the expression of RARα and RARE-dependent transcriptional activity is similar to that reported previously (12, 13). Both dbcAMP and 8-bcAMP have been shown to suppress the tRA-induced mRNA expression of all three retinoic acid receptors (RARα, -β, and -γ) and the transcriptional activity of RARE-luciferase reporter gene in B16 melanoma and F9 teratocarcinoma cells (12, 13). It was determined that the RARα mRNA stability was not altered but that the binding affinity of the receptor to its RARE had decreased by 75% (12), suggesting that the effect of dbcAMP or
8-bcAMP on decreasing RAR mRNA levels is probably at the transcriptional level. However, the effect of dbcAMP on decreasing RAR mRNA levels may also occur at an earlier event, at the level of nuclear trafficking of RAR. In this report, we demonstrate for the first time that both dbcAMP and FSH inhibit the nuclear localization of RARα, and this inhibition influences the transcriptional activity of RARα and the expression of endogenous RARα in mouse Sertoli cell lines. The inhibitory effects of dbcAMP and FSH are dominant over the stimulatory effect of tRA and are apparently mediated by PKA, because treatment with PKA-selective antagonists relieved the FSH and dbcAMP-induced inhibitory effects on tRA-induced nuclear localization and transcriptional activity. Previously, protein kinase C has been shown to be involved in the regulation of RARα subcellular localization (10). The inhibition of protein kinase C activity decreased the tRA-induced RARα nuclear localization and transcriptional activity in COS-7 cells (10). Thus, we report that PKA is an additional cellular kinase that regulates the subcellular localization of RARα, subsequently altering its transcriptional activity.

The mode of PKA regulation of RARα may be a common form of signaling cross-talk because the involvement of PKA as an antagonist to the physiological effects of retinoic acid has been described previously in other cells. It has been demonstrated that PKA may down-regulate tRA-induced neural differentiation (11). Scheibe et al. (11) reported that tRA induced neuronal differentiation in two mutant PC12 cell lines that were deficient in PKA but not in the parental PC12 cell line harboring the PKA activity. More significantly, the mutant cells lines that were deficient in PKA had an increased expression of retinoic acid receptors, which may be explained by our results.

However, other investigators have shown that overexpression of the catalytic subunit of PKA in CV-1, HeLa, and COS-1 cells enhanced a tRA-induced RARE-dependent RARα transcriptional activity (14, 15). This disparity between their results and our data may be due to overexpression of the catalytic subunit of PKA, which could potentially disrupt normal PKA or RARα signaling mechanism within the cell. Alternatively, differential regulation of the transcriptional activity of RARα by PKA may be a cell type-dependent event.

The signaling cross-talk between the retinoid receptor and the FSH receptor may have significant implications during testis development. FSH stimulation of cAMP production in rat testes was reported to start at embryonic day 19 and reach a maximum (23-fold) on day 10 postpartum, followed by a rapid decline, remaining constant thereafter (34). Interestingly, we have demonstrated that RARα mRNA expression in the developing rat testis does not appear to reach a maximum until 10 days of age (30) and that the protein is not translocated into the nucleus of Sertoli cells until 20 days of age (35). Together, these data suggest that FSH plays a significant role in Sertoli cells early in testis development, possibly playing a role in Sertoli cell proliferation (36, 37), while simultaneously inhibiting the transcriptional activity of RARα. It is only when the FSH action diminishes after 10 days of age in rats that RARα plays a dominant role, possibly in Sertoli cell differentiation. It has been well established that retinoic acid signaling induces cell differentiation in many cell types (1, 11, 38-40). Thus, we postulate that the interplay between FSH and RARα signaling may initiate an important biological switch in Sertoli cells from proliferation in early testis development to differentiation during later stages of development.

In summary, we demonstrate that the ligand for RARα, tRA, positively regulates the nuclear localization, transcriptional activity, and the protein expression of RARα in the mouse Sertoli cell lines, MSC-1 and MSC-1F. We also identify FSH stimulation of the FSH receptor as one upstream physiological event that leads to the down-regulation of RARα signaling in mouse Sertoli cells. Thus, FSH represents a potentially important modulator of RARα activity that may have significant physiologic implications in the testis. In addition, results utilizing PKA-selective antagonists suggest that PKA may be involved in FSH-mediated down-regulation of RARα. With PKA signaling networks found in a myriad of different cell types, it is possible that the modulation of RARα by PKA is a common regulatory mechanism. Further studies will be neces-
sary to determine the exact role of PKA with regards to the down-regulation of RARα.

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REFERENCES
1. Gudas, L. J., Sporn, M. B., and Roberts, A. B. (1994) The Retinoids: Biology, Chemistry, and Medicine (Sporn, M. B., Roberts, A. B., and Goodman, D. S., eds) 2nd Ed., pp. 443–520, Raven Press, New York
2. De Luca, L. M. (1991) FASEB J. 5, 2924–2933
3. Napoli, J. L. (1996) FASEB J. 10, 993–1001
4. Chambon, P. (1996) FASEB J. 10, 940–954
5. Guiochon-Mantel, A., Delabre, K., Lescop, P., and Milgrom, E. (1996) J. Mol. Endocrinol. 16, 743–750
6. Akner, G., Wikstrom, A. C., and Gustafsson, J. A. (1995) J. Steroid Biochem. Mol. Biol. 53, 1–16
7. DeFranco, D. B. (1997) Biochem. Soc. Trans. 25, 593–597
8. Chinetti, G., Griglio, S., Antonucci, M., Torra, I. P., Delerive, P., Majd, Z., Fruchart, J. C., Chapman, J., Najib, J., and Staels, B. (1998) J. Biol. Chem. 273, 25573–25580
9. Sestgawa, K., and Fuji, K. Y. (1997) Biochemistry 222, 1075–1079
10. Tahayato, A., Lefebvre, P., Formstecher, P., and Dautrevaux, M. (1993) Mol. Endocrinol. 7, 1642–1653
11. Scheibe, R. J., Ginty, D. D., and Wagner, J. A. (1991) J. Cell Biol. 113, 1173–1182
12. Xiao, Y., Desai, D., Quick, T. C., and Niles, R. M. (1996) J. Cell. Physiol. 167, 413–421
13. Hu, L., and Gudas, L. J. (1990) Mol. Cell. Biol. 10, 391–396
14. Hugtenburg, J. I., and Collard, M. W., Kim, Y. W., and Sharma, R. P. (1993) Mol. Endocrinol. 7, 743–750
15. Bochette-Egly, C., Oulad-Abdelghani, M., Staub, A., Pfister, V., Scheuer I., Chambon, P., and Gaub M. (1995) Mol. Endocrinol. 9, 860–871
16. Orth, J., and Christensen, A. (1997) Endocrinology 100, 103–107
17. Russell, L. D., and Griswold, M. D. (1993) The Sertoli Cell, pp. 493–509, Cache River Press, Clearwater, FL