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Gene Expression of Basic Helix-Loop-Helix Transcription Factor, SHARP-2, Is Regulated by Gonadotropins in the Rat Ovary and MA-10 Cells[1]

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ABSTRACT

Basic helix-loop-helix (bHLH) proteins regulate transcription from the E box sequence (5′-CANNTG-3′) located in the regulatory region of most gene promoters. The rat enhancer of split- and hairy-related protein 2 (SHARP-2) is a member of the bHLH protein family. To analyze the possible role of SHARP-2 in the rat ovary, the regulation of the expression of the *SHARP-2* gene was examined, and the SHARP-2 protein was characterized. Northern blot analysis revealed that the level of SHARP-2 mRNA abruptly and temporarily increases as the result of the action of LH, i.e., eCG or hCG treatment alone or hCG after eCG treatment, in the rat ovary, as indicated by the treatment of primary cultured rat granulosa cells with hCG after FSH treatment or of mouse Leydig MA-10 cells with hCG or 8-bromoadenosine 3′,5′-cyclic monophosphate. An in situ hybridization analysis showed that eCG treatment increases the level of the SHARP-2 transcript in theca interna cells and that hCG treatment, after the administration of eCG, increases the level of the SHARP-2 transcript in granulosa cells. Furthermore, transfection experiments with green fluorescence protein (GFP) expression vectors into primary cultured granulosa cells and MA-10 cells revealed that the entire coding sequence of SHARP-2 fused to the GFP is localized in the nucleus. The transcriptional activity of SHARP-2 also was examined using transient DNA transfection experiments. When an expression vector encoding the full length of SHARP-2 was cotransfected with thymidine kinase promoter-luciferase reporter plasmids, with or without E box sequences, into MA-10 cells, the luciferase activity was decreased in an E box-dependent manner. We conclude that the level of SHARP-2 mRNA is regulated by gonadotropins and that SHARP-2 functions as a transcriptional repressor localized in the nucleus.

cyclic adenosine monophosphate, gene regulation, granulosa cells, ovary, theca cells

INTRODUCTION

Gonadotropins FSH and LH regulate ovarian functions, including folliculogenesis, ovulation, and luteinization [1]. FSH stimulates cell growth and differentiation in granulosa cells of small antral to preovulatory follicles, thus leading to follicular development. LH induces steroidogenesis in theca interna cells and acts on preovulatory follicles, resulting in ovulation and luteinization. The expression of genes involved in these ovarian function is regulated by the specific *cis*-acting elements upstream from the genes. The E box sequence (5′-CANNTG-3′) of these elements is located on the transcriptional regulatory region of a number of genes and controls the transcription of the genes in biological events, including development, cell differentiation, cell growth, and oncogenesis [2]. Basic helix-loop-helix (bHLH) proteins, with or without a leucine zipper motif, bind to and regulate transcription from the E box. To understand the molecular mechanisms underlying cell type-specific transcription and its hormonal regulation in the rat ovary, we initiated a search for E box-binding proteins that are expressed in granulosa cells. We previously reported on the molecular cloning and characterization of rat upstream stimulatory factor (USF) 2 cDNA [3]. However, USF2 is a ubiquitous transcription factor, and thus its expression is not regulated by gonadotropins in the rat ovary [3].

The rat enhancer of split- and hairy-related protein 2 (SHARP-2) is a member of the bHLH protein family [4]. Mouse and human orthologs have been designated stimulation of retinoic acid 13 (Stra13) and differentiated embryo chondrocytes 1 (DEC1), respectively [5, 6]. It has been reported that the level of SHARP-2 mRNA is induced by nerve growth factor, retinoic acid, cAMP, serum starvation, transforming growth factor (TGF) β, and hypoxia in various cells and tissues [5–9]. We also recently reported that the expression of SHARP-2 mRNA is rapidly increased by insulin in the rat liver [10]. Defective T-cell activation and the genesis of autoimmune disorders have been reported in aging *Stra13*-deficient mice, and DEC1 and a closely related protein DEC2 are expressed in the suprachiasmatic nucleus in a circadian fashion and are regulators of the mammalian molecular clock [11, 12]. In knockout mice, other phenotypes, including those showing effects on ovarian function, have not yet been reported. Therefore, the issue of whether the *SHARP-2* gene is expressed in reproductive tissues or is regulated by gonadotropins remains unknown.

As the first step in elucidating the physiological role of SHARP-2 in the rat ovary, we examined the expression of rat SHARP-2 mRNA under various conditions. In both animals and cultured cells, we found that the expression of SHARP-2 mRNA is regulated by gonadotropins. We also identified the transcriptional activity and subcellular localization of SHARP-2.

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MATERIALS AND METHODS

Materials

The eCG, ovine FSH (oFSH), and hCG were obtained from the National Hormone and Pituitary Distribution Program (Bethesda, MD). Human CG was also obtained from Sankyo (Tokyo, Japan). Diethylstilbestrol (DES), Waymouth MB752/1 medium, and 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP) were purchased from Sigma Chemical Co. (St. Louis, MO). The Trizol reagent, Superscript II, and Lipofectamine PLUS were purchased from Invitrogen Corp. (Carlsbad, CA), and the Biodyne membrane was obtained from PALL (ICN Biomedicals, Glen Cove, NY). The ExpressHyb solution and pEGFP-C1 were purchased from Clontech (Palo Alto, CA), and [α-^{32}P]deoxycytidine triphosphate (dCTP, 110 TBq/mmol) was purchased from Amersham Biosciences (Cleveland, OH). The BcaBest DNA labeling kit and ExTaq DNA polymerase were purchased from Takara Biomedicals (Kyoto, Japan), and α-^{35}S cytidine triphosphate (CTP, 46.2 TBq/mmol) was purchased from NEN Life Science Products (Wilmington, DE). T7 and T3 RNA polymerases and FuGENE6 transfection reagent were obtained from Roche Diagnostics (Basel, Switzerland). The pCI-neo, pGEM-T Easy, and pRL-CMV vectors and dual luciferase reporter assay system were purchased from Promega (Madison, WI), and the Big Dye terminator cycle sequencing kit FS was purchased from Applied Biosystems Japan (Tokyo, Japan). The Invisorb plasmid kit was purchased from Invitek (Berlin, Germany).

Animals and Treatment

Immature female Kwl:Wistar rats (21 days old) were used in this study. The rats were treated with 30 IU eCG and/or 30 IU hCG, and the ovaries were removed at the indicated times. Animal care and procedures followed the National Institute of Health guidelines.

Cells and Cell Culture

Rat granulosa cells were isolated from DES-treated immature female rat ovaries and cultured as described previously [13]. MA-10 cells, a mouse Leydig cell line, was a generous gift from Dr. Mario Ascoli (University of Iowa, Iowa City, IA). Cells were grown in Waymouth MB752/1 medium supplemented with 15% horse serum and antibiotics at 37°C in a 5% CO_2 incubator.

Northern Blot Analysis

Total RNA was prepared from gonadotropin-primed immature rat ovaries and MA-10 cells treated with or without 100 ng/ml hCG or 1 mM 8-Br-cAMP for the indicated times using the Trizol reagent. Total RNA (10 μg/lane) was subjected to denaturating agarose gel electrophoresis and then transferred to a Biodyne membrane. The filter was prehybridized in ExpressHyb solution at 68°C for 30 min and then hybridized with a ^{32}P-labeled probe and 20 μg/ml heat-denatured herring testis DNA for 1 h. After washing at 50°C in 0.1× saline sodium citrate and 0.1% SDS, the filter was exposed to a imaging plate (Fujix, Kanagawa, Japan). Hybridization signals were detected with the BAS-2000 image analyzing system (Fujix).

The pF3 and pGEM-T Easy 36B4 were described previously [10]. A 1.1-kilobase (kb) *Eco*RI/*Mlu*I fragment of the pF3 and a 954-base pair (bp) *Eco*RI fragment of the pGEM-T Easy 36B4 were used as the probes for SHARP-2 and 36B4 ribosomal protein, respectively [10]. Probe DNAs were labeled with α-^{32}P-dCTP using the BcaBest DNA labeling kit.

Reverse Transcription Polymerase Chain Reaction Analysis

Total RNA was isolated from primary cultured rat granulosa cells using the Trizol reagent. Reverse transcription and polymerase chain reaction (RT-PCR) was performed as described previously [14]. Primers used were 5′-CACGG ACGCA GGTTC ACCGT GG-3′ and 5′-CCGGT CTAGA TTAGT CTTTG GTTTC TAAGT TTAAA GG-3′ oligonucleotides for SHARP-2 and 5′-GAACG GGAAG CTCAC TGGCA-3′ and 5′-TCCAC CACCC TGTTG CTGTA-3′ oligonucleotides for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The PCR was conducted at 90°C for 3 min, followed by 29 cycles (for SHARP-2) or 24 cycles (for GAPDH) of 90°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec, and then a final extension at 72°C for 5 min. The reaction mixture was subjected to electrophoresis in a 2% agarose gel, and bands were visualized by staining with ethidium bromide.

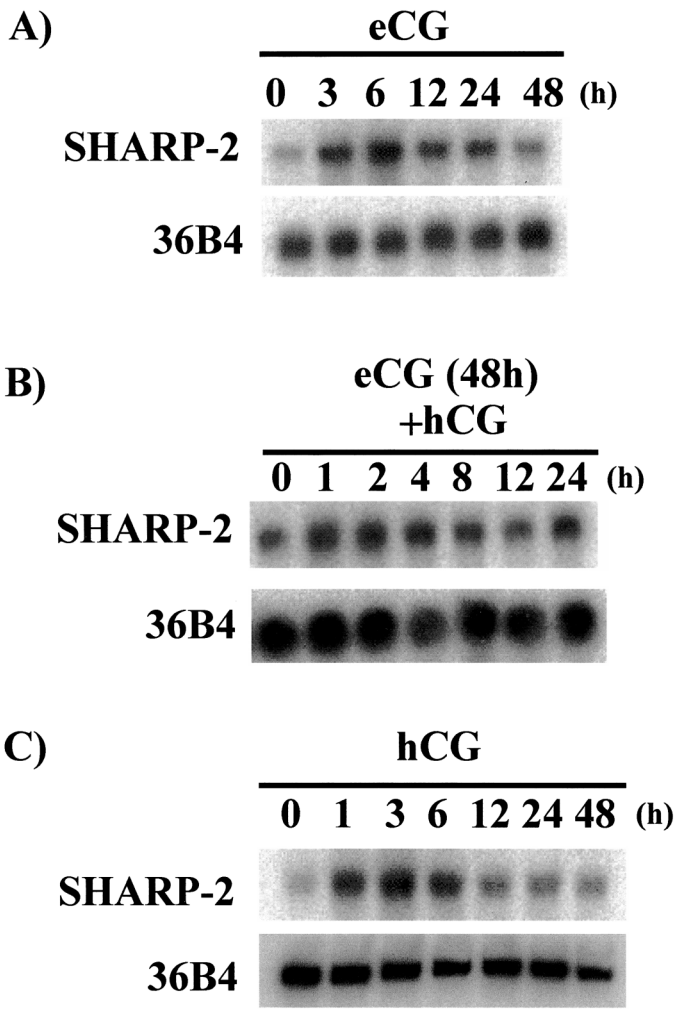

FIG. 1. Northern blot analysis of SHARP-2 mRNA in the rat ovary. Total RNA (10 μg) from immature rat ovaries was treated with eCG (**A**), hCG after treatment with eCG for 48 h (**B**), or hCG for various times (**C**). Samples were subjected to 1% denaturating agarose gel electrophoresis and then transferred to a nylon membrane. The membranes were hybridized with ^{32}P-labeled SHARP-2 or 36B4 probes (left). Each experiment was carried out twice.

In Situ Hybridization

In situ hybridization was performed as described previously [15], using pBSII-SHARP-2EM as described previously [10]. A 860-base antisense or sense ^{35}S CTP-labeled RNA probe was synthesized using T7 or T3 RNA polymerase. The samples were hybridized and washed at high stringency and autoradiographed with an NTB2 emulsion (Eastman Kodak Co., Rochester, NY). All slides were counterstained with hematoxylin, dehydrated, and mounted.

Plasmids

The tk/Luc and E box-tk/Luc plasmids were described previously [3]. A 1.1-kb *Eco*RI/*Mlu*I fragment of the pF3 was subcloned into the *Eco*RI/*Mlu*I sites of the pCI-neo to obtain pCI-neo-SHARP-2EM.

Total RNA from rat livers was prepared using the Trizol reagent according to the manufacturer's protocol. RT-PCR was performed as described previously with minor modifications [16]. Combinations of oligonucleotides, 5′-CACGG ACGCA GGTTC ACCGT GG-3′ and 5′-CCGGT CTAGA TTAGT CTTTG GTTTC TAAGT TTAAA GG-3′, and 5′-CCGGG AATTC CCATG GAGCG GATCC CCAGC-3′ and 5′-GGTGG GGGGC TCTTC AGATT C-3′, were employed as primers. Each PCR product was subcloned into the pGEM-T Easy vector to obtain

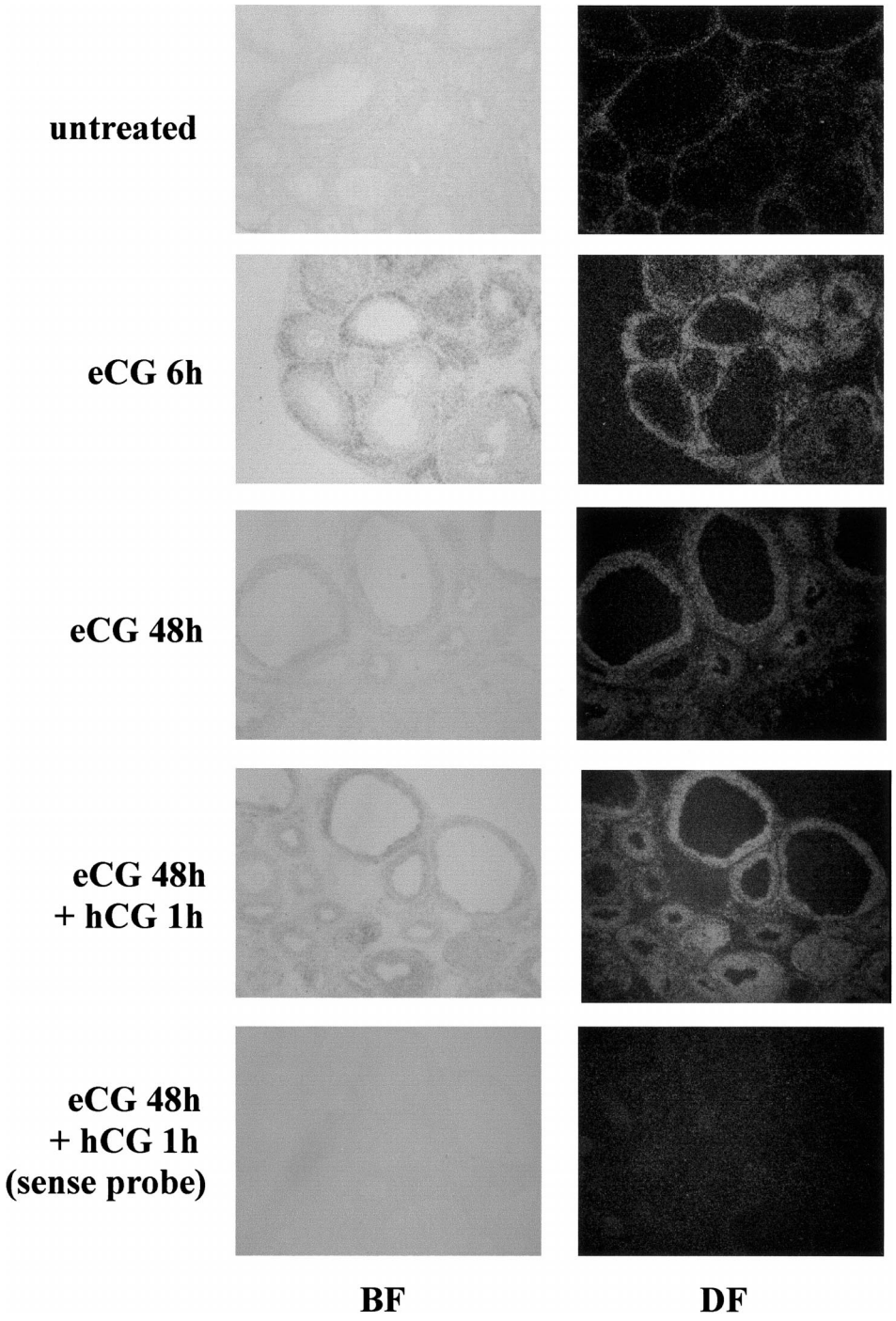

FIG. 2. In situ hybridization of SHARP-2 in the rat ovaries. Ovaries from 21-day-old rats treated with or without eCG or eCG followed by hCG were dissected, sectioned, and hybridized with ^{35}S-labeled antisense SHARP-2 probes (untreated, eCG 6 h, eCG 48 h, eCG 48 h + hCG 1 h) or sense SHARP-2 probes (eCG 48 h + hCG 1 h): untreated, immature female rat ovary; eCG 6 h; 30 IU eCG for 6 h; eCG 48 h, 30 IU eCG for 48 h; eCG 48 h + hCG 1 h, 30 IU hCG for 1 h after treatment with 30 IU eCG for 48 h. Brightfield (BF) and darkfield (DF) images of same field are shown (left and right, respectively). Each experiment was carried out twice. Original magnification ×100.

pGEM-T Easy SHARP-2MX or pGEM-T Easy SHARP-2EM, respectively. A 410-bp *Mlu*I/*Xba*I fragment of the pGEM-T Easy SHARP-2MX was subcloned into the pCI-neo-SHARP-2EM to give pCI-neo-SHARP-2.

Oligonucleotides 5′-AATTG CGAAT TCCCG G-3′ and 5′-GATCC CGGAA TTCGC-3′ were annealed, phosphorylated, and subcloned into the *Hin*dIII/*Sal*I sites of the pEGFP-C1 to obtain pEGFP-C1E2. A 410-bp *Mlu*I/*Xba*I fragment of the pGEM-T Easy SHARP-2MX and an 860-bp *Eco*RI/*Mlu*I fragment of the pGEM-T Easy SHARP-2EM were subcloned into the *Eco*RI/*Xba*I sites of the pEGFP-C1E2 to produce pGFP-SHARP-2.

The nucleotide sequences of all inserts were confirmed using a DNA Sequencer 3100 (Applied Biosystems Japan).

DNA Transfections and Luciferase Assays

All plasmids used for transfection were prepared using the Invisorb plasmid kit, followed by CsCl gradient ultracentrifugation. DNA transfections to primary cultured rat granulosa cells were carried out using the Lipofectamine PLUS reagent according to the protocol provided by the supplier. DNA transfections to MA-10 cells using FuGENE6 have been described previously [17]. For observation of green fluorescence protein (GFP) fusion protein, 300 ng (for primary cultured rat granulosa cells) or 400 ng (for MA-10 cells) of the indicated GFP plasmid was used. For determination of the transcriptional activity of SHARP-2, the luciferase reporter plasmid (200 ng), pRL-CMV (1 ng), and the expression plasmids (100 ng) were used for transfection. After 36 h, the cells were observed with an Olympus IX-70 fluorescence microscope (Tokyo, Japan) or subjected to a luciferase assay. Before observation of subcellular localization, cells were stained with Hoechst 33342 (Sigma Chemical Co., St. Louis, MO) to visualize the nuclei. Firefly and sea pansy luciferase assays were carried out according to the manufacturer's recommended protocol. Luciferase activities were determined using a Berthold Lumat model LB 9501 (Wildbad, Germany). Firefly luciferase activities (relative light units) were normalized relative to sea pansy luciferase activities.

RESULTS

Regulation of SHARP-2 Gene Expression by Gonadotropins

We initially examined the expression and regulation of SHARP-2 mRNA in an attempt to analyze the possible roles of SHARP-2 in the rat ovary. Either eCG or hCG or a combination of these were administered to immature female rats, and their ovaries were then collected at various times. Administration of eCG to the immature female rat leads to the stimulation of follicular development. A SHARP-2 transcript was detected in the untreated rat ovary (Fig. 1A). The level of SHARP-2 mRNA was temporarily induced after eCG treatment, and the maximum induction was observed at 6 h after treatment with eCG. Administration of hCG 48 h after the administration of eCG to the immature female rat leads to ovulation and luteinization. The expression of SHARP-2 mRNA in these ovaries was transiently increased within 2 h. The administration of hCG to the immature female rat leads to the activation of theca cells. The level of SHARP-2 mRNA was temporarily induced after hCG treatment and the maximum induction was observed at 3 h after treatment with hCG. In contrast, the levels of 36B4 mRNA remained unchanged in these rat ovaries. These results indicate that SHARP-2 mRNA is induced by gonadotropins in the rat ovary.

In Situ Hybridization Analysis

To determine the cellular localization and induction of *SHARP-2* expression, an in situ hybridization analysis was performed. In the immature female rat ovary, *SHARP-2* was expressed in the whole ovary and was strongly induced in theca interna cells by administration of eCG (Fig. 2). Equine CG has both LH- and FSH-like activities. These results indicate that eCG activates SHARP-2 gene expression in the ovary through LH receptors on the theca interna cells. An induction was also observed in granulosa cells by the administration of hCG after eCG treatment. The in situ hybridization results were consistent with those of the Northern blot analyses.

Human CG Induces Expression of SHARP-2 mRNA in Primary Cultured Rat Granulosa Cells

To confirm that SHARP-2 mRNA is induced by hCG in differentiated granulosa cells, we employed an RT-PCR method. The total RNAs from primary cultured rat granulosa cells treated with hCG for various times after treatment with oFSH for 48 h were analyzed. Under these conditions, the cells were differentiated and expressed LH receptors. The SHARP-2 mRNA was induced at 4–8 h after treatment with hCG (Fig. 3A). In contrast, the level of GAPDH mRNA remained unchanged (Fig. 3B).

Both hCG and cAMP Induce Expression of SHARP-2 mRNA in MA-10 Cells

The expression of SHARP-2 mRNA was induced by hCG in theca cells (Figs. 1C and 2). We then examined the regulation of the SHARP-2 gene expression in mouse Leydig MA-10 cells, the male counterpart of ovarian theca cells. A SHARP-2 transcript was detected in MA-10 cells and the level was increased by treatment with hCG for 4 h (Fig. 3B). We then examined the effect of cAMP, the second messenger of gonadotropins, on *SHARP-2* expression. MA-10 cells were treated with 8-Br-cAMP, and the expression of SHARP-2 mRNA was analyzed. The level of SHARP-2 mRNA was temporarily increased after 8-Br-cAMP treatment and the maximum increase was observed at 2 h after treatment (Fig. 3B). In contrast, the levels of 36B4 mRNA under all conditions remained unchanged.

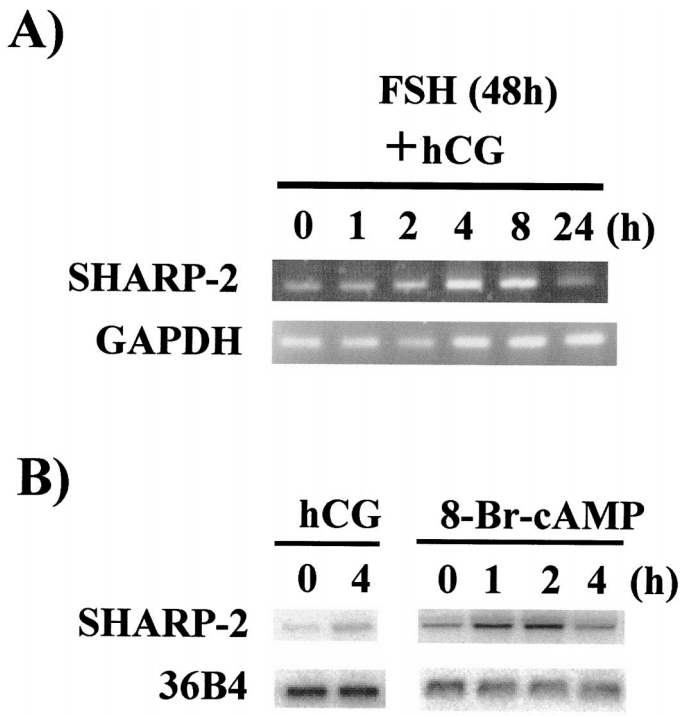

FIG. 3. Expression of SHARP-2 mRNA in primary cultured rat granulosa cells and MA-10 cells. **A**) RT-PCRs were performed using total RNA prepared from primary cultured rat granulosa cells treated with 30 ng/ml oFSH for 48 h, followed by treatment with 30 ng/ml hCG for various times. Total RNA (1 μg) was reverse transcribed, and a portion of the product was subjected to PCR for the specific amplification of SHARP-2 and GAPDH (left). The reaction mixture was subjected to 2% agarose gel electrophoresis, and bands were visualized by staining with ethidium bromide. **B**) Northern blot analysis of the expression of SHARP-2 mRNA in MA-10 cells. Total RNA (10 μg) from MA-10 cells treated with or without 100 ng/ml hCG for 4 h (left) or 1 mM 8-Br-cAMP for various times (right) were subjected to 0.8% denaturating agarose gel electrophoresis and then transferred to a nylon membrane. The membrane was hybridized with ^{32}P-labeled probes (left). Each experiment was carried out twice.

These results indicate that SHARP-2 mRNA is also expressed in male reproductive cells and that expression is upregulated by both hCG and cAMP.

Determination of the Subcellular Localization and Transcriptional Activity of Rat SHARP-2

To determine the subcellular localization of the SHARP-2 protein, we prepared a GFP-SHARP-2 fusion protein expression plasmid. When the pEGFP-C1E1 plasmid, encoding GFP alone, was transfected into primary cultured rat granulosa cells or MA-10 cells, the corresponding protein was observed in the whole cell (Fig. 4). In contrast, when the pGFP-SHARP-2 plasmid, in which an entire coding sequence of SHARP-2 was fused to the C-terminal of GFP, was transfected, the fusion protein was observed only in the nucleus in both cells (Fig. 4). These results indicate that SHARP-2 is also able to become localized in the nucleus as a GFP fusion protein.

Thus far, ovarian target genes of SHARP-2 are unknown. It has been reported that DEC1, the human ortholog of SHARP-2, binds to the E box sequence [8]. To evaluate the

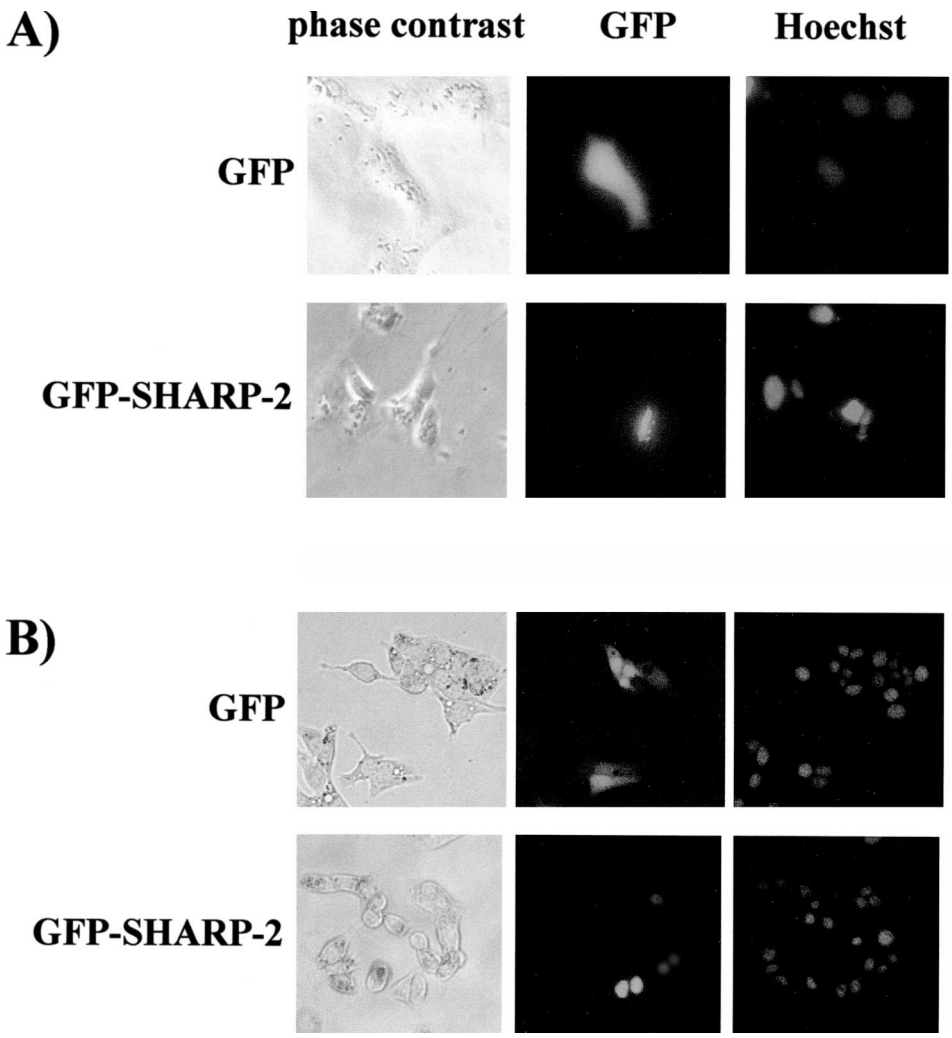

FIG. 4. Subcellular localization of SHARP-2. Expression plasmids encoding GFP alone or an entire coding sequence of SHARP-2 fused to the C-terminal of GFP were transfected into primary cultured rat granulosa cells (**A**) and MA-10 cells (**B**). Thirty-six hours after transfection, subcellular localization of GFP fusion protein was observed. Left: phase contrast micrographs. Middle and right: fluorescence and Hoechst 33342 staining micrographs of the same fields. Each experiment was carried out twice. Original magnification ×100.

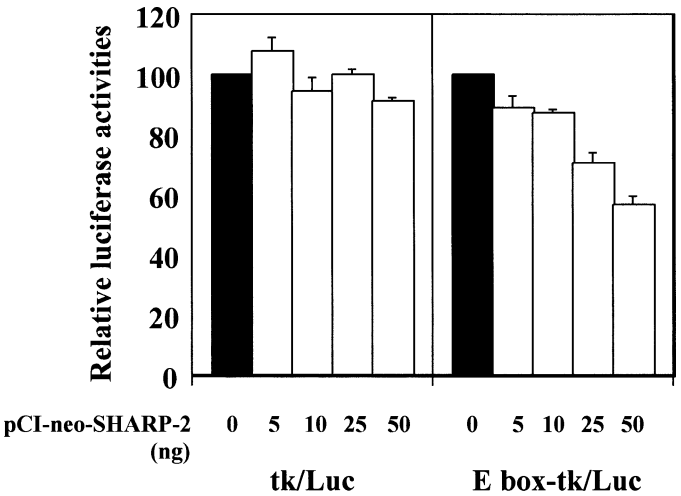

FIG. 5. SHARP-2 is a transcriptional repressor. MA-10 cells were cotransfected with 1 ng of pRL-CMV, an indicated amount of cytomegalovirus enhancer/promoter-directed SHARP-2 expression vector, and 200 ng of the tk/Luc or E box-tk/Luc reporter plasmid. The total amount of plasmid (301 ng) was adjusted by the addition of the pCI-neo. The pCI-neo is an empty vector, and pCI-neo-SHARP-2 expresses the entire coding sequence of SHARP-2. Thirty-six hours after transfection, the cells were harvested, and both firefly and sea pansy luciferase activities were determined. A value of 100% was assigned to the promoter activity of the reporter plasmid in the presence of 100 ng of the pCI-neo. Each column and bar represents the mean and SEM of at least three transfection experiments.

transcriptional role of SHARP-2 in rat reproductive tissues and cells, we employed transient DNA transfection experiments. Two reporter plasmids, tk/Luc and E box-tk/Luc, were used. The tk/Luc plasmid contains a thymidine kinase (tk) promoter linked to the firefly luciferase gene. The E box-tk/Luc harbored three copies of the consensus E box sequence just upstream of the tk promoter [3]. The luciferase activity of each reporter plasmid in the presence of the pCI-neo was normalized relative to a value of 100%. When the tk/Luc reporter plasmid was cotransfected with various concentrations of the pCI-neo-SHARP-2 into MA-10 cells, luciferase activities remained unchanged (Fig. 4). In contrast, when the E box-tk/Luc was cotransfected with various concentrations of the pCI-neo-SHARP-2, the luciferase activities were decreased in a dose-dependent manner and the activity was decreased to 56.7%. These results indicate that SHARP-2 represses promoter activity in an E box-dependent manner and that the activity is dose dependent.

DISCUSSION

The regulation of the expression of *SHARP-2* in the rat ovary and MA-10 cells was examined. The SHARP-2 transcript was detected in the untreated immature female rat ovary (Fig. 1A). The level of SHARP-2 mRNA rapidly and temporarily increased after the administration of either eCG or hCG to immature female rats (Fig. 1, A and C). In this

time, gonadotropins mainly regulated the gene expression of SHARP-2 in theca interna cells. In situ hybridization revealed that expression of SHARP-2 transcript was induced in theca interna cells under the same conditions (Fig. 2). However, eCG treatment for 48 h induced LH receptors in granulosa cells of large antral follicles. Therefore, granulosa cell genes become responsive to hCG stimulation. Expression was also observed during ovulation after the administration of hCG following eCG treatment or after treatment of primary cultured rat granulosa cells with hCG following treatment with oFSH for 48 h (Figs. 1B and 3A). In situ hybridization also revealed that expression of the SHARP-2 transcript was induced in granulosa cells under the same conditions (Fig. 2). In addition, the level of SHARP-2 mRNA rapidly and temporarily increased after treatment of MA-10 cells with either hCG or 8-Br-cAMP (Fig. 3B). Thus, SHARP-2 is a gonadotropin-inducible bHLH transcription factor. This is the first observation of a bHLH protein, the production of which is regulated by gonadotropins in the rat ovary and a mouse Leydig cell line.

Various hormones and growth factors, such as parathyroid hormone, insulin, TGFβ, and nerve growth factor, cause an increase in the level of SHARP-2 mRNA [4, 8, 10]. Based on this study, gonadotropins should be added to the list of inducers of *SHARP-2* expression. It has also been reported that expression of SHARP-2 mRNA is induced by cAMP in other cell types [18]. The level of SHARP-2 mRNA in MA-10 cells was induced by cAMP (Fig. 3B), suggesting that an induction of SHARP-2 mRNA by gonadotropins may be mediated by cAMP. However, expression was not induced by cAMP in primary cultured rat hepatocytes. Thus, the level of SHARP-2 mRNA stimulated by cAMP may be regulated in a cell type-specific manner [10].

The GFP-SHARP-2 fusion protein was localized in the nuclei of both primary cultured rat granulosa cells and MA-10 cells (Fig. 4). In a previous report, the nuclear localization signal (NLS) was mapped to a cluster of basic amino acid residues [19]. Although the basic region of the bHLH proteins functions as the DNA-binding domain, whether a region of the SHARP-2 protein is required for nuclear localization remains to be determined.

Cotransfection assays indicated that SHARP-2 functions as a transcriptional repressor in MA-10 cells, that it represses promoter activity in an E box-dependent manner, and that the activity is dose dependent (Fig. 5). The C-terminal region of SHARP-2 contains a proline-rich domain. It is well known that a proline-rich region is involved in transcriptional activation or transcriptional repression. It has been reported that SHARP-2 interacts with the histone deacetylase HDAC1 via the proline-rich region or with the basal transcription factor TFIIB to repress gene transcription in other cells [7].

Which genes are regulated by SHARP-2 in reproductive tissues and cells? The E box sequence is essential for cell type-specific transcription or the gonadotropin-dependent transcriptional regulation of the Ad4-binding protein/steroidogenic factor 1, FSH receptor, and the prostaglandin endoperoxide synthase 2 genes in the rat ovary [20–22]. Generally, E box-binding proteins consist of two classes: one that encompasses ubiquitous transcription factors such as USF1, USF2, E12/E47, and Myc and another that encompasses cell type-specific factors, including NeuroD/β-cell E-box trans-activator 2, Hairy and Enhancer of Split, MyoD, and myogenin [2]. The USF transcriptional activator proteins, USF1 and USF2, are key regulators of the E box of the above three genes [21–23]. However, the USF1 and USF2 proteins are expressed ubiquitously, and their levels are not regulated by gonadotropins [3, 24]. Therefore, modifications of USF proteins or the expression of an interacting cofactor(s) of USF proteins or other E box-binding proteins would be required for gonadotropin-dependent regulation of the genes. At least two possibilities can hypothesized to account for this regulation. One possibility is that SHARP-2 directly binds to the E box element, repressing the transcription of the gene in a gonadotropin-dependent manner. In this case, the binding of SHARP-2 to the element should be subject to competition by other E box-binding proteins, including USF proteins. It has been reported that Stra13, the mouse ortholog of SHARP-2, antagonizes transactivation by USF2 via a direct protein-protein interaction [25]. Therefore, it is likely that SHARP-2 interacts with endogenous E box-binding proteins or interferes with its binding to the E box in a gonadotropin-dependent manner.

What is the physiological role of SHARP-2? Because SHARP-2 is a transcriptional repressor, its induction may result in alterations of cellular function in a gonadotropin-dependent manner. In theca cells and differentiated granulosa cells, steroidogenesis is an important function. Therefore, SHARP-2 may counteract inhibition of steroidogenesis in these cells in a gonadotropin-dependent manner. Further studies are required for the identification of target genes of SHARP-2 and to clarify the physiological role of SHARP-2 in reproductive tissues and cells and the mechanism of transcriptional regulation and to provide a detailed mapping of NLS and repressor domains to characterize the properties of the SHARP-2 protein.

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