Cloning and Uterus/Oviduct-specific Expression of a Novel Estrogen-regulated Gene (ERG1)*

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The steroid hormone estrogen profoundly influences growth and differentiation programs in the reproductive tract of cycling and pregnant mammals. It is thought that estrogen exerts its cellular effects by regulating the expression of specific target genes. We utilized a messenger RNA differential display method to identify the genes whose expression is modulated by estrogen in the preimplantation rat uterus. Here we report the cloning of a novel gene (ERG1) that is tightly regulated by estrogen in two key reproductive tissues, the uterus and oviduct. Spatio-temporal analyses reveal that ERG1 mRNA is expressed in a highly stage-specific manner in the uterus and oviduct, and its expression is restricted to the surface epithelium of both of these tissues. Nucleotide sequence analysis of the full-length ERG1 cDNA indicates that it has an open reading frame of 1821 nucleotides encoding a putative protein of 607 amino acids with a single transmembrane domain and a short cytoplasmic tail. The extracellular part of the protein contains several distinct structural motifs. These include a zona pellucida binding domain, which is present in a number of proteins such as the zona pellucida sperm binding proteins, and uromodulin. In addition, there is a repeat of a motif called CUB domain, which exists in a number of genes involved in development and differentiation such as bone morphogenetic protein 1 (BMP1). Although the precise function of ERG1 eludes us presently, its unique pattern of expression in the uterus and oviduct and its regulation by estrogen, a principal reproductive hormone, lead us to speculate that this novel gene plays an important role in events during the reproductive cycle and early pregnancy.

The steroid hormone estrogen modulates the structure and function of the female reproductive tissues, such as the uterus and oviduct, by eliciting an array of biochemical responses in these tissues. Estrogen critically influences the transport of the fertilized egg through the oviduct into the uterus (1, 2). Estrogen also promotes the growth, differentiation, and remodeling of the uterus at various physiological states, such as the reproductive cycle and pregnancy (3–6). The cellular actions of this hormone are mediated through its nuclear receptors, which function as ligand-inducible transcription factors (7–10). Previous studies in rodents employing immature and ovariectomized model systems have demonstrated that in the uterus, estrogen modulates the expression of genes that are likely to be involved in the regulation of cell growth and proliferation. These include the genes encoding protooncogenes, such as c-fos and c-myc, growth factors, such as epidermal growth factor and insulin-like growth factor-1, and their receptors (11–21). The identity of the majority of estrogen-regulated genes that mediate the uterotropic hormonal responses to estrogen, however, remains largely unknown. In order to understand the role of estrogen in complex physiological processes, such as the changes in the endometrium during the reproductive cycle, uterine receptivity, zygote transport, implantation, stromal cell differentiation, and parturition, it is essential to identify the spectrum of genes that are regulated by estrogen in the reproductive tract.

It is known that in the rodents, the preovulatory ovarian estrogen is important for uterine cellular proliferation and differentiation of epithelium during early stages (days 1 and 2 post-fertilization) of pregnancy (22–24). This transformation of the uterus in response to estrogen is critical for subsequent uterine preparation for embryonic implantation and successful establishment of pregnancy (22–24). To identify genes that mediate estrogen action in the uterus during early pregnancy, we employed the mRNA differential display (DD)† procedure devised by Liang and Pardee (25). Using this approach, we compared mRNAs obtained from uteri of non-pregnant (estrus stage) and pregnant (day 5) rats and isolated several cDNA clones representing genes that are either up- or down-regulated during the preimplantation stage of pregnancy.

In this study, we report the isolation of a novel gene that is expressed in the uterus in a stage-specific manner during the ovarian cycle and early pregnancy. The expression of this gene is induced in the uterus of ovariectomized or immature rats upon administration of estrogen and is abolished in the presence of antiestrogens. We, therefore, termed this gene as estrogen-regulated gene 1 or ERG1. ERG1 is also highly expressed in the oviduct and this expression is under estrogenic control. ERG1 mRNA is present predominantly in the surface epithelium of the uterus and oviduct. The full-length cDNA of ERG1 codes for a putative protein of 607 amino acids harboring a single transmembrane domain. Nucleotide sequence analysis of the ERG1 cDNA clone revealed the presence of several interesting structural features. The amino terminus of the predicted ERG1 protein harbors a tandem repeat of a CUB domain, which

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† The abbreviations used are: DD, differential display; ZP, zona pel- lucida; PCR, polymerase chain reaction; DIG, digoxigenin; bp, base pairs; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
ERG-1 Expression in the Uterus and Oviduct

ERG-1 expression exhibits striking homology to the one that exists in the bone morphogenetic protein 1 (BMP1) (26, 27). The carboxyl terminus of ERG1 consists mostly of a highly conserved ZP domain that is also found in the zona pellucida sperm binding proteins and other proteins, such as uromodulin (28, 29).

**Experimental Procedures**

**Reagents**—Progesterone and 17β-estradiol were purchased from Sigma. Mifepristone (RU 38486) and ICI 182,780 were kind gifts of Roussel-Uclaf, France and Zeneca Pharmaceuticals, Cheshire, United Kingdom.

**Animals**—All experiments involving animals were conducted according to National Institutes of Health standards for the care and use of experimental animals. Virgin female rats (Sprague-Dawley, from Charles River, Wilmington, MA; 60–75 days of age) in proestrus were mated with adult males. The different stages of the cycle in the nonpregnant rats were ascertained by examining vaginal smears. The presence of a vaginal plug after mating was designated as day 1 of pregnancy. The animals were killed at various stages of gestation and the uteri collected. In some experiments, animals were ovarioctomized and 14 days later were injected subcutaneously with either estradiol (2 µg/kg body weight), progesterone (40 mg/kg body weight), or a combination of both hormones or vehicle (sesame oil) as described under "Results." To study the effects of decidualization, immature rats were killed 16 h after final injections. For experiments immature rats (21 days old, 40–65 g) were injected with estradiol (40 µg/kg body weight) in sesame oil. Control rats received an injection of sesame oil only.

**DD**—Total RNAs were extracted from pregnant (day 5) and nonpregnant (estrus) uteri using an RNAlater isolation system (Promega, Madison, WI). RNA samples were freed of DNA after treatment with DNase I (Genehunter Corp., Brookline, MA) and subjected to differential display with random primers as described previously (25, 30, 31) with several modifications. Briefly, 2 µg of DNA-free total RNA were reverse-transcribed with 200 units of Moloney murine leukemia virus reverse transcriptase (Promega) in the presence of 1 mM T<sub>4</sub>MA, T<sub>4</sub>MC, or T<sub>4</sub>MG primer (Genehunter Corp.), where M is a mixture containing dG, dA, and dC. The reaction was performed at 37 °C for 1 h. One tenth of this reaction was then used in a PCR amplification reaction containing 2 mM each of dNTPs, 10 µCi of [35S]dATP (Amersham Pharmacia Biotech), and two primers: 1 mM T<sub>3</sub> oligonucleotide and 0.2 mM of one of the five arbitrary decamers, AP-1 (5'-AGCCAGCGCA-3'); AP-2 (5'-GACCGTTGTG-3'); AP-3 (5'-AGGTGACGCG-3'); AP-4 (5'-GCTACTCCAC-3'); AP-5 (5'-GGTCTGAGTC-3'). These reactions also contained 1 unit of AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT). The cycling parameters for PCR were: 94 °C for 30 s, 40 °C for 1 min, 72 °C for 30 s for 40 cycles. After PCR amplification samples were analyzed on a 6% polyacrylamide sequencing gel, dried without fixation, and exposed to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) for 72 h. Bands exhibiting differential expression were cut out of the gel, and DNA was eluted by boiling as described before (25, 30, 31). Eluted DNA samples were then amplified by PCR using the corresponding pair of primers under the same conditions as described above, except that neither 25 mM dNTP nor radioisotope was used. The PCR products were cut from 2.5% low melting point agarose gels, subeluted into TA vector (Invitrogen), and subjected to nucleotide sequence analysis.

**Northern Blot Analysis**—For Northern analysis 20 µg of total RNA was separated by formaldehyde-agarose gel electrophoresis and transferred to Duralon membrane (Stratagene). Blots were prehybridized in 50 mM NaPO<sub>4</sub>, pH 6.5, 5% SSC, 5% Denhardt’s, 50% formamide, 0.1% SDS, and 100 µg/ml salmon sperm DNA for 4 h at 42 °C. Hybridization was carried out overnight in the same buffer containing 10<sup>6</sup> cpm/ml of a 32P-labeled ERG1 cDNA fragment. The filters were washed twice for 15 min in 1× SSC, 0.1% SDS at room temperature, then twice for 20 min in 0.2× SSC, 0.1% SDS at 55 °C and the filters were exposed to x-ray films for 24–72 h. The intensities of signals on the autoradiograms were estimated by densitometric scanning. To correct for RNA loading, the obtained signals were normalized with respect to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal in the same blot. For this, the filters were stripped of the radioactive probe by washing for 10 min in 0.2× SSC, 0.1% SDS at 55 °C and the filters were re-probed with a 32P-labeled GAPDH probe (CLONTECH) as described above.

In Situ Hybridization—Uterine or oviductal tissues from pregnant animals were collected and frozen. Tissues were fixed in 4% paraformaldehyde at 4 °C. Cryostat sections were cut at 8 µm and attached to 3-aminopropyl triethyliamine (Sigma) coated slides. In situ hybridization was performed with digoxigenin (DIG)-labeled sense or antisense RNA probes complimentary to ERG1 cDNA. DIG-labeled RNA probes were synthesized from ERG1 cDNA using T3 or T7 RNA polymerase and DIG-labeled nucleotides according to manufacturer’s specifications (Roche Molecular Biochemicals). Prehybridization was carried out in a damp chamber at 55 °C for 60 min in hybridization buffer (50% formamide, 5× SSC, 2% blocking reagent, 0.02% SDS, 0.1% N-laurylsarcosine). Hybridization was carried out at 55 °C in a damp humidified chamber. To develop the substrate, sections were sequentially washed in 2× SSC, 1× SSC, and 0.1× SSC for 15 min in each buffer at 37 °C. Sections were then incubated with anti-DIG alkaline phosphatase-conjugated antibody. Excess antibody was washed away and the color substrate (nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate) was added. Slides were allowed to develop in the dark, and the color was visualized under light microscopy until maximum levels of staining were achieved. The reaction was stopped and the slides counterstained in Nuclear Fast Red for 5 min. The slides were washed in water, dehydrated, and coverslipped. Control incubations utilized a DIG-labeled RNA sense strand and were performed under identical conditions.

**Results**

Cloning of ERG1—To isolate the uterine genes that are regulated by steroid hormones in the preimplantation phase of pregnancy, we employed the messenger RNA differential display method (25). We compared RNA samples prepared from uteri of nonpregnant (estrous) and pregnant (day 5 morning prior to implantation) rats. Several differentially displayed bands representing cDNA clones corresponding to genes whose expression in the uterus was up- or down-regulated during the preimplantation phase were isolated. One of these bands, which was present in the uteri of nonpregnant rats but disappeared on day 5 of pregnancy (marked by an arrow in Fig. 1, left lane), was selected for further characterization. The cDNA present in this band was recovered from the gel and amplified by PCR (40 cycles). The cDNA fragment was labeled with 32P and used to probe Northern blots of mRNAs obtained from uteri of nonpregnant (estrous stage) and pregnant (day 5) rats (Fig. 1, right panel). A strong signal corresponding to 2.2 kilobase pairs emerged in the RNA isolated from the nonpregnant uteri, while no detectable signal was observed in the RNA from pregnant uteri. Hybridization of the blot with a control probe (ferritin light chain) indicated that the difference in intensities of the signals in the lanes did not arise due to unequal loading of mRNAs in these lanes. These results indicated that the gene we isolated is indeed down-regulated in the uteri of pregnant rats. Using this cDNA fragment as a probe, we isolated a full-length cDNA from a rat uterus cDNA library. Nucleotide sequence analysis of the isolated cDNA and comparison with the GenBank<sup>®</sup> data base indicated it to be a novel gene (GenBank<sup>®</sup> accession no. AF167170). Subsequent characterization of this gene in our laboratory revealed that it is regulated by estrogen. Therefore, it has been designated “estrogen-regulated gene 1” or ERG1.

The ERG1 cDNA harbors an open reading frame of 1821 base pairs in length. The conceptual translation of the cDNA predicts a protein of 607 amino acids (Fig. 2A). A search of GenBank<sup>®</sup> protein data bases showed that ERG1 exhibits homology to two kinds of protein families. In the amino-terminal region, it possesses a repeat of a 100-amino acid motif termed CUB domain that exhibits ~40% amino acid identity to a similar sequence in bone morphogenetic type-1 protein, while its carboxy-terminal half contains a single 16-amino acid transmembrane domain. The ERG1 carboxyl-terminal domain is also found in the zona pellucida sperm binding proteins. Excess antibody was washed away and the color substrate (nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate) was added. Slides were allowed to develop in the dark, and the color was visualized under light microscopy until maximum levels of staining were achieved. The reaction was stopped and the slides counterstained in Nuclear Fast Red for 5 min. The slides were washed in water, dehydrated, and coverslipped. Control incubations utilized a DIG-labeled RNA sense strand and were performed under identical conditions.

ERG1 mRNA Expression Is Restricted to the Uterus and Oviduct—As a first step in understanding the biological function of ERG1, we analyzed the expression pattern of this gene...
in a variety of tissues (Fig. 3A). Using Northern blot analysis, we observed a band of about 2.2 kilobases corresponding to the ERG1 mRNA in the uterus (estrus stage, lane 10). We failed to detect any ERG1 mRNA in several other tissue such as heart, brain, lung, liver, kidney, skeletal muscle, and testes (Fig. 3A, lanes 1–9). We also studied its expression in other female reproductive tissues such as the ovary and oviduct (Fig. 3B). While no ERG1 mRNA could be detected in the ovary, it was expressed abundantly in the oviduct. By our estimation, the level of ERG1 expression in the oviduct appeared to be at least 2-fold higher than in the uterus. Taken together, these results indicate that ERG1 is expressed predominantly in two reproductive tissues, uterus and oviduct, at a high level.

**ERG1 Expression Is Modulated in the Uterus and Oviduct during the Reproductive Cycle**—To examine the pattern of ERG1 expression in the uterus and oviduct during estrus cycle, we performed Northern blot analysis of RNA isolated from uteri of nonpregnant rats at proestrus, estrus, and diestrus stages. As shown in Fig. 4, when the blot was hybridized with a 32P-labeled ERG1 cDNA probe, a strong 2.2-kilobase message was seen in the uterine RNA sample at the proestrus stage of the cycle (lane 1). The signal corresponding to ERG1 mRNA declined at estrus (lane 2) and disappeared at the diestrus stage of the cycle (lane 3). The magnitude of the decline at the estrus phase was estimated to be about 40% compared with proestrus stage of the cycle. In the oviduct, the expression of ERG1 was high in both proestrus and estrus but declined significantly at diestrus (Fig. 4, lanes 4–6). The expression of the ERG1 message in both uterus and oviduct is therefore modulated in a stage-specific manner during the reproductive cycle.

**Localization of ERG1 mRNA in the Surface Epithelia of the Uterus and Oviduct**—The site of expression of ERG1 mRNA in the uterus and oviduct was investigated by employing in situ hybridization (Fig. 5). Uterine and oviductal sections from nonpregnant animals at proestrus stage were hybridized with a 500-base pair digoxigenin-labeled antisense or sense RNA probe containing sequences from ERG1 cDNA. While control uterine sections hybridized with the sense RNA probe did not exhibit any significant signal (Fig. 5A, panel b), we observed a strong hybridization signal in the luminal epithelial cells of these uterine sections upon hybridization with the antisense probe (panels a and c). A strong hybridization signal in the

**FIG.1. Profiles of differentially expressed mRNAs in uterine tissues of nonpregnant and pregnant rats.** Left panel, total RNA (2 µg) isolated from uteri of nonpregnant (estrus) and pregnant (day 5) rats was subjected to differential display. Right panel, Northern blot analysis of uterine mRNAs of nonpregnant and pregnant rats. Poly(A⁺) RNA (1 µg/lane) was analyzed by RNA gel blot analysis. The upper panel shows the pattern of signals obtained after hybridization with a 32P-labeled cDNA fragment that was isolated from the DD gel shown in the left panel (indicated by arrowhead). The position of this mRNA is indicated by ERG1 mRNA. The lower panel shows the same blot after hybridization with a control 32P-labeled ferritin light chain probe.

**FIG.2. Structure and deduced amino acid sequence of ERG1.** A, the complete nucleotide sequence of rat ERG1 cDNA has been submitted to the GenBank (accession no. AF167170). The longest open reading frame of ERG1 yields a deduced amino acid sequence of 607 residues. The positions of the CUB and ZP domains are indicated in a linear structure. The position of the signal peptide is shown at the amino-terminal end and a putative transmembrane domain, indicated by TM, is depicted at the carboxyl-terminal region. B, description of CUB and ZP domains of ERG1, bone morphogenetic protein 1 (BMP1), or uromodulin (UROM) is indicated.
ovulational epithelial cells was also observed upon hybridization with the antisense probe (Fig. 5B, panels a and c). These results demonstrate that the surface epithelium is the primary site of synthesis of ERG1 mRNA in rat uterus and oviduct.

Profile of ERG1 mRNA Expression in the Uterus and Oviduct during Early Pregnancy—We then investigated the pattern of ERG1 mRNA expression in rat uterus at various stages of early pregnancy. We performed Northern blot analysis of RNA isolated from uteri of pregnant animals at days 1–6 of gestation. As shown in Fig. 6A, a high level of ERG1 mRNA was observed in the uterus of day 1 pregnant animals (lane 1). The level of this mRNA declined dramatically on day 2 of pregnancy (lane 2). No ERG1 expression was observed on days 3, 4, 5, and 6 of gestation (lanes 3, 4, 5, and 6, respectively). In situ hybridization of uterine sections at different days of gestation further confirmed the down-regulation of ERG1 mRNA expression during early pregnancy (Fig. 6B). The signal corresponding to ERG1 mRNA was intense at the luminal epithelium of uterus on day 1 (panel A), diminished on day 2 (panel B), and disappeared on days 5 and 6 of pregnancy (panels C and D, respectively). Collectively, these results show that ERG1 is expressed in a highly stage-specific manner in the luminal epithelial cells of the uterus during early pregnancy.

We also examined the expression of ERG1 in the oviduct during early pregnancy by Northern blot analysis (Fig. 7A). In pregnant rats, ERG1 mRNA level was high in the oviduct on days 1 and 2 but declined significantly on days 3 and 4. The magnitude of this decline on days 3–4 was estimated to be about 50% compared with either day 1 or 2 of pregnancy (Fig. 7B). These results indicate that, as in the uterus, the expression of ERG1 in the oviduct is also down-regulated during early pregnancy.

Estrogen Up-regulates and Progesterone Down-regulates ERG1 Gene Expression in the Uterus—To determine whether ERG1 expression in the uterus is regulated by steroid hormones, we administered estrogen (E), progesterone (P), or a combination of both to ovariectomized rats. Uteri were collected from animals 16 h after the injection, and mRNAs were isolated from these tissues for Northern blot analysis. The blot was probed with 32P-labeled ERG1 cDNA. As shown in Fig. 8A, the signal representing ERG1 mRNA was barely detectable in the uteri of ovariectomized rats (lane 1). While treatment with progesterone (lane 2) failed to induce ERG1 mRNA, estrogen alone (lane 2) markedly increased the level of ERG1 mRNA in the uterus. The level of this mRNA declined (by more than 60%) significantly when progesterone was administered together with estrogen to ovariectomized animals (Fig. 8, A and B, lane 4). These results demonstrate that estrogen alone can trigger the accumulation of ERG1 mRNAs in the uteri of ovariectomized animals. These results also clearly show that progesterone antagonizes the action of estrogen on induction of ERG1 expression in the uterus.

We next examined the time course of ERG1 gene induction by estrogen. Ovariectomized rats were injected with estrogen for 4, 8, 16, and 24 h, and a Northern blot analysis was carried out using mRNAs isolated from the uteri. As shown in Fig. 9A, the amount of ERG1 mRNA increased progressively with the duration of estrogen treatment. A significant increase (10-fold) in the level of ERG1 mRNA was observed after 8 h of estrogen treatment. Estrogen injections up to 16 or 24 h led to a further enhancement in the level of ERG1 mRNA. We also performed in situ hybridization analysis of uterine sections from ovariectomized animals treated with estrogen (Fig. 9B). Consistent with the results of Northern blot analysis, no ERG1 mRNA expression was observed in the luminal epithelial cells of ovariectomized animals (panel A). While ERG1 mRNA was barely visible at the surface epithelial cells 4 h after estrogen treatment (panel B), this mRNA became detectable 8 h after estrogen treatment (panel C). The level of ERG1 mRNA increased further after 16 or 24 h of estrogen treatment (panels D and E, respectively). Taken together, these results show that the level of ERG1 mRNA at the surface epithelial cells of the uterus increases progressively with the duration of estrogen treatment.

An Antagonist of ER Blocks ERG1 mRNA Expression in the
Uterus and Oviduct—We further investigated the estrogenic regulation of ERG1 mRNA expression by employing an antiestrogen, ICI 182,780, which is known to severely impair the transcriptional activity of estrogen receptors (32). Animals were ovariectomized and injected with vehicle or estrogen or a combination of estrogen and ICI 182,780. As depicted in Fig. 10A, ERG1 expression was not observed in vehicle-treated ovariectomized animals (lane 1). As expected, administration of estrogen induced ERG1 in the uterus (lane 2). Treatment of animals with estrogen in combination with ICI 182,780 (lane 3), however, caused a drastic decline in the level of ERG1 mRNA but did not affect the expression of GAPDH mRNA. These results strongly suggest that ICI 182,780 switched off the ERG1 gene expression in the uterus by inhibiting the estrogen receptors.

We also studied the effects of ICI 182,780 on ERG1 expression in the uterus of cycling rats by in situ hybridization (Fig. 10B). Animals were injected at proestrus stage with either vehicle (control) or ICI 182,780, and the uteri were isolated at estrus stage for in situ hybridization. As shown in Fig. 10B, a high level of ERG1 mRNA was observed in the uterine epithelial cells of animals at estrus stage (panel A). However, a single dose of ICI 182,780 dramatically reduced the level of this mRNA in the uterine epithelial cells (panel B). These studies further confirm the role of estrogen in regulating ERG1 expression in the uterus.

We next examined the regulation of ERG1 mRNA by estrogen in the oviduct of cycling animals. Animals were injected at proestrus stage with either vehicle (control) or ICI 182,780. Uteri were isolated and a Northern blot analysis was performed. As shown in Fig. 11, a single dose of ICI 182,780 reduced the level of ERG1 mRNA in the oviduct. By our estimate, the level of ERG1 mRNA was reduced to about 50% of that of the control within 16 h of treatment. These results indicate that, as in the uterus, estrogen regulates ERG1 expression in the oviduct.

Progestosterone Suppresses ERG1 Expression in the Uterus during Early Pregnancy—Since our results indicated that progesterone antagonizes estrogen-induced ERG1 expression in the uterus (Fig. 8), we considered the possibility that the down-regulation of ERG1 expression following day 1 of pregnancy could be caused by the surge of progesterone at the onset of pregnancy. The level of progesterone increases markedly on day 2 and remains high until the end of gestation. To test this hypothesis, animals were injected on day 3 of pregnancy either with a vehicle or a dose of RU486, which is known to block the action of progesterone receptors in the uterus (33, 34). As shown in Fig. 12A, ERG1 was not detected in the uterus of day 4 pregnant animals (lane 1). A single dose of RU486, however, induced a remarkable expression of ERG1 mRNA in the uterus within 24 h of treatment (lane 2). The RU486-induced expression of ERG1 mRNA in the uterus was also examined by in situ hybridization. Consistent with the results of the Northern blot analysis, RU486 treatment led to an increase in the accumulation of ERG1 mRNA in the epithelial cells relative to the signal seen in the uteri of vehicle-treated pregnant (day 4) animals (Fig. 12B, compare panels B and C). Collectively, these results confirm the role of progesterone in down-regulating estrogen-induced ERG1 gene expression in the uterus of pregnant rats.

Estrogen Induces ERG1 Gene Expression in the Uterus of Immature Rats—Estrogen-induced proliferation of immature rodent epithelium is a long standing and widely used model of uterine actions of estrogen (35–37). We, therefore, examined whether ERG1 is expressed in the uterus of immature rats in response to estrogen. Immature rats were treated with estrogen for 4, 8, and 24 h; mRNA was isolated and subjected to Northern blot analysis using ERG1 cDNA probe. As shown in Fig. 13A, ERG1 mRNA was not detected in the uteri of immature rats (lane 1). However, treatment with estrogen alone for 4 or 8 h dramatically increased the level of this mRNA (lanes 2 and 3). The level of ERG1 mRNA increased further after 24 h of estrogen treatment.

We then performed in situ hybridization to localize estrogen-induced expression of ERG1 mRNA in the uterine compartments of immature rats. As shown in Fig. 13B (panel A), no signal corresponding to ERG1 mRNA was observed in the uterus of immature rats. A specific signal corresponding to ERG1 mRNA, however, appeared in the uterus after 24 h of estrogen treatment (panel B), which was restricted to the luminal epithelial cells. These results indicate that, as in the adult rats, estrogen is the primary inducer of ERG1 mRNA expression at the surface epithelium of immature rats.

DISCUSSION

In the present study, we have isolated a full-length cDNA of ERG1 from rat uterus. In the mRNA differential display, ERG1 cDNA appeared to represent a gene that is down-regulated during early pregnancy (Fig. 1). Indeed, spatio-temporal analyses of ERG1 expression in the endometrium confirm that its expression in the surface epithelium is strongly repressed as gestation progresses from day 2 to day 5. The level of ERG1 mRNA is high on day 1 of pregnancy, declines on day 2, and is barely detectable on days 3, 4, 5, or 6 of gestation. ERG1 is also expressed in the uterus in a stage-specific manner during the ovarian cycle. While maximal expression of ERG1 occurred at the proestrus stage of the ovarian cycle coincident with the estrogen-induced uterine cell proliferation, we observed mini-
mal, if any, ERG1 expression at the diestrus stage of the cycle. The tight spatio-temporal regulation of ERG1 expression during the reproductive cycle and early pregnancy suggest that it might have a critical regulatory role(s) in these processes.

Recently Kasik reported the isolation of a cDNA that is highly expressed in mouse uterus during late pregnancy (38). This cDNA exhibits 82% homology with the rat ERG1 clone isolated by us. The investigator performed Northern blot analysis using ERG1 (upper panel) or GAPDH (lower panel) cDNA probes. The results are representative of two independent experiments. The investigator performed Northern blot analysis using ERG1 (upper panel) or GAPDH (lower panel) cDNA probes. B, the intensities of the signals corresponding to the ERG1 transcript was quantitated by densitometric scanning of the autoradiogram and normalized with respect to the GAPDH signals of the Northern blot shown in panel A. The results are representative of two independent experiments.

Our studies also indicate that the ERG1 mRNA level in the uterus is regulated by progesterone. While estrogen induces ERG1 expression, progesterone inhibits estrogen-mediated ERG1 expression in the uterus. The estrogen concentration remains low from pregnancy day 2 onward, except for a transitory rise on day 4 immediately prior to implantation (22–24).
progesterone level, on the other hand, starts to increase by day 2 and remains high until the end of gestation (39). It is likely that the estrogen-induced ERG1 expression in the uterus on day 2 and subsequent days of pregnancy is antagonized by the rising progesterone level. This concept is supported by the observation that treatment of animals with RU486, which antagonizes the action of progesterone, dramatically enhances ERG1 expression on day 4 of gestation (Fig. 12). ERG1 expression in the uterus is therefore regulated by a complex interplay of estrogen and progesterone.

Besides uterus, ERG1 is also highly expressed in the oviduct. In mammals, oviduct is the site at which fertilization of the egg and early development of the zygote occurs. The zygote travels through the oviduct and enters the uterus to arrive at the site of implantation. Previous studies indicated that the correct programming of egg or zygote transport through the oviduct is regulated by estrogen and progesterone (1, 2). It was shown in a number of animal models that estrogen accelerates egg/zygote transport and progesterone blocks this estrogen-mediated transport. Allen showed that estrogen stimulates differentiation of the oviductal epithelium in the rhesus monkey (40, 41). Since the receptors for steroid hormones are present in oviducal tissue, it is likely that the actions of estrogen and progesterone on gamete transport and differentiation of the epithe-
lium are mediated by receptor-activated mechanisms. Despite the well known regulatory role of estrogen in the oviduct, to the best of our knowledge, there is only one published report of an estrogen-regulated gene in the oviduct (42). Therefore, our finding that estrogen regulates ERG1 expression in the epithelial cells of the oviduct establishes a novel marker of estrogen action in this tissue. The expression level of ERG1 in the oviduct, however, is significantly higher than that in the uterus. We therefore cannot rule out the possibility that another factor(s) in addition to estrogen modulates ERG1 expression in the oviduct.

The biological implication of ERG1 expression in the uterus and oviduct is unclear. The ERG1 gene product is predicted to exhibit several distinct structural motifs. In the carboxyl-terminal region, a stretch of 260 amino acids harbors a ZP domain. The ZP domain was first identified in ZP1, ZP2, and ZP3, major glycoproteins of the extracellular matrix surrounding oocytes (28). These proteins are involved in sperm-egg recognition. The ZP domain was later found in several other receptor-like proteins, such as uromodulin, and the major zymogen granule membrane glycoprotein (GP-2), defining a new family of distantly related proteins involved in binding functions (28, 29). Uromodulin, a major glycoprotein secreted by the mammalian kidney, functions as an immune suppressor and prevents urinary tract infections by binding mannose-sensitive fimbriated microorganisms. The homologous region common to all ZP domain proteins contains eight strictly conserved cysteines, which are likely to form disulfide bridges. In addition to the conserved cysteines, these proteins also harbor few aromatic or hydrophobic amino acids that are highly conserved among the family members. Interestingly, in each of these proteins the common domain occurs next to the putative membrane-spanning regions suggesting a possible conserved biological role of the domain. Consistent with the ascribed features of the ZP family proteins, ERG1 possesses the characteristics of an integral membrane protein since it harbors a single transmembrane signal near the carboxyl terminus. Future studies will determine whether ERG1 exists in a soluble or secreted form and displays specific binding functions.

The nucleotide sequence of ERG1 cDNA also reveals the presence of two CUB domains at the amino-terminal end of the predicted protein. The CUB domain is a 110-amino acid module that was first identified in the complement subcomponents C1s and C1r (C1s/C1r) (26). Later on, a similar domain was found in bone morphogenetic protein 1 (BMP1) and an embryonic sea urchin protein Uegf. Recently, several other proteins containing CUB domain have been reported (26, 27). These include the Drosophila dorso-ventral patterning gene product tolloid, the sea urchin blastula protein Bp10, and a family of sperm adhesins (27). In all the proteins identified so far, four cysteine residues are conserved in all CUB modules, which display a typical antiparallel β-sheet structure. Although a common structural framework exists for all CUBs, there is no clear indication for functional relations. Nevertheless, there are several indications of an involvement of all CUB domain proteins in the developmental and differentiation processes. It is in-

![Fig. 10. ERG1 mRNA in the uterus of nonpregnant rats declines after treatment with antiestrogen ICI 182,780. A, total RNA (20 µg/lane) was subjected to Northern blot analysis and hybridized with a 32P-labeled ERG1 (upper panel) or GAPDH (lower panel) probes. Lane 1, RNA from uteri of animals injected with vehicle only after ovariectomy; lane 2, RNA from uteri of animals injected with estrogen; lane 3, RNA from uteri of animals injected with estrogen (2 µg/kg body weight) and ICI 182,780 (1 mg/kg body weight). B, in situ hybridization of uterine sections from nonpregnant animals at the estrus stage (panel A) and following treatment with a single dose of antiestrogen ICI 182,780 (1 mg/kg body weight) (panel B).](image)

![Fig. 11. ERG1 mRNA in the oviduct of nonpregnant rats declines after treatment with antiestrogen ICI 182,780. Total RNA (20 µg/lane) was subjected to Northern blot analysis and hybridized with a 32P-labeled ERG1 (upper panel) or S16 RNA (lower panel) probe. Lanes 1 and 2 represent oviductal RNA at the estrus stage and upon treatment with a single dose of antiestrogen ICI 182,780 (1 mg/kg body weight), respectively.](image)
triguing that ERG1 shows maximal similarity to BMP1, a factor that induces mesenchymal cells to differentiate into cartilage-forming cells and further into bone cells. Interestingly, ERG1 is induced in the uterine epithelium of immature rodents during estrogen-induced cell proliferation and differentiation. Future studies will determine whether ERG1 expression in response to estrogen plays a role in the proliferation and differentiation of epithelial cells during the estrus cycle and early pregnancy.

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**REFERENCES**

1. Croxatto, H. B. (1996) in *Reproductive Endocrinology, Surgery, and Technology* (Adashi, E. Y., Rock, J. A., and Rosenwaks, Z., eds) Vol. 1, pp. 385–402, Lippincott-Raven, New York

2. Harper, M. J. K. (1994) in *The Physiology of Reproduction* (Knobil, E., and Neill, J. D., eds) pp. 123–187, Raven Press, New York

3. Jensen, E. V., and DeSombre, E. R. (1972) *Annu. Rev. Biochem.* **41**, 203–230

4. Katzenellenbogen, B. S., and Gorski, J. (1975) in *Biochemical Actions of Hormones* (Litwack, G., ed) Vol. 3, pp. 187–243, Academic Press, New York

5. Anderson, J. N., Peck, E. J., Jr., and Clark, J. H. (1975) *Endocrinology* **96**, 160–167

6. Psychovas, A. (1973) *Vitam. Horm.* **31**, 205–255

7. Katzenellenbogen, B. S., and Korach, K. S. (1997) *Endocrinology* **138**, 861–862

8. Evans, R. M. (1988) *Science* **240**, 889–895

9. Beato, M. (1989) *Cell* **56**, 335–344

10. O’Malley, B. W. (1990) *Mol. Endocrinol.* **4**, 363–369

11. Murphy, L. J., Murphy, L. C., and Freisen, H. G. (1987) *Endocrinology* **120**, 1882–1888

12. Travers, M. T., and Knowler, J. T. (1987) *FEBS Lett.* **211**, 27–30

13. Murphy, L. J., Murphy, L. C., and Freisen, H. G. (1987) *Mol. Endocrinol.* **1**, 445–450

14. DiAugustine, R. P., Petrusz, P., Bell, G. I., Brown, C. F., Korach, K. S.,...
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McLachlan, J. A., and Teng, C. T. (1988) Endocrinology 122, 2355–2363
15. Lingham, R. B., Stancel, G. M., and Loose-Mitchell, D. S. (1988) Mol. Endocrinol. 2, 230–235
16. Weisz, A., and Bresciani, F. (1988) Mol. Endocrinol. 2, 816–824
17. Loose-Mitchell, D. S., Chiappetta, C., and Stancel, G. M. (1988) Mol. Endocrinol. 2, 946–951
18. Ghahary, A., and Murphy, L. J. (1989) Endocrinology 125, 597–604
19. Chiappetta, C., Kirkland, J. L., Loose-Mitchell, D. S., Murthy, L., and Stancel, G. M. (1992) J. Steroid Biochem. Mol. Biol. 41, 113–123
20. Nelson, K. G., Takahashi, T., Lee, D. C., Luetteke, N. C., Bossert, N. L., Ross, K., Eitzman, B. E., and McLachalan, J. A. (1992) Endocrinology 131, 1657–1664
21. Cullinan-Bove, K., and Koos, R. D. (1993) Endocrinology 133, 829–837
22. Finn, C. A. (1971) in Biology of the Uterus (Wynn, R. M., ed) pp. 246–308, Plenum Press, New York
23. Psychoyos, A. (1973) in Handbook of Physiology (Greep, R. O., Astwood, E. G., and Geiger, S. R., eds) pp. 187–215, American Physiological Society, Washington, DC
24. Yoshinaga, K., Hawkins, R. A., and Stocker, J. F. (1969) Endocrinology 85, 103–112
25. Liang, P., and Pardee, A. B. (1992) Science 257, 967–971
26. Bork, P. (1991) FEBS Lett. 282, 9–12
27. Bork, P., and Beckmann, G. (1993) J. Mol. Biol. 231, 539–545
28. Bork, P., and Sander, C. (1992) FEBS Lett. 300, 237–240
29. Pennica, D., Kohr, W. J., Kaang, W.-J., Glaister, D., Aggarwal, B. B., Chen, E. Y., and Goeddel, D. V. (1987) Science 236, 83–88
30. Utans, U., Liang, P., Wyner, L. R., Karnovsky, M. J., and Russell, M. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6463–6467
31. Aiello, L. P., Robinson, G. S., Lin, Y. W., Nishio, Y., and King, G. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6231–6235
32. Wakeling, A. E., and Bowler, J. (1988) J. Steroid Biochem. 36, 141–148
33. Baulieu, E. E. (1989) Science 245, 1351–1357
34. Baulieu, E. E. (1991) Trends Endocrinol. Metab. 2, 233–239
35. Kaye, A. M., Shertzky, D., and Lindner, H. R. (1972) Biochim. Biophys. Acta 261, 475–486
36. Kirkland, J. L., LaPointe, L., Justin, E., and Stancel, G. M. (1979) Biol. Reprod. 21, 269–272
37. Quarmby, V. E., and Korach, K. S. (1984) Endocrinology 114, 694–702
38. Kasik, J. W. (1998) Biochem. J. 330, 947–950
39. Wiest, W. G. (1970) Endocrinology 87, 43–48
40. Allen, E. (1928) J. Morphol. 46, 479–520
41. Allen, E. (1928) Am. J. Anat. 42, 467–487
42. Donnelly, K. M., Fazleabas, A. T., Verbage, H. G., Mavrogianis, P. A., and Jaffe, R. C. (1991) Mol. Endocrinol. 5, 356–364