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Regulation of Laminin Chain Gene Expression by Ovarian Steroid Hormones in Uterine Tissues of Ovariectomized Mice

Chanseob Shim, Dongho Geum, Donchan Choi, and Kyungjin Kim*

Department of Molecular Biology and Research Center for Cell Differentiation, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea

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To precisely analyze the role of ovarian steroids in the regulation of laminin chain gene expression in mouse uterine tissues, the ovariectomized mouse model was used. Ovariectomized mice received a single injection of steroid hormones and total RNA was isolated from whole uterine tissues. Messenger RNA levels of each laminin chain (A, B1, and B2) were determined by competitive RT-PCR procedures. Estradiol decreased mRNA levels of laminin B1 chain about two-fold, and B2 chain rather moderately. Estradiol-induced inhibition of laminin B1 and B2 chain mRNA levels were completely blocked by pretreatment with estrogen receptor antagonist tamoxifen. Estriol, a short acting estrogen which cannot induce hyperplastic responses of rodent uterine tissues, also showed an inhibitory effect on B1 and B2 chain mRNA levels, while estrone, an inactive estrogen, failed to influence either B chain mRNA levels. Effects of steroids on A chain mRNA level were quite different from those on B chains. Laminin A chain mRNA level was slightly increased by estradiol treatment, but negatively affected by progesterone. Progesterone treatment greatly increased both B chain mRNA levels, but slightly decreased A chain mRNA level compared to the control. The effect of progesterone on laminin chain-specific mRNA levels was further increased by co-injection of estradiol in a time-dependent manner. Progesterone-induced B1 and B2 chain mRNA transcription was inhibited by RU486, a synthetic anti-progesterone /anti-glucocorticoid. The present study demonstrates for the first time that steroids are able to regulate laminin gene expression in mouse uterine tissues, indicating that steroid-regulated laminin gene expression is involved in uterine growth and probably differentiation.

In rodents, the overall changes in uterine growth and differentiation are regulated through well-coordinated actions of estrogen and progesterone (Weitlauf, 1994). Preovulatory increase in ovarian estrogen stimulates uterine luminal and glandular epithelial cell proliferation, whereas postovulatory progesterone from newly formed corpora lutea initiates the proliferation of stroma cells for implantation of embryos to the uterus. Estrogenic stimulation induces a series of well-defined uterine growth responses. These responses include increased DNA and protein synthesis (Moulton and Koenig, 1985), increased vascular permeability leading to active nutrient transport (Glasser, 1972; Kennedy, 1979), and re-organization of basement membrane by regulating the deposition of extracellular matrix (ECM) components (Glass et al., 1983, Wewer et al., 1985). Many reports indicate that uterine proliferative responses to ovarian steroids are mediated by locally produced growth factors (Das et al., 1992; Kapuret al., 1992; reviewed by Weitlauf, 1994). However, the precise molecular events leading to steroid-induced proliferation and differentiation in the uterus are not well understood.

The expression of a wide variety of ECM molecules including laminin, fibronectin, collagen, and heparin sulfate proteoglycan not only supports an extracellular skeletal architecture, but also mediates a large body of biological functions (reviewed by Adams and Watts, 1993). ECM molecules can regulate a cellular proliferation and differentiation of various cells and tissues (Engel, 1992). Increasing evidence suggests that ECM molecules are involved in the mediation of growth factor's function and gene expression, although the molecular and cellular basis of action mechanism is poorly understood. It is evident that ECM molecules can regulate the differentiated phenotype of epithelial cells and their expression is influenced by several growth factors (Drago et al., 1991).

The previous reports using immunohistochemistry and *in situ* hybridization demonstrated the presence of ECM molecules and their mRNAs in uterine

* To whom correspondence should be addressed.
Tel: 82-2-880-6694, Fax: 82-2-872-1993

tissues (Wewer et al., 1986; Glasser et al., 1987; Senior et al., 1988; Clark et al., 1992), indicating their possible involvement in reproductive events such as implantation and pregnancy (Carson et al., 1988; Sutherland et al., 1988; O'Shea et al., 1990; Yelian et al., 1995). However, the regulation patterns of ECM expression and deposition in uterus by ovarian steroids are not well established. The present study was performed to examine the regulation of laminin chain-specific gene expression by ovarian steroids in uterine tissues of ovariectomized adult mice *in vivo*.

Materials and Methods

Animals

ICR mice (5-6 weeks old) were obtained from Seoul National University Animal Breeding Center and maintained under a 14 h light and 10 h dark photocycle (light on at 06:00) with water and food supplied *ad libitum*.

To determine the effects of steroid hormones, adult female mice (6-8 weeks old) were ovariectomized without regard to the stage of estrus cycle. After at least 2 weeks of post-operative recovery, mice received a single injection of steroids subcutaneously (sc) at 14:00 hour. Twenty four hour after steroid treatment, uteri were dissected and transferred to microfuge tubes followed by immediate snap-freezing in liquid nitrogen and stored at -70℃ until use. Steroids were dissolved in sesame oil at appropriate concentrations: estrone (100 ng/0.1 ml), 17β-estradiol (100 ng/0.1 ml), estriol (100 ng/0.1 ml), progesterone (1 mg/0.1 ml), dexamethasone (1 mg/0.1 ml), and testosterone (1 mg/0.1 ml). To examine the possible involvement of steroid receptors in mediating the steroid actions on laminin chain mRNA expression, an estrogen antagonist, tamoxifen (100 ng/0.1 ml in ethanol) and a progesterone antagonist, RU486 (1 mg/0.1 ml in sesame oil) were administered intraperitonealy (ip) and sc, respectively, 1hr before steroid injection.

Total RNA isolation

Total RNA was isolated by acid guanidinium phenol-chloroform method (Chomzynski and Sacchi, 1987). The organs were homogenized with tissue homogenizer in 600 μl homogenization buffer (4 M guanidinium isothiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% lauryl sarcosine, and 0.1 M 2-mercaptoethanol) on ice. Subsequently, 0.1 volume of 2 M sodium acetate (pH 4.0), 1 volume of water-saturated phenol, and 0.2 volume of chloroform-isoamyl alcohol (49:1) were added to the homogenates. After thorough mixing with vigorous agitation, the mixture was incubated on ice for 10 min. Aqueous-phase solution containing total RNA was then fractionated by centrifugation at 12,000 rpm for 30 min at 4℃ and RNA was precipitated in the presence of 1 volume of isopropanol. The pellet was washed twice with 75% ethanol and reconstituted in distilled water. Unless otherwise mentioned, diethyl pyrocarbonate-treated water was used in all experiments. Concentration of total RNA was assessed by absorbance at 260 nm.

Mutant cDNA construction and in vitro transcription for a competitive reverse transcription-polymerase chain reaction (RT-PCR)

Template cDNAs for mutant construction were obtained by RT-PCR amplification of ovarian total RNA. RT-PCR amplicons specific for each laminin chain were cloned into pGEM4Z vector, and direction and sequence specificity of inserted cDNAs were confirmed by sequence analyses (Shim et al., 1996). Deletion mutant cDNAs for each laminin chain were constructed as follows.

Laminin A-pGEM4Z vector was first digested with *Eco*RI and *Hin*dIII and the cDNA fragments containing the PCR amplicon were isolated. Digestion of the A chain cDNA fragments with *Alu*I produced three fragments: a 72 bp containing the upper primer binding site, 94 bp with the middle portion, and 86bp containing the lower priming site. *Alu*I-digested cDNA fragments were then re-ligated without further purification, ligates were inserted into the *Eco*RI/*Hin*dIII-digested pGEM4Z vector, and bacterial clone containing mutant cDNA without a middle portion of 94 bp fragment was selected by PCR amplification of plasmid DNAs. A deletion mutant cDNA of laminin A chain was 159 bp in size.

The mutant cDNA of laminin B1 chain was obtained by digesting laminin B1-pGEM4Z vector with *Sty*I and *Bgl*II, followed by re-ligation after trimming protruding ends with T4 polymerase (Promega). It contained a 249 bp fragment of PCR amplicon truncating a 121 bp fragment.

The laminin B2 chain cDNA-containing vector was digested with *Pst*I and *Xba*I and resulted 3' and 5' protruding ends were removed by T4 polymerase to make blunt end. Blunt ended vector was ligated and transformed to bacteria. Digestion with *Pst*I and *Xba*I followed by T4 polymerase treatment yielded 163 bp in size without a middle 89 bp fragment of B2-pGEM4Z vector.

To synthesize mutant cRNA transcripts *in vitro*, vectors containing each laminin chain mutant cDNA were linearized by appropriate restriction enzymes and *in vitro* transcription was performed according to the manufacturer's instructions using SP6 or T7 viral RNA polymerase (Boehringer Mannheim). After *in vitro* transcription, cRNAs were purified by phenol/chloroform extraction and dissolved in diethylpyrocarbonate-treated water at a concentration of 1 μg/ml.

RT-PCR

RT was primed with random hexanucleotide (Boehringer Mannheim) unless otherwise stated. Total RNA (1 μg) was denatured in the presence of 100 pmol random hexanucleotide in a final volume of 10.75 μl at 75℃ for 10 min. For competitive RT-PCR, *in vitro* transcribed cRNA mutants were added before random priming reaction. After brief centrifugation at 4℃, 9.25 μl of master mix [200 U RNaseH⁻-MMLV reverse transcriptase, Promega; 4 μl dNTP mix (2.5 mM each), 0.25 μl RNasin, Promega; 26 U/μl; and 4 μl of 5×RT buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 10 mM dithiothreitol)] was added in ice and incubated at 37℃ for 1h. Temperature was raised to 95℃ for 5min to terminate reaction. PCR amplification was carried out with 2 μl of RT reaction mixture in 40 μl reaction mixture containing 4 μl 10× PCR buffer (supplied by Perkin Elmer Cetus), 3.2 μl dNTP mix (2.5 mM each), 0.3 μCi α[^{32}P]-dCTP (3,000 Ci/mmol, Amersham), and 1U Ampli-Taq polymerase (Perkin Elmer Cetus). In each reaction, 10pmols of PCR primers were added. The sample was then overlaid with 40 μl mineral oil (Sigma) and subjected to amplification on PCR cycler (Pharmacia LKB, Gene ATAQ controller). PCR was performed under following parameters: 94℃ for 1 min, 60℃ for 1 min, and 72℃ for 1.5 min. The last cycle at 72℃ was extended to 10min. In case of competitive RT-PCR, second amplification step (85℃ for 1 min, 60℃ for 1 min, and 72℃ for 1.5 min) was included to minimize native/mutant hetero-duplex (Seong et al., 1995). The 6 μl radio-labeled PCR products were then analyzed by 5% polyacrylamide gel electrophoresis without a further purification step. Gels were dried and directly exposed to X-ray film for 2 h. The resulting autoradiogram was analyzed with densitometric scanning. Oligonucleotides used for PCR amplification were designed based on published cDNA sequences and obtained from Inter-University Center for Natural Science Research Facility, Seoul National University.

Northern blot hybridization analysis

For Northern blot hybridization, RNA solution (20 μg) was denatured in the presence of 50% formamide, 2.2 M formaldehyde, 20 mM MOPS (3-[N-morpholino] propanesulfonic acid), 4 mM sodium acetate, and 0.5 mM EDTA at 65℃ for 10 min. Denatured RNA was then fractionated in 1.2% agarose gel containing 2.2 M formaldehyde. After RNA transfer onto nylon membrane (Nytran, 0.45 μm pore size; Schleicher and Schuell) by capillary action under 10x SSPE (1x SSPE: 0.18 M NaCl, 10 mM Na_2HPO_4, pH 7.7, and 1 mM EDTA). The RNA transfer and loading efficiency were estimated by staining extra membrane with 0.1% methylene blue. RNA intactness was estimated by comparing the ratio between 18 and 28 S ribosomal RNA stain. The membrane to hybridize was washed with 6x SSPE for 5 min and air dried and RNA was permanently attached to the membrane by UV illumination for 1 min. Hybridization was performed overnight in a polyethylene heat sealable bag containing 40 ml hybridization buffer (5x SSPE, pH 7.4, 5xDenhardt's solution, 0.5% SDS, 0.2 mg/ml heat-denatured salmon sperm DNA, and 50% formamide) and hybridization probe. We used ^{32}P-labeled antisense RNA transcripts (5×10^8 cpm/μg RNA) as hybridization probes. Template cDNAs for *in vitro* transcription were obtained by cloning RT-PCR products in pGEM4Z (Promega) vector by bluntend ligation method. The orientation of cloned cDNAs was determined by sequence analysis and *in vitro* transcription was performed by either SP6 or T7 polymerase according to the methods in the manufacturer's instructions (Boehringer Mannheim).

Data analysis

Signals for each laminin chain-specific RT-PCR product on X-ray film were quantified by densitometric scanning (Hoefer Scientific Instruments). The data (mean ± SEM) were evaluated using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference test for a post-hoc comparison. Statistical significance was set at $P < 0.05$.

Results

Tissue-specific expression patterns of laminin chain-specific mRNA transcripts

Expression patterns of laminin chain-specific mRNAs

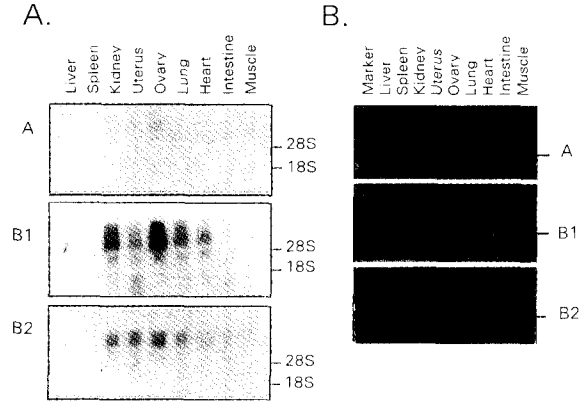

Fig. 1. Tissue-specific expression patterns of laminin chain-specific mRNAs. A: Northern blot hybridization was performed with total RNAs (20 μg) isolated from various organs of mice. Laminin chain-specific antisense cRNA was labeled with α[^{32}P]-UTP and used as a hybridization probe. B: RT-PCR analysis of laminin chain mRNAs. Total RNA (1 μg) was reverse transcribed by random hexamer priming and subjected to RT-PCR with laminin chain-specific primer sets. After thirty PCR amplifications, PCR products were analyzed on 2% agarose gel electrophoresis and visualized by ethidium bromide staining. A 100 bp ladder marker (M) was used as the size standard.

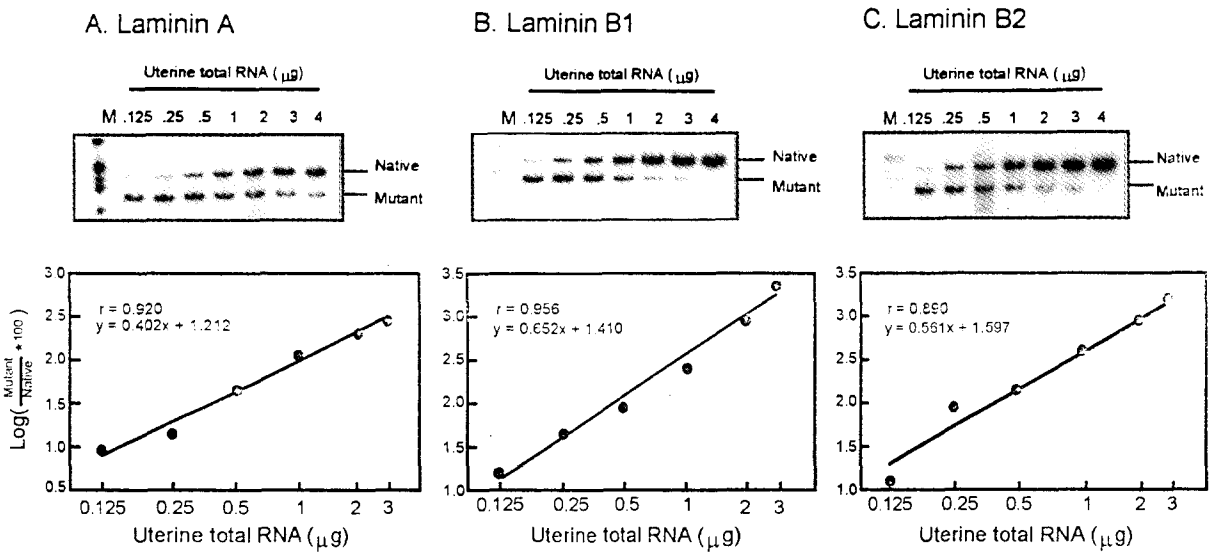

Fig. 2. Evaluation of competitive RT-PCR to quantify the laminin chain-specific mRNAs. Serially diluted total RNAs (0.125-4 μg) isolated from mouse whole uterine tissues were co-reverse transcribed in the presence of fixed amounts of mutant cRNAs (0.1 pg, 10 pg and 80 pg for A, B1, and B2, respectively), and PCR amplification was performed in the presence of $\alpha[^{32}P]$-dCTP. Relative densities between native and mutant bands were calculated after densitometric scanning. Standard plots were constructed as a function of mRNA input (lower panels).

in various organs were determined by Northern blot hybridization and RT-PCR (Fig. 1). Northern blot hybridization specifically and reproducibly detected laminin A, B1, and B2 chain mRNAs with about 10-, 6-, and 8-kb in size, respectively. B1 and B2 chain mRNAs were found in all tissues and readily detected in tissues including the kidney, uterus, ovary, lung, and heart, while laminin A chain mRNA was barely detected in all tissues except for the ovary (Fig. 1A). Although Northern hybridization failed to detect A chain mRNA in uterus, RT-PCR showed that there were A chain mRNA transcripts in uterus (Fig. 1B).

Optimization of competitive RT-PCR to quantify laminin chain-specific mRNA levels

To precisely examine the expression patterns of laminin chain-specific mRNAs in mouse uterus, competitive RT-PCR procedures for each chain were developed. Comparison of relative densities between native and mutant cDNA bands indicates that A, B1, and B2 chain mutant cRNAs effectively competed with native mRNAs (in 1 μg of total RNA preparation) at the concentrations of about 0.5, 10, and 100 pg of mutant RNAs, respectively (data not shown). Standard plots were then constructed for quantification of endogenous mRNA copies. Increasing amounts of RNA samples (0.125-4 μg) were co-reverse transcribed with a fixed amounts of mutant cRNA and and subjected to PCR amplification (Fig. 2). The linear regression of log ratio of mutant/native bands against RNA inputs was established. With these competitive RT-PCR protocols, we are able to precisely calculate a copy number of mRNA transcripts present in uterine samples.

Effect of various steroid hormones on laminin chain-specific mRNA levels in uterine tissues of ovariectomized mice

Ovariectomized mice were treated with estrogens,

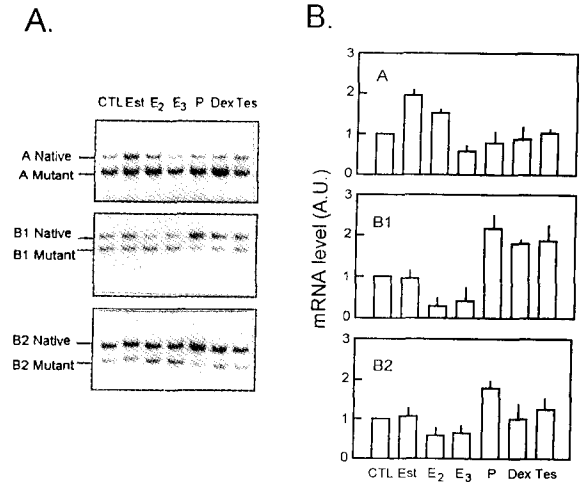

Fig. 3. Effects of various steroid hormones on laminin chain-specific mRNA levels in the uterine tissues of ovariectomized adult female mice. Ovariectomized mice were administered with a single injection of steroids [CTL; vehicle only, Est; estrone (100 ng/mouse), E_2; estradiol (100 ng/mouse), E_3; estriol (100 ng/mouse), P; progesterone (1 mg/mouse), Dex; dexamethasone (1 mg/mouse) and Tes; testosterone (1 mg/mouse)]. Twenty four hours after hormone treatments, whole uterine tissues were dissected and total RNA was isolated. A: Representative autoradiograms of RT-PCR amplification analysis of laminin chain-specific mRNAs. B: Relative changes in laminin chain-specific mRNA levels were calculated. Data were presented in an arbitrary unit (A.U.) relative to the control group (mean ± SE, n=4).

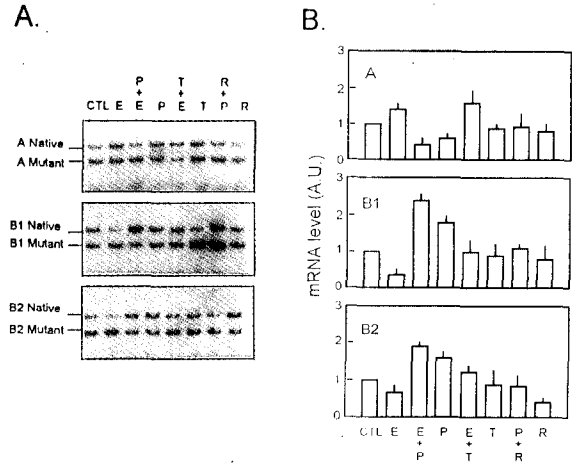

Fig. 4. Regulation of laminin chain-specific mRNA levels by estradiol (E, 100 ng/mouse) and/or progesterone (P, 1 mg/mouse) in the uteri of ovariectomized mice. To examine the involvement of steroid receptors in estradiolor progesterone-induced laminin chain mRNA expression, tamoxifen (T, 100 ng/ mouse) or RU486 (R, 1 mg/mouse) were pretreated 1 h before hormone treatment. A single injection of estradiol and/or progesterone were given to ovariectomized mice and uterine tissues were dissected 24 h after treatment. A: Representative autoradiograms of RT-PCR amplification analysis of laminin chain-specific mRNAs. B: Relative changes in laminin chain-specific mRNA levels in each treatment group were presented in an arbitrary unit (A.U.) relative to the control group (mean ± SE, n=3).

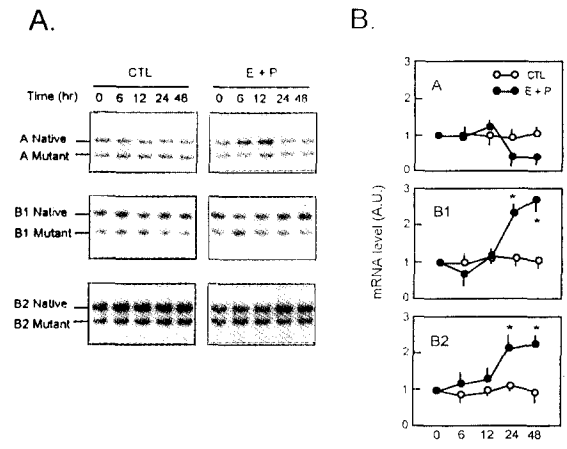

Fig. 5. Time-dependent changes of laminin chain mRNAs by a combined treatment of estradiol (E) and progesterone (P) in ovariectomized mouse uterus. In the control experiment, single injection of vehicle (0.1 ml of sesame oil) was administered to ovariectomized mice and uteri were dissected at different time points. A competitive RT-PCR was performed using 1 g of total RNA isolated from uterine tissues. Autoradiograms of three different experiments were shown (A). Relative changes of laminin chain mRNA levels were calculated and expressed as a mean ± SE (n=3) in an arbitrary unit (A.U.) relative to the control group (B).

progesterone, testosterone or dexamethasone and total RNAs isolated from uterine tissues were subjected to competitive RT-PCR to analyze the effects of steroids on laminin chain-specific mRNA levels in mouse uterus. Fig. 3 shows differential changes in laminin chain mRNA levels by steroid hormones. Laminin A chain mRNA level was increased by estrone and estradiol (Fig. 3B), but not by any other steroids used in the present study. Estradiol reduced laminin B1 chain mRNA level by one third and B2 chain mRNA level by half. Estriol, a short acting estrogen which is known to be unable to induce hyperplastic responses of rodent uterine tissues (Pastore et al., 1992), also produced inhibitory effects on both B chain mRNA levels. However, estrone, an inactive estrogen, failed to affect B chain mRNA level.

In contrast, progesterone greatly increased laminin B1 and B2 chain mRNA levels. Dexamethasone and testosterone treatment increased the B1 chain mRNA level, but not the B2 chain mRNA level.

Effect of steroid receptor antagonists on steroid-induced laminin chain mRNA levels

To examine the possible involvement of steroid receptors in steroid-induced changes in laminin chain-pecific mRNA levels, ovariectomized mice were pretreated with tamoxifen (100 ng/mouse) or RU486 (1 mg/mouse) 1 h before estradiol and/or progesterone treatments. While tamoxifen, an estrogen receptor antagonist, alone did not produce any notable changes in laminin chain-specific mRNA levels, pretreatment of tamoxifen blocked inhibitory effects of estradiol on laminin B1 and B2 chain mRNA levels (Fig. 4). However, estradiol-induced laminin A chain mRNA level was not affected by tamoxifen treatment. RU-486, a potent progesterone receptor antagonist, reduced progesterone-induced elevation of B1 and B2 chain mRNA levels, indicating that the progesterone receptor may be involved in the regulation of laminin B chain mRNA expression.

Effect of combined treatment of estradiol and progesterone on laminin chain mRNA levels

As shown in Fig. 4, progesterone when treated with estradiol, reduced estradiol-induced changes in laminin chain mRNA levels. Both laminin B chain mRNA levels were significantly ($P < 0.01$) increased by combined treatment of estradiol and progesterone. In contrast, laminin A chain mRNA level was completely suppressed by this co-treatment regimen. This is also evident in the time-course experiment. Laminin B1 can B2 chain mRNA levels were increased 24 h after hormone treatment, while laminin A chain mRNA level was decreased. These time-dependent alterations were sustained up to 48 h (Fig. 5).

Discussion

Although there are much data on the distribution of ECM molecules and their transcripts in mouse uterine tissues (Wewer et al., 1986a; Glasser et al., 1987; Senior et al., 1988; Clark et al., 1992), it is largely unknown how the synthesis and deposition of ECM are regulated. The present study clearly

demonstrates that the laminin chain gene expression in uterine tissues is under the control of ovarian steroids. Expression of both B1 and B2 chain mRNAs in uterine tissues was negatively regulated by estradiol, but positively altered by progesterone in ovariectomized mice. When estrogen was administered at the same time with progesterone, progesterone-induced B1 and B2 chain mRNA expression was further potentiated by estrogen. The effect of estrogen and/or progesterone on laminin B chain mRNA levels was in a time-dependent manner. Since anti-estrogens and anti-progesterone inhibited the effects of estrogen and progesterone on laminin B1 and B2 chain mRNA expression, it is tempting to speculate that the steroidal modulation of laminin B chain mRNA expressions may be mediated through their receptors.

Increasing evidence indicated that activated receptor-ligand complexes can bind to the specific regulatory DNA sequences and then regulate transcription of target genes (Adler et al., 1988; Yang-Yen et al., 1990). Genomic DNA analysis of human laminin B1 chain reveals that there are several putative steroid hormone responsive, *cis*-acting elements in the 5'-flanking region of DNA; these include the partial estrogen responsive element, glucocorticoid responsive element, and multiple AP2 sites (Vuolteenaho et al., 1990). In the 5'-flanking region of human B2 chain gene, no notable steroid responsive elements are present. However, multiple AP2 sites are present, which may be involved in transcriptional regulation by steroid hormones (Kallunki et al., 1991). There are many similarities in the 5'-flanking region of B1 and B2 chain genes. TATA and CAAT boxes, but not the multiple GC box, were present in both B1 and B2 chains. This is the common feature of basement membrane-forming glycoproteins such as collagen type IV genes. However, the information about the 5'-flanking region of A chain gene is not available yet at present. The present results, therefore, offer an indirect evidence that laminin chain expression can be regulated by steroid hormones at the level of transcriptional activation.

Another possible explanation for regulation of laminin chain mRNA expression by steroid hormones is that steroid hormones may act by modulating the expression of early response genes (Loose-Mitchell et al., 1988; Weisz and Bresciani, 1988; Suva et al., 1991; Kirkland et al., 1992; Nephew et al., 1993; Bigsby and Li, 1994) and several growth factors (Das et al., 1992; Kapur et al., 1992). An emerging concept is that steroid hormone actions are mediated in part by autocrine/paracrine effects of growth factors in the uterus (Huet-Hudson et al., 1990; Nelson et al., 1991; Kapur *et al* ., 1992). Recently, several reports have shown that extracellular matrix protein synthesis may be regulated by growth factors such as TGF and FGF (Drago et al., 1991) at the transcriptional level in various cells.

In most mammals, ovarian steroids are directly involved in the regulation of uterine growth and differentiation. Estrogen administration to ovariectomized adult mice induces a marked hyperplasia of the luminal epithelium, while it produces little proliferative effect on the stroma and myometrium (Martin et al., 1970). Progesterone blocks the proliferative effect of estrogen on the epithelium when the two steroids are administered simultaneously. Laminin has long been speculated to be involved in the epithelial cell differentiation in various tissues (reviewed by Adams and Watts, 1993). Deposition of laminin in basement membrane is a key event leading to epithelial cell differentiation. However, whether laminin chain-specific synthesis, which is inhibited by estrogen but up-regulated by progesterone, is directly involved in the regulation of uterine growth and differentiation needs to be explored.

In summary, the present results demonstrate for the first time that steroid hormones regulate laminin gene expression in a chain-dependent manner in mouse uterus.

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References

Adams J and Watts F (1993) Regulation of development and differentiation by the extracellular matrix. *Development* 117: 1183-1198.

Adler S, Waterman ML, He X, and Rosenfeld MG (1988) Steroid receptor-mediated inhibition of rat prolactin gene expression does not require the receptor-DNA binding domain. *Cell* 52: 685-695.

Bigsby RM and Li A (1994) Differentially regulated immediate early genes in the rat uterus. *Endocrinology* 134: 1820-1826.

Carson DD, Tang J, and Gay S (1988) Collagens support embryo attachment and outgrowth *in vitro*: Effects of the arg-gly-asp sequence. *Dev Biol* 127: 367-385

Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156-159.

Clark DE, Hurest PR, Myers DB, and Spears GF (1992) Collagen concentration in dissected tissue compartments of rat uterus on days 6, 7 and 8 of pregnancy. *J Reprod Fertil* 94: 169-175.

Das SK, Flanders KC, Andrews GK, and Dey SK (1992) Expression of transforming growth factor-β isoforms (β2 and β3) in the mouse uterus: Analysis of the periimplantation period and effects of ovarian steroids. *Endocrinology* 130: 3459-3466.

Drago J, Nurcombe V, Pearse MJ, Murphy M, and Baltlett PF (1991) Basic fibroblast growth factor upregulates steady-state levels of laminin B1 and B2 chain mRNA in cultured neuroepithelial cells. *Exp Cell Res* 196: 246-254.

Engel J (1992) Laminins and other strange proteins. *Biochemistry* 31: 0643-10651.

Glass RH, Aggeler J, Spindle A, Pederson RA, and Werb Z (1983) Degradation of extracellular matrix by mouse trophoblast outgrowths: A model for implantation. *J Cell Biol* 96: 108-1116.

Glasser SR (1972) The uterine environment and implantation and decidualization. In: Balin H and Glasser SR (eds), Reproductive Biology, Exceptra Medica, Amsterdam, pp 776-973.

Glasser SR, Lampelo S, Munir MI, and Julian J (1987) Expression of desmin, laminin and fibronectin during *in situ* differentiation (decidualization) of rat uterine stromal cells. *Differentiation* 35: 132-142.

Huet-Hudson YM, Chakraborty C, De SK, Suzuki Y, Andrews GK, and Day SK (1990) Estrogen regulates synthesis of EGF in mouse uterine epithelial cells. *Mol Endocrinol* 4: 510-523.

Kallunki T, Ikonen J, Chow LT, Kallunki O, and Tryggvason K (1991) Structure of the human laminin B2 chain gene reveals extensive divergence from the laminin B1 chain gene. *J Biol Chem* 266: 221-228.

Kapur S, Tamada H, Dey SK, and Andrews GK (1992) Expression of insulin-like growth factor-I (IGF-I) and its receptor in the peri-implantation mouse uterus, and cell-specific regulation of IGF-I gene expression by estradiol and progesterone. *Biol Reprod* 46: 208-219.

Kennedy TG (1979) Prostaglandins and increased endometrial vascular permeability resulting from the application of an artificial stimulus to the uterus of the rat sensitized for the decidual cell reaction. *Biol Reprod* 20: 399-407.

Kirkland JL, Murphy L, and Stancel GM (1992) Progesterone inhibits the estrogen-induced expression of c-*fos* messenger ribonucleic acid in the uterus. *Endocrinology* 130: 3223-3230.

Loose-Mitchell DS, Chiappetta C, and Stancel GM (1988) Estrogen regulation of c-*fos* messenger ribonucleic acid. *Mol Endocrinol* 2: 946-951.

Martin L, Finn CA, and Carter J (1970) Effects of progesterone and oestrogen on the luminal epithelium of the mouse uterus. *J Reprod Fertil* 21: 37-56.

Moulton BC and Koenig BB (1985) Uterine deoxyribonucleic acid synthesis during preimplantation in precursors of stromal cell differentiation during decidualization. *Endocrinology* 115: 1302-1307.

Nelson KG, Takahashi T, Bossert NL, Walmer DK, and McLachlan JA (1991) Epidermal growth factor replaces estrogen in the stimulation of female genital track growth and differentiation. *Proc Natl Acad Sci USA* 88: 21-25.

Nephew KP, Webb DK, Akcali KC, Moulton BC, and Khan SA (1993) Hormonal regulation and expression of the *jun*-D protooncogene in specific cell types of the rat uterus. *J Steroid Biochem Mol Biol* 46: 281-287.

O'Shea KS, Liu LH, Kinnunen LH, and Dixit VM (1990) Role of the extracellular matrix protein thrombospondin in the early development of the mouse embryo. *J Cell Biol* 111: 2713-2723.

Pastore GN, Dicola LP, Dollahon NR, and Gardner RM (1992) Effect of estriol on the structure and organization of collagen in the lamina propria of the immature rat uterus. *Biol Reprod* 47: 83-91.

Senior PV, Critchely DV, Beck F, Walker RA, and Varley JM (1988) The localization of laminin mRNA and protein in the postimplantation embryo and placenta of the mouse: An *in situ* hybridization and immunohistochemical study. *Development* 104: 431-446.

Seong JY, Jarry H, Kuhnemuth S, Leonhardt S, Wuttke W, and Kim K (1995) Effect of GABAergic compounds on gonadotropin-releasing hormone receptor gene expression in the rat. *Endocrinology* 136: 2587-2593.

Shim C, Kwon HB, and Kim K (1995) Differential expression of laminin chain-specific mRNA transcripts during mouse preimplantation embryo development. *Mol Reprod Dev* 44: 44-55.

Sutherland A, Calarco PG, and Damsky CH (1988) Expression and function of the integrin family of cell surface extracellular matrix receptors in mouse blastocyst attachment and outgrowth. *J Cell Biol* 106: 1331-1348.

Suva LJ, Harm SC, Gardner RM, and Thiede MA (1991) *In vivo* regulation of Zif268 messenger RNA expression by 17 β-estradiol in the rat uterus. *Mol Endocrinol* 5: 829-835.

Vuoteenaho R, Chow LT, and Tryggvason K (1990) Structure of the human laminin B1 chain gene. *J Biol Chem* 265: 15611-15616.

Weisz A and Bresciani F (1988) Estrogen induces expression of c-*fos* and c-*myc* protooncogenes in rat uterus. *Mol Endocrinol* 2: 816-824.

Weitlauf HM (1994) Biology of implantation. In: Knobil E, and Neil JD (eds.), The Physiology of Reproduction, Raven Press, New York, pp 391-448.

Wewer UM, Faber M, Liotta LA, and Albrechtsen R (1985) Immunochemical and ultrastructural assessment of the nature of the pericellular basement membrane of human decidual cells. *Lab Invest* 53: 624-633.

Wewer UM, Damjanov A, Weiss J, Liotta LA, and Damjanov I (1986) Mouse endometrial stromal cells produce basement-membrane components. *Differentiation* 32: 49-58.

Yang-Yen HF, Chambard JC, Sun Y, Smeal T, Schmidt T, Drouin J, and Karin M (1990) Transcriptional interference between c-*jun* and the glucocorticoid receptor: Mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62: 1205-1215.

Yelian FD, Yang Y, Hirata JD, Schultz JF, and Armant DR (1995) Molecular interactions between fibronectin and integrins during mouse blastocyst outgrowth. *Mol Reprod Dev* 41: 435-448.

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