In Vivo Regulation of Syndecan-3 Expression in the Rat Uterus by 17β-Estradiol

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The immature rat uterus has been extensively used as an in vivo model system to study the molecular mechanisms of steroid hormone actions. In this study, we demonstrated the regulated expression of syndecan-3 in the rat uterus by the steroid hormone 17β-estradiol. Administration of a single physiological dose of 17β-estradiol (40 μg/kg) to ovariectomized immature animals induced a rapid and transient increase in uterine syndecan-3 mRNA. Transcript levels reached a peak elevation of 3-fold above saline control tissues 4 h after hormone administration. Inhibition of message up-regulation by actinomycin D but not cycloheximide indicated a hormone response dependent on RNA transcription but not new protein synthesis. The estrogenic ligands estriol and tamoxifen were also effective at raising syndecan-3 mRNA levels; however, nonestrogenic ligands, including progesterone, 5α-dihydrotestosterone, and dexamethasone, failed to stimulate a change in mRNA levels. Hormone-induced changes in mRNA led to transient changes in syndecan-3 protein content and significant alteration in the temporal and spatial expression in endometrial epithelial cells. Collectively, these data show that the steroid hormone 17β-estradiol, regulates transcription of the syndecan-3 gene in the uterus via an estrogen receptor-dependent mechanism. This estrogen-regulated expression of syndecan-3 may play an important role in changes in tissue ultrastructure crucial for proper uterine growth.

It has been known for some time that ovarian hormones induce changes in expression of surface proteins on epithelial cells lining the uterine lumen believed to be crucial for blastocyst reception (1). Included in this group of modified surface proteins are the heparan sulfate proteoglycans (HSPGs). HSPGs are nearly ubiquitous components of mammalian cell membranes that exhibit binding interactions with a variety of extracellular ligands. Molecular cloning and analysis has led to identification of multiple forms of membrane-anchored proteoglycans. Those best characterized are the membrane intercalated molecules, four of which display significant homologies in core protein structure and are classified as members of the same gene family. These related molecules are called the syndecans and include syndecans-1–4. Syndecans contain a small core protein with distinct functional domains to which are linked multiple glycosaminoglycan (GAG) chains, predominantly of the heparan sulfate variety. Homology of syndecan family members is most striking in the transmembrane and cytoplasmic domains, showing >50% amino acid sequence similarity. In contrast, the extracellular ectodomains show little homology (2). Sequence-specific serine residues in the ectodomain regions serve as target sites for GAG attachment during proteoglycan synthesis. Variation in number and position of GAG attachment sites within the nonhomologous ectodomains leads to substantial differences in syndecan structure and potentially produces differential functions for each of the family members.

Precise functions of each syndecan family member have not fully been determined. Experimental observations indicate involvement of these compounds in cell adhesion, cell morphogenesis, and regulation of cell responsiveness to soluble growth-regulatory compounds (3). These functions are attributed primarily to the glycan moieties. Both basic fibroblast growth factor (4, 5) and vascular endothelial growth factor (6) have binding affinities for heparan sulfate, and evidence indicates that HS PGs function as coreceptors for these compounds at the cell surface. By virtue of their HSPG associations, binding of growth factors to specific cell surface receptors is facilitated, thereby enhancing effective signal transduction in target cells. Heparan sulfate GAG chains also provide cell membrane binding sites for extracellular matrix adhesion proteins, including fibronectin (7, 8) and laminin (9). Such binding interactions may facilitate or reinforce attachment of matrix proteins to their specific cell surface integrin receptors. Evidence that cell surface HSPGs have a role in focal adhesion formation in fibroblast and Chinese hamster ovary cells (10) and the specific localization of syndecan-4 to focal adhesions in a variety of cell types (11, 12) further supports syndecan facilitation of organized cell-matrix interactions. Syndecan-3 has been implicated as an important matrix adhesion molecule in regulated neurite outgrowth activity during development in the central nervous system (13). Additional observations that neuronal cell adhesion molecule, a homophilic cell-cell adhesion protein, exhibits binding affinity for heparan sulfate also suggest potential HSPG involvement in cell-cell anchorage (14). A direct assessment of differential binding affinities of the various membrane proteoglycans has not yet been completed. Therefore, the functional significance of the diverse forms of syndecan family members remains unclear.

Given the potential functional variation of the syndecans, it is not surprising that regulated expression of the family members has been observed. Although data are incomplete, synde-
cans appear to be expressed in tissue-specific patterns, although some crossover is apparent (for review, see Ref. 15). Syndecan-3, although initially characterized by its high degree of expression in the nervous system during early postnatal development (16), is also observed in vascular smooth muscle cells and stratified epithelia. Regulation of expression in these various cell types, however, is poorly understood, although evidence indicates both temporal and spatial changes during a variety of development and cell differentiation processes. Such changes in expression often correlate with intervals when cell morphology is altered and major tissue ultrastructural reorganizations are occurring.

Limited information is available concerning the molecular mechanisms of syndecan gene regulation. Induction of syndecan-1 expression has been observed in response to basic fibroblast growth factor in cultured populations of epithelial cells (17) and in vascular smooth muscle cells by exposure to platelet-derived growth factor (18). Likewise, syndecan-4 expression in vascular smooth muscle cells is induced by basic fibroblast growth factor (19). Ovarian hormones have been shown to regulate expression of syndecan-1 in mouse uterine tissue (20). In this study, histochemical analysis to detect binding of anti-syndecan-1 antibodies demonstrated a dramatic change in luminal epithelial cell localization during the estrous cycle involving repositioning from a predominantly apical location during intervals of low hormone secretion to an exclusively basolateral position during the peak fertility phase. Estrogen has also been shown to change the total content of cell surface HSPGs and to stimulate their turnover in the mouse uterine epithelium (21). It is widely believed that such changes in epithelial cell HSPG expression are important for establishment of the blastocyst-receptive uterine state. The binding interactions of HSPGs in cell-matrix adhesion may indicate that estrogen-regulated changes in HSPG expression have an important role in directing endometrial matrix reorganization and cell spatial arrangements that accompany growth of the organ. However, little is known about the role of the syndecans in hormone-regulated uterine growth or about specific mechanisms that regulate their expression in the uterus.

In this study, we investigated the regulatory effect of estrogen on syndecan-3 expression in the rat uterus. Analysis of syndecan-3 mRNA and protein indicate that transient changes in syndecan-3 expression occur in response to estrogen treatment. Immunohistochemical analysis of uterine tissue sections showed high levels of expression in epithelial cells that line the uterine lumen and a change in cell surface localization of the protein in these cells in response to estrogen treatment.

**EXPERIMENTAL PROCEDURES**

**Uterine Growth and Heparan Sulfate Proteoglycans**

**RNA Extraction**—Total RNA was extracted from pooled uterine samples (approximately three to five uteri/sample) using Tri Reagent solution (22). Frozen tissue was homogenized in Tri Reagent using a Polytron homogenizer (Brinkmann Instruments), and the homogenates were separated by addition of chloroform and centrifugation at 12,000 g. RNA was precipitated from the aqueous phase with 100% isopropanol and pelleted by centrifugation at 12,000 × g. The final RNA pellet was washed once with 75% ethanol, briefly dried, and dissolved in Formazol (Molecular Research Center). Total RNA was quantified by spectrophotometric analysis at an absorbance of 260 nm. **Northern Blot**—Total RNA was resolved in a 1% formaldehyde-agarose gel by electrophoresis at 70 V in a 1× 4-morpholineethanesulfonic acid buffer (20 mM 4-morpholineethanesulfonic acid, 8.3 mM NaOAc, 1 mM EDTA, pH 7.0). RNA samples contained ethidium bromide (1 µg) for later visualization under ultraviolet light. An RNA ladder (0.24–9.5 kb; Life Technologies, Inc.) was loaded into one lane of each gel as molecular weight standard. Gels were photographed under UV illumination (Eagle Eye System; Stratagene), and the images were used for densitometric analysis of the 18S rRNA band to correct for loading inequalities between samples. Resolved RNA was transferred to Magna 0.45-µm nylon membrane (Magna Separation Industries) using a downward capillary transfer technique in 10× SSC as transfer buffer. RNA was fixed by baking at 80 °C for 15 min, followed by UV cross-linking (GS Gene Linker, Bio-Rad). **Northern Blot Analysis**—32P-Radiolabeled nucleic acid probes were prepared from a 1-kb EcoRI fragment of cloned rat syndecan-3 cDNA (16). 23 ng of purified cDNA fragment were used for probe synthesis by random primer labeling (Prime-It II kit; Stratagene) with α[32P]dCTP (3000 Ci/mmol; Amersham Pharmacia Biotech) as isotope. Northern blots were prehybridized at 65 °C in a solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 0.5% SDS, 100 µg/ml denatured salmon sperm DNA, and 5× Denhardt’s reagent. Prehybridized blots were incubated at 65 °C in a hybridization solution (5× saline/sodium phosphate/EDTA, 0.5% SDS, 100 µg/ml denatured salmon sperm DNA, 5× Denhardt’s reagent, 10% dextran sulfate) and the 32P-labeled probe (∼12–21 × 105 dpm/µg DNA). Membranes were washed under standard conditions and exposed to x-ray film (Fuji RX). For quantitative comparisons of mRNA levels, hybridization signals were quantified by direct measurement of membrane-bound radioactivity by phosphorimage analysis (Molecular Dynamics). Nonspecific background counts per minute in each lane were subtracted from the total counts per minute for radiolabeled mRNA bands to yield net counts per minute. Net counts were used for quantification of mRNA levels and direct comparison between RNA samples. **Protein Extraction**—Protein was extracted from pooled samples of frozen uteri (three to six uteri/sample) by homogenization in a buffer containing 0.1 M Tris (pH 7.5) using a Polytron homogenizer. Homogenates were centrifuged at 17,000 × g for 30 min at 4 °C, supernatants were saved, and the pellets were resuspended in buffer containing 0.1 M Tris (pH 7.5) plus 1% Triton X-100. Pellet extracts were centrifuged as before, and the supernatants were removed and pooled with the supernatants from the initial extraction step. Aliquots of mixed supernatant samples were quantified by spectrophotometric analysis using a colorimetric dye assay (Bio-Rad). **Immunoblotting**—Extracted protein (25 µg) was denatured at 100 °C in a solution containing 0.5 M Tris-HCl (pH 6.8), 2% SDS, 2% mercaptoethanol, 10% glycerol, and 0.002% bromphenol blue. Samples were applied to a 6% SDS-polyacrylamide gel and resolved by electrophoresis (Mini Protein II apparatus; Bio-Rad) at 100 V in a buffer containing 2.5 mM Tris base (pH 8.3), 192 mM glycine, and 0.1% SDS. After electrophoresis the proteins were transferred to an Immobilon-P membrane (Millipore) with a 100-V current. Membranes were soaked in a blocking solution of 5% nonfat dry milk, 0.1% Tween 20, and then incubated in a 1:250 dilution of 17β-estradiol (E2; 40 µg/kg in a 1× 4-morpholineethanesulfonic acid buffer (20 mM 4-morpholineethanesulfonic acid, 8.3 mM NaOAc, 1 mM EDTA, pH 7.0). RNA samples contained ethidium bromide (1 µg) for later visualization under ultraviolet light. An RNA ladder (0.24–9.5 kb; Life Technologies, Inc.) was loaded into one lane of each gel as molecular weight standard. Gels were photographed under UV illumination (Eagle Eye System; Stratagene), and the images were used for densitometric analysis of the 18S rRNA band to correct for loading inequalities between samples. Resolved RNA was transferred to Magna 0.45-µm nylon membrane (Magna Separation Industries) using a downward capillary transfer technique (23) in 10× SSC as transfer buffer. RNA was fixed by baking at 80 °C for 15 min, followed by UV cross-linking (GS Gene Linker, Bio-Rad). **Immunohistochemistry—**Uteri were harvested, and the horns were separated at the cervix. Each horn was embedded in OCT tissue me-
**RESULTS**

Changes in Uterine Syndecan-3 mRNA Levels in Response to \( E_2 - E_2 \)-induced changes in syndecan-3 mRNA content were assessed by Northern blot analysis of RNA samples extracted from ovariecotomized animals over a range of post-hormone treatment intervals from 0 to 48 h that included both the early and late uterine growth phases. Hybridization of Northern blots with radiolabeled rat syndecan-3 probe identified one major 5.9-kb transcript in the uterus (Fig. 1, top). Phosphorimage analysis of hybridized blots indicates that levels of syndecan-3 mRNA increased as early as 2 h and reached a peak value 3-fold higher 4 h after \( E_2 \) administration compared with levels in saline control animals (Fig. 1, bottom). Transcript levels remained elevated at 18 and 24 h after hormone treatment and then returned to the saline control level by 48 h.

There were no changes detected in syndecan-3 mRNA content in 24- or 48-h saline-injected control animals. These data clearly indicate a transient increase in syndecan-3 mRNA content in response to estrogen treatment with peak changes in mRNA occurring during the early uterine growth phase.

**Effect of 17\( \beta \)-Estradiol on Syndecan-3 mRNA Is Dose Dependent**—To determine whether the effect of \( E_2 \) on syndecan-3 mRNA is dose dependent and thus indicative of a true hormone-regulated process, ovariecotomized animals were treated with increasing doses of \( E_2 \), and levels of syndecan-3 mRNA were analyzed by Northern blot. Because the initial Northern blot analysis (Fig. 1) indicated that the induced change in syndecan-3 mRNA content was greatest 4 h after hormone treatment, all uterine tissues were extracted at this time point after either \( E_2 \) or saline control injections. Little or no change in syndecan-3 mRNA was detected at \( E_2 \) doses of <0.4 \( \mu g/kg \) (Fig. 2, top). However, mRNA levels increased to 43% of the maximum hormone response at a dose of 0.4 \( \mu g/kg \) (Fig. 2, bottom). Doses of 4 and 40 \( \mu g/kg \) produced equivalent increases in mRNA, indicating a maximum response level had been reached. The characteristic dose-response curve from the analysis (Fig. 2, bottom) is consistent with a response that is controlled by physiological levels of estradiol.

**\( E_2 \)-induced Changes in Uterine Syndecan-3 mRNA Content Are Translation but Not Translation Dependent**—Although the changes in syndecan-3 mRNA are indicative of a direct hormone-regulated process, it was of interest to determine whether the increase in mRNA after hormone treatment is the result of a direct change in transcription of the syndecan-3 gene. To study the transcription-dependent nature of syndecan-3 mRNA changes, ovariecotomized animals were pretreated with the transcription inhibitor actinomycin D or saline as
control before E$_2$ administration 2 h later. Tissues were harvested 4 h after hormone challenge to determine the effect of the inhibitor at the time point when mRNA content was estimated to be at the peak level after estrogen treatment. Hybridization results are shown in Fig. 3. Syndecan-3 mRNA content in animals pretreated with actinomycin D and challenged with E$_2$, (Act D + E$_2$) were significantly reduced 2-fold from transcript levels observed in animals treated with E$_2$ alone (saline + E$_2$). This decrease in mRNA content by the transcription-inhibitory compound thus indicates that the syndecan-3 gene is regulated at the level of transcription.

The E$_2$-induced changes in syndecan-3 mRNA, however, were not similarly affected by pretreatment of animals with the protein translation inhibitor cycloheximide. Animals were pretreated with cycloheximide (4 mg/kg) or saline vehicle 2 h before E$_2$ administration, and mRNA levels were measured in uterine tissue 4 h after hormone challenge. Syndecan-3 mRNA content was increased ~2–3-fold in cycloheximide-pretreated animals (Fig. 3, CHX + E$_2$), which was similar to the level of change observed in animals pretreated with saline (saline + E$_2$).

**Changes in Uterine Syndecan-3 mRNA Are Steroid Hormone Specific**—To more specifically investigate hormone responsiveness of the syndecan-3 gene, ovariectomized animals were treated with various estrogenic and nonestrogenic steroid hormones, and mRNA content was determined by Northern blot analysis. Animals were treated with the estrogenic compounds E$_2$, estradiol, and tamoxifen or nonestrogenic steroid compounds, including progesterone, 5α-dihydrotestosterone, and dexamethasone at the indicated effective dosages (Fig. 4). Uterine tissues were harvested from all treatment groups 4 h after hormone injections and processed for Northern blot analysis. Of the steroid hormones administered, only the estrogenic compounds E$_2$, estradiol, and tamoxifen induced increases in syndecan-3 mRNA content that were ~2–3-fold above levels measured in saline control animals for all three compounds (Fig. 4, bottom). The nonestrogenic sex steroid compounds progesterone and 5α-dihydrotestosterone caused no change in syndecan-3 mRNA over the same interval. However, treatment with the glucocorticoid agonist dexamethasone produced a decrease in mRNA levels, an ~80% reduction, compared with levels in tissues from saline control animals.

**E$_2$-induced Changes in Syndecan-3 mRNA Levels Are Specific to the Uterus**—Because the observed changes in uterine syndecan-3 mRNA clearly indicate hormone regulation of gene transcription, it was of interest to assess whether E$_2$ effected similar changes in other tissues known to contain the estrogen receptor, including heart (24), liver (25), lung (26), and spleen (27). All tissues were harvested from the same hormone-treated animals whose uteri were extracted and used for the initial syndecan-3 Northern blot analysis shown in Fig. 1. The syndecan-3 mRNA transcript was detected in all tissues analyzed; however, there were no detectable changes in mRNA levels after E$_2$ administration in any of these tissues at any postinjection interval (Fig. 5).

**Estradiol-induced Temporal and Spatial Changes in Uterine...**
Syndecan-3 Expression—To assess changes in expressed syndecan-3 protein during the E₂-induced uterine growth response, Western blot analysis of total uterine protein extracts was completed. Anti-syndecan-3 antibodies identified a broad high molecular mass band in the range of 190–250 kDa (Fig. 6). This is consistent with previous observations of the protein in other rat tissues and likely reflects varying degrees of glycanation at potential GAG attachment sites on the core protein (16). Protein content clearly increased by 8 h and remained elevated at 20 h after hormone treatment. Levels then appeared to decline and return to that measured in saline control animals by 48 h. These changes in syndecan-3 protein content therefore initiate during the hypertrophic phase but peak early in the hyperplastic phase of uterine growth and clearly demonstrate transient regulation of syndecan-3 expression by the estrogenic hormone.

Previous work in the mouse uterus has shown regulated changes in syndecan-1 expression in vivo during the normal fertility cycle in adult animals (20). To better characterize the cell type-specific expression of syndecan-3 and assess changes in the pattern of uterine syndecan-3 expression, immunohistochemical localization was completed. The same anti-syndecan-3 antibodies were used to visualize location of the protein in sections of uteri harvested from saline control and hormone-treated animals. Results show that syndecan-3 is expressed in epithelial cells of glands in the endometrial stroma, smooth muscle cells of the myometrium, and epithelial cells of the perimetrium (Fig. 7). The protein is also highly expressed in columnar epithelial cells of the endometrium (Fig. 8). Changes in staining intensity in all cell types indicate that protein levels increase after hormone treatment; however, the most pronounced changes in expression pattern were observed in the epithelial cells of the endometrium that line the uterine lumen.

The staining pattern in uteri of saline-treated control animals indicated a diffuse expression of syndecan-3 along all cell borders apical to basal (Fig. 8). However, 8 h after E₂ treatment, syndecan-3 was diminished significantly at apical cell borders but was higher in concentration along basolateral membrane regions. By 20 h, the protein was nearly entirely localized to the basal cell surfaces of these epithelial cells. At 48 h after hormone treatment, when the tissue growth response had been completed, the staining profile was again similar to that observed in saline-treated animals, with syndecan-3 evident on both apical and basal cell surfaces.
are controlled by estrogen in the immature rat uterus and that syndecan proteins are expressed in spatially specific areas in epithelial cells of the endometrium.

Administration of a single physiological dose of E2 increased uterine syndecan-3 mRNA and protein steady-state levels. Tissue levels of the transcript reach a maximum at 4 h after hormone administration, a time point that falls within the early phase of uterine growth. Similar analyses of E2 effects on uterine expression of syndecan-1 and 2 also clearly indicate transient increases in mRNA and protein content, however, with distinct temporal patterns. These observations clearly indicate specific E2-regulated expression of multiple syndecan family members and suggest a role for these molecules in uterine fertility.

The rapid induction of syndecan-3 mRNA suggests that the syndecan-3 gene is an early growth response gene in the uterus. Analysis of the dependence of mRNA changes on new gene transcription or protein translation events supports a direct effect of E2 on syndecan-3 gene transcription. Pretreatment of animals with actinomycin D, a transcription inhibitor, significantly blocked the E2-induced increase in mRNA. However, the translation inhibitor cycloheximide failed to affect the hormone-induced mRNA response. The dosages of actinomycin D and cycloheximide used in these experiments have been shown to be effective in inhibition of accumulation of other mRNAs and proteins in response to E2 administration (30, 37) and to block growth-related uterine morphological changes (36).

Regulated expression of the syndecan-3 gene has been best characterized during development of the central nervous system in which dramatic temporal changes in expression correlate with intervals of cell differentiation (13). Similarly, temporal changes in expression have been observed during chondrocyte growth intervals in avian limb formation (38). The molecular factors that regulate proteoglycan gene expression in these systems have not been elucidated. Although we have shown in the present studies that syndecan-3 gene transcription is hormone regulated in the uterus, the specific mechanism by which E2 elicits this effect is unclear. Complete DNA sequence analysis of the 5′-flanking region of the rat syndecan-3 gene has not been completed. Therefore, information on the presence of an estrogen response element is lacking. Without evidence for specific target sites for binding of activated steroid hormone receptors, the exact mechanism of E2 regulation is unknown. It is possible that E2 induces a change in activity of existing transcription factors, whereby increasing mRNA transcription, or that the hormone significantly affects syndecan-3 mRNA stability, thus elevating mRNA levels through an increase in transcript half-life. Such forms of control have been described for regulated expression of the vitellogenin gene in Xenopus hepatocytes after E2 exposure (39, 40). Effects of actinomycin D on E2 induction of uterine syndecan-3 mRNA indicate that a change in transcript stability alone does not account for the observed mRNA increase and that mRNA changes in the uterus are, at least in part, transcription dependent.

The present studies show that the syndecan-3 response is hormone specific. Only steroid ligands that bind the estrogen receptor, including estriol and tamoxifen, induce increased transcript levels in the uterus. Despite evidence of receptors for nonestrogenic steroids in the uterus (28, 41, 42), their lack of effect indicates a transcription response specific to activation of the estrogen receptor. The glucocorticoid dexamethasone induced a decrease in mRNA content below that measured in saline control tissues. Dexamethasone has been shown to down-regulate specific mRNAs in other systems, such as the PTHrP gene transcript in a human C-cell line (43). Dexamethasone treatment of SJ115 mammary carcinoma cells induces a dramatic change in cell morphology that is accompanied by a loss of syndecan-1 protein (44). This effect suggests repression of syndecan-1 gene expression by the glucocorticoid and is consistent with the observed syndecan-3 mRNA down-regulation observed here in the uterus.

In addition to the steroid-specific nature of the uterine syndecan-3 response, there is also tissue specificity. Analysis of other estrogen-responsive rat tissues clearly indicated the presence of the major 5.9-kb transcript in heart, liver, lung, and spleen; however, mRNA levels remained constant 2–48 h after E2 treatment. The lack of a hormone response in these tissues is not known. This could reflect specific regulatory mechanisms
or differential tissue levels of the estrogen receptor. Although a complete explanation for differential tissue responsiveness is lacking, it is clear that the response in the uterus is significant in magnitude and occurs with a physiological dose of the hormone equivalent to in vivo circulating levels in cycling adult animals.

Uterine syndecan-3 protein content also increased transiently during the growth response, with a peak elevation occurring at ~8–20 h after hormone administration. This temporal change in protein correlates well with the time course of change in mRNA and further indicates that regulation of expression is mainly at the level of transcription. Immunohistochemical staining to visualize syndecan-3 protein in intact uterine sections revealed a high level of expression in endometrial epithelial cells that line the uterine lumen, with additional expression detected in glandular epithelial cells of the endometrial stroma, as well as smooth muscle cells of the myometrium. Hormone-induced changes in staining intensity in these cells indicate up-regulation of the protein during the tissue growth response. However, the most dramatic changes in temporal and spatial expression occurred in luminal epithelial cells, where the protein changed from a more uniform distribution on apical and basolateral cell surfaces in saline-treated tissues to a nearly complete basal localization 20 h after E2g administration. The increase in syndecan-3 levels corresponded to initiation of altered spatial localization in epithelial cells. Similar changes in the histochemical distribution of syndecan-1 have been described in the mouse uterus during the normal fertility cycle and early pregnancy (20). Syndecan-1 became localized to the basal border of endometrial epithelial cells during estrous cycle intervals when circulating levels of ovarian hormones are highest. These observations were made in animals with an intact pituitary-ovary axis; therefore, changes in syndecan-1 expression could not be attributed solely to estrogen. However, estrogen has been specifically shown to respond to initiation of altered spatial localization in epithelial cells during estrous cycle intervals when circulating levels of ovarian hormones are highest. These observations were made in animals with an intact pituitary-ovary axis; therefore, changes in syndecan-1 expression could not be attributed solely to estrogen. However, estrogen has been specifically shown to respond to initiation of altered spatial localization in epithelial cells during estrous cycle intervals when circulating levels of ovarian hormones are highest. These observations were made in animals with an intact pituitary-ovary axis; therefore, changes in syndecan-1 expression could not be attributed solely to estrogen. However, estrogen has been specifically shown to respond to initiation of altered spatial localization in epithelial cells during estrous cycle intervals when circulating levels of ovarian hormones are highest.

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