

# Stromal–epithelial interaction mediates steroidal regulation of metalloproteinase expression in human endometrium

(cell–cell interactions/matrilysin/stromelysin/progesterone/estrogen)

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**ABSTRACT** The hallmark of the menstrual cycle is extensive steroid-dependent tissue turnover. Estrogen mediates endometrial cell growth and structural remodeling, whereas progesterone suppresses estrogen-dependent proliferation and promotes cellular differentiation. In nonfertile cycles, tissue degradation and menstruation occur as a consequence of steroidal deprivation as the ovarian corpus luteum fails. Stromal–epithelial interactions are recognized as a necessary component in mediating steroid-induced endometrial turnover. Specific mRNAs for metalloproteinases of the stromelysin family are expressed during endometrial growth and menstrual breakdown but are absent in the progesterin-dominated secretory phase. This expression pattern suggests involvement of stromelysins in remodeling the extracellular matrix of the endometrium during tissue growth and breakdown and implicates progesterone in the suppression of these enzymes. We examined the regulation of endometrial stromelysins in explant cultures and found no acute effect of estradiol on their expression, whereas progesterone was a potent inhibitor of stromelysin expression. Progesterone also suppressed stromelysin expression in cultures of isolated stromal cells, but epithelial cells were progesterone insensitive. Coculture of recombined stromal and epithelial cells restored steroidal suppression of the epithelial-specific metalloproteinase. Our data confirm that progesterone inhibits endometrial stromelysins and further demonstrate the necessity for a stromal-derived factor(s) as a mediator of steroid suppression of an epithelial metalloproteinase.

The human endometrium undergoes extensive estradiol-induced growth and remodeling during the proliferative phase of the menstrual cycle, followed by secretory maturation in response to postovulatory progesterone (1). This rapid and extensive degree of steroid-mediated tissue development, which rivals that of many neoplasias, appears to be a necessary component of providing an environment suitable for sustaining hemochorial placentation (2). In the absence of implantation and the continued progestational environment of pregnancy, the superficial functionalis region of the endometrium undergoes degradation and is expelled with menstrual blood flow.

Several laboratories have recently described the expression of matrix metalloproteinases (MMPs) in the normal, cycling human endometrium (3–6). These enzymes degrade many components of the extracellular matrix, including proteoglycans, glycoproteins, and basement membrane collagens (7). Our studies (5, 6) identified a cell type-specific expression pattern of mRNAs coding for members of the stromelysin family only during the proliferative and premenstrual/menstrual stage of the cycle; none of the enzymes

were identified during the progesterone-dominated secretory menstrual interval. This pattern of expression suggests an active role for stromelysins during growth-associated structural remodeling as well as during the extensive tissue breakdown associated with menstruation.

MMPs of the stromelysin family are also known to be involved in other reproductive-tract functions, including ovulation, implantation, parturition, and postpartum involution of the uterus (8–14). The stromelysins also play a role in nonreproductive-tissue processes, such as organ morphogenesis during embryonic development (15–17), and in the pathological process of tumor invasion and metastasis (18–22).

The absence of endometrial stromelysins during the secretory interval strongly suggests that progesterone suppresses their expression. Progesterone has been reported to regulate the activity of collagenase and gelatinases in endometrial explants (4). However, it is not known whether steroids can affect the expression of endometrial stromelysins. Neither is it understood whether the suppression of MMPs during the menstrual cycle reflects a direct response to steroidal signals or the participation of local autocrine or paracrine mechanisms. A principal role for cell–cell interactions has long been recognized in mediating aspects of steroid action during growth and differentiation in numerous adult tissues, including the endometrium (23, 24). The cellular language by which cell–cell interactions can occur is complex, involving growth factors (25–27), cytokines (28), extracellular matrix components (29), and direct cell contact (30).

In this report, we describe *in vitro* studies which explore the role(s) of estradiol and progesterone in the regulation of stromelysins in the human endometrium. Explants of intact endometrium, as well as isolated and recombined cell types, were cultured in the presence or absence of these steroids. While estradiol had no acute effect on endometrial stromelysin expression, progesterone suppressed the secretion of both an epithelial- and a stromal-specific stromelysin in explants of endometrium. Isolated endometrial stromal cells were also progesterone sensitive in culture, but isolated epithelial cells continued to secrete an epithelial-specific MMP in the presence of progesterone. Coculture of recombined epithelial and stromal cells separated by an adjacent permeable membrane or in a physically separated culture environment restored progesterone suppression of the epithelial-specific stromelysin.

## MATERIALS AND METHODS

**Explant Cultures of Intact Endometrium.** Proliferative and secretory endometrium was obtained from diagnostic endo-

Abbreviation: MMP, matrix metalloproteinase.

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metrial biopsies or hysterectomies performed for benign conditions. The use of all specimens was approved by Vanderbilt University's Institutional Review Board through its Committee for the Protection of Human Subjects. The tissue was dissected into uniform,  $1 \times 2$ -mm<sup>2</sup> explants, 8–10 of which were suspended within tissue culture inserts near the surface of the air/culture medium interface (Millipore). Explant cultures were maintained in phenol red-free Dulbecco's modified Eagle's medium/Ham's F-12 (DMEM/F12) (Sigma) supplemented with 1% ITS<sup>+</sup> and 0.1% Excyte (Collaborative Biomedical Products, Bedford, MA), and incubated at 37°C in a humidified chamber with an atmosphere of 95% air/5% CO<sub>2</sub>. Treatments included control (no hormones), estradiol (10 nM), and progesterone (500 nM). Explant cultures were treated for a total of 30 hr, after which secreted proteins were metabolically labeled for 18 hr with [<sup>35</sup>S]methionine.

**Endometrial Cell Purification and Culture.** Isolation of endometrial epithelial and stromal cells was accomplished by sequential enzymatic dissociation and differential sedimentation (31). In a modification of our previous method, epithelial cell clusters were further dissociated into single cells with 0.05% trypsin/EDTA in phenol red-free Hanks' balanced salt solution. Cell purity was assessed by immunocalization of cytokeratins and vimentin; <5% contamination by epithelial or stromal cells was routine within either purified cell population. Epithelial cells were cultured on biomatrix-coated nitrocellulose tissue culture inserts with a 3.0- $\mu$ m pore size (Biocoat; Becton Dickinson Labware). Stromal cells were cultured on a dry coating of type I rat tail collagen (Becton Dickinson Labware) either on the bottom surface of tissue culture inserts or directly on the bottom of tissue culture wells. Epithelial or stromal cells ( $3 \times 10^5$  per well) were allowed to attach to the culture matrix for 24–48 hr in DMEM/F12 with 5% charcoal-stripped, heat-inactivated calf serum before initiation of serum-free conditions. In coculture experiments, stromal cells were allowed to attach for 3–4 hr to the bottom of collagen-coated inverted tissue culture inserts or to a collagen coating on the bottom of the culture well before epithelial cells were transferred to the wells in the interior of culture inserts. After cell attachment, each culture was maintained in serum-free DMEM/F12 supplemented with 1% ITS<sup>+</sup> and 0.1% Excyte. As with explant cultures, treatments of isolated cells included control (no hormones), estradiol (10 nM), and progesterone (500 nM).

For standardization, initiation of serum-free conditions was considered day 0 of culture. Media were changed at 2- to 3-day intervals except during the last 18 hr, at which time proteins were labeled with [<sup>35</sup>S]methionine.

**Analysis of Protein Expression *in Vitro*.** Secreted proteins from explant and monolayer cultures of isolated cells were labeled for 18 hr at 37°C in methionine-free medium supplemented with [<sup>35</sup>S]methionine (100  $\mu$ Ci, 3700 kBq). Before each labeling period, cultures were preincubated for 4 hr in methionine-free medium without label. Labeled proteins were collected by trichloroacetic acid (10%, wt/vol) precipitation and uniform counts (10<sup>5</sup> cpm) from each treatment group were selectively immunoprecipitated with rabbit polyclonal antibodies raised against human matrilysin (directed toward a glutathione S-transferase–matrilysin fusion protein containing the last 100 amino acids of matrilysin; refs. 20 and 32), or rat stromelysin 1 (directed toward a synthetic peptide corresponding to 23 amino acids at the carboxyl terminus of rat stromelysin 1; refs. 21 and 33). Each antibody was coupled to protein A-Sepharose and analyzed by SDS/PAGE and autoradiography.

**Northern Analysis.** To accumulate adequate numbers of cells for Northern hybridization studies, stromal cells were isolated as described above and passaged two or three times in medium containing 3% charcoal-stripped calf serum and 10 nM estradiol. Subsequently, cells were cultured under serum-free conditions for 6 days without further steroid treatment (control) or with medium containing 10 nM estradiol or 500 nM progesterone. Cultures were maintained at 37°C in 95% air/5% CO<sub>2</sub> with medium changes every other day. For Northern blot analysis, poly(A)<sup>+</sup> RNA was isolated, separated electrophoretically according to size, and transferred to a nitrocellulose filter (34). RNA (4  $\mu$ g) was hybridized with random-primed <sup>32</sup>P-labeled probes corresponding to sequences specific for human stromelysin 1 (nucleotides 1206–1758, from pStrome-1; ref. 20) and human stromelysin 3 (nucleotides 928–2105, from pZIV; ref. 33). The same blot was stripped and hybridized to a probe for cyclophilin, whose mRNA is constitutively expressed (35).

## RESULTS

The *in vivo* expression pattern of endometrial stromelysins from our previous work (5, 6) is graphically summarized in Fig. 1 relative to changing blood levels of ovarian steroids

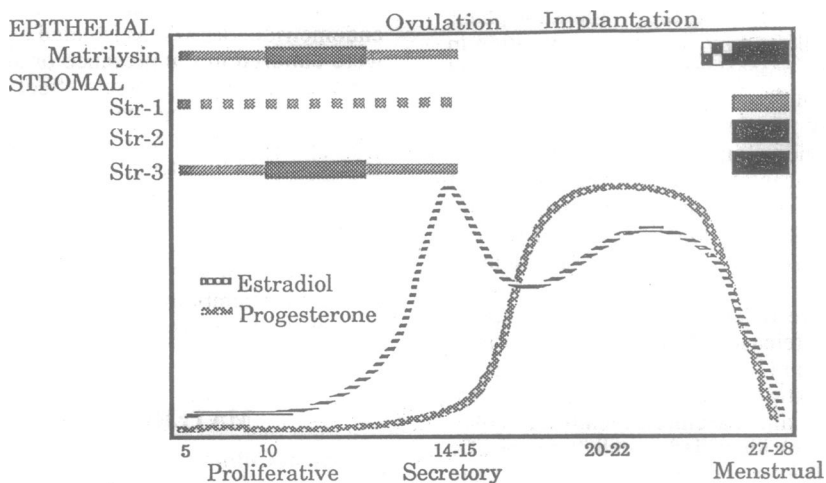
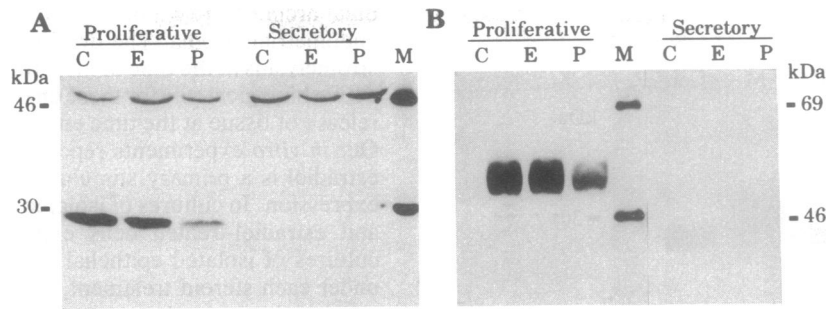


FIG. 1. Summary of endometrial expression of specific RNA transcripts for metalloproteinases of the stromelysin (Str) family during the human menstrual cycle. The expression patterns reflect data from published *in situ* hybridization studies (5, 6) and are summarized here relative to circulating levels of ovarian estradiol and progesterone. The relative intensities of detected mRNAs for epithelial-specific matrilysin, and stromal-specific stromelysins 1, 2, and 3 are denoted by the thickness of horizontal bars. The bar on the upper right indicates variability of expression: checked, moderate; solid, intense. Dotted lines represent low levels of mRNA expression or mRNA not detectable in all samples examined at a particular stage of the menstrual cycle.



**FIG. 2.** Detection and steroidal regulation of promatrilysin (A) and prostromelysin (B) in explants of human endometrium. Promatrilysin (30 kDa) and prostromelysin (60 kDa) from representative experiments with proliferative and secretory endometrial tissues are shown. Similar results were obtained from independent experiments (proliferative tissue,  $n = 6$ ; secretory tissue,  $n = 5$ ). Lane M,  $^{14}\text{C}$ -labeled markers; lanes C, control; lanes E, estradiol-treated; lanes P, progesterone-treated.

during an idealized menstrual cycle. The mRNA for matrilysin (EC 3.4.24.23, also known as pump-1 and MMP-7) localized to luminal and glandular epithelium during the proliferative and premenstrual/menstrual stage of the cycle. The mRNA for matrilysin is absent during the progesterone-dominated secretory phase. The mRNAs for stromelysin 1 (EC 3.4.24.17, MMP-3) and stromelysin 3 (MMP-11) display a similar temporal pattern of expression but localize exclusively to the stromal component of the endometrium. Specific hybridization with antisense RNA probes for stromelysin 2 (EC 3.4.24.22, MMP-10) was also observed exclusively in the stroma, but only during the premenstrual and menstrual interval.

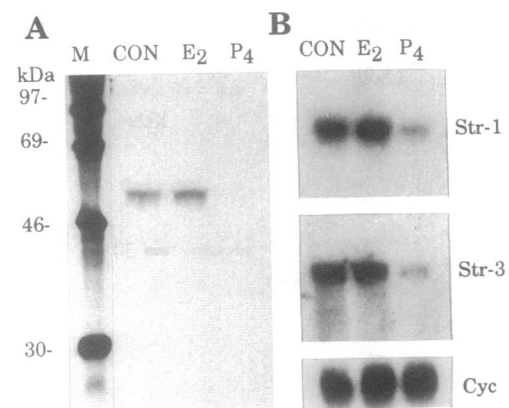
The patterns of endometrial stromelysin expression shown in Fig. 1 strongly suggested steroidal regulation of these enzymes. To test this possibility, endometrial tissue was obtained during the proliferative and secretory stage of the menstrual cycle and cultured as explants in the presence or absence of steroid hormones. Radiolabeled stromelysins secreted by the explants were analyzed after immunoprecipitation with specific antibody. A distinct 30-kDa band representing promatrilysin protein (20, 32) was detected in explants of proliferative-phase endometrium cultured under control conditions or in the presence of estradiol (Fig. 2A). Treatment of explants from the proliferative phase with progesterone markedly inhibited promatrilysin secretion. Explant cultures of tissue acquired during the secretory phase produced negligible promatrilysin *in vitro*, which is consistent with the lack of matrilysin mRNA expression observed during this phase of the cycle (5). The expression of a stromal-specific MMP, stromelysin, was similar to that of the epithelial-specific matrilysin in explant cultures of endometrium, again reflecting the *in vivo* expression pattern for stromal-specific MMPs (6). Immunoprecipitation with the anti-stromelysin antibody revealed a 59- to 60-kDa protein corresponding to prostromelysin (21, 33) in control or estradiol-treated explants of proliferative-phase, but not secretory-phase, endometrium (Fig. 2B). Progesterone treatment markedly reduced the level of prostromelysin produced in endometrial explants established from tissue obtained during the proliferative phase. Over the time course of these endometrial explant cultures (48 hr), no clear changes in the amount of either prostromelysin or promatrilysin were observed following treatment with exogenous estradiol, relative to untreated tissues.

To determine whether the suppression of prostromelysin secretion by endometrial explants *in vitro* reflected a direct progesterone effect on each principal cell type, we examined the regulation of these enzymes in cultures of isolated stromal and epithelial cells. Stromal cells were isolated from proliferative-phase biopsy specimens, cultured in plates for 6 days, and analyzed for steroid-sensitive prostromelysin secretion. A suppression of the 60-kDa prostromelysin protein was

observed following progesterone treatment, relative to control or estradiol-treated stromal cells (Fig. 3A). Progesterone treatment also suppressed the levels of mRNA for both stromelysin 1 and stromelysin 3, whereas estradiol treatment did not significantly affect the message level for either stromelysin examined (Fig. 3B).

The regulation of matrilysin, which is specifically expressed only in the glandular epithelium of the endometrium, was similarly analyzed. Epithelial cells were isolated from proliferative-phase biopsy specimens and established as primary cultures on biomatrix-coated tissue culture inserts. Following attachment to this biomatrix in serum-containing medium, we have previously demonstrated endometrial epithelial cells to maintain an *in vivo*-like morphology over 6–9 days under serum-free conditions (36). Cultures of isolated epithelial cells secreted the 30-kDa promatrilysin for 5–9 days (Fig. 4). As opposed to the results obtained with explant cultures of intact endometrium and monolayer cultures of isolated stromal cells, the secretion of promatrilysin by isolated epithelium *in vitro* was not steroid sensitive (Fig. 4). Neither progesterone nor estradiol affected promatrilysin protein secretion by cultures of isolated endometrial epithelium as compared with control cultures.

Since progesterone readily suppressed prostromelysin secretion by stromal cells but failed to suppress matrilysin secretion by isolated epithelial cells *in vitro*, we examined the possibility that stromal cells may secrete a paracrine factor, in response to progesterone, that affects epithelial promatrilysin secretion. Cocultures of isolated and recombined stro-



**FIG. 3.** Detection and steroidal regulation of radiolabeled prostromelysin (A) and mRNA for stromelysins 1 and 3 (B) in cultures of isolated human endometrial stromal cells. Cells were cultured under serum-free conditions with no additions (control, CON), 10 nM estradiol ( $\text{E}_2$ ), or 500 nM progesterone ( $\text{P}_4$ ). Lane M,  $^{14}\text{C}$ -labeled markers. In B, the same blot was stripped and hybridized to a probe for cyclophilin (Cyc) mRNA, which is constitutively expressed.

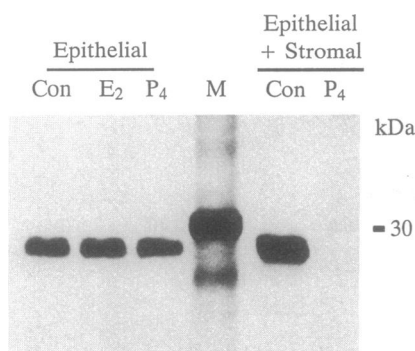


FIG. 4. Detection and steroidal regulation of radiolabeled promatrilysin in isolated human endometrial epithelial cells and isolated epithelial cells recombined with stromal cells. Cultures were maintained for 5–9 days under serum-free conditions with no additives (Con) or treated with 10 nM estradiol ( $E_2$ ) or 500 nM progesterone ( $P_4$ ). Lane M,  $^{14}C$ -labeled markers. Results of a representative experiment from a total of five independent experiments are shown.

mal and epithelial cells were conducted with Biocoat tissue culture inserts. In initial experiments, stromal cells and epithelial cells were maintained in culture attached to either side of biomatrix/collagen-coated permeable membranes. Under these conditions, a limited degree of direct cell–cell contact could occur through the membrane pores. Progesterone suppressed secretion of radiolabeled promatrilysin in a representative coculture experiment (Fig. 4). Similar coculture experiments were subsequently conducted with stromal cells attached to the bottom of culture wells (where no direct cell–cell contact across the permeable membrane would be possible). Suppression of promatrilysin was also observed in these cultures (Fig. 5), indicating that a soluble factor(s) is the active stromal signal that can suppress promatrilysin expression by isolated epithelial cells.

## DISCUSSION

The endocrine-directed cyclic growth, differentiation, and breakdown of the human endometrium represents perhaps the most dynamic example of steroid-driven tissue turnover in the adult. Recent reports from our laboratory and others (3–6) have indicated that MMPs, which degrade and remodel the extracellular matrix, may play a significant role(s) in this complex reproductive tissue. The expression of MMPs of the stromelysin family during the proliferative phase of the cycle (5, 6) may be required for the development of normal glan-

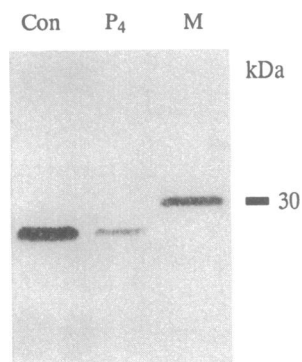


FIG. 5. Steroidal regulation of radiolabeled promatrilysin in isolated human endometrial epithelial cells cultured with isolated stromal cells with no physical contact between cell types. Cultures were maintained for 5–7 days under serum-free conditions with no additives (Con) or treated with 500 nM progesterone ( $P_4$ ). Lane M,  $^{14}C$ -labeled markers. Results of a representative experiment from a total of seven independent experiments are shown.

dular architecture within the dense extracellular matrix of the endometrial stroma. The dramatic reexpression of multiple stromelysins at the time of menstruation (5, 6) also implies an active biological role for these enzymes in the breakdown and release of tissue at the time endocrine support is withdrawn. Our *in vitro* experiments reported here do not indicate that estradiol is a primary stimulus for endometrial stromelysin expression. In cultures of isolated stromal cells, both control and estradiol-treated cells expressed prostromelysin and cultures of isolated epithelial cells expressed promatrilysin under each steroid treatment. Possibly, a prior exposure to estrogen *in vivo* or the mechanical disruption associated with cell purification was responsible for activating the genes necessary for the expression of these enzymes *in vitro*. However, the suppression of both stromal-specific and epithelial-specific MMP secretion *in vitro* by progesterone suggests that the rising levels of this hormone during the secretory menstrual phase is responsible for the loss of stromelysin mRNA and protein expression we have observed *in vivo* (5, 6). The repression of stromelysins during the progesterone-dominated secretory phase may be necessary to stabilize the endometrium in preparation for normal implantation and placentation. Progesterone suppression of interstitial collagenase, gelatinase A, and gelatinase B has also been reported in explant cultures of human endometrium (4). It is noteworthy that inadequate postovulatory progesterone support to the endometrium is associated with infertility and recurrent spontaneous abortion (37).

Stromal–epithelial interactions are complex and can involve direct cell–cell contact, extracellular matrix interactions, and soluble paracrine factors (25–30). While a principal role for the stroma has been often proposed in mediating steroid action during growth and differentiation in numerous adult tissues (23), including the endometrium (24), our study demonstrates that stromal cells can mediate progesterone suppression of an epithelial-specific protein. Additionally, our results indicate that a soluble factor(s) produced by the stroma in response to progesterone may be the principal signal that suppresses epithelial cell production of promatrilysin. The nature of this soluble factor is, at present, only speculative; however, numerous cytokines and growth factors that are known to regulate the expression of MMPs are prevalent in the human endometrium (28, 38).

It is unlikely that the requirement for the participation of the stroma in mediating epithelial MMP expression is a relationship unique to the normal cycling endometrium. MMPs are involved in extracellular matrix turnover during many reproductive processes (8–14), as well as during normal nonreproductive functions (15–17). These potent enzymes also play an important role in the pathological process of tumor invasion and metastasis (18–22). Clearly, the ability and apparent requirement of stromal participation in the regulation of epithelial matrilysin expression during endometrial remodeling presents the possibility for a role for stromal–epithelial interactions in regulating MMPs in other important biological processes.

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