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Expression Pattern of Metalloproteinases and Tissue Inhibitors of Matrix-Metalloproteinases in Cycling Human Endometrium[1]

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ABSTRACT

The cyclic growth, differentiation, and cell death of endometrium represents the most dynamic example of steroid-driven tissue turnover in human adults. Key effectors in these processes—matrix metalloproteinases (MMPs) and their specific inhibitors (TIMPs)—are regulated by ovarian steroids and, locally, by cytokines. We used reverse transcription-polymerase chain reaction to evaluate the expression of both transcriptionally regulated molecules such as estrogen receptor-α, progesterone receptor, and prolactin and a large array of MMPs and TIMPs (MMP-1, -2, -3, -7, -8, -9, -11, -12, -19, -26, MT1-MMP, MT2-MMP, MT3-MMP, TIMP-1, -2, -3). Altogether, three distinct patterns of MMP and two patterns of TIMP expression were detected in cycling endometrium: 1) MMPs restricted to the menstrual period (MMPs-1, -3, -8, -9, -12); 2) MMPs and TIMPs expressed throughout the cycle (MMP-2, MT1-MMP, MT2-MMP, MMP-19, TIMP-1, and TIMP-2); 3) MMPs predominantly expressed during the proliferative phase (MMP-7, MMP-11, MMP-26, and MT3-MMP); and 4) TIMP-3, which, contrary to the other TIMPs, shows significant modulations, with maximum expression during the late secretory and menstrual phases. These specific patterns of MMP expression associated with each phase of the cycle may point to specific roles in the processes of menstruation, housekeeping activities, angiogenesis, tissue growth, and extracellular matrix remodeling.

female reproductive tract; gene regulation; menstrual cycle

INTRODUCTION

The endometrium is a unique dynamic tissue undergoing extensive cyclic changes in response to fluctuations in steroid hormone concentrations. In the course of the normal menstrual cycle, it displays a regular sequence of growth, differentiation, and programmed breakdown [1]. The human genome sequence has revealed more than 500 genes that encode proteases or protease-like proteins [2]. Among these the matrix-metalloproteinases (MMPs), a group of 24 enzymes that degrade the extracellular matrix (ECM) and basement membrane and process bioactive mediators, have been the focus of much anticancer research and clinical trials.

In the endometrium, MMPs are involved in matrix remodeling associated with perimenstrual phases [3, 4]. They are synthesized as inactive zymogens, are either secreted or expressed as transmembrane proteins, are inhibited by tissue inhibitors of MMPs (TIMPs), and can hydrolyze ECM and non-ECM proteins (such as adhesion molecules, growth factors, and cytokines). They can be divided into several distinct subgroups based on substrate specificity or structural similarities: collagenases (MMP-1, -8, -13); gelatinases (MMP-2, -9); stromelysins (MMP-3, -7, -10, -11); and membrane-type MMPs (MT-MMP1 to 6). An additional miscellaneous group of MMPs includes MMP-12, MMP-18, and MMP-19 to -26 [5, 6].

Beyond their clinical connective tissue-degrading functions, MMPs regulate the activities of bioactive molecules by proteolytic processing [7, 8]. For example, MMPs mediate cell surface-receptor cleavage and release, cytokine and chemokine activation and inactivation, and the release of apoptotic ligands. They also inactivate growth factor-binding proteins and release mitogenic molecules from matrix proteins. These cellular processes are all involved in promoting aspects of endometrial cell proliferation, adhesion, mesenchymal to epithelial transition during deciduas formation, differentiation, angiogenesis, apoptosis, menstrual breakdown, and host defense. MMP inhibitors are therefore potentially useful for blocking endometrial remodeling and bleeding [9, 10].

In most adult tissues, the expression of MMPs is very low [11], but it can be rapidly and drastically induced during active tissue remodeling. Their proteolytic activities are regulated by gene transcription, zymogen activation, and specific inhibition by physiologic tissue inhibitors (TIMPs) [12–16]. The expression profile of ''classical'' MMPs (MMP-1, -2, -3, -7, -9, -11, and membrane-type [MT]1-MMP), which were the first described and the most extensively studied to date, and TIMPs has been determined in endometrium by in situ hybridization and Northern blot, and the proteins have been localized using immunohistochemistry [17–21]. They are expressed at specific phases during the menstrual cycle. Most MMPs show a maximal expression during the perimenstrual phase and are undetectable during the proliferative and secretory phases, but few of them are expressed throughout the cycle [22].

Recently new members of the MMP family have been

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described, such as MMP-19, MMP-26 (endometase or matrilysin-2), and MT-MMPs (1 to 6) [23–28]. MMP-26 has marked structural similarities with MMP-7 but distinct substrate specificity [29]. These MMPs are expressed in epithelia and epithelial tumor cells [26, 27, 30]. It has therefore been suggested that MMP-26 could play an important role in ECM remodeling associated with rapid epithelial growth and angiogenesis [27, 31].

MMP-19 expression in normal tissue is ubiquitous [23, 32] and could be involved in acute inflammatory angiogenesis [33]. Moreover, MMP-19 shows an unusual downregulation in invasive cancer.

MT-MMPs comprise a family of six transmembrane MMPs. They are able to degrade various ECM components, including native collagens [9]. The expanding recognized roles of MMPs in complex regulatory and remodeling processes has stimulated expression studies in various normal tissues and pathological states (for a review see [7, 34]).

Because MMPs and TIMPs form a complex network of interacting enzymes, sensitive and accurate determination of their expression profile is required to evaluate their regulation and specific roles. In this study, we used quantitative reverse transcription-polymerase chain reaction (RT-PCR) to precisely delineate the temporal expression patterns of six recently described MMPs and compare them with that of the interstitial collagenase (MMP-1), stromelysin 1 (MMP-3), and gelatinase B (MMP-9), which culminate during menstruation or with that of housekeeping gene permanently expressed such as gelatinase A (MMP-2).

MATERIALS AND METHODS

Tissues

Endometrial tissues were collected from healthy women with spontaneous, regular menstrual cycles (26–33 days) on a voluntary basis. Patients with endometriosis, infertility, or irregular menstrual cycles and those on hormonal treatment or oral contraception were excluded. Endometrial biopsy samples were obtained with a Pipelle curette or by curettage. Patients gave their informed consent, and the protocol was approved by the University Ethics Committee. Each sample was divided in two. One fragment was fixed in buffered formalin for histological dating (Noyes criteria) by a pathologist (V.F.) experienced in endometrial studies. The second was immediately snap frozen and stored in liquid nitrogen. Samples were excluded when the histological dating was inconsistent with the date of the patient's last menstrual period. Thirty-three patients aged 20–45 yr (mean 33 yr) were included. The samples were classified according to classical histologic criteria as early proliferative phase (EP, n = 6); late proliferative phase (LP, n = 6); early (ES, n = 6), mid- (MS, n = 5), and late (LS, n = 5) secretory phase; and menstrual phase (ME, n = 5). We also validated the histological tissue dating by comparison with the known profiles of gene products that are hormonally regulated during the cycle. Indeed, mRNA encoding the estrogen-α and progesterone receptors is downregulated [35], but prolactin mRNA is upregulated during the late secretory phase [36]. In our samples, the expression pattern of prolactin (PRL), progesterone receptor, and estrogen receptor-α, measured by RT-PCR was identical to that reported for the corresponding proteins, throughout the cycle [35, 37–39] (data not shown).

RNA Extraction

Endometrial tissue biopsies were immediately snap frozen and stored in liquid nitrogen until analysis. The frozen tissues were pulverized using a Dismembrator (B. Braun Biotech International, GmBH, Melsungen, Germany) and suspended in lysis buffer (5 M guanidine thiocyanate, 25 mM sodium citrate, 17 mM *N*-lauroylsarcosine and 0.1 M 2-mercaptoethanol [pH 7]). Total RNA was purified by cesium chloride ultracentrifugation according to the procedure of Chirgwin et al. [40].

RT-PCR Amplification

RT-PCR amplification was performed using the GeneAmp Thermostable rTth reverse transcriptase RNA PCR kit (Perkin-Elmer, Branchburg,

TABLE 1. Primers sued to perform quantitative RT-PCR.*

		Forward primer (5′-3′)	Reverse primer (5′-3′)	Expected size (bp)		No. of cycles
				sRNA	mRNA	
MMP-1	Interstitial Coll	5′-GAGCAAACACATCTGAGGTACAGGA-3′	5′-TTGTCCCGATGATCTCCCCTGACA-3′	267	185	30
MMP-2	Gelatinase A	5′-AGATCTTCTTCTTCAAGGACCGGTT-3′	5′-GGCTGGTCAGTGGCTTGGGGTA-3′	271	225	27
MMP-3	Stromelysin-1	5′-GATCTCTTCATTTTGGCCATCTCTTC-3′	5′-CTCCAGTATTTGTCCTCTACAAAGAA-3′	272	246	30
MMP-7	Matrilysin	5′-CCCCCTGCATTTCAGGAA-3′	5′-TCCTGGCCCATCAAATGG-3′	NA	100	30
MMP-8	Neutrophil coll	5′-CCAAGTGGGAACGCACTAACTTGA-3′	5′-TGGAGAATTGTCACCGTGATCTCTT-3′	267	200	30
MMP-9	Gelatinase B	5′-GCGGAGATTGGGAACCAGCTGTA-3′	5′-GACGCGCCTGTGTACACCCACA-3′	266	208	30
MMP-11	Stromelysin-3	5′-ATTTGGTTCTTCCAAGGTGCTCAGT-3′	5′-CCTCGGAAGAAGTAGATCTTGTTCT-3′	268	155	30
MMP-12	MetalloElastase	5′-ACATTTCGCCTCTCTGCTGATGAC-3′	5′-CAGAAACCTTCAGCCAGAAGAACC-3′	245	196	30
MMP-14	MT1-MMP	5′-GGATACCCAATGCCCATTGGCCA-3′	5′-CCATTGGGCATCCAGAAGAGAGC-3′	269	221	30
MMP-15	MT2-MMP	5′-CTTGCAGAGATGCAGCGCTTCTAC-3′	5′-CTGGATGCTAAAGGTCAGATGGTG-3′	269	202	30
MMP-16	MT3-MMP	5′-GGTTGGATTTCGTGCATCATTCGG-3′	5′-ATAGAACTGCTGCATGGCAGCTAG-3′	269	209	30
MMP-19	RAS1-1	5′-CCGCCAAAGCTCGTACTGTT-3′	5′-CGTCTTCGTCGAAGTGGCACA-3′	NA	101	30
MMP-26	Endometase	5′-CCCCTGCTGCAGACCATAAA-3′	5′-GGGCTGGTGTAGCAGAGCAT-3′	NA	179	30
TIMP-1		5′-CATCCTGTTGTTGCTGTGGCTGAT-3′	5′-GTCATCTTGATCTCATAACGCTGG-3′	271	170	30
TIMP-2		5′-CTCGCTGGACGTTGGAGGAAAGAA-3′	5′-AGCCCATCTGGTACCTGTGGTTCA-3′	269	155	29
TIMP-3		5′-CTCTGCAACTCCGACATCGTGAT-3′	5′-CAGCAGGTACTGGTACTTGTTGAC-3′	269	210	29
Prolactin		5′-GGTGACCCTTCGAGACCTGGTT-3′	5′-GGAAGAAGTGTGGCAGCTGTT-3′	NA	150	30
PR		5′-AGCCAGAGCCCACAATACAGCT-3′	5′-TTTCGACCTCCAAGGACCATG-3′	NA	260	30
ER-α		5′-GTTTCCCCCCACTCAACAGCGT-3′	5′-ACTTCCCTTGTCATTGGTACTGGC-3′	NA	234	30
28S		5′-GTTCACCCACTAATAGGGAACGTGA-3′	5′-GATTCTGACTTAGAGGCGTTCAGT-3′	269	212	18

*Coll, collagenase; MT-MMP, membrane-type metalloproteinase; PR, progesterone receptor; ER, estrogen receptor; NA, not available; sRNA synthetic RNA; RT-PCR, reverse transcription-polymerase chain reaction.

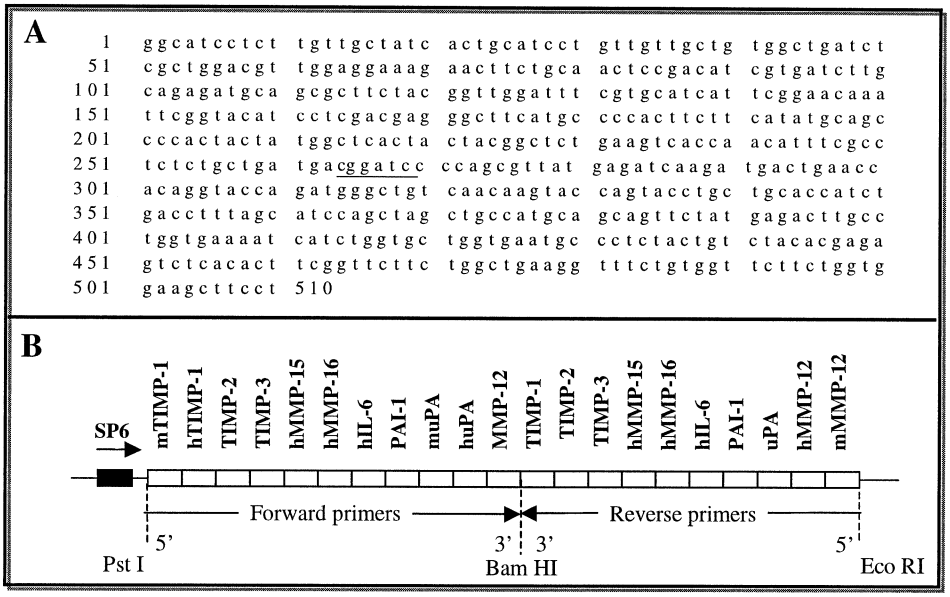


FIG. 1. Synthetic DNA standard used as a template for mssRNA. **A**) Sequence of the synthetic DNA standard. **B**) Succession of the selected forward and reverse primer pairs that allow reverse transcription-polymerase chain reaction amplification of mRNA coding for TIMP-1, -2, -3, MMP-15, -16 (open boxes). The black box corresponds to the SP6 polymerase initiation site in the pSPT18 plasmid.

NJ), specific pairs of primers (5 pmol each; Gibco BRL-Life Technologies, Carlsbad, CA) (Table 1), 10 ng of total RNA and, when available, a known copy number of an internal standard (mssRNA, see below) per 25 μl of reaction mixture (final volume). Reverse transcription was performed at 70°C for 15 min, followed by 2 min at 95°C to denature RNA-DNA heteroduplexes. For most transcripts, PCR amplification was run as follows: 15 sec at 94°C, 20 sec at 68°C, and 10 sec at 72°C (number of cycles as indicated in Table 1), followed by a final 2-min extension step at 72°C. For MMP-3 and -11, the cycling conditions consisted of 15 sec at 94°C, 30 sec at 63°C, and 30 sec at 72°C (for 30 cycles). RT-PCR products were resolved on 10% polyacrylamide gels and analyzed using a Fluor-S MultiImager (BioRad, Hercules, CA) after staining with Gelstar dye (FMC BioProducts, Rockland, ME).

Selection of Primers for RT-PCR

Pairs of primers (Table 1) for RT-PCR amplification were designed according to the following criteria: (1) a theoretical melting temperature of 72°C (4°C for G or C and 2°C for A or T); (2) minimum complementarity between the forward and reverse primer sequences; (3) primers were reverse and forward located on different exons to permit electrophoretic discrimination between genuine RT-PCR products and products amplified from contaminating DNA; (4) the length of the RT-PCR product ranged between 155 and 270 bp, a size both permitting easy discrimination between specific and artifactual amplification products and producing good yields. We also conducted BLASTn (National Center for Biotechnology Information, Bethesda, MD) searches against dbEST and the nonredundant set of GenBank, EMBL, and DDBJ database sequences to confirm the total gene specificity of the nucleotide sequences chosen for the primers. The specificity of the amplified PCR products was finally confirmed either by restriction digest or sequencing.

The efficiency of the selected primer pairs for each human mRNA was tested by RT-PCR using 10 ng of total RNA extracted from human placenta or human dermal fibroblasts. All the RT-PCR products had the expected size. The assay was also performed using 50 ng of human DNA. When detected, the reaction products were larger than the amplification products obtained from cellular RNA, owing to the presence of intronic sequences (not illustrated). No signal was detected in control samples free of nucleic acid.

Construction and Use of Multistandard Synthetic RNAs

Synthetic RNAs were created to monitor, in each sample, possible variations in the efficiency of RT-PCR amplification. These synthetic RNAs have two main characteristics. First, they can be amplified by primers used for RT-PCR amplification of the endogenous mRNA of interest. Second, the size of their amplification product is different from the size of the RT-PCR product amplified from the mRNA, permitting their discrimination after electrophoresis. Generation of the synthetic standard RNA for MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-11, MMP-14 mRNA, and 28S rRNA was done as previously described [41, 42]. MMP-12, MMP-15, MMP-16, TIMP-1, TIMP-2, and TIMP-3 mRNA were monitored with a second multistandard synthetic RNA (mssRNA). For the construction of this multistandard RNA, double-stranded synthetic DNA (Fig. 1a) was synthesized as follows: the first half (bases 1 to 264) and the second half (bases 271 to 510) were generated separately by annealing and ligating eleven (first half) and nine (second half) partially overlapping phosphorylated oligonucleotides. The relevant oligonucleotides (1 pmol each) were mixed and incubated at 80°C for 5 min before slow cooling to room temperature to permit sequential annealing. Ligation (T4 DNA ligase; Invitrogen Life Technologies, Merelbeke, Belgium) was performed at 24°C for 3 h. The ligation mixture was submitted to 25 cycles of PCR (94°C for 20 sec, 66°C for 20 sec, 72°C for 45 sec).

For the first half of the final construction, the following oligonucleotides flanked by *Pst* I and *Bam* HI restriction sites (underlined) were used for PCR amplification: 5′-AAAACTGCAGGGCATCCTCTTGTTGCTATCACTG-3′ and 5′-TATAGGATCCGTCATCAGCAGAGAGGCGAAATGT-3′. For the second half, the oligonucleotides 5′-TTAAGGATCCCCAGCGTTATGAGATCAAGATGAC-3′ and 5′-AAAAGAATTCAGGAAGCTTCCACCAGAAGAACCA-3′ were used, amplifying a PCR product flanked by *Bam* HI and *Eco* RI restriction sites (underlined). After cleavage with *Pst* I and *Bam* HI (first half) or *Bam* HI and *Eco* RI (second half), the PCR products were purified on agarose gel and sequentially cloned into pSPT18 transcription vector (Roche Diagnostics Belgium, Vilvoorde, Belgium) (Fig.1b). A sequence-verified construct was then linearized, purified, and used as template for RNA synthesis (SP6 polymerase, SP6/T7 transcription kit; Roche Diagnostics Belgium). The resulting mssRNA was then purified and quantified. RT-PCR amplification of this mssRNA with the different pairs of primers generated amplification products larger (245 to 271 bp) than those obtained from the cellular RNA (155 to 210 bp, see Table 1), enabling them to be discriminated by electrophoresis.

As previously described [41], some preliminary assays were performed to define the number of copies of the mssRNA to be added to each sample to monitor variations in efficiency of the RT. The noncompetitive conditions, as defined by Freeman et al. [43], allowed quantification of the various RNAs. We validated our RT-PCR quantification assay by performing RT-PCR reactions with a defined and constant number of copies of mssRNA and increasing amounts of cellular RNA (0.5 to 10 ng). The ratio between the signals for a specific mRNA and the mssRNA was shown to increase linearly with the amount of cellular RNA. In all assays, the actual concentration of the specific mRNA (calculated as the mssRNA copy number equivalent) was expressed per unit of 28S rRNA measured in the same RNA solution.

No mssRNA was available for RT-PCR quantification of MMP-7, MMP-19, MMP-26, PRL, progesterone receptor, and estrogen receptor α mRNA. Their level of expression was thus normalized to 28S mRNA quantified in the same sample.

Statistical Analysis

For each RNA sample, RT-PCR quantification was performed three times. Data expressed in the figures represent the means ± SEM for each

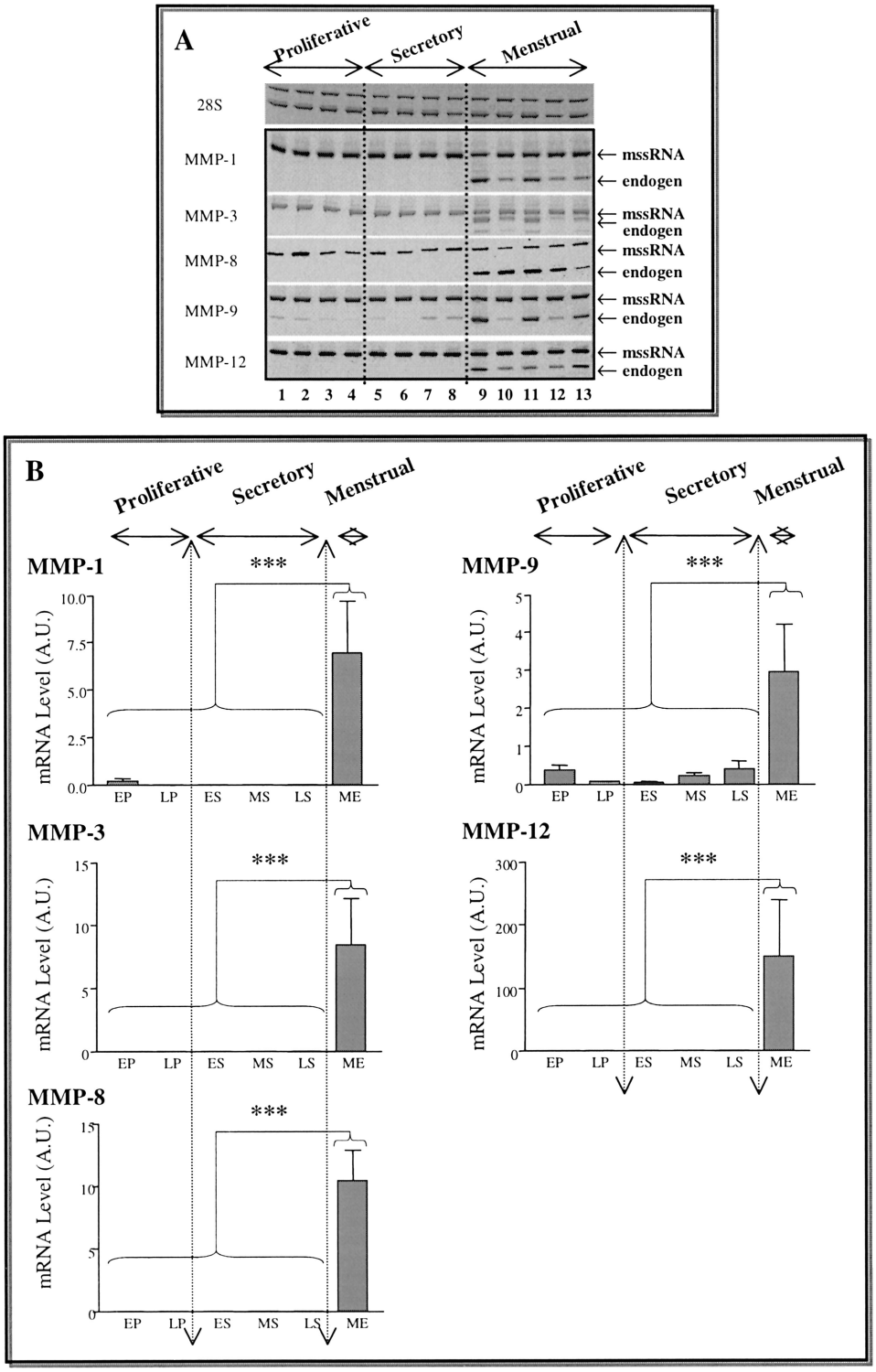


FIG. 2. Analysis of RT-PCR of MMPs that are upregulated during the menstrual phase (MMP-1, -3, -8, -9, -12) in endometrial tissue from regularly cycling women throughout the menstrual cycle. **A**) Representative gels of samples from the early proliferative (EP: lanes 1 and 2), late proliferative (LP: 3 and 4), early secretory (ES: 5 and 6), late secretory (LS: 7 and 8), and menstrual phases (ME: lanes 9 to 13). Endogenous target mRNAs and mssRNA are coamplified with the same primers, but their products differ in size. Arrows indicate RT-PCR products from the mssRNA and the endogenous mRNA. **B**) Densitometric quantification of the mRNA levels normalized with 28S rRNA. Results are means ± SEM. The actual concentration of the specific mRNA (calculated as the mssRNA copy number equivalent) was expressed per unit of 28S rRNA measured in the same RNA solution. The menstrual phase was compared with the values of all other phase (EP, LP, ES, MS, and LS) and found to be significantly different (***$P <$ 0.0001).

group ($n \geq 5$). Results for the different groups were compared using the Kruskal-Wallis test, and any significant differences were further analyzed by pairwise comparison using the Mann-Whitney test. P values $< 5\%$ were considered statistically significant.

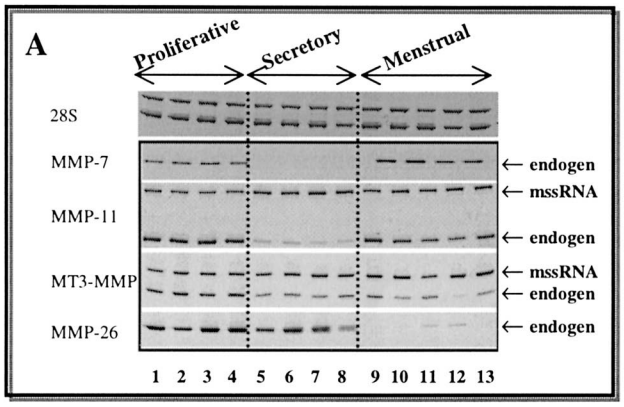
RESULTS

MMP Expression

We used RT-PCR to examine the expression of a large array of MMPs and TIMPs in the cycling endometrium. In addition to its high sensitivity, RT-PCR permits the detection of minute amounts of specific mRNA. Moreover, by using an internal RNA standard to monitor the entire RT-PCR amplification process, the method becomes semiquantitative [42, 44].

We examined the expression, at the mRNA level, of a large array of MMPs and TIMPs (listed in Table 1) during the endometrial cycle. An example of the expression profile of MMPs and TIMPs analyzed is shown in Figure 2A to 5A for representative samples of each phase of the cycle.

Three patterns of expression were observed. First, A ''menstrual'' pattern included MMPs almost exclusively ex-



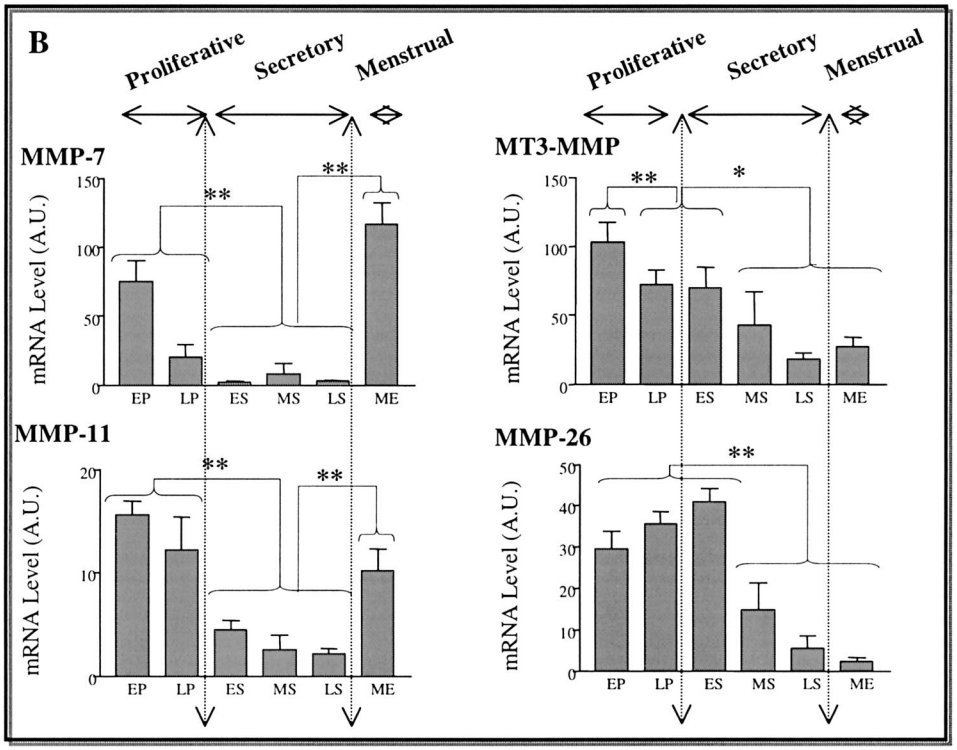


FIG. 3. Analysis of RT-PCR of late proliferative MMPs that are upregulated during the proliferative phase (MMP-7, -11, -MT3, -26). **A**) Representative gels as described in Figure 2A. **B**) Densitometric quantification of the RT-PCR products as described in Figure 2B. The early proliferative (EP) phase was compared with the early secretory (ES), midsecretory (MS), and late secretory (LS) phases for MMP-7 and -11 (**$P < 0.001$) and with late proliferative (LP), ES, MS, and LS phases for MT3-MMP (*$P < 0.05$) and MMP-26 (**$P < 0.001$).

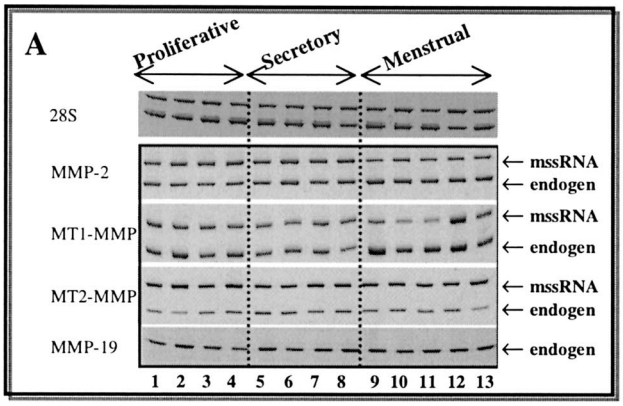
pressed during menstruation. This included mRNAs for MMP-1, MMP-3, MMP-8, MMP-9, and MMP-12, which were not or minimally detected during the proliferative and secretory phases. These MMPs showed markedly increased expression during the menstrual phase (more than 10-fold for MMP-9, $P < 0.0001$) (Fig. 2). Second, a ''proliferative'' pattern involved MMPs largely expressed during the proliferative phase. Transcripts of MMP-7, MMP-11, MT3-MMP, and MMP-26 were detected permanently but with significant variations throughout the cycle (Fig. 3). The levels of MT3-MMP and MMP-26 mRNAs were maximal during the proliferative phase and subsequently declined to reach basal values during the late secretory phase and menstruation. On the contrary, MMP-7 and MMP-11 exhibited a repression at midcycle (more than 10-fold for MMP-7, $P < 0.0001$ and 2- to 3-fold for MMP-11, $P < 0.001$). Third, a ''housekeeping gene'' (permanent) profile concerned MMP mRNAs expressed throughout the cycle, with less than 2-fold variation. A moderate increase in the mRNA levels of MMP-2, MT1-MMP, and MMP-19 was observed during the menstrual phase (Fig. 4). Only the 2-fold in MT1-MMP mRNA levels during the menstrual phase was found to be statistically significant (Fig. 4).

TIMP Expression

TIMP-1, -2, and -3 mRNAs were detected throughout the cycle (Fig. 5) but with distinct profiles: TIMP-1 mRNA levels increased during the secretory and menstrual phases in comparison with the proliferative phase, but these changes were not statistically significant. TIMP-2 levels were stable. By contrast, TIMP-3 mRNA levels increased significantly (2- to 3-fold) during the late secretory and menstrual phases when compared with the proliferative and early secretory phases ($P < 0.001$).

DISCUSSION

The cyclic proliferation, neoangiogenesis, secretory differentiation, predecidua formation, inflammatory cell infiltration, extensive apoptosis, and tissue breakdown of the endometrial functional layer are associated with a coordinated selective up- and downexpression of at least 13



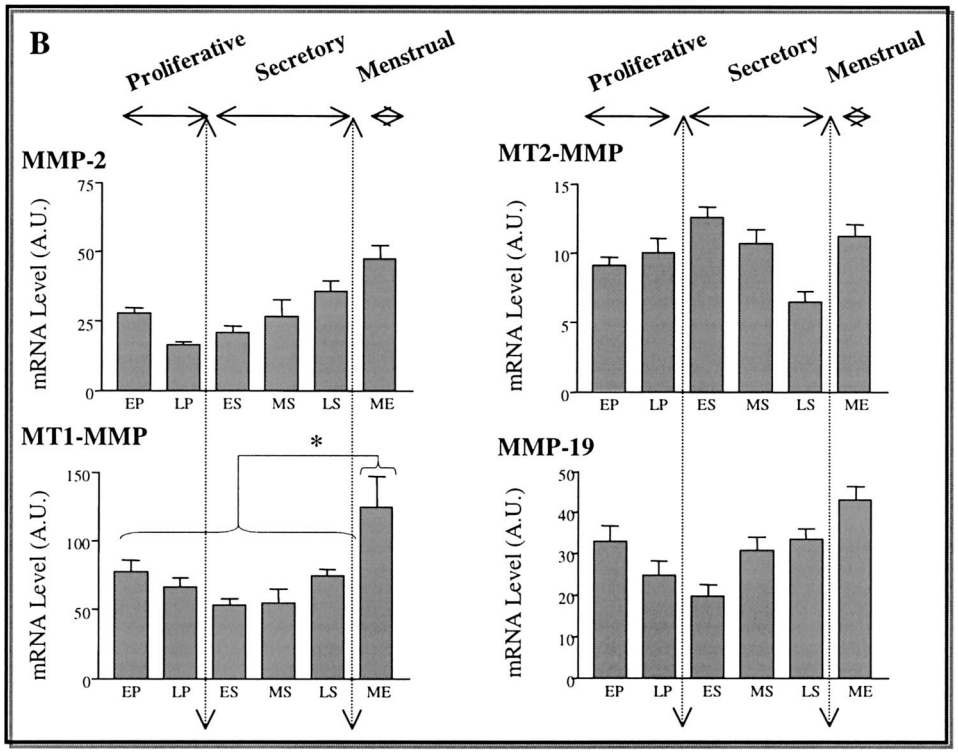


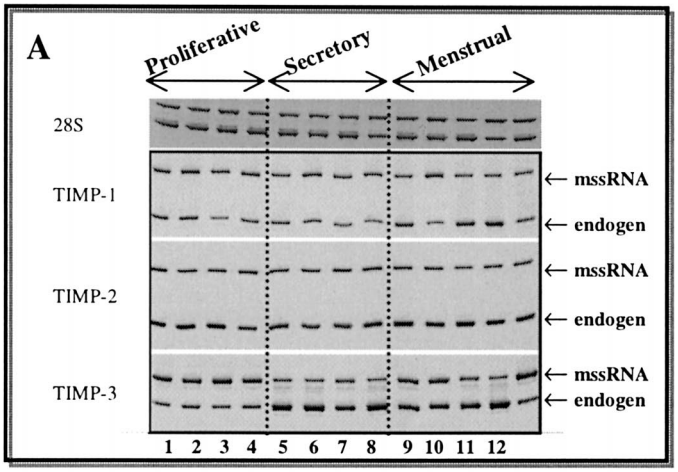
FIG. 4. Analysis of RT-PCR of MMPs that are expressed throughout the cycle, with minor variation (MMP-2, MT1-, MT2-MMP, and MMP-19). **A**) Representative gels as described in Figure 2A. **B**) Densitometric quantification of the RT-PCR products as described in Figure 2B. Results are means ± SEM. For MT1-MMP, a significant increase of mRNA level was observed in the menstrual phase, compared with the late proliferative (LP) and all secretory phases of the endometrial cycle ($P < 0.005$).

MMPs and their physiological inhibitors, TIMPs. Three patterns of MMPs and two of TIMPs expression were distinguished.

The first pattern is that of ''classical'' MMPs expressed only during the menstrual phase (MMP-1, MMP-3, MMP-8, MMP-9, and MMP-12). Our data concerning MMP-1, -3, and -9 confirm previous studies performed by Northern blot, in situ hybridization, or immunolocalization [3, 18, 19, 45]. This pattern of expression in normal endometrium highlights the pivotal role of MMPs in matrix degradation associated with menstruation [46]. Provided that corresponding changes in protein levels follow a similar pattern, a proteolytic imbalance promoting tissue breakdown would occur during menstruation because TIMP-1 and -2 expression remained essentially unchanged.

To our knowledge, this is the first report of the expression of MMP-8 and MMP-12 in the endometrium. The precise cellular origin of these MMPs remains to be elucidated by appropriate in situ hybridization and immunohistochemical studies. However, their expression during the menstrual phase coincides with infiltration of the functionalis layer by transient immune cells, and particularly by neutrophils, eosinophils, and macrophages, that are known to produce these enzymes [47]. In vitro studies have previously shown that MMPs-1, -3, and -9 are abundantly secreted by endometrial stromal cells on progesterone withdrawal [3, 48]. These cells might also secrete MMP-8 and -12 because inflammatory cytokines present in menstrual endometrium are known to induce their expression by stromal cells during the acute phase of inflammatory reactions in other settings [49].

The second pattern was that of MMPs expressed throughout the cycle (MMP-2, MMP-19, MT1-MMP, and MT2-MMP). Except for MT1-MMP, the mRNA levels of these factors remained unchanged throughout the cycle. A moderate increase was consistently observed during menstruation but was only statistically significant for MT1-MMP. In contrast to the menstrual MMPs (pattern 1), these MMP mRNAs were not downregulated during the secretory phase. These enzymes may thus exert housekeeping activities. Previous immunolocalization and in situ hybridization studies [18, 21] have shown that MT1-MMP and MMP-2



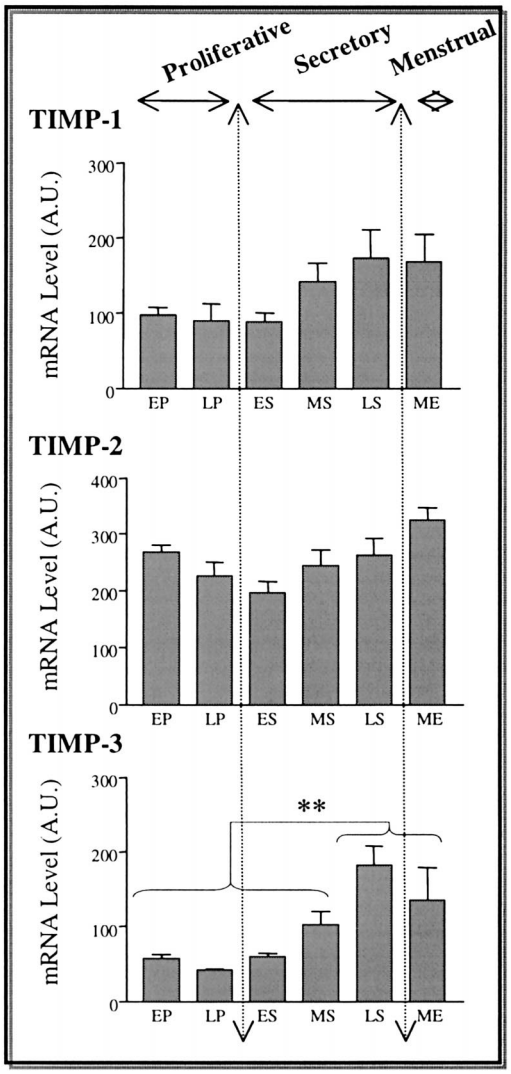


FIG. 5. Analysis of RT-PCR of TIMPs-1, -2, and -3. **A**) Representative gels as described in Figure 2A. **B**) Densitometric quantification of the RT-PCR products as described in Figure 2B. Results are means ± SEM. For tissue inhibitors of MMP-3, a significant increase of the mRNA level was detected in the late secretory (LS) and menstrual phase (ME), compared with the proliferative and early secretory (ES) or midsecretory (MS) phase ($P < 0.001$).

are predominantly produced by stromal fibroblasts in other tissues. Because MT1 and MT2-MMPs are physiological activators of pro-MMP-2, their coordinate expression suggests that these proteases could collectively be produced in active form and contribute to the matrix remodeling throughout cycle. Despite their marked molecular and structural homologies, the MT1 to MT3 MMPs are differentially regulated. Our study confirms previous immunohistochemical observations that MT1-MMP is predominantly expressed during menses in coordination with most other MMPs, but MT2-MMP is expressed throughout the cycle [21]. MMP-19 has previously been localized in other tissues in vascular endothelial and smooth muscle cells [33] and has therefore been assumed to play a role in angiogenesis [32]. Blood vessel growth and remodeling in the endometrium are detectable throughout the cycle. MMP-19 expression throughout the menstrual cycle is thus compatible with its documented angiogenic role [50, 51].

The ''proliferative'' MMPs (MMPs-7, -11, -26, MT3-MMP) represent the third expression pattern. Our data confirm previous observations that MMP-7 and -11 expression is drastically downregulated by progesterone and strongly upregulated during menses [52, 53]. In addition we show here that MT3-MMP and MMP-26 are poorly expressed during the mid- and late secretory phases. Their expression is not reactivated on progesterone withdrawal during menses, indicating that these enzymes are probably not associated with cellular apoptosis and tissue breakdown during menstruation [54].

MMP-26 is structurally related to MMP-7. Their expression is restricted to epithelial tumor cells and epithelial cells of the endometrium [27, 29, 31, 55]. We show here that it is enhanced during the proliferative phase of the cycle (Fig. 3). It remains to be determined whether MMP-26 plays specific roles in the cell proliferation, rapid glandular growth, stromal expansion, and angiogenesis observed in the preovulatory follicular phase. The requirement of growth factors and cytokines release from the endometrial ECM by MMPs supports this hypothesis [5].

The pattern of MT3-MMP mRNA expression—minimal during menses and maximal during the early proliferative phase—is compatible with its documented morphogenetic and angiogenic role in the embryo [56, 57].

Finally, among the TIMPs, only TIMP-3 showed significant cyclical variations. TIMP-1 and TIMP-2 were expressed at comparable levels throughout cycle. Maximal TIMP-3 expression occurred in the late secretory phase, when endometrial stromal cells underwent predecidual differentiation. Although TIMP-3 has been considered a molecular marker for decidualization that would limit trophoblast invasion [58, 59], others have shown in addition that TIMP-3 is also produced in endometrium by nondecidualized stromal and endothelial cells [10, 20].

MMPs are tightly regulated at the transcriptional and posttranscriptional levels and are also controlled at the protein level by their activators and inhibitors. This study focused only on MMP transcript levels, which do not necessarily correspond to protein levels, and the latent proenzyme zymogen is not necessarily secreted and activated. Furthermore, regulation of activated MMP enzymes by TIMPs adds to the complexity of the situation. A great deal of work remains to understand the roles of MMPs in endometrial physiology, but this study provides the groundwork for future experiments designed to determine which of the MMPs are locally transcribed during the different phases of the cycle.

MMPs have functions other than matrix remodeling and endometrial breakdown. Their substrates, in addition to components of the EM encompass cytokines-chemokines and their ligands (either in soluble forms or bound to the cell surface) as well as cell-adhesion molecules of the cadherins and integrins family, their own zymogen forms, and other MMPs and proteinase inhibitors such as serpins (reviewed in [34]). Our study gives a comprehensive view of the pattern of endometrial MMPs throughout the menstrual cycle. It further supports the concept that MMPs expression is associated with multiple biological activities by several cell types. In addition to their precise identification by immunohistochemical and in situ hybridization, the recently developed methodology of protease degradomics—the application of genomic and proteomic approaches to identify the protease and protease-substrate repertoires or ''degradomes''—on the endometrium would be most useful to better delineate the multiple roles of MMPs in endometrial physiology in vivo [2]. This knowledge will ultimately facilitate the identification of new pharmaceutical targets to treat MMP-related disease such as endometriosis, adenomyosis, shedding, or unwanted bleedings.

In conclusion, this study highlights the complex regulation of the MMP family in the endometrium. It points to specific roles of MMPs that are differentially expressed during the various phases of the cycle. MMPs expressed during the proliferative phase may exert functions associated with rapid tissue growth and angiogenesis, and ''menstrual MMPs'' degrade ECM components, leading to tissue breakdown on progesterone withdrawal.

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