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Research Article

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Patterns of Matrix Metalloproteinase Expression in Cycling Endometrium Imply Differential Functions and Regulation By Steroid Hormones

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Abstract

Matrix metalloproteinases are a highly regulated family of enzymes, that together can degrade most components of the extracellular matrix. These proteins are active in normal and pathological processes involving tissue remodeling; however, their sites of synthesis and specific roles are poorly understood. Using in situ hybridization, we determined cellular distributions of matrix metalloproteinases and tissue inhibitor of metalloproteinase-1, an inhibitor of matrix metalloproteinases, in endometrium during the reproductive cycle. The mRNAs for all the metalloproteinases were detected in menstrual endometrium, but with different tissue distributions. The mRNA for matrilysin was localized to epithelium, while the others were detected in stromal cells. Only the transcripts for the 72-kD gelatinase and tissue inhibitor of metalloproteinases-1 were detected throughout the cycle. Transcripts for stromelysin-2 and the 92-kD gelatinase were only detected in late secretory and menstrual endometrium, while those for matrilysin, the 72-kD gelatinase, and stromelysin-3 were also consistently detected in proliferative endometrium. These data indicate that matrix metalloproteinases are expressed in cell-type, tissue, and reproductive cycle-specific patterns, consistent with regulation by steroid hormones, and with specific roles in the complex tissue growth and remodeling processes occurring in the endometrium during the reproductive cycle. (*J. Clin. Invest.* 1994. 94:946–953.) Key words: in situ hybridization • reproductive cycle • matrilysin • stromelysin • RNA

Introduction

Remodeling of the extracellular matrix is an integral part of normal tissue growth and differentiation, as well as pathological processes involving abnormal growth and tissue destruction. Various members of a large family of matrix metalloproteinases (MMPs)[1] have been shown to be active in these processes (for reviews see references 1–4). The MMPs are collagenases, gelatinases, and stromelysins, with distinct but partially overlapping substrate specificities. Together they are capable of degrading most, if not all, of the components of the extracellular matrix. Most have been shown to be transcriptionally regulated by various oncogenes, cytokines, tumor promotors, growth factors, and other biologically active agents. All are secreted as proenzymes and require activation for proteolytic activity. MMP activity can be inhibited by another family of proteins, the tissue inhibitors of metalloproteinases (TIMPs), consisting of the related proteins TIMP-1 (5), TIMP-2 (6), and ChIMP-3 (7). TIMP-1 expression is highly regulated and has been associated with the same tissues and cells expressing MMPs (2, 8). Thus, the regulation of MMP and TIMP activity in tissues is complex and occurs at many levels.

MMP activity, synthesis, or secretion by a variety of cell types has been extensively studied and implicated as important in numerous pathological processes involving tissue destruction, including wound healing (8), arthritis (9), and tumor invasion and metastasis (10). These proteins' roles in normal tissue formation and remodeling has been less frequently studied. MMP activity or expression has been detected in ovulation (11), embryo implantation (12), and mammary gland involution following lactation (13), all highly orchestrated events which are tightly regulated by hormones. MMPs have also been implicated in endometrial remodeling (14–17). One of the stromelysins, matrilysin, was initially isolated from involuting rat uteri (17). Conditioned media of endometrial explants has been shown to release matrix metalloproteinase activity in vitro, and the activity was shown to be inhibited by progesterone (Osteen, K., W. Rodgers, M. Gaire, J. Hargrove, F. Gorstein, and L. Matrisian, manuscript submitted for publication) (15, 16).

Recently, using in situ and Northern hybridization and immunohistochemistry, we determined that matrilysin expression is restricted to the epithelium of the endometrium and that its expression varies during the menstrual cycle in a pattern consistent with regulation by progesterone (14). Matrilysin mRNA, localized by in situ hybridization, was always associated with matrilysin antigen in epithelial cells; while matrilysin antigen was also detected in stromal tissue during the menstrual phase. These data were consistent with diffusion of matrilysin antigen throughout stroma during the menstrual phase and suggested that matrilysin was a likely effector of the tissue breakdown occurring during menstruation. These data also indicated that detection of MMP mRNA by in situ hybridization was a better

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1. *Abbreviations used in this paper:* MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

indicator of cellular MMP expression than immunohistochemical localization of MMP antigen due to the likely diffusion of matrilysin antigen in tissues.

The understanding of the roles played by MMPs and TIMPs in normal and pathological processes has been hampered by a lack of information on the expression of individual members of the MMP and TIMP families in these tissues. To place our observations of endometrial matrilysin expression in perspective of the spectrum of expression of other members of the MMP and TIMP families, we extended our localization studies to examine, at the level of metalloproteinase RNA expression, the patterns of expression of most of the known MMPs and TIMP-1 to provide insight into the roles MMPs and TIMP may play in the dramatic tissue remodeling and degeneration occurring in the cycling endometrium and in normal tissues in general.

Methods

Tissues. This study was conducted in accordance with institutional committees for the protection of human subjects, using tissue in excess of that required for diagnostic purposes. The majority of the tissues used in this study were those of Rodgers et al. (14). Endometrial samples were obtained at the time of endometrial biopsy or hysterectomy, from women, ages 20 to 40, without a history of endometrial dysfunction, as determined by review of medical records. Tissues for RNA isolation were immediately frozen in liquid nitrogen and stored at −80°C. Hysterectomy specimens were placed on ice until examined and fixed in 10% neutral buffered formalin. Endometrial biopsy specimens were immediately fixed in 10% neutral buffered formalin. Endometrium were examined histologically, dated from hematoxylin and eosin stained sections according to the criterion of Noyes et al. (18) and grouped according to the stage of an idealized 28-d reproductive cycle as: proliferative (days 7–15), secretory (days 16–24), late secretory (days 25–27), and menstrual (days 28–6). Sections were cut at 5 μm and mounted on 3-aminopropyltriethoxysilane-coated slides (Sigma Chemical Co., St. Louis, MO) (19).

Hybridization probes. Hybridization probes were prepared from cloned human MMP cDNAs. All cDNAs were cloned in pGEM vectors (Promega, Madison, WI) except the stromelysin-3, and gelatinase cDNAs which were in Bluescript vectors (Stratagene, La Jolla, CA). The cDNAs for stromelysin-1 (transin, MMP-3, EC 3.4.24.27), stromelysin-2 (MMP-10, EC 3.4.24.22), and matrilysin (PUMP-1, MMP-7, EC 3.4.24.23) are those used by McDonnell et al. (20) and are 552, 530, and 849 bp, respectively. The cDNA for interstitial collagenase (Type I collagenase, MMP-1, EC 3.4.24.7) is a 640 bp ClaI/Bg1III fragment of pHCOLL from Muller et al. (21) and the cDNA for stromelysin-3 (467 bp) is from Basset et al. (22). The cDNA for human 72-kD gelatinase (72-kD Type IV gelatinase, MMP-2, EC 3.4.24.24), is the 884-bp PvuII–BamHI fragment from position 910–1794 of pBS-GEL (23). The cDNA for human 92-kD gelatinase (92-kD Type IV gelatinase, MMP-9, EC 3.4.24.35) is a 900-bp SmaI–HindIII fragment from position 852–1751 of pBS-92 (24). The cDNA for TIMP-1 is an 810 bp EcoRI fragment containing the entire coding region of TIMP-1 (25) cloned in pGEM-3Z (8). For in situ hybridization, full-length RNA transcripts from the sense and antisense strands were synthesized from the linearized plasmids by in vitro transcription using 3H-UTP (New England Nuclear, Boston, MA) to specific activities of approximately 10^8 dpm/μg. For Northern blot hybridizations, single stranded RNA probes were prepared using ^{32}P-UTP (14). The MMP probes used did not cross hybridize with other MMP cDNAs or RNAs by Northern, Southern, and in situ hybridization (20, 26).

In situ hybridization. In situ hybridization was performed as described in Rodgers et al. (14). Briefly, 5-μm sections were treated with proteinase K, acetylated with acetic anhydride in triethanolamine, treated with hybridization solution (50% formamide; 0.3 M NaCl, 20 mM Tris-HCl, 5 mM EDTA, pH 8.0; 0.2% BSA, ficoll, and polyvinylpyrolidone; 10% dextran sulfate; 500 μg/ml yeast RNA, 100 ng/ml UTP) without probe at 50–55°C for 1 h, and covered with hybridization solution containing 0.1–0.3 μg/ml of probe. Sections were heated to 100°C and incubated at 50–55°C for 18 h. After hybridization the slides were washed in 4× SSC, treated with 30 μg/ml RNase A and 1 U/ml ribonuclease T1 in (0.5 M NaCl, 0.01 M Tris, 1 mM EDTA, pH 8.0; 37°C, 30 min), washed in 2× SSC at room temperature, 0.1× SSC at 65°C for 60 mins, and washed in 0.1× SSC at room temperature for 1 h. The sections were dehydrated, dipped in Kodak NTB-2 emulsion, exposed for 4 and 8 wk at 4°C, and developed in D-19 (Eastman Kodak Co., Rochester, NY). Control hybridizations using the sense transcript were done on adjacent sections in all experiments. Hybridization signals were scored by comparing patterns of silver grain deposition between the antisense (messenger RNA) probe hybridizations and sense (control) probe hybridizations using dark field illumination and a 10× objective. Intensity of hybridization was semi quantitated as 0 (negative) or 1, 2, or 3 (weakly to strongly positive). The majority of positive cases had moderate to strong (2 to 3) hybridization scores. Counting of grains in positive areas on selected cases indicated that these scores represented ~ 3 (score of 1) to greater than 10-fold (score of 3) more grains/positive cell using the antisense probe than the sense probe. All tissues were assayed at least two times with each probe. Identification of hybridized cells was confirmed using 40× objective with transmitted illumination. All tissues studied exhibited positive patterns of hybridization with at least one and usually two or more of the probes.

Northern analysis. Northern blots used in Rodgers et al. (14) were stripped and reprobed for this study. Blots were stripped by adding 100°C 0.1 × SSC, 5% SDS to the blots and shaking for 30 min at 65°C, repeating and rinsing with water. Hybridization was in 4× SSPE, 50% formamide buffer with 1% SDS, 0.5% nonfat dried milk, 0.5 mg/ml denatured carrier DNA, and 0.25 mg/ml yeast tRNA and 2 × 10^6 cpm of probe/ml. All hybridizations were at 60°C overnight, followed by two brief rinses in 2× SSC/0.1% SDS at room temperature, two 30-min washings in 1× SSC/0.1% SDS at 67°C, and two 30 min washings in 0.2× SSC/0.1% SDS at 67°C. RNA retention in all blots was checked by hybridization of stripped blots to a chicken β-actin probe.

Results

In situ hybridization of sections of endometrial tissues permitted us to determine the cellular sites of synthesis of MMP and TIMP-1 RNAs while preserving the cellular relationships within the tissue. Endometrial tissues were either immediately fixed in formalin at the time of biopsy or rapidly fixed after dissection from hysterectomy specimens which were obtained on ice and rapidly processed. No differences in the results from these tissues were obtained. Table I summarizes the hybridization results. Differences in the numbers of specimens studied for different probes is due to exhaustion of tissue blocks of some specimens before all MMPs or TIMP-1 were assayed. Representative photomicrographs of the hybridization reactions are in Figs. 1 and 2. All tissues were positive for at least one probe. MMP antisense (mRNA) probes reacted with either epithelial cells (Figs. 1, *A* and *L*, and 2 *H*) or stromal cells (e.g., Fig. 1, *D* and *P*) within tissues. Sense probes yielded minimal background hybridization (e.g., Fig. 1, *F, H, J,* and *N*). The hybridization signals in positive tissues was usually obvious (3× to greater than 10× background) and present in large areas of the tissues. Occasionally, individual cells or small groups of cells (< 5% of the tissue) were positive (e.g., Fig. 2 *G*)—these cases are indicated as focally positive in Table I. Weak hybridization was repeatedly obtained in 2 of 8 proliferative endometrial specimens with antisense probe to stromelysin-1 and in 2 of 6 late secretory specimens with antisense probe to TIMP-1. In these cases the antisense (mRNA) probe hybridizations contained less than 2× the background number of grains as

Table I. Results of In situ Hybridization Assays for Matrix Metalloproteinases and TIMP-1

Endometrium	Antisense hybridization probe (No. positive/No. assayed)							
	Mat	Strom-1	Strom-2	Strom-3	Int Col	72-kD G	92-kD G	TIMP-1
Proliferative								
Epithelium	9/9	0/8	0/8	0/8	0/7	0/7	0/7	2/7*
Stroma	0/9	2/8§	0/8	8/8	2/7§	6/7	0/7	7/7
Secretory								
Epithelium	0/12	0/12	0/12	0/12	0/12	0/10	0/10	0/12
Stroma	0/12	0/12	0/12	0/12	0/12	8/10	0/10	9/12‡
Late Secretory								
Epithelium	10/11*	0/6	0/6	0/6	0/5	0/10	0/10	2/6§
Stroma	0/11	0/6	4/6*	3/6*	0/5	9/10	0/10	6/6
Menstrual								
Epithelium	9/9	0/8	0/8	0/8	1/7*	2/8*	0/8	8/8
Stroma	0/9	8/8	8/8	8/8	7/7*	7/8	6/8*	8/8

* Focal positivity; ‡ prominent perivascular and periglandular distribution; § weak positivity. *Mat,* matrilysin; *Strom,* Stromelysin; *Int Col,* interstitial collagenase; *kD G,* kD gelatinase.

the same cells in the sense probe hybridizations. This level of hybridization is difficult to visualize and may represent either low levels of mRNAs in these tissues or false positive interpretation. The other patterns of in situ hybridization of probes were obvious and consistent for different tissues in the same stage of the reproductive cycle.

Cellular distributions of MMP and TIMP-1 RNAs. Matrilysin RNA expression was restricted to epithelial cells (Figs. 1, *A* and *L* and 2 *H*). All other MMPs were detected predominantly or exclusively in stromal tissue (e.g., stromelysins-3 and -2, Fig. 1, *D* and *P*). The 72-kD gelatinase and interstitial collagenase RNAs were focally detected in epithelium in 2 of 8 and 1 of 7 menstrual specimens respectively. The 72-kD gelatinase RNA was predominantly detected in the stromal tissue of endometrium from all stages of the cycle (Fig. 1 *G* and 2 *I*). In proliferative endometrium, different MMP probes yielded differences in intensity but not distribution of hybridization signal, with the majority of typical stromal cells containing MMP RNA (e.g., Fig. 1 *D*). In early menstrual samples subtle differences in intensity of hybridization of stromal cells throughout the same tissue sections probed for different MMPs were observed. Antisense probe for stromelysin-1 was most abundantly reactive with stromal elements immediately adjacent to glandular epithelium (Fig. 2 *C*), while probes for the 72-kD gelatinase (Fig. 2 *A*), and stromelysin-2 (Fig. 1, *O* and *P*), and stromelysin-3 (Fig. 1 *M*) were distributed throughout the stromal tissue. No specific localization of MMP probes was observed relative to vascular tissues.

TIMP-1 RNA was detected in both epithelium and stroma with different proportions of stroma and epithelium reacting, depending on the stage of the cycle. TIMP-1 RNA was abundant in small arteriolar and capillary vascular tissue in the secretory endometrium (Fig. 1 *I*), and in both the epithelium (Fig. 2 *P, top left*) and periglandular stroma (Fig. 2 *P, bottom right*) of different areas of the same section of menstrual endometrium.

Temporal expression of MMP and TIMP-1 RNAs. MMP RNAs were expressed in the endometrium in reproductive stage specific groups, summarized in Fig. 3. Matrilysin and stromelysin-3 RNAs were detected in proliferative (Fig. 1, *A, B,* and *D*), late secretory (Fig. 1, *L, M,* and *N*) and menstrual (Fig. 2, *H* and *J*) specimens; they were not detected in early or mid secretory endometrium. The 72-kD gelatinase (Figs. 1, *E* and *G*, and 2, *A, B,* and *I*) and TIMP-1 (Figs. 1, *I*, and 2, *E* and *P*) RNAs were detected throughout the cycle; indeed, they were the only RNAs detected in the early to mid secretory endometrium (Figs. 1, *G–K*). Stromelysin-2 (Figs. 1, *O* and *P*, and 2 *O*) and, except for weak expression in a few samples of proliferative endometrium, stromelysin-1 (Fig. 2 *C*) RNAs were detected only in late secretory and menstrual endometrium. The 92-kD gelatinase (Fig. 2, *M* and *N*) and interstitial collagenase (Fig. 2, *G* and *L*) RNAs were focally detected predominantly in menstrual endometrial stroma.

In previous studies, matrilysin RNA was focally abundant in restricted isolated endometrial glands in some specimens of late secretory endometrium (see reference 14). In this study, stromelysin-2, and stromelysin-3 are also focally distributed in the stroma of late secretory endometrium, but not in the exact region exhibiting matrilysin expression (data not shown). Stromelysin-1 RNA was not detected in late secretory endometrium. The 72-kD gelatinase RNA was more widely distributed and more frequently detected in the stromal cells from the late secretory period than the RNAs for stromelysin-3 or stromelysin-2. The intensity of the hybridization of the 72-kD gelatinase probe was greatest in menstrual tissues. Stromal cells expressing MMP RNAs were more widely distributed in these tissues than glands expressing matrilysin RNA (data not shown). The intensity of TIMP1 probe hybridization paralleled that of the 72-kD gelatinase probe.

In menstrual endometrium all MMPs were detected, but in different tissue distributions. Matrilysin was present throughout the epithelium in tissue from the functionalis of early menstrual endometrium (Fig. 1 *L*). The surrounding stromal cells contained abundant stromelysin-3 (Fig. 1 *M*), stromelysin-2 (Fig. 1, *O* and *P*), 72-kD gelatinase (Fig. 2 *A* and *B*), and somewhat less abundant stromelysin-1 (Fig. 2*C*) RNAs. Interstitial collagenase (Fig. 2 *G*) and 92-kD gelatinase (data not shown) RNAs were focally detected in stroma, in the same tissue regions in either the same or adjacent cells. In endometrial specimens from hysterectomies in the menstrual phase, the cells of the basalis endometrium express MMP RNAs in the same pattern found

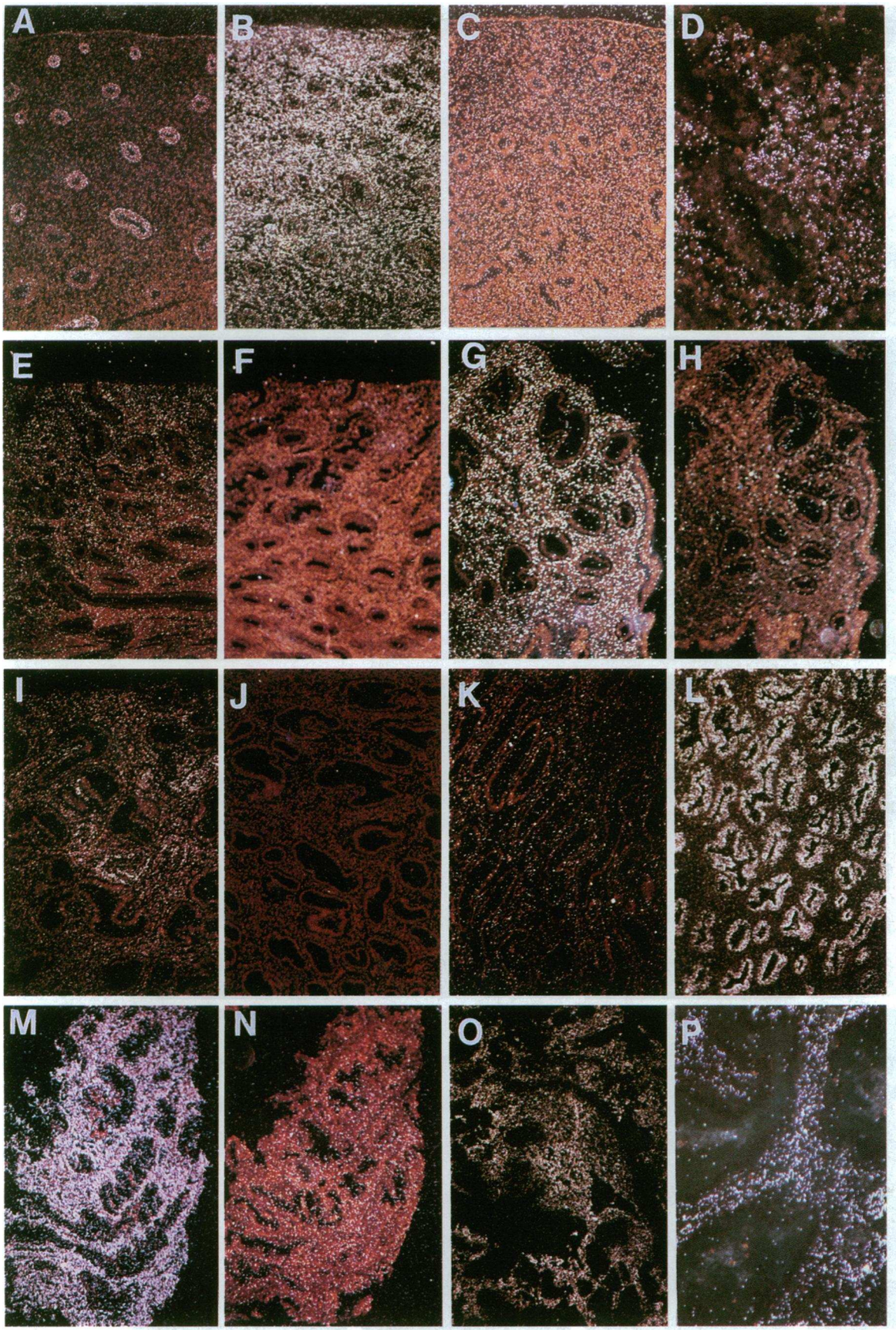

Figure 1. MMP and TIMP-1 mRNA expression in proliferative, secretory and early menstrual endometrium. Dark field photomicrographs of in situ hybridizations of riboprobes to adjacent sections of proliferative phase (hysterectomy, *A–F*), secretory phase (endometrial biopsy, *G, H*; hysterectomy, *I–K*) and early menstrual (day 27–28 with focal hemmhorage, endometrial biopsy, *L–P*) endometrium with the following probes: (*A*) matrilysin antisense, (*B*) stromelysin-3 antisense, (*C*) stromelysin-3 sense (control; slightly overexposed to show tissue), (*D*) stromelysin-3 antisense (high power of *B*), (*E* and *G*) 72-kD gelatinase antisense, (*F* and *H*) 72-kD gelatinase sense, (*I*) TIMP-1 antisense, (*J*) TIMP-1 sense, (*K*) matrilysin antisense (showing lack of matrilysin hybridization to secretory endometrium), (*L*) matrilysin antisense, (*M*) stromelysin-3 antisense, and (*N*) stromelysin-3 sense, (*O*) stromelysin-2 antisense, (*P*) stromelysin-2 (high power of *O*). *A–C, E–O* (25×). *D* and *P* (×100).

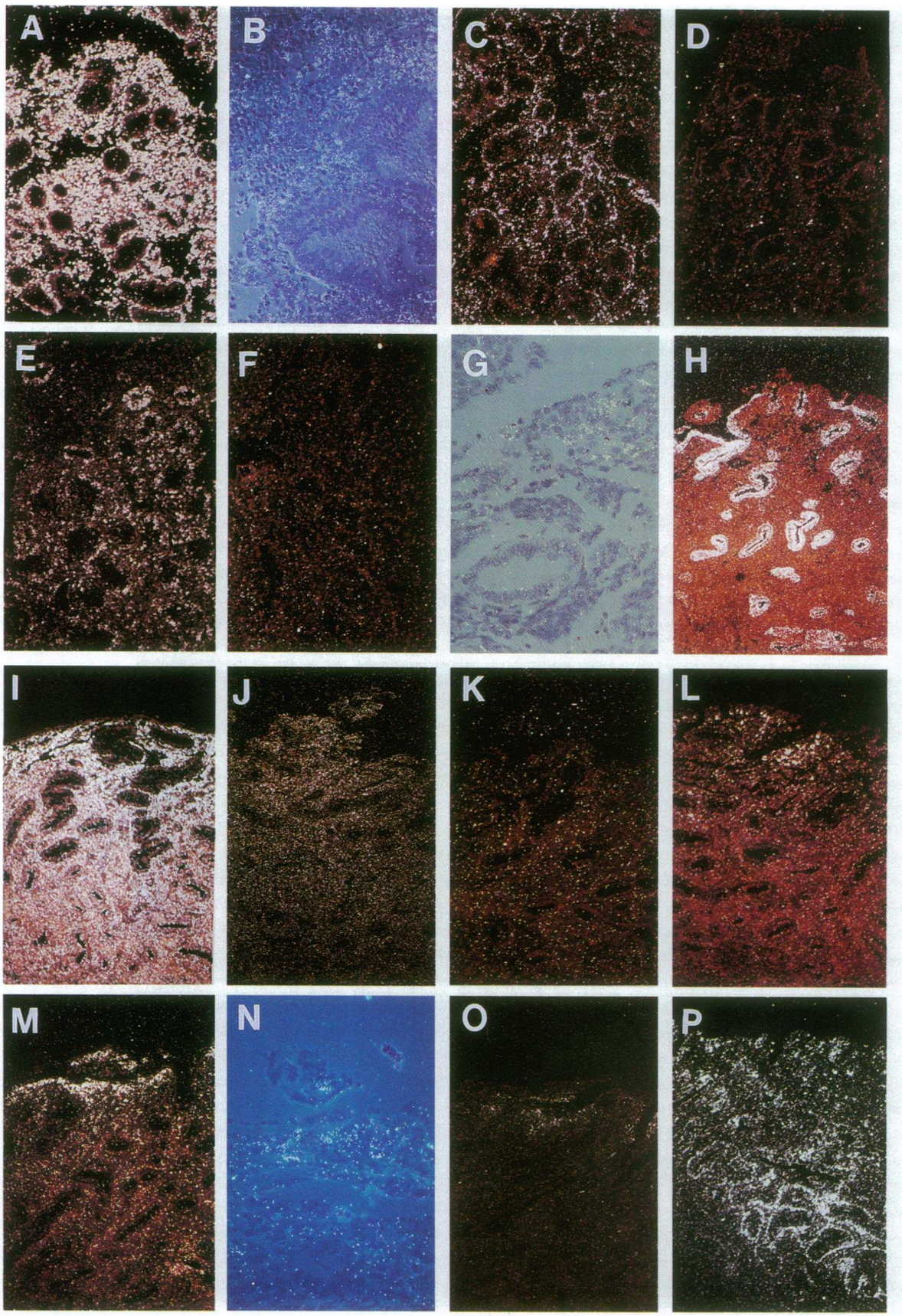

Figure 2. MMP and TIMP-1 mRNA expression in menstrual endometrium. Photomicrographs of in situ hybridizations of riboprobes to adjacent sections of early menstrual (day 27–28 with focal hemmhorage, endometrial biopsy, *A–F*) and menstrual (biopsy, *G*; hysterectomy, *H–P*) endometrium with the following probes: (*A, B*) 72-kD gelatinase antisense, (*C*) stromelysin-1 antisense, and (*D*) stromelysin-1 sense (control), (*E*) TIMP-1 antisense, (*F*) TIMP-1 sense, (*G*) interstitial collagenase antisense, (*H*) matrilysin antisense, (*I*) 72-kD gelatinase antisense, (*J*) stromelysin-3 antisense, and (*K*) stromelysin-3 sense, (*L*) interstitial collagenase antisense, (*M* and *N*) 92-kD gelatinase antisense, (*O*) stromelysin-2 antisense, (*P*) TIMP-1 antisense. *A, C, D–F, H–M,* and *O–P* (×25, dark field). *B, G,* and *N* (×100, DIC).

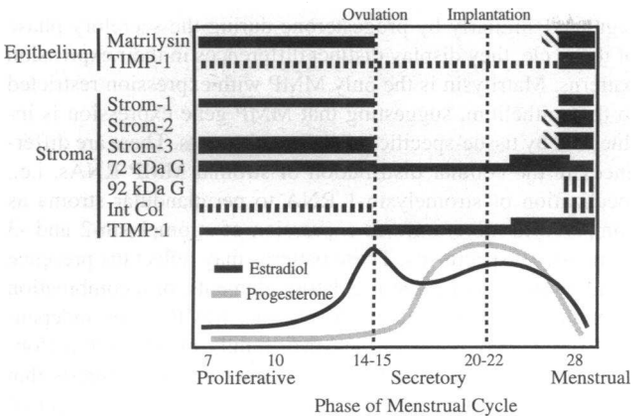

Figure 3. Summary of in situ hybridization studies of MMPs and TIMP-1 expression in endometrium. Height of bars reflects relative intensity of hybridization signal. Patterns of bars indicate fraction of specimens scored as positive: vertical dashes, < 25%; slanted dashes, 25 to 80%; solid bars, > 80%. Shaded curves show serum estradiol and progesterone during an idealized 28-d reproductive cycle.

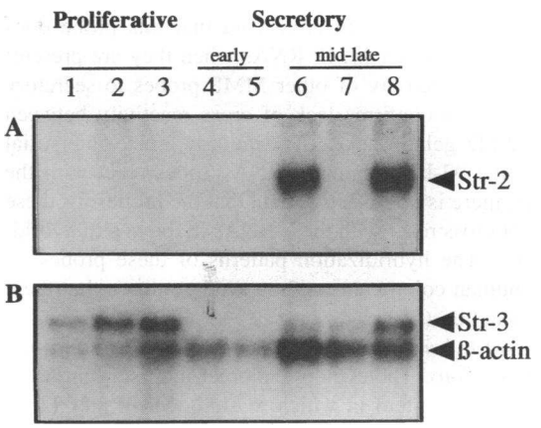

Figure 4. Northern blot analysis of stromelysin-3 and stromelysin-2 RNAs in endometrium. (*A*) Hybridization of stromelysin-2 antisense probe. (*B*) Hybridization of stromelysin-3 and actin antisense probes to stripped membrane used in the experiment shown in *A*. 5 μg of total RNA from the following tissues were analyzed: (lanes *1–3*) early, mid and late proliferative endometrium, (lanes *4* and *5*) early secretory endometrium, (lanes *6–8*) mid and late secretory endometrium.

in proliferative endometrium—i.e., matrilysin (Fig. 2 *H*), 72-kD gelatinase (Fig. 2 *I*) and stromelysin-3 (Fig. 2 *J*) are found in the epithelium and stromal cells respectively from the basalis endometrium (bottom of the sections) to the luminal menstrual tissue. The stromal cells of the luminal surface of the menstrual basalis endometrium showed intense hybridization in a band-like distribution for stromelysin-2 (Fig. 2 *O*), 92-kD gelatinase (Fig. 2, *M* and *N*), stromelysin-1 and interstitial collagenase (Fig. 2 *L*) RNAs. Stromelysins-2, the 92-kD gelatinase and interstitial collagenase expression was particularly interesting in that the mRNA for these metalloproteinases was identified in the stromal cells of virtually all specimens only during the menstrual phase of the reproductive cycle. In menstrual specimens, collagenase and 92-kD gelatinase expression was much more focal (Fig. 2 *G*) than the expression of the other MMPs, being localized only to select regions of the stroma and not observed throughout the entire biopsy samples.

TIMP-1 RNA was detected in both stromal and epithelial cells in menstrual specimens in complicated patterns which varied greatly from region to region in the tissue. In some areas the epithelium had an intense hybridization signal while in other areas the stroma was most intense (Fig. 2 *P*).

Northern hybridization analysis. To confirm that the in situ hybridization method is sufficiently sensitive to exclude expression of RNAs which were not detected in the proliferative and secretory tissues, Northern hybridization was performed on stripped blots of endometrial RNAs isolated from endometrial biopsies used in previous studies of matrilysin RNA expression (14). Stromelysin-2 RNA (Fig. 4 *A*) was not detected in all three specimens of RNA from early proliferative endometrium and was detected in 2 of 3 specimens from mid-late secretory endometrium. Positive hybridization for stromelysin-3 RNA (Fig. 4 *B*) was obtained in all proliferative RNA samples and in the same mid-late secretory samples reacting with stromelysin-2 probe. Positive hybridization for the actin probe (Fig. 4 *B*) confirmed the presence of hybridizable RNA on the blots. 72-kD gelatinase RNA was detected in all specimens, and stromelysin-1 RNA was not detected in any of the specimens from the same blot (data not shown).

Discussion

Sensitivity and specificity of the in situ hybridization assay. The concordance of results from three independent assays of matrilysin expression (in situ hybridization, Northern blot analysis and immunolocalization) in Rodgers et al. (14) and the concordance between Northern and in situ hybridization results for stromelysins-2 and -3 and the 72-kD collagenase in this report indicate that the in situ hybridization method is as sensitive as Northern hybridization for assay of MMP RNAs, even for determination of the absence of RNA in endometrium. The in situ hybridization method is theoretically able to detect as few as 10 transcripts/cell (27), which is considerably less sensitive than the Northern hybridization method. So far this sensitivity appears adequate for determination of MMP transcription in these tissues. Presumably, this is due to relatively large changes in cellular RNA concentrations which are likely associated with MMP expression in these tissues. When the RNAs are not detected in the secretory phase, the RNA is presumably rapidly degraded below the level of detection of either method. When MMPs are being produced, their RNAs are sufficiently abundant so that they can be detected by either hybridization method.

Analysis of patterns of hybridization with the different MMP probes confirms that they do not cross react with other MMP RNAs in the in situ hybridization assays; thus, the patterns of hybridization with the various probes reflect biologically plausible distributions of MMP mRNAs within the tissues. The localization of hybridization of matrilysin probes to epithelium in tissues where other MMPs are present in stroma and not detected in epithelium confirms lack of cross reaction of the matrilysin probe with other MMP RNAs and lack or reaction of other MMP probes with matrilysin RNA. Lack of hybridization of the stromelysin-2 probe to proliferative phase stroma, which contains stromelysins-1 and -3, and 72-kD gelatinase RNAs, confirms lack of cross reactivity of this probe with these MMP RNAs. Lack of hybridization of stromelysin-1 probe to proliferative endometrium reacting strongly with stromelysin-3 confirms lack of cross reactivity between these reagents. Because the 72-kD gelatinase is detected in stroma of all endome-

trial samples, there is no objective data that this probe does not cross react with other MMP RNAs when they are present; however lack of reactivity of other MMP probes to secretory endometrial tissues confirms lack of cross reactivity between them and 72-kD gelatinase RNA. Similarly, since interstitial collagenase and 92-kD gelatinase RNA probes react with the same tissues, there is no objective data that the probes for these MMPs do not cross react with these RNAs in the in situ hybridization assays. The hybridization patterns of these probes on samples of human colon cancers, however, confirms that there is no cross-reaction (26).

Patterns of hybridization suggest steroidal regulation and differential functions. The cycling endometrium is an informative and attractive system in which to study MMP and TIMP expression. It is a dynamic tissue, composed of tubular epithelial glands contiguous with surface epithelium and a dynamic connective tissue stroma, which contains specialized stromal cells, fibroblasts, vascular elements, and a variable population of transient and resident inflammatory cells. The endometrium undergoes monthly cycles of rapid growth, remodeling, and differentiation, directly or indirectly in response to changes in ovarian steroid hormones, principally estradiol (18, 28). After ovulation, the rapid growth of the proliferative phase ceases abruptly, and secretory differentiation occurs principally under the influence of progesterone. In the absence of pregnancy, progesterone declines, coincident with breakdown of the functionalis region of the endometrium, which is expelled with the menstrual flow. Estradiol rises at the end of menstruation, coincident with a new cycle of tissue renewal originating from the intact basalis region of the endometrium. The mechanism of action of these hormones and other factors on endometrium is complex and remains to be completely elucidated.

The lack of expression of all of the MMPs except for the 72-kD gelatinase in secretory endometrium, suggests that the mRNAs for the proenzymes of these proteins may be regulated by steroid hormones, in particular, that they may be negatively regulated by progesterone. This hypothesis has been tested in explant cultures of human endometrium confirming that progesterone inhibits the expression of matrilysin, stromelysin-1 and stromelysin-3 protein or RNA in vitro (Osteen, K., W. Rodgers, M. Gaire, J. Hargrove, F. Goldstein, and L. Matrisian, manuscript submitted for publication). Similarly, progesterone has been shown to inhibit the expression of the 72- and 92-kD gelatinase and interstitial collagenase enzyme activities in endometrial explants (16). In addition, progesterone withdrawal, equivalent to the late secretory/early menstrual phase, promotes secretion of the 72-kD gelatinase by endometrial stromal cells decidualized in vitro (data not shown, L. Giudice). The intensity of the hybridization signal for 72-kD gelatinase is less intense in specimens in the secretory interval and dramatically increases in late secretory and early menstrual endometrium indicating that quantitative regulation of expression of this enzyme by progesterone may also occur. Thus, it is likely that the expression of the majority of MMPs in endometrium may be inhibited by progesterone. This hypothesis is consistent with the structure of MMP genes. The matrilysin (29) and stromelysin (30) promotors have sequences resembling steroid hormone-response elements. Glucocorticoids have been shown to repress collagenase expression (31) and retinoic acid to repress stromelysin expression (32) via an interaction with these steroid hormone receptors and factors recognizing the AP-1 site in the promoters of these genes.

Although the members of the MMP family appear to be regulated similarly by progesterone during the secretory phase of the cycle, they display distinct differences in their expression patterns. Matrilysin is the only MMP with expression restricted to the epithelium, suggesting that MMP gene expression is influenced by tissue-specific regulatory elements. There are differences in the cellular distribution of stromal MMP RNAs, i.e., localization of stromelysin-1 RNA to periglandular stroma as compared to more uniform expression of stromelysin-2 and -3 in menstrual specimens. These patterns may reflect the presence of autocrine or paracrine regulatory elements, or a combination of influences that regulate the various MMP genes independently. The expression of the MMP family members in a globally coordinated but individually distinct manner suggests that each enzyme makes an important contribution to a concerted effort that mediates localized changes in endometrial extracellular matrix.

The patterns of expression of MMPs within endometrial tissues suggest potential roles played coordinately by these enzymes. Matrilysin, stromelysin-3 and the 72-kD gelatinase RNAs are abundant in proliferative endometrium and in basalis menstrual endometrium, which is the tissue which proliferates to replace tissue lost during menstruation, suggesting that these MMPs play roles in tissue growth and remodeling. Lack of expression of all MMPs except the 72-kD gelatinase in proliferative endometrium suggests that differentiation of endometrium does not require a diverse set of MMPs. MMPs may also be inhibited in mid secretory endometrium to facilitate embryo implantation. All MMPs examined demonstrated a high level of RNA expression during the early menstrual phase, suggesting that these enzymes play a role in the breakdown and loss of this tissue. The mRNAs for stromelysin-2 and the 92-kD gelatinase were expressed only in menstrual tissues, suggesting that these enzymes may be particularly critical for this activity. The expression of stromelysin-1 and interstitial collagenase RNA was most prominent in menstrual tissue as well, implicating their activity in menstruation.

Interestingly, the 92-kD gelatinase and interstitial collagenase RNA are found most abundantly in the surface of the basalis endometrium. This observation, and the observation that these MMPs are only detected focally, but with strong hybridization signals, in curettings from menstrual endometrium suggests that their expression may be localized within the endometrium, perhaps to a band of tissue at the interphase between the endometrium destined to be lost during menstruation and the basalis endometrium, from which the endometrium regenerates. If this is the case, this band of tissue may be the only region in the endometrium expressing all MMPs in abundance with a potential combined matrix substrate specificity capable of rapid tissue breakdown. Alternatively, the focal expression of these MMPs may reflect tissue remodeling/wound healing which is occurring at the surface of the basalis endometrium and in other regions of endometrium simultaneously with breakdown of functionalis endometrium.

The apparent lack of temporal regulation of TIMP-1 mRNA expression during the menstrual cycle and the complex and variable patterns of TIMP-1 RNA expression in different areas and cells of the same endometrial tissue is interesting. It is possible that the hybridization patterns with the TIMP-1 probes may not necessarily reflect only TIMP-1 RNA expression. The probe for TIMP-1 is a transcript of the full length TIMP-1 cDNA. Whether this probe cross reacts with RNAs with sequence homology with TIMP-1 mRNA (perhaps other members of the TIMP family) is not known. Alternatively, the complex

patterns of TIMP-1 RNA expression may reflect complex paracrine or autocrine mechanisms of regulation of TIMP-1 RNA by individual stromal and epithelial cells within tissue microenvironments in the endometrium.

The RNA for stromelysin-3 is abundant in normal proliferative and menstrual endometrium. This MMP was originally isolated as a novel transcript of the stroma adjacent to invasive mammary carcinomas (22, 33). Its abundant expression in endometrium suggests a role in normal tissue growth, which should be taken into account in evaluating its role in neoplastic processes. Similarly, the patterns of MMP and TIMP-1 expression in endometrium provide clues for the regulation of MMP activity in endometrium and for the roles played by various MMPs. Further study of metalloproteinase gene expression in the cycling human endometrium may shed light on the molecular mechanisms of steroidal regulation of MMPs and an understanding of how MMP regulation in normal and abnormal endometrium effects tissue remodeling and growth.

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