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The Matrix Metalloproteinase System: Changes, Regulation, and Impact throughout the Ovarian and Uterine Reproductive Cycle

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The ovary and uterus undergo extensive tissue remodeling throughout each reproductive cycle. This remodeling of the extracellular environment is dependent upon the cyclic hormonal changes associated with each estrous or menstrual cycle. In the ovary, tissue remodeling is requisite for growth and expansion of the follicle, breakdown of the follicular wall during the ovulatory process, transformation of the postovulatory follicle into the corpus luteum, as well as the structural dissolution of the corpus luteum during luteal regression. In the uterus, there is extraordinary turnover of the endometrial connective tissue matrix during each menstrual cycle. This turnover encompasses the complete breakdown and loss of this layer, followed by its subsequent regrowth. With implantation, extensive remodeling of the uterus occurs to support placentation. These dynamic changes in the ovarian and uterine extracellular architecture are regulated, in part, by the matrix metalloproteinase (MMP) system. The MMP system acts to control connective tissue remodeling processes throughout the body and is comprised of both a proteolytic component, the MMPs, and a regulatory component, the associated tissue inhibitors of metalloproteinases. The current review will highlight the key features of the MMPs and tissue inhibitors of metalloproteinases, focus on the changes and regulation of the MMP system that take place throughout the estrous and menstrual cycles, and address the impact of the dynamic tissue remodeling processes on ovarian and uterine physiology. (*Endocrine Reviews* 24: 428–465, 2003)

I. Introduction

THE EXTRACELLULAR MATRIX (ECM) has become recognized as a key regulatory component in cellular physiology, providing an environment for cell migration, division, differentiation, anchorage, and, in some cases, an ultimate fate between cell survival or cell death (1). The highly regulated control of ECM turnover and homeostasis occurs, in part, by the action of a specific class of proteolytic enzymes known as the matrix metalloproteinases (MMPs). The MMPs and their associated endogenous inhibitors act in concert to control the site and extent of ECM turnover throughout the body. This regulation by the MMP system impacts numerous, yet diverse biological processes such as embryonic development, organ morphogenesis, angiogenesis, cartilage remodeling, bone growth, wound healing, periodontal integrity, and corneal repair (1–3). Loss of the exquisite control of the MMP system leads to extensive and often destructive degradation of the ECM as seen in arthritis and cancer.

The MMP system also controls aspects of reproductive function. In the ovary and uterus, the MMP system has been postulated to regulate the dynamic structural changes that occur throughout the menstrual or estrous cycle. These structural changes in tissue architecture, which are orchestrated by various hormones, growth factors, and cytokines, are crucial for normal ovarian and uterine physiology. The present review will focus on the changes and regulation of the MMP system that occur in the ovary and uterus throughout the reproductive cycle.

A. Metalloproteinases

One of the first descriptions of an enzyme capable of degrading the ECM was the 1962 observation of Gross and Lapière (4) with frogs undergoing metamorphosis. These authors observed that the resorbing tail of metamorphosing frogs released an enzyme capable of degrading collagen. Subsequently, the enzyme responsible for this resorption

Abbreviations: ADAMTS-1, A disintegrin and metalloproteinase with thrombospondin-like motifs; AP-1, activator protein-1; CL, corpus luteum; DNP, dinitrophenyl peptide; ECM, extracellular matrix; EGF, epidermal growth factor; hCG, human chorionic gonadotropin; MMP, matrix metalloproteinase; MT-MMP, membrane type MMP; PCOS, polycystic ovarian syndrome; PEA-3, polyoma virus enhancer A3; PG, prostaglandin; PMSG, pregnant mare serum gonadotropin; PRL, prolactin; PROM, premature rupture of the membranes; TIMP, tissue inhibitors of metalloproteinase; uPA, urokinase plasminogen activator; VEGF, vascular endothelial growth factor.

was identified as collagenase, thereby opening investigation into a field of proteolytic enzymes responsible for connective tissue remodeling. To date, more than 200 known metalloproteinases have been described (3) that can be subdivided on the basis of their three-dimensional conformation as well as their evolutionary structure into 40 different metalloproteinase families (5). The current review will focus on one of these metalloproteinase families (M10, the interstitial collagenases), commonly referred to as the "matrix metalloproteinases" or MMPs.

The class of MMPs continues to grow, with new members being described with increasing frequency. Currently, the MMP family encompasses at least 25 related proteolytic enzymes (1, 2, 6, 7) that include four broad classes: the collagenases, gelatinases, stromelysins, and membrane type enzymes (MT-MMPs). However, there are a number of family members that are classified outside of these four broad classes. A listing of the MMP family members and their substrates is detailed in Table 1. These proteinases exhibit numerous structural and functional similarities (Fig. 1). Common features of the MMP family include: 1) the presence of zinc in the active site of the catalytic domain, 2) synthesis of the MMPs as preproenzymes that are secreted in an inactive form, 3) activation of the latent zymogen in the extracellular space, 4) recognition and cleavage of the ECM by the catalytic domain of the enzyme, and 5) inhibition of enzyme action by both serum-borne and tissue-derived metalloproteinase inhibitors in the extracellular environment. Although similarities exist in the structure of the MMPs, there are also distinct differences in the recognition and specificity for components of the ECM (reviewed in Refs. 1–3 and 6 and outlined in Table 1). For example, the collagenases (MMP-1, MMP-8, and MMP-13) are able to cleave fibrillar collagens, such as collagen types I, II, III, V, and XI, as well as nonfibrillar collagens, such as collagen types IX, XII, and XIV. Cleavage of the triple helical collagen molecule by these members of the MMP family changes the stability and solubility properties of collagen that result in the denaturation of the collagen molecule into gelatin. Gelatin is susceptible to a wide range of tissue proteinases, including the gelatinases and stromelysins. The gelatinases (MMP-2 and MMP-9) contain a fibronectin-like sequence within their catalytic domain (Fig. 1), which results in a potent ability for these MMPs to bind to and cleave gelatin. The stromelysin enzymes (MMP-3, MMP-7, MMP-10, and MMP-11) act on a broad and diverse array of ECM substrates. Both the gelatinases and stromelysins are capable of degrading major constituents of basement membranes, including type IV collagen, laminin, and fibronectin. The MT-MMPs contain a transmembrane domain near their carboxy-terminal region that anchors these proteinases to the plasma membrane (Fig. 1). An extracellular domain directs the proteolytic component of the enzyme to the exterior surface of the cell. One important role of the MT-MMPs is the activation of MMP-2 (discussed in detail below). In addition to degrading the ECM, the MMPs and especially the stromelysins exhibit activity toward other MMPs, growth factors, and cytokines such as IGF binding proteins, epidermal growth factor (EGF), TNF-α, and substance P (3, 8). The ability of these enzymes to cleave binding proteins as well as cleave active extracellular domains of growth factors expands the repertoire of MMP actions to include modulation of cell growth. This growth promoting action of MMPs may occur either directly by controlling cell–matrix interactions or indirectly by regulating growth factor bioavailability.

Although the different classes of MMPs are capable of cleaving diverse ECM substrates, differences also exist in the ability of members of the same class of MMPs to act on the ECM. An example is seen in the collagenases. Although all three members of the collagenase class cleave fibrillar collagen, these proteinases have different affinities toward type I, type II, or type III collagen and have different mechanisms of reaching the extracellular environment (2, 9, 10). MMP-1 has a 15-fold higher affinity for type II collagen compared with MMP-8, which in turn preferentially cleaves type I collagen (9, 10). MMP-8 is synthesized and stored in granules within neutrophils until needed, whereas MMP-1 and MMP-13 are produced in response to varying stimuli and secreted without being stored. These differences in the substrate specificity of the collagenases and the MMPs as a family allow these proteinases to act on the same matrix components (such as collagen) or different components at selective times and locations to regulate connective tissue remodeling (9, 10).

Degradation of the ECM is an exquisitely controlled process and, as such, MMP synthesis and activation are critical points of regulation of ECM homeostasis (Fig. 2 and Refs. 1–3). The regulation of MMP synthesis can be both tissue- and MMP-specific and will be discussed in the following sections on the ovary and uterus. Subsequent to MMP synthesis, activation of the pro-MMPs in the extracellular space occurs via proteinases, including other MMPs, serine proteinases such as the plasminogen activator/plasmin system, cysteine proteinases, as well as by nonproteolytic agents such as reactive oxygen species, sulfhydryl reactive agents, and denaturants. The plasminogen activator–plasmin system has been proposed to activate MMPs at the cell surface through the urokinase plasminogen activator (uPA), uPA receptor/plasminogen cascade (11). Thus, the activation of MMPs can be regulated by a balance between serine proteinases, such as uPA, and the plasminogen activator inhibitors (2, 11). However, MMPs that are resistant to serine proteinase activation, such as MMP-2, are activated via a novel mechanism involving the MT-MMPs and tissue inhibitors of metalloproteinase (TIMP)-2. One means of activation of MMP-2 is brought about by the ability of the C-terminal domain of TIMP-2 to bind to pro-MMP-2 to form a noninhibitory pro-MMP-2–TIMP-2 complex (Fig. 3A). This complex can be recognized and bound by the extracellular domain of MT1-MMP or MMP-14. Alternatively, TIMP-2 may be bound to the MT1-MMP in such a manner as to form a receptor for the latent MMP-2. Irrespective, the trimolecular complex of MT1-MMP/TIMP-2/pro-MMP-2 is localized to the cell surface. Adjacent unoccupied MT1-MMPs cleave the prodomain of MMP-2 and activate the gelatinase (Fig. 3A). Thus TIMP-2, in conjunction with the MT-MMPs, acts to regulate the specific site of MMP-2 activation. Yet a delicate balance exists in the activation of MMP-2. The exposure of exogenous TIMP-2 to cells expressing MT1-MMP enhances the activation of MMP-2, whereas either an absence of TIMP-2 (Fig. 3B)

TABLE 1. Summary of the nomenclature and actions of MMPs

MMP no.	Common name	Collagen	Additional substrates
1	Collagenase-1, interstitial collagenase, fibroblast collagenase	I, II, III, VII, VIII, X, XI	Aggrecan/gelatin/fibrin/MMP-2, MMP-9/Pro-TNFα/IGFBPs/IL-1β
2	72kDa Gelatinase, gelatinase A, 72 kDa type IV collagenase	I, III, IV, V, VII, X, XI	Aggrecan/elastin/fibronectin/ gelatin/laminin/IGFBPs/ IL-1β/MMPs 9, 13/ProTGFβ/ProTNFα/substance P
3	Stromelysin-1, transin-1, procollagenase, PTR1 protein	II, III, IV, V, VII, IX–XI	Aggrecan/elastin/fibronectin/ gelatin/laminin/IL-1β/MMPs 7, 8, 13/ProTNFα/substance P
4–6	(Obsolete)		
7	Matrilysin, matrin, PUMP-1, uterine metalloproteinase	I, IV, X	Aggrecan/elastin/fibronectin/ gelatin/laminin/MMPs 1, 2, 9/ProTNFα
8	Collagenase-2, neutrophil collagenase, PMNL collagenase	I, II, III, V, VIII, X	Aggrecan/fibronectin/gelatin/laminin/substance P
9	92kDa Gelatinase, gelatinase B, 92kDa type IV collagenase	IV, V, VII, XIV	Aggrecan/elastin/fibronectin/ gelatin/ProTGFβ/ProTNFα/substance P
10	Stromelysin-2, transin-2	III, IV, V	Aggrecan/elastin/fibronectin gelatin/laminin/MMPs 1, 8
11	Stromelysin-3		Aggrecan/fibronectin/laminin/IGFBPs
12	Macrophage metalloelastase	I, IV	Elastin/fibronectin/gelatin/laminin/ProTNFα
13	Collagenase-3	I, II, III, IV	Aggrecan/fibronectin/gelatin
14	Membrane type 1 metalloproteinase, MT1	I, II, III, IV	Aggrecan/elastin/fibronectin/ gelatin/laminin/MMPs 2, 13/ProTNFα
15	Membrane type 2 metalloproteinase, MT2		Fibronectin/gelatin/laminin/MMP-2
16	Membrane type 3 metalloproteinase, MT3	III	Fibronectin/MMP-2
17	Membrane type 4 metalloproteinase, MT4		
18	*Xenopus* collagenase-4, xCol4	I	
19	RASI-1	I, IV	Fibronectin/gelatin
20	Enamelysin		
21	*Xenopus* MMP, XMMP		
22	Chicken MMP, CMMP		
23	Cysteine array MMP, CA-MMP		
24	Membrane type 5 metalloproteinase, MT5		
25	Membrane type 6 metalloproteinase, MT6		
26	Matrilysin-2, endometase	IV	Fibronectin/gelatin
27	Human paralog of MMP-22		
28	Epilysin		

The MMP no., corresponding common name, and partial listing of proposed substrates are shown. [Modified with permission from T. E. Curry and K. Osteen: Biol Reprod 64: 1285–1296, 2001 (193).]

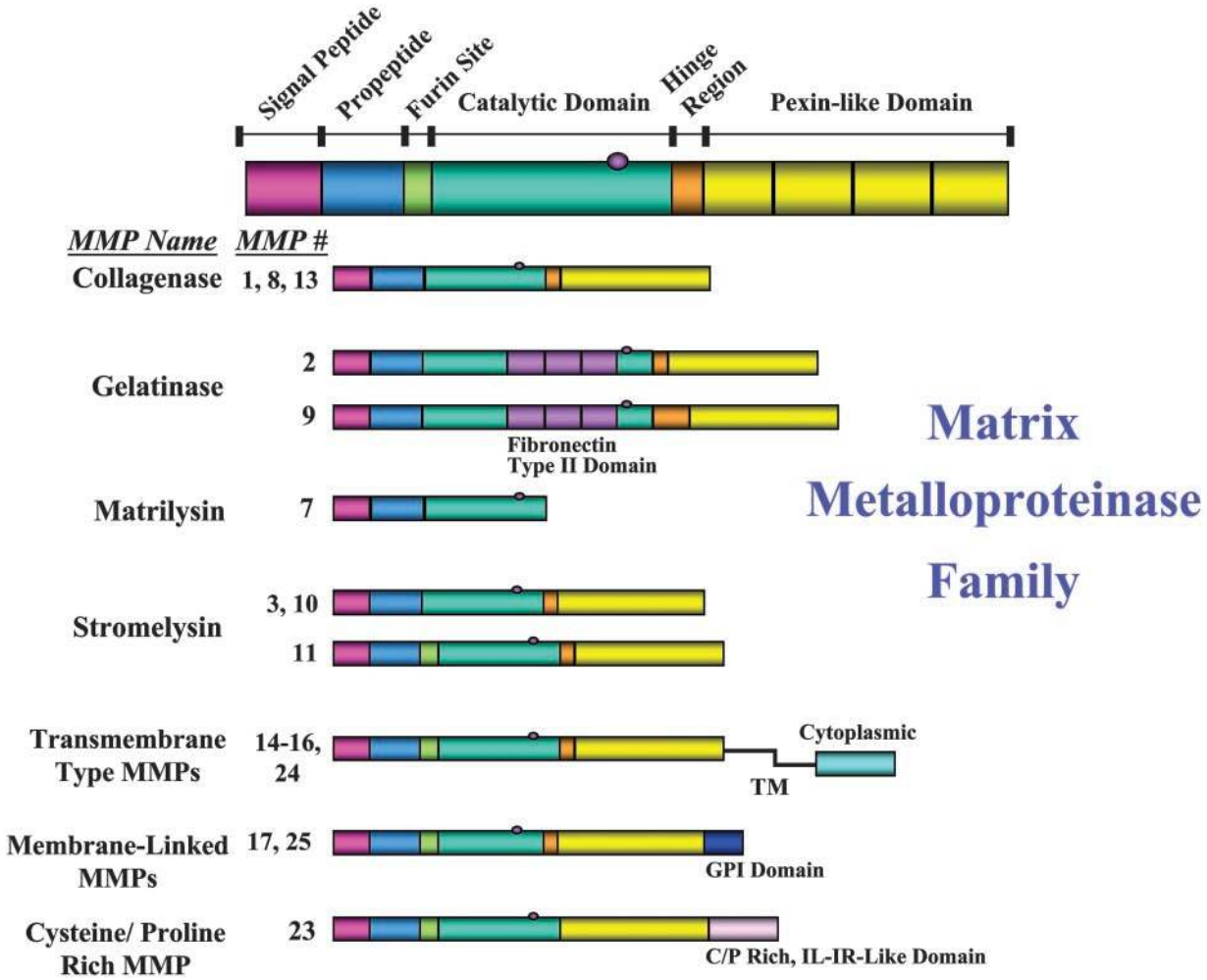

FIG. 1. Schematic representation of the MMP family. A general model of some of the more common MMPs is presented. The common name of the MMP and its corresponding MMP number are denoted. The MMPs contain a signal peptide, a propeptide domain that must be cleaved for activation, a catalytic domain that contains the zinc binding site, and a hemopexin-like domain. The gelatinases contain a fibronectin type II domain, whereas certain other MMPs contain a furin-susceptible site that allows intracellular activation. The transmembrane type MMPs contain a transmembrane (TM) linker to a cytoplasmic domain, whereas the membrane-linked MMPs possess a glycophosphatidyl inositol anchoring domain (GPI). MMP-23 contains a cysteine-proline rich IL-1 receptor (IL-1R) like domain. [Derived from Refs. 8 and 193.]

or high concentrations of exogenous TIMP-2 (Fig. 3C) decreases MMP-2 activation (12). This process has been proposed to occur because low levels of TIMP-2 increase the formation of the trimolecular complex (*i.e.*, MT1-MMP/TIMP-2/pro-MMP-2) resulting in the activation of MMP-2, whereas high concentrations of TIMP-2 bind all the available MT1-MMP such that there is no free MT1-MMP available to activate MMP-2 (12). Therefore, activation of the latent form of MMP-2 can be differentially regulated by the presence or absence of MT1-MMP on the cell surface and the concentration of TIMP-2 in the extracellular space. This mechanism of MMP-2 activation may also occur for the activation of other MMPs, such as MMP-13, although other inhibitors may be involved (11, 13). One notable exception to the extracellular activation of pro-MMPs has been reported for MMP-11 (*i.e.*, stromelysin-3). This MMP contains a small region after the propeptide domain containing a furin-like enzyme recognition motif (Fig. 1) that is cleaved intracellularly by protein-processing enzymes known as furins. This intracellular cleavage of the propeptide domain by furins or furin-like enzymes results in MMP-11 being secreted in an active form (7). Similar to MMP-11, the MT-MMPs also contain a recognition sequence in the propeptide domain that is recognized by furins, suggesting that this class of MMPs may also be activated intracellularly (3, 14).

B. Tissue inhibitors of metalloproteinases

In addition to the regulation of MMP synthesis and activation, MMP activity in the extracellular environment is rigorously controlled by MMP inhibitors (Fig. 2). Two major classes of MMP inhibitors are generally distinguished, serum-borne and tissue-derived inhibitors (reviewed in Refs. 15 and 16). The serum-borne inhibitors, which include the macroglobulins, have a potent ability to inhibit a broad range of proteinases including the MMPs. The macroglobulins are large molecular proteins (180–725 kDa) that include α2-macroglobulin, α1-macroglobulin, α1-inhibitor-3, pregnancy

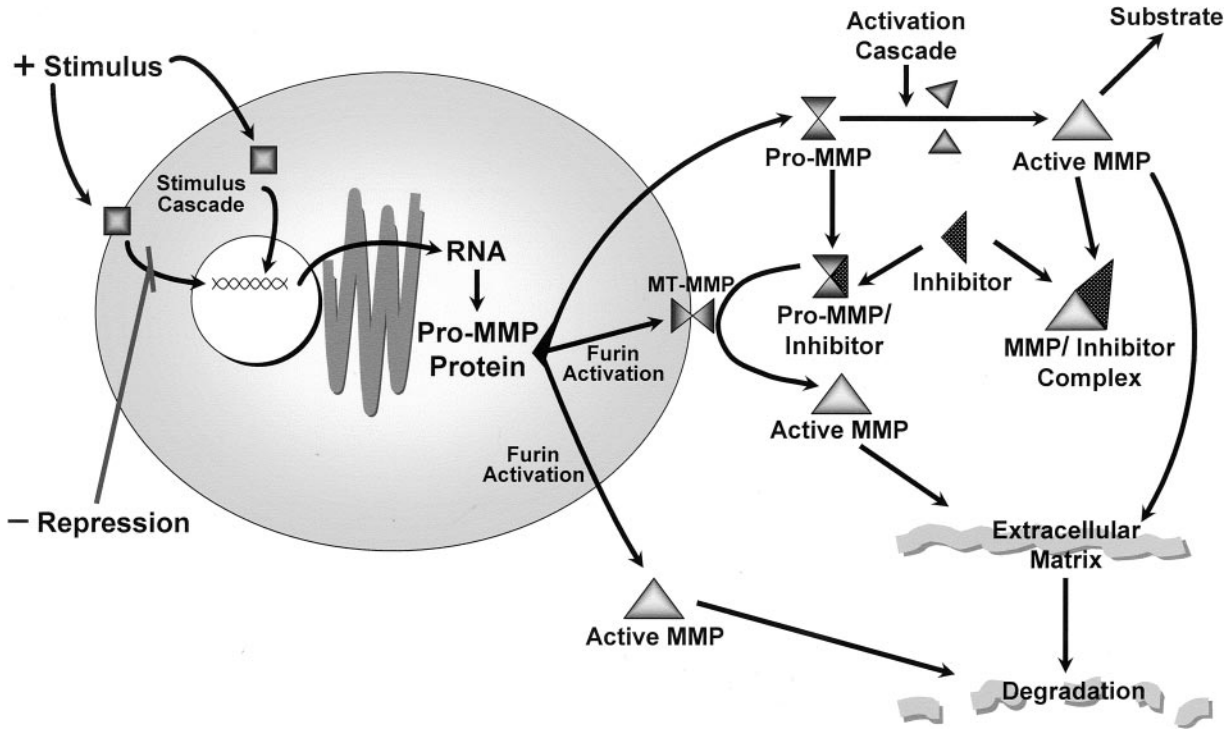

FIG. 2. Schematic representation of the regulation of the MMP system. A general model is presented in which a stimulus, working through membrane-bound or intracellular receptors, sets in motion an intracellular signaling cascade resulting in the synthesis of specific MMP mRNA. This mRNA would be translated into a latent or pro form of the MMP protein. Certain MMPs, such as the MT-MMPs and MMP-11, are activated intracellularly via a furin proteolytic processing pathway and are secreted or inserted into the membrane in an active form. The majority of pro-MMPs, however, are secreted in a latent form that requires activation in the extracellular space by other proteinases. This activation cascade can occur via other MMPs, such as the MT-MMPs, serine proteinases such as the plasmin-plasminogen activator pathway, or other proteinases. Once the MMP is active, it can cleave the ECM, resulting in focal degradation. Alternatively, the active MMP can be bound by MMP inhibitors, such as the TIMPs, resulting in an inhibition of MMP action.

zone protein, and the ovomacroglobulins (*e.g.*, ovostatin). Interestingly, the MMPs, especially MMP-1, have approximately a 150-fold higher affinity for α2-macroglobulin than type I collagen, suggesting that this class of inhibitors is a major regulator of collagenolysis in serum and tissue fluids (3). The second group of inhibitors, referred to as "tissue inhibitors of metalloproteinases" or TIMPs, are locally produced and specifically inhibit MMPs. Although the two classes of inhibitors were originally distinguished as serum-borne or tissue-derived predominantly on the basis of the site of action, findings that the tissue-derived inhibitors are present in serum and that the serum-borne inhibitor α2-macroglobulin is produced in the ovary (17, 18) have blurred the nomenclature distinction between these two different classes of inhibitors. The present review will explore the changes in the TIMPs because this class of inhibitor is highly abundant in reproductive tissues, locally produced, hormonally regulated, and has been hypothesized to coordinate numerous ovarian and uterine processes including matrix turnover, cell growth, and steroidogenesis.

The ability of a small molecular weight protein to inhibit collagenase activity was first reported in 1975 by two separate investigators (19, 20). Subsequently, this protein, TIMP-1, has been shown to be a secreted glycoprotein (29 kDa) that binds to and inhibits the active form of MMPs on a 1:1 stoichiometric basis. The ability of TIMPs to inhibit MMP action occurs through the interaction of the N-terminal domain of TIMP with the active site on the catalytic domain and the substrate binding groove of the MMP. Since the initial discovery of TIMP-1, other TIMPs have been identified including TIMP-2, TIMP-3 (which is glycosylated), and TIMP-4. TIMP-2 is differentially regulated from TIMP-1 and has been proposed to act selectively on different MMPs (15, 21, 22). For example, TIMP-2 has a high affinity for MMP-2, whereas TIMP-1 preferentially binds to MMP-9. TIMP-3 also exhibits differential preference for the MMPs, having a high affinity for MMP-9 and being able to inhibit MT1-MMP, unlike TIMP-1, which cannot act on MT1-MMP. However, unlike TIMP-1 or TIMP-2, TIMP-3 is secreted and then bound to the ECM. By residing in the ECM as opposed to being free in the extracellular fluid, TIMP-3 has been suggested to act as an additional regulatory stop point by acting at the site of MMP action (23, 24). TIMP-4 has recently been cloned and shown to be present in reproductive tissues (25–28). The preliminary information regarding the specificity of this inhibitor and mode of action suggests that TIMP-4 has traits similar to TIMP-2 (15, 16, 25). TIMP-4 has been shown to act on numerous MMPs, leading Stratmann *et al.* (29) to postulate that this TIMP is a good inhibitor for all classes of MMPs without remarkable preference for special MMPs.

Recent evidence indicates that TIMPs, in addition to their classical ability to regulate MMP action, function to regulate many aspects of cellular physiology, including cell growth and steroidogenesis (30). This capability is readily apparent

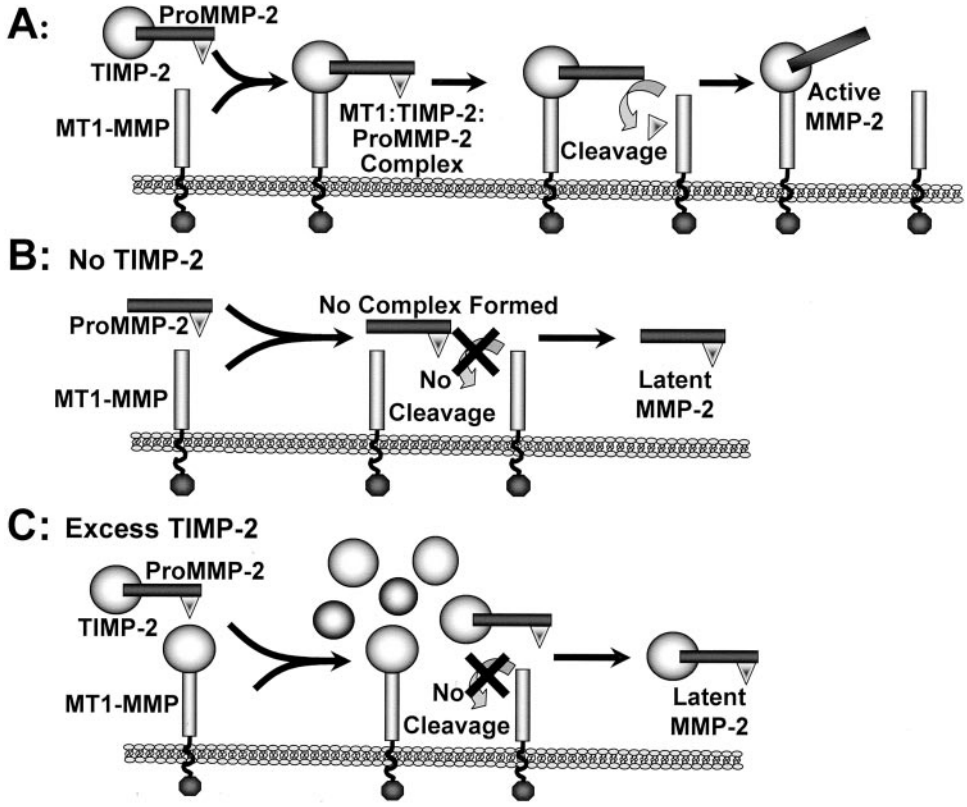

FIG. 3. Activation and regulation of MMP-2 by membrane type-1 metalloproteinase (MT1-MMP). A complex of TIMP-2 and proMMP-2 binds to MT1-MMP (A). An adjacent MT1-MMP is able to cleave the propeptide domain of MMP-2, resulting in activation of MMP-2. This activation of MMP-2 depends on a delicate balance between proMMP-2, TIMP-2, and MT1-MMP. For example, in the absence of TIMP-2 (B), proMMP-2 is not bound by MT1-MMP. As such, proMMP-2 cannot be activated by adjacent MT1-MMPs and remains in a latent state. In the presence of excess TIMP-2 (C), proMMP-2 is not bound by MT1-MMP due to binding of TIMP-2 to both MMP-2 and MT1-MMP. Excess TIMP-2 does not permit the docking of proMMP-2 to MT1-MMP. Thus, proMMP-2 cannot be activated by adjacent MT1-MMPs and remains in a latent state.

from the initial description of TIMP-1 whereby TIMP-1 was isolated and sequenced before it was discovered that this inhibitor had already been described as an erythroid potentiating factor produced by human lymphocytes (31). Further support for the concept that TIMPs may act in nonclassical methods (*i.e.*, other than as MMP inhibitors) and actually be multifunctional is based on reports that TIMPs promote embryo growth and development (32), are antiangiogenic agents (33, 34), stimulate cell growth in a variety of tissues (35), influence apoptosis (36), and recruit quiescent cells into the cell cycle (37). A possible role for TIMPs as nuclear transcription factors comes from the observation that a TIMP-1/green fluorescent protein chimera binds to the surface of MCF-7 breast carcinoma cells and is translocated to the nucleus (38). These findings suggest that TIMPs may act as autocrine/paracrine factors in reproductive processes involving cellular proliferation, differentiation, and neovascularization. Such a postulate is supported by reports that a TIMP-1 like protein stimulates steroid production in the testis and ovary (39), that granulosa cell estradiol production is stimulated by TIMP-1 *in vitro* (40), that TIMP-1-deficient mice have altered uterine morphology as well as reduced serum levels of progesterone during the estrous cycle (41), that progesterone levels are diminished during luteal development in mice lacking TIMP-1 (42), and that human granulosa-luteal cell progesterone secretion is inversely related to the MMP-9/TIMP-1 ratio (43). Finally, there are correlative reports of TIMP-3 mRNA expression associated with healthy follicular development in the rat (44). Thus, the MMPs and TIMPs can be viewed in the classical sense of regulating ECM turnover in reproductive processes. However, the ability of MMPs to cleave binding proteins, release growth factors, and activate cytokines in conjunction with the actions of the TIMPs on cell growth expand the repertoire of the MMP system beyond the classical actions on the ECM to include additional key regulatory roles in ovarian and uterine physiology.

II. Ovary

A. Ovulation

Ovulation is a dynamic, orchestrated process that is set in motion by the midcycle surge of LH (45). LH initiates and synchronizes a series of biochemical events that culminate in the breakdown of the follicle wall and extrusion of the oocyte. These biochemical events include synthesis and secretion of progesterone, prostaglandins (PGs), growth factors, cytokines, and proteolytic enzymes such as the plasmin-plasminogen activator system and the MMPs. In the ovulatory process, proteolysis has been postulated to degrade the apical follicular connective tissue, changing the structural integrity of the follicular wall and thereby facilitating oocyte release (45). There is a body of morphological and biochemical literature supporting this postulate and promoting a paramount role for the MMPs in follicular rupture. Morphologically, there is a breakdown of the granulosa cell basement membrane (46) and fragmentation of the collagenous matrix at the follicular apex (47). In conjunction with these morphological changes in the ovarian ECM, biochemical analysis has revealed a decrease in ovarian and follicular collagen after the LH surge (45, 48, 49) especially at the apex (45, 49,

50). These alterations in the follicular ECM are postulated to occur via the action of a cascade of proteolytic events involving an increase in ovarian MMP activity before ovulation. Support for such a proteolytic regulatory step in follicular rupture is forthcoming from experiments in which ovulation is inhibited by blocking MMP activity with exogenous chemical MMP inhibitors or antibodies against the MMPs (51–53).

1. Metalloproteinases in the ovulatory process. One of the earliest proposals that proteolysis may play a role in ovarian function came from Schochet (54) who suggested in 1916 that proteolytic enzymes are active in digesting the connective tissue matrix at the apex of the Graffian follicle to allow for follicular rupture. Subsequent experiments have validated Schochet's initial hypothesis of a proteolytic component in the mechanism of ovulation. Early studies demonstrated that injection of bacterial collagenase into rabbit follicles *in situ* resulted in follicular rupture, supporting a role for collagenase in the ovulatory process (55). Later studies, however, were unable to measure collagenase or neutral proteinase activity using synthetic substrates in rat (56), porcine, or rabbit follicles (57). In the rodent, Morales *et al*. (48, 58) validated a microassay for collagenase activity using the release of hydroxyproline fragments, a major component of collagen, as a measure of increased collagenase activity. These authors observed an increase in hydroxyproline release, suggesting an increase in collagenolysis, in Graafian follicles collected at 7–12 h after an endogenous LH surge in pregnant mare serum gonadotropin (PMSG)-primed immature rats (48). Ensuing studies used various substrates, such as radiolabeled collagen, colorimetric peptides, and gelatin zymography, to examine the changes in and regulation of collagenase and gelatinase activity. The overwhelming consensus is that there is an increase in collagenase (59–63) and gelatinase (64–67) activity in intact ovaries, follicles, follicular fluid, conditioned medium from cultured follicles (49, 50, 68–71), and isolated granulosa and thecal cells (72, 73) in species such as the rat, mouse, ovine, and human. This increase in activity is dependent on an LH stimulus (59, 60, 74). As an example, there is a 4- to 5-fold increase in gelatinase (64) and collagenase activity (60) after LH/human chorionic gonadotropin (hCG) stimulation in the rat, which can be blocked by inhibiting the endogenous LH surge with Nembutal (61). In the human, gelatinolytic activity in follicular fluid increases 4-fold between menstrual cycle d 5 and 13, and there is an increase in the active form of MMP-2 during the periovulatory period (71). Similar increases in gelatinolytic activity for MMP-2 and MMP-9 have been observed in ovine follicles or follicular fluid before ovulation (67, 75).

Although there are numerous reports of LH/hCG-induced MMP activity, total enzyme activity is not always reflected by these activity assays. Analysis of enzyme activity is compromised by tissue extraction procedures, culture conditions, as well as the substrate used to determine enzyme activity. In attempting to measure MMP activity in intact tissues such as the ovary or uterus, tissue extraction procedures artificially introduce MMPs to substrates and inhibitors not found in the immediate cellular environment, resulting in a loss or diminution of activity. As an example, MMPs in the endometrium may be exposed to inhibitors present in the myometrium upon tissue homogenization, resulting in an artificial decline in their activity. Attempting to measure MMP activity *in vitro* can only be achieved by disrupting the extracellular architecture supporting the cell as well as cell-cell interactions, thereby altering normal cellular responses in addition to cellular reaction to exogenous stimuli. Finally, the ability to measure MMP activity can be substrate dependent. For instance, early studies used radiolabeled collagen as a substrate for collagenase activity. The amount of radiolabeled fragments released upon incubation with a sample was used as an index of collagenase activity. However, if the telopeptide regions that represent the cross-linking ends of the collagen molecule were present, these regions could be cleaved by nonmetalloproteinases resulting in the false interpretation of collagenase activity. Thus, the often confusing literature surrounding enzyme activity may be a reflection of technical difficulties introduced with tissue extraction, cell culture models, or the type of substrate used to determine enzyme activity.

The advent and development of molecular biology techniques have allowed the changes in mRNA expression patterns to be investigated, overcoming some of the shortfalls of enzyme activity assays. The changes in mRNA levels, for the most part, however, correspond with the changes reported for collagenase and gelatinase activity. In the rodent, for example, mRNA expression of MMP-1 (76), MMP-2 (76, 77), MMP-9 (66), MMP-13 (78), and MT1-MMP (79) all increase after the endogenous LH surge or administration of hCG to immature PMSG-primed animals similar to previous reports for enzyme activity (summarized in Table 2). Although there are many similarities in the expression patterns of the MMPs between the rat and mouse, there are differences in the expression patterns of MMP-2 and MMP-9. In the rat, MMP-2 mRNA expression is stimulated by LH, whereas MMP-9 expression is unchanged throughout the periovulatory period (77). In contrast, MMP-9 mRNA increases after a LH stimulus in the mouse, whereas MMP-2 is unchanged (66). In addition to the collagenases and gelatinases, there is a periovulatory increase in other MMP family members. Using subtractive hybridization, Espey *et al*. (80) identified a periovulatory increase in ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin-like motifs that belongs to a different metalloproteinase family, the adamalysins) that was subsequently localized to the granulosa cells of mature follicles. Hägglund *et al*. (81) explored the expression of 11 different MMPs during the periovulatory period in PMSG/hCG-treated mice and observed an increase in only MMP-19. The mRNAs for MMP-2, MMP-11, and MT1-MMP were constitutively expressed, whereas mRNAs for MMP-3, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13, and MMP-17 were not detected by Northern blot analysis of 20 μg of total ovarian RNA (81). Although the levels of MT1-MMP mRNA were unchanged, the pattern of mRNA expression displayed unique changes in cellular localization (81). Before hCG administration, MT1-MMP was present in the granulosa and thecal cell layers. After hCG treatment, expression was down-regulated in the granulosa cells of large preovulatory follicles while appearing to increase in the theca-interstitial cells surrounding the preovulatory follicle, suggesting a po-

TABLE 2. Summary of the general changes in the MMP system in the ovary during the estrous or menstrual cycle

	Ovulation	Follicular	Luteal formation	Luteal regression
MMP-1	↑ Rat (76)		nc Porcine (176)	↑ Porcine (176)
	↑ Primate (83)		nc Human (169)	nc Human (169)
MMP-2	↑ Rat (76, 77)	↑ Rat (140, 141)	↑ Rat (165–167)	nc Rat (165, 167)
	nc Mouse (66, 81)	↑ Ovine (143)	nc Bovine (168)	↑ Rat (171)
	↑ Primate (83)	↑ Porcine (75)	nc Porcine (176)	↑ Porcine (176)
	nc Bovine (82)	↑ Bovine-atresia (144)	nc Human (169)	↑ Ovine (180)
MMP-3	Ab in Mouse (81)			
MMP-7	↑ Primate (83)			
MMP-8	Ab in Mouse (81)			
MMP-9	↑ Mouse (66)	↑ Rat (140, 141)	↑ Bovine (168)	nc Rat (165)
	nc Rat (77)	↑ Ovine (143)	nc Porcine (176)	↑ Porcine (176)
	Ab in Mouse (81)	↑ Porcine (75)	nc Human (169)	
	↑ Primate (83)	↑ Bovine (144)		
MMP-10	Ab in Mouse (81)			
MMP-11	nc Mouse (81)	↑ Mouse (147)		
MMP-12	Ab in Mouse (81)			
MMP-13	↑ Rat (78)	↑ Rat (141)	nc Rat (165)	↑ Rat (165)
	Ab in Mouse (81)			
MMP-14	nc Mouse (81)	nc Rat (79, 167)	nc Rat (79)	↑ Rat (171)
	↑ Rat (79)		↑ Ovine (178)	nc Bovine-RNA (174)
	↑ Bovine (82)			↑ Bovine-protein (174)
MMP-17	Ab in Mouse (81)			
MMP-19	↑ Mouse (81)	nc Rat (personal observation)		
	↑ Rat (personal observation)			
Collagenase activity	↑ Rat (59–63)	↑ Rat (141)	↑ Rat (165)	nc Rat (165)
	↑ Ovine (49, 69, 70)	↑ Caprine (142)		
	↑ Human (68)			
Gelatinase activity	↑ Rat (64–66, 72)	↑ Rat (141)	↑ Rat (165, 166)	nc Rat (165)
	↑ Ovine (67)		↑ Human (169)	↑ Rat (163, 170, 171)
	nc Bovine (82)			↑ Human (169)
	↑ Human (50, 68, 71)			↑ Bovine (174)
TIMP-1	↑ Mouse (81, 85)	↑ Rat (149)	↑ Rat (167, 183)	↑ Rat (167, 183)
	↑ Rat (76, 86, 87)		nc Ovine (88)	↑ Mouse (172)
	↑ Ovine (88, 91, 92)		↑ Bovine (98, 186)	↓ Ovine (180)
	↑ Porcine (75, 90)		↑ Porcine (176)	Ovine ↓ early, ↑ late (184)
	↑ Primate (83)		nc Human (169, 188)	↑ Bovine (187)
	↑ Human (89)			nc Porcine (176)
	Human ↓ PCOS (135)			↓ Primate (97)
				nc Human (169, 188)
TIMP-2	nc Mouse (81, 85)	nc Rat (149)	↑ Rat (166)	↑ Rat (166)
	nc Rat (99)		nc Rat (183)	↑ Mouse (172)
	nc Ovine (100)		↑ Ovine (100)	↓ Ovine (100, 180)
	↑ Bovine (82, 98)		nc Bovine (98)	↑ Ovine (184)
	↑ Primate (83)		↑ Porcine (176)	nc Bovine (98)
			nc Human (169)	nc Porcine (176)
				nc Human (169)
TIMP-3	nc Mouse (81)	↓ Rat (149)	↑ Rat (166)	↑ Rat (183)
	↑ Mouse (85)		↓ Rat (44)	↑ Ovine (184)
	↑ Rat (44)			
TIMP-4	↓ Rat (26, 101)	↑ Rat (26)		

Representative changes in MMP and TIMP protein or mRNA during follicular growth, ovulation, luteal formation, and luteal regression. An increase in expression is indicated as ↑, a decrease in expression is shown as ↑, no change in expression is depicted as "nc", and if the MMP or TIMP has been examined but not observed, this is noted as "Ab" representing the MMP or TIMP being absent.

tential role in the ovulatory process (81). These observations underscore the importance of understanding the cellular localization pattern of the MMP system in light of the findings that the quantitative levels of MT1-MMP in whole ovarian extracts was unchanged.

The changes in mRNA expression patterns of the MMP system have been investigated in other species that display some striking similarities to the rodent but also some notable differences. For example, in the bovine the expression of mRNA for MT1-MMP and TIMP-2 was elevated at 24 h and 6 h after the preovulatory gonadotropin surge, respectively. MMP-2 mRNA was constitutively expressed, and MMP-2 activity in follicular fluid was unchanged (82). Interestingly, MT1-MMP mRNA was localized primarily to the theca before the gonadotropin surge but was increased in granulosa cells at 12 and 24 h after the induction of ovulation (82), the opposite pattern observed in the mouse (81).

In the primate, MMP expression has been studied in isolated macaque granulosa cells by RT-PCR after hCG administration (83). Expression of MMP-1 mRNA was undetectable in granulosa cells at the time of hCG administration but significantly increased by 12 h after hCG. Similarly, the

mRNA for MMP-2 and MMP-7 increased 5-fold and 12-fold by 12 h after hCG, respectively, whereas an increase in MMP-9 mRNA occurred at 36 h after hCG (83). The stimulation of a number of MMPs, as outlined in Table 2, would be expected if degradation of the components of the follicle wall, such as the various types of collagen, fibronectin, and laminin, is to take place. In the human, the majority of information about the MMPs has come from preovulatory follicular fluid and luteinizing granulosa cells from assisted reproduction techniques such as *in vitro* fertilization. It is difficult, therefore, to gain an appreciation of the dynamic changes in the MMPs during the preovulatory process in the human. Data on the changes in clinical conditions such as polycystic ovarian syndrome (PCOS) is discussed in the *Section II.A.3* on MMP regulation, whereas data on cultured granulosa-luteal cells is discussed in *Section II.C.2* in the context of the forming corpus luteum (CL).

Further evidence for an obligatory role of the MMPs in follicular rupture is forthcoming from experiments in which ovulation is blocked by exogenous chemical MMP inhibitors both *in vitro* (51, 52) and *in vivo* (59), as well as by antibodies against the MMPs (53). In the hamster, ovulation was completely blocked (100%) by incubating explanted proestrous ovaries in the collagenase inhibitor talopeptin (51). Administration of cysteine via intrabursal injection blocked ovulation and breakdown of ovarian collagen in a dose-dependent manner in proestrous rats (59). Using a perfused ovary model, Brannstrom *et al*. (52) noted that oocyte release was completely blocked or inhibited to varying degrees with the metalloproteinase inhibitors phenanthroline or SC 40827 (a Searle compound). This blockade of ovulation by phenanthroline, however, was confounded by the fact that high concentrations of phenanthroline (1 mM) also diminished progesterone secretion. The SC 40827 compound resulted in approximately a 64% reduction in oocyte release without compromising progesterone production (52). Further studies with a more selective MMP inhibitor (SC 44463) in ovaries perfused *in vitro* confirmed the role of the MMPs in follicular rupture but interestingly demonstrated a time dependence for when the inhibitor needed to be present (84). Inhibition of ovulation could be achieved by adding the inhibitor to culture medium as late as 7 h after LH (ovulation occurring at 9–12 h after LH), but there was no significant inhibition when it was added at 9 h. Butler *et al*. (84) suggested that the major collagenolytic events occur beyond 7 h after stimulation by LH. Although these observations suggest that follicular rupture requires focal degradation of the apical ECM, the crucial MMP(s) involved and their interplay in follicular rupture remains to be fully understood. In the ewe, injection of a bioactivity-neutralizing MMP-2 monoclonal antibody into the antral cavity of preovulatory follicles resulted in the formation of luteinized unruptured follicles (53). These unruptured follicles lacked the hallmarks of early luteal formation such as the numerous connective tissue projections that provide a framework for cellular migration and angiogenesis (53). The findings of Gottsch *et al*. (53) would suggest that MMP-2 is a pivotal MMP in ovulation and luteal formation in the ewe.

2. TIMPs in the ovulatory process. In conjunction with the periovulatory increase in MMPs, LH/hCG stimulates an increase in mRNA expression and inhibitor activity for some of the TIMPs (Table 2). TIMP-1 mRNA expression increases after an LH stimulus in the mouse (81, 85), rat (76, 86, 87), ewe (88), nonhuman primate (83), and human (89). Associated with the increase in TIMP-1 mRNA levels is an increase in TIMP-1 protein in preovulatory follicles, especially the granulosa cell compartment (90–92). In conjunction with the rise in TIMP-1 mRNA and protein, there is an increase in inhibitor activity in whole ovaries (86, 93), isolated granulosa cells (86, 94), and follicular fluid (75, 95). The inhibitor activity reflects TIMP-1 (75) or TIMP-like activity (75, 86, 93, 95) as well as activity from the macroglobulin class of inhibitors (89, 93, 95).

The periovulatory increase in TIMP-1 concomitant with the LH-induced increase in MMPs would at first appear paradoxical. One would anticipate that for proteolytic breakdown of the follicular wall to occur, inhibitor expression and activity would decrease as MMP expression and activity increased. However, parallel regulation of MMPs and their inhibitors has been reported in numerous tissues and has been postulated to maintain proteolytic homeostasis (1, 2, 6). Thus, the periovulatory increase in TIMPs may act to precisely coordinate the actions of MMPs, regulating the location and extent of ECM remodeling of the follicular apex during the ovulatory process (discussed in detail in *Section II.A.3* and in Fig. 4). Alternatively, the stimulation of TIMP expression and activity may play an autocrine/paracrine role in ovulatory processes involving cellular proliferation, differentiation, or steroidogenesis as noted previously. Of particular interest is the observation that TIMP-1 mRNA localization switches from a thecal origin to a granulosa cell-derived pattern in preovulatory follicles of rat and marmoset

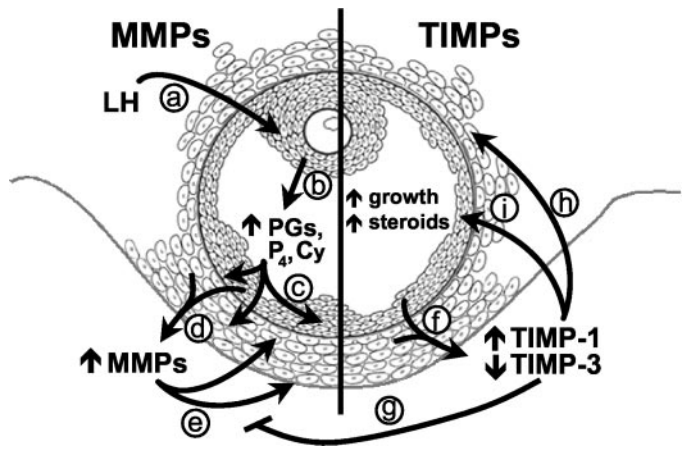

FIG. 4. A working model for the MMP system in the ovulatory process. LH initiates a series of biochemical events (path a) that stimulate progesterone (P_4), PGs, and cytokines (Cy; path b). These mediators act on the granulosa and theca (path c) to induce the MMPs (path d) and the TIMPs (path f). MMP action would degrade the follicular apex (path e) allowing oocyte release. The TIMPs may act to control MMP action at the follicular apex (path g) while protecting the basal portion of the follicle (path h). Additionally, the TIMPs may be involved in stimulating cellular proliferation, neovascularization, or steroidogenesis as the postovulatory follicle remodels to form the CL (path i).

ovaries, suggesting a potential functional change in the action or role of TIMP-1 (96, 97).

For the other members of the TIMP family, there are differences and/or conflicting reports on the periovulatory mRNA expression patterns (Table 2). For TIMP-2, the mRNA has been reported to increase after a LH/hCG stimulus in bovine periovulatory follicles (98) and nonhuman primate granulosa cells (83) but is unchanged in mouse (81, 85) and rat ovaries (99) as well as ovine preovulatory follicles (100). These differences may be species specific, tissue specific, or they may reflect the more sensitive detection of TIMP-2 mRNA by RT-PCR in nonhuman primate granulosa cells *vs.* Northern blot analysis used in the other studies. For TIMP-4, there are reports of a trend toward a decrease in TIMP-4 mRNA and protein expression in ovaries from immature gonadotropin-primed rat (26, 101). After an ovulatory dose of hCG, both TIMP-4 mRNA and protein decreased about 30% (26), whereas Udoff *et al*. (101) reported approximately a 65% decline in TIMP-4 mRNA by 4 h post hCG. For TIMP-3, there are reports that the mRNA for this inhibitor increases before the LH surge during early proestrus in cycling mice (85) and rats (44) but is unchanged or slightly decreases after hCG administration to PMSG-primed immature mice (81) and rats (99). Driancourt *et al*. (75) demonstrated that not only are there differences in expression of the MMPs and TIMPs between species, there are also distinct patterns of expression in different breeds within species. In porcine follicular fluid collected from the Large White breed, levels of TIMP-1 protein increased after hCG administration, whereas TIMP-1 was unchanged in follicular fluid from the Meishan breed. Follicular fluid levels of TIMP-2 were higher in follicles from the Large White breed, yet an increase after hCG was only observed in follicles from the Meishan breed. Similarly, there were distinct differences in MMP-9 activity between the different breeds. Follicular fluid levels of MMP-9 activity increased 7-fold after hCG administration to the Meishan breed, whereas MMP-9 activity was only 1.5-fold higher in the follicular fluid of the Large White breed (75). Thus, some of the differences in expression patterns of the MMPs and TIMPs may be species specific, breed specific, cell specific (*e.g*., isolated granulosa cells *vs.* intact ovaries), or they may reflect different model systems such as naturally cycling *vs.* gonadotropin-primed animals. Disparity in expression patterns may also be manifest in the means of detection such as Northern blot analysis compared with RT-PCR.

3. Regulation of the MMP system during ovulation. The regulation of the MMP system is extremely complex. Signals that may coordinately regulate one MMP may differentially regulate other MMPs. Likewise, signals that up-regulate a certain MMP in one setting may have no effect or even an opposite effect depending on the absence or presence of other signals, hormones, *etc*. Irrespective, it is readily apparent that those members of the MMP system that increase during the periovulatory period do so through the cascade of events initiated by LH. For example, blocking the endogenous surge of LH with Nembutal or phenobarbital inhibits the increase in MMP-13 mRNA as well as collagenase activity (59, 60, 74, 77, 102). Recent and current investigations have begun to examine many of the potential signal cascade pathways mediated by LH, such as progesterone, PGs, and cytokines, and their role in regulating the periovulatory increase in the MMP system. *Section II.B* will cover some of these findings, with more detailed information on regulation at the level of transcription below (*Section III.B.3*).

The LH surge stimulates an increase in progesterone and progesterone receptor induction that is critical for the ovulatory process. The pivotal role of progesterone in follicular rupture is supported by studies in which ovulation is compromised when progesterone synthesis is inhibited (reviewed in Ref. 45), progesterone action is blocked by treatment with the progesterone antagonist RU486 (62, 103–106), or progesterone receptors are lacking (107). A role for progesterone in regulating the MMP system during the ovulatory process is forthcoming from reports that: 1) progesterone increases the distensibility of porcine follicles (108), 2) progesterone stimulates collagenase-like activity in ovine and hen follicular tissue (109, 110), 3) inhibition of progesterone synthesis or blocking progesterone action with RU486 decreases MMP activity in the rat (62) and ewe (109), 4) RU486 decreases inhibitor activity in human and rat granulosa cells (111), and 5) mice lacking progesterone receptors have a decrease in ADAMTS-1 and a lysosomal cysteine protease, cathepsin L (66). In the primate, treatment with the 3β-hydroxysteroid dehydrogenase inhibitor trilostane decreased the mRNA expression of MMP-1, MMP-9, MMP-7 (matrilysin), TIMP-1, and TIMP-2. Administration of exogenous progesterone to trilostane-treated macaques restored expression of MMP-1 and TIMP-1 to control levels (83). Similar findings that progesterone regulates the MMP system were observed in the ewe. Administration of an inhibitor of progesterone synthesis (isoxazol) injected into the follicular antrum of ewes blocked the LH-induced increase in collagenase activity, which could be restored by exogenous administration of progesterone and, interestingly, $PGF_{2\alpha}$ (109). These observations provide strong support that the proteolytic cascade associated with ovulation is regulated, in part, by progesterone.

Another potential LH-stimulated regulator of the MMPs system is PGs. The involvement of the preovulatory increase in PGs in follicular rupture is well documented using experimental models in which PG synthesis is inhibited (reviewed in Ref. 45) or PG synthase is absent (such as knockout models; Ref. 112). Inhibition of PG synthesis blocks the periovulatory increase in the mRNA for MMP-1 and MMP-13 as well as collagenase activity without affecting the gelatinases (76, 77, 102, 109, 113). In the ewe, inhibition of the preovulatory increase in PGs by administration of indomethacin suppressed the periovulatory rise in collagenase activity at 24 h after a LHRH stimulus. This suppression of collagenolysis could be restored with exogenous PGE or $PGF_{2\alpha}$ (109). In the rabbit, administration of indomethacin altered the pattern of collagenase activity associated with follicular rupture (113). Specifically, the periovulatory increase in neutral collagenase activity [as determined by dinitrophenyl peptide (DNP)-peptidase activity] was blocked. However, the periovulatory increase in acidic collagenase activity (as measured by α-*N*-benzoyl-DL-Arg-β-naphthylamide hydrolase activity) was elevated to the same extent in the presence of indomethacin, but the peak of activity was delayed 3–4 h

(113). In the human, addition of PGE_2 or $PGF_{2\alpha}$ to cultures of the follicle wall was without effect on DNP-peptidase activity (an index of collagenolysis), whereas both relaxin and oxytocin stimulated DNP-peptidase activity (114). Although administration of indomethacin to inhibit PG synthesis blocked the increase in MMP-13 mRNA in the rat (76, 77), indomethacin did not inhibit the preovulatory increase in the activity of an enzyme extracted in a latent form that was capable of degrading radiolabeled collagen (61). The significance of an increase in latent collagenolytic activity despite the fact that MMP-13 mRNA does not increase is unclear. The possibility exists, however, that the latent enzyme may require a PG-mediated step for activation or that the radiolabeled activity assay is detecting a fibrillar collagenase other than MMP-13. For the TIMPs, blockage of PG production does not effect the mRNA expression patterns for TIMP-1, TIMP-2, and TIMP-3 in intact rat ovaries (77), nor did indomethacin administration effect production of MMP inhibitor activity in conditioned medium from rat granulosa cells (94).

In addition to progesterone and PGs, LH stimulates an increase in cytokines, such as IL and TNFα, which may act independently or in concert with other signal pathways to regulate MMPs and inhibitors. The ovary has a complete, compartmentalized, and hormonally regulated IL-1 system that has been postulated to play a role in ovulation (115, 116). The preovulatory LH surge increases levels of IL-1 (117, 118), IL-1 stimulates PG biosynthesis (119), IL-1 induces ovulation in perfused ovaries (120), an IL-1 receptor antagonist inhibits oocyte release (121), and IL-1 stimulates MMPs in numerous tissues (122, 123). Support for the concept that ILs may regulate the ovarian MMP system is forthcoming from studies by Hurwitz *et al*. (65) who demonstrated that IL-1 stimulated MMP-9 activity. In these experiments, addition of IL-1β to enriched theca-interstitial cultures from immature rats increased MMP-9 activity in a dose-dependent manner that could be blocked with an IL-1 receptor antagonist (65). IL have also been reported to stimulate MMP inhibitor activity. Addition of IL-1β to rat granulosa cells in the absence of LH results in an increase in inhibitor activity in the conditioned medium; however, in the presence of LH there is no further augmentation by IL-1β (124).

Similar to the IL, TNF-α has been shown to be present in the ovary (125) and stimulate the events associated with ovulation (126, 127). Induction of ovulation in the ewe was associated with an elevation in TNF-α that was related to an increase in the activity of collagenase and MMP-2 as well as collagen breakdown (67, 70). Further support for this association of TNF-α and induction of the MMP system is evident from experiments in which TNF-α addition to follicular tissues stimulated collagenase and MMP-2 activity, and this action could be blocked by antibodies to TNF-α (67, 70). Murdoch (127) postulated that the preovulatory gonadotropin surge stimulates TNF-α production by the oocyte cumulus complex, which in turn diffuses to the follicular wall to increase the activity of the MMPs. The increase in MMPs would bring about breakdown of collagen in the theca and tunica albuginea, facilitating oocyte release as well as assisting in luteal formation. However, there may be species differences in the role of TNF-α in the ovulatory process because addition of TNF-α to perfused rat ovaries inhibited steroidogenesis and ovulation (128).

In addition to many of the classical LH-mediated pathways, there are reports of other periovulatory regulators of the MMP system such as relaxin and prolactin (PRL). For example, intrabursal injection of an antibody to relaxin resulted in a decrease in the number of oocytes released (72), whereas administration of relaxin to isolated rat theca or granulosa cells stimulated gelatinolytic activity (72). Similarly, addition of relaxin to cultured pieces from the wall of human follicles or rat granulosa cells results in an increase in collagenase or proteoglycanase activity (114, 129). In the horse, relaxin treatment of ovarian stromal cells increased the activity of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the conditioned medium (130). These findings would suggest that relaxin may facilitate ovulation by modulating the degradation of ECM in the granulosa and thecal compartments.

In certain species, there is a periovulatory increase in PRL. Initially, Hirsch *et al*. (131) reported that PRL does not regulate the preovulatory increase in collagenase activity. In this experiment, surgical disruption of the LH surge in rats that had a normal proestrous PRL surge did not exhibit an increase in collagenase activity. More recently, Hirsch *et al*. (132) reported that PRL modulates MMPs in studies investigating the changes in mRNA expression of MMP-1 and TIMP-1 in rats in which the preovulatory PRL surge was blocked by bromocryptine for three consecutive estrous cycles. Bromocryptine treatment inhibited the preovulatory increase in MMP-1 and TIMP-1 mRNA that could be restored by exogenous PRL. Similarly, exogenous PRL treatment to cultured rat granulosa cells resulted in an increase in TIMP-1 mRNA as well as inhibitor activity in the conditioned medium (87). These findings would support a role for the stimulation of the MMP system by the preovulatory PRL surge. However, there may exist a delicate equilibrium between the levels of PRL and the regulation of the MMP system. For instance, sulpiride-induced hyperprolactinemia inhibits hCG-induced ovulation in rabbit (133). Associated with this inhibition of follicular rupture is a decrease in progesterone as well as collagenase activity as determined by DNP-peptidase and α-*N*-benzoyl-DL-Arg-β-naphthylamide hydrolase assays (133).

In the human, alteration of the hormonal milieu results in changes in the ovarian MMP system. PCOS is associated with anovulatory infertility, hyperandrogenism, decreased insulin sensitivity, and an increase in the ratio of LH to FSH, although the primary etiology remains enigmatic. Fluid from preovulatory follicles of women with PCOS contained elevated levels of MMP-2 and MMP-9 activity (134). Cultured granulosa-lutein cells from follicles of PCOS women produced more MMP-2 and MMP-9 protein and contained elevated activity compared with cells from normal ovulatory women. Levels of TIMP-1 protein meanwhile were unchanged (134). In contrast to these findings, Lahav-Baratz *et al*. (135) did not observe any differences in the activity of MMP-2 or MMP-9 or the content of MMP-1 in follicular fluid from PCOS patients. The levels of TIMP-1 protein, however, were decreased in the PCOS population. The differences in these studies may be related to the follicle selected for analysis (135). Irrespective of the differences in the findings be-

tween the two laboratories, both groups of investigators observed changes in the ratio of the MMPs to their inhibitors and proposed that the MMP/TIMP balance is shifted toward an increase in MMP activity in women with PCOS that may compensate for the physical properties of the thickened ovarian capsule associated with PCOS (134, 135).

Many of the previous studies have attempted to explore the changes in the patterns of mRNA expression and activity in intact follicles or ovaries. Although such information is critical to our understanding of which MMPs may be important in the ovulatory process, such data in intact tissues do not elucidate the site of action of the MMP system. This has begun to be addressed by studies exploring the cellular localization of mRNA and protein for the MMPs and TIMPs. There is, however, a delicate balance between MMP action and inhibition by the various TIMPs that cannot be fully appreciated by documenting the presence of mRNA or protein. Studies have attempted to understand this equilibrium by examining the active cellular localization of ECM degradation using techniques such as *in situ* fibrinolysis or *in situ* zymography. With *in situ* fibrinolysis, ovarian sections were overlaid on slides coated with fibrin, and then zones of fibrin degradation were identified during ovulation in the rat (136). In these studies, fibrin degradation was first observed at the apex, peaked at 2 h before ovulation, but was not present in the follicular cavity (136). The majority of this activity, however, may reflect a serine proteinase because the activity was absent when fibrin slides were incubated with a serine proteinase inhibitor (136). Our laboratory has used the technique of *in situ* zymography to determine the ovarian compartments in which type IV collagen, a major component of the granulosa cell and germinal epithelial cell basement membranes, is degraded during the periovulatory period. *In situ* zymography uses fluorescently labeled substrates that are activated upon cleavage to allow the union of enzyme activity and localization to provide insight into the activity at a cellular level. In these studies, degradation of a type IV collagen substrate results in an increase in fluorescence at the apex of preovulatory rat follicles at 12 h after hCG (Fig. 5). These findings would suggest that, although both the MMPs and TIMPs are stimulated by the preovulatory gonadotropin surge, the balance is tipped in favor of the MMPs at the follicular apex.

In summary, the LH surge induces an increase in the expression and activity of specific MMPs during the ovulatory process. As a generalized schematic model, LH (or ad-

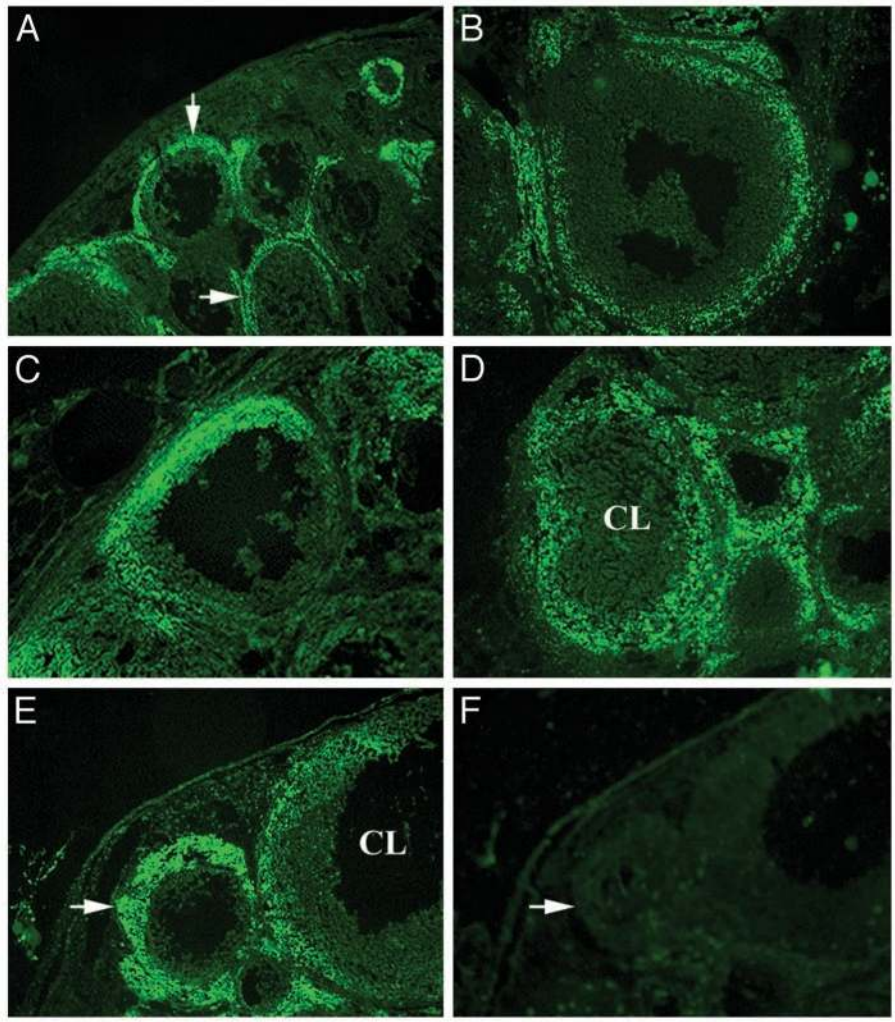

FIG. 5. *In situ* zymography of gelatinase activity in the rat ovary. Representative photomicrographs of gelatinolytic activity during follicular growth, the ovulatory period, and luteal formation. Gelatinolytic activity is indicated by regions of intense fluorescence. Gelatinase activity was observed predominately in the theca encompassing developing follicles at the time of gonadotropin (PMSG) administration (A, *arrows*). In a developing preovulatory follicle, intense fluorescence was observed surrounding the follicle primarily in the thecal layer (B). At 12 h after hCG, gelatinase activity was present in the apical region of the preovulatory follicle (C). In the forming CL, fluorescence was observed throughout the CL (D and E). Adjacent sections were incubated in the absence or the presence of EDTA to block gelatinase activity. Intense fluorescence observed encircling the follicle (E, *arrows*) was absent in the sections incubated in EDTA (F). Original magnification, ×100. [Reproduced with permission from T. E. Curry *et al.*: *Biol Reprod* 65:855–865, 2001 (96).]

ministration of exogenous hCG to mimic LH action) would initiate and synchronize a series of biochemical events such as an increase in progesterone, PGs, and cytokines (Fig. 4). In turn, these LH-stimulated mediators would induce the expression and activity of the MMPs and the TIMPs. This postulate is supported by the findings that the LH surge induces an increase in the mRNA for the collagenases (MMP-1, MMP-13), the gelatinases (MMP-2, MMP-9), MMP-19, and ADAMTS-1 in a temporal pattern consistent with breakdown of the follicular wall in the rat (59, 60, 64, 76, 77), mouse (81), ewe (109), and nonhuman primate (83) during the follicular rupture. The MMPs would degrade the apical follicular connective tissue (Fig. 4), thereby facilitating oocyte release (45). The TIMPs may act to control the extent and location of MMP action, or the TIMPs may be involved in stimulating cellular proliferation, neovascularization, or steroidogenesis as the ruptured follicle remodels to form the CL (Fig. 4).

B. Follicular development

Although the proposal that the MMP system plays a role in ovarian function was initially investigated during the periovulatory period, there is considerable evidence that the MMPs and TIMPs function during follicular growth. During the development of the ovarian follicle there is extensive cellular proliferation and remodeling of the ECM as the follicle grows and differentiates along the path from a small primordial follicle to a large preovulatory Graafian follicle (137, 138). This process is characterized by proliferation of the granulosa cells, differentiation of the granulosa and thecal compartments, and the deposition of a basement membrane separating the avascular granulosa cells from the theca cells. The thecal cell layer, unlike the granulosa compartment, is well vascularized and contains circumferential collagen bundles. As the follicle grows and develops, the granulosa cells secrete a mucopolysaccharide-rich fluid that coalesces to form the antral cavity in the secondary follicle. With continued growth, the resultant mature follicle is approximately 400-fold larger than the initial primordial follicle and rests in an extracellular environment of collagen, laminin, proteoglycan, and fibronectin (137, 138). This extracellular environment undergoes cyclic changes in its composition. In the mouse, Oksjoki *et al*. (139) observed cyclic expression patterns of the mRNAs encoding type III, IV, and VI collagens as well as the proteoglycans biglycan, syndecan-1, and osteonectin, suggesting that the ovarian ECM changes during follicular growth as well as luteal formation and regression.

1. Metalloproteinases during follicular growth. The extensive changes in the ovarian extracellular environment during folliculogenesis have been postulated to occur, in part, by the actions of the MMP system. Although a definitive causative effect has yet to be demonstrated, there is correlative data implicating the MMP system in follicular development. For example, the patterns of cellular localization and expression of the MMP system change in concordance with the dynamic ovarian structural changes associated with follicular growth (summarized in Table 2). In the rat, Bagavandoss (140) noted that the pattern and extent of MMP-2 and MMP-9 immunolocalization was markedly increased at the latter stages of follicular development. In these experiments, MMP-9 immunostaining in the neonatal rat ovary was absent, whereas MMP-2 immunoreactivity was observed in the granulosa cells and the surface epithelium during early follicular development. Administration of PMSG to induce follicular growth resulted in an increase in the cellular expression of MMP-2 and MMP-9 compared with ovaries from neonatal rats. After PMSG, immunoreactive MMP-2 was present in the granulosa and theca of preovulatory follicles, whereas MMP-9 was observed in the thecal and interstitial tissue (140). The increase in immunoreactive MMPs during the latter stages of follicular growth corresponds with an increase in mRNA expression patterns as well as an increase in gelatinolytic and collagenolytic activity (141) after PMSG administration to immature rats. The levels of mRNA for MMP-2 and MMP-9 in whole ovaries increased within 12–24 h after PMSG administration, and the mRNA expression for MMP-13 increased at 36–48 h after gonadotropin treatment (141). Gelatinolytic and collagenolytic activity was elevated at 36–48 h after PMSG stimulation (141). Localization of MMP-2 and MMP-9 transcripts during induction of follicular growth by PMSG administration revealed MMP-2 and MMP-9 mRNA in the theca of developing follicles as well as in the stroma (96). Although the mRNA and activity increased in whole ovarian extracts during follicular growth and the cellular localization of MMP-2 and MMP-9 corresponds with sites that must be remodeled as the follicle grows, definitive proof that indeed there is cellular activity of the MMPs in the appropriate sites to allow follicular remodeling has been lacking. Using *in situ* zymography to explore the cellular activity of the MMPs, initial findings have demonstrated a pattern of gelatinolytic activity that corresponds with the localization of MMP-2 and MMP-9 mRNA around developing follicles (Fig. 5 and Ref. 96), further supporting a role for the MMP system in follicular growth.

Changes in the MMPs have been reported during follicular growth in species other than the rodent. These changes in MMP expression during follicular differentiation are summarized in Table 2. In the goat, collagenase activity increased with increasing follicular size (142) as also reported in the rat (141). Ovine follicular fluid contains both MMP-2 and MMP-9 activity (143), whereas only MMP-9 activity was observed in porcine follicular fluid (75). In bovine follicular fluid collected from follicles between 2 and 20 mm in diameter, pro-MMP-2 activity was present and elevated in atretic follicles, whereas MMP-9 activity was elevated in follicles larger than 25 mm in diameter (144). These observations led Imai *et al*. (144) to suggest that MMP-2 could be a marker for follicular health, whereas MMP-9 may reflect the presence of a follicular cyst. In the horse, MMP-2 and MMP-9 activity was reported in the conditioned medium from cultured equine ovarian stromal cells (130). Although the role of MMPs during follicular growth in the human has received limited attention, we have observed expression of MMP-2 mRNA encircling small developing follicles (our unpublished observation), suggesting a similar pattern of expression in the human. Postawski *et al*. (145) also observed an abundance of MMP-2 activity compared with MMP-9 activ-

ity in human ovarian samples. In these studies, collagen content was decreased, whereas collagenase activity was increased 2-fold in the apical tunica albuginea compared with areas of the ovarian capsule that were devoid of follicles (145). In contrast, the levels of MMP-1 protein were decreased in the apical wall of atretic follicles compared with areas devoid of follicles (146), suggesting that MMP-1 regulates ECM remodeling in the tunica albuginea associated with follicular growth in the human. Thus, it is readily apparent that in numerous species the MMPs are present within the follicle, are stimulated by the events associated with follicular development, and that their localization and expression patterns coincide with the changes in the granulosa and thecal cell layers that occur during follicular remodeling. Of particular interest is the proposal by Huet *et al*. (143) that the MMPs may play a role not only in follicular growth but also in follicular atresia. In the sheep, hypophysectomy resulted in an increase in MMP-2 and MMP-9 activity in the follicular fluid concomitant with the induction of follicular atresia (143). Hägglund *et al*. (147) noted an association between expression of MMP-11 (stromelysin-3) and atresia in the mouse. This MMP, however, does not appear to be obligatory for initiation or completion of the atretic process because atresia appears normal in mice lacking MMP-11. MMP-11 mRNA is found in the oocytes of small follicles and this MMP is lost as the follicles grow in the freshwater teleost medaka fish (148). *In toto*, the observations that MMPs are stimulated during induction of follicular growth, that mRNA is present in the theca surrounding the follicle, and that gelatinolytic activity is detected around growing follicles supports a role for the MMPs in follicular growth.

2. TIMPs during follicular growth. The emerging data for TIMP mRNA expression patterns during follicular growth suggest that the expression of TIMP-1 parallels the change in MMPs (Table 2). In the rat, TIMP-1 mRNA levels increase after PMSG administration (149), as does mRNA expression for certain members of the MMP family (141). For the other TIMPs, PMSG treatment of immature rats results in no change in TIMP-2 mRNA or a slight decline in TIMP-3 mRNA levels (149). However, the mRNA and protein for TIMP-4 increase after gonadotropin stimulation (26). Thus, the parallel regulation of MMPs and TIMP-1 and TIMP-4, as well as the basal expression of TIMP-2 and TIMP-3, may act to maintain a proteolytic balance during follicular growth that provides localized control of ECM degradation, thereby regulating the location and extent of follicular remodeling.

The localization of TIMP mRNA and TIMP protein has begun to be elucidated during follicular growth. In the rat, the mRNAs for TIMP-1, -2, and -3 were localized to the stroma and theca of developing follicles, although the pattern of expression in the theca varied depending upon the inhibitor. For TIMP-1, mRNA was present in the outer layers of the theca, whereas TIMP-2 and TIMP-3 were present throughout the theca (96). In the granulosa cell layer, expression of the mRNA for TIMP-1 and TIMP-2 was at low levels, in some cases equivalent to that of the background (44, 96). Regardless of this lack of *in situ* reaction product, granulosa cells of rat antral follicles contain TIMP-1 mRNA as demonstrated by laser capture microdissection of the granulosa cell layer and RT-PCR (44). Of physiological interest was the observation that TIMP-3 mRNA was present in the granulosa cells of certain follicles but was absent in granulosa cells of adjacent follicles. Although the significance of this differential expression pattern of TIMP-3 is unclear, Simpson and colleagues (44) demonstrated low levels of TIMP-3 mRNA expression in granulosa cells of healthy follicles but a lack of TIMP-3 mRNA in adjacent atretic follicles (Fig. 6). With continued follicular development, TIMP-3 mRNA becomes highly expressed in the granulosa cells of healthy preovulatory follicles (96). For TIMP-4, the levels of mRNA are extremely low, although expression was reported in the theca in the rat ovary (26) and in the oocyte, granulosa, and theca of follicles in the mouse (27). In conjunction with the changes in mRNA, ovarian TIMP-1 protein has been identified in the rat by immunohistochemistry. In 7-d-old neonatal ovaries, TIMP-1 immunofluorescence was found in the oocyte cytoplasm. In ovaries from immature rats treated with PMSG, TIMP-1 immunostaining was localized to the theca, ovarian surface epithelium, and interstitial blood vessels (140) similar to localization of TIMP-1 mRNA (44, 96, 150).

Distinct differences have been reported in the cellular localization patterns of the TIMPs between the rat and the mouse. Using colorimetric *in situ* hybridization, Inderdeo *et al*. (85) observed TIMP-1 mRNA in the oocyte of developing follicles in ovaries collected from adult mice at 1 d postpartum. These investigators did not detect TIMP-1 mRNA in the theca or granulosa cells at any stage of development. In contrast, TIMP-1 mRNA was localized to the oocyte, theca, and granulosa cells of small and large follicles in the rat (44, 96, 150). These species differences are also apparent in the ewe where immunoreactive TIMP-1 was present in the oocyte, theca, and granulosa cell layers of all healthy nonpreovulatory follicles with a greater intensity in the basal granulosa layer (91). There was no difference in the staining pattern associated with the different stages of atresia (91). The pattern of TIMP-1 mRNA expression in the nonhuman primate, however, is similar to that described in the rat in certain aspects, but unique differences are also apparent. Duncan *et al*. (97) observed that follicles smaller than 200 μm in diameter did not contain TIMP-1 mRNA, but with continued follicular growth TIMP-1 mRNA expression was present in the theca but absent in the granulosa compartment. With the onset of atresia, TIMP-1 mRNA was intensely expressed at the granulosa-thecal interface (97).

Further understanding of the cellular localization of MMP and TIMP production has been forthcoming from cell isolation and culture experiments. *In situ* hybridization has revealed TIMP-1 mRNA present in the germinal epithelium of the rat ovary (44, 96, 150); however, nonspecific *in situ* reaction product can become trapped at this epithelial layer interface between the ovarian tissue and the microscope slide, leading to erroneous interpretation of epithelial edge effects (151). Vigne *et al*. (152) have convincingly demonstrated that indeed the germinal epithelium is a source of TIMP-1 production in cultured cells from the bovine ovary. In addition to the germinal epithelium, TIMP-1 has been shown to be secreted by ovine granulosa cells (153), porcine granulosa cells (90), and equine ovarian stromal cells (130) *in vitro*. Shores and Hunter (154) reported on the relative dis-

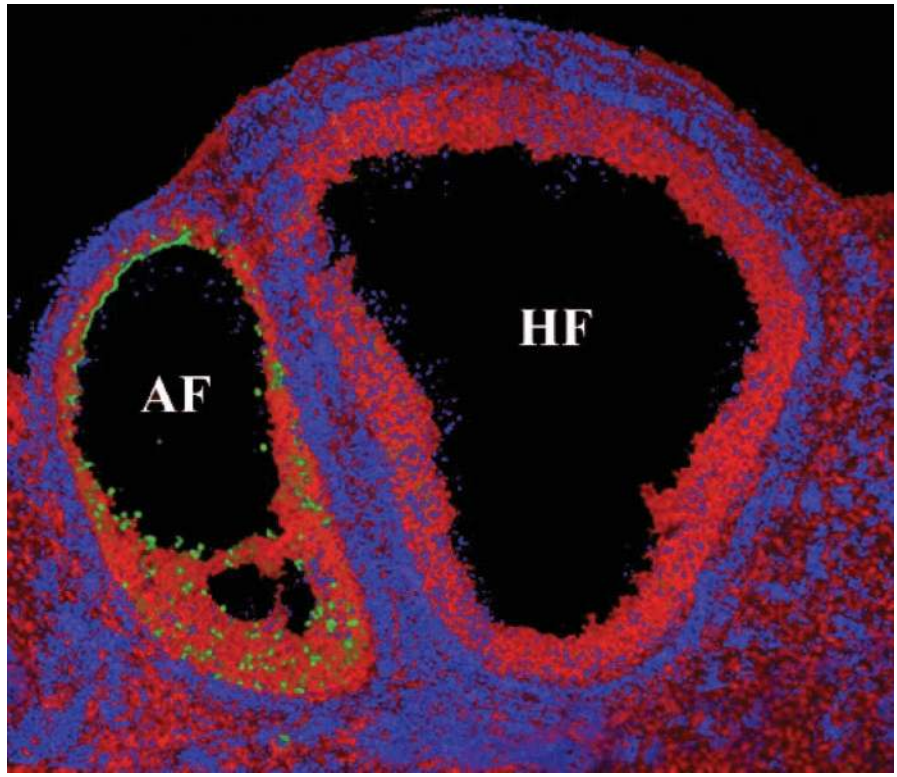

FIG. 6. Tricolor composite photomicrograph of TIMP-3 *in situ* hybridization, DNA fragmentation, and cellular DNA. *In situ* reaction product for TIMP-3 mRNA in a rat ovary collected at proestrus is represented by the blue reaction product. DNA fragmentation of apoptotic nuclei fluoresce green, whereas cellular DNA was stained with the red fluorescent dye, propidium iodide. An atretic follicle (AF) is identified by the high level of green fluorescence in the granulosa cells denoting apoptosis. A healthy follicle (HF) did not exhibit DNA fragmentation. *In situ* localization exhibited a higher level of TIMP-3 mRNA in the granulosa cells of the healthy follicle. Magnification, ×55. [Adapted with permission from K.S. Simpson *et al*.: *Endocrinology* 142:2058–2069, 2000 (44). © The Endocrine Society.]

tribution of TIMP-1, TIMP-2, and TIMP-3 protein in ovine follicular fluid, granulosa cells, and theca cells. The concentration of the different TIMPs was tissue type dependent, with the highest concentrations in the theca, and there was an interaction between the size of the follicle and the tissue distribution for TIMP-1 and TIMP-2 (154).

Although the definitive role of the MMP system in follicular growth awaits discovery, it is readily apparent that the MMPs and TIMPs are in the appropriate cellular compartments, are regulated by the hormonal signals that stimulate follicular development, and are associated with an increase in follicular growth in various species. A working model for the ovarian MMP system in follicular growth would encompass the precise coordination of MMPs and inhibitors to regulate remodeling of the ECM during growth of the ovarian follicle (Fig. 7). Theoretically, factors that stimulate follicular development, such as FSH, would act on the granulosa or theca cell compartments to increase the MMPs and regulate TIMP expression. The MMPs would facilitate remodeling of the granulosa cell basement membrane as well as the thecal ECM and allow follicular expansion. The MMPs may act directly to control ECM remodeling or they may have indirect actions to stimulate follicular growth. For example, the MMPs are known to activate growth factors and cytokines as well as cleave IGF binding proteins; thus, the MMPs may act to regulate the availability of growth factors and cytokines, thereby impacting follicular growth (8). The TIMPs may provide control for the extent of MMP action. However, the concept that MMP inhibitors exist only to regulate ovarian proteolysis may be overly simplistic. Ovarian TIMPs may function independently of their inhibitory capacity as noted previously and act as autocrine/paracrine

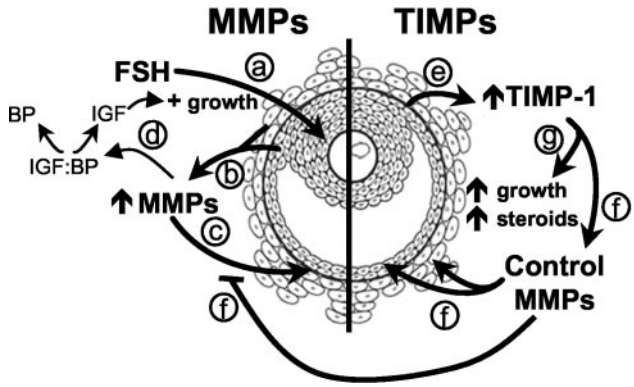

FIG. 7. A working model for the ovarian MMP system in follicular growth. FSH would stimulate granulosa cells (path a), which in turn stimulates MMP (path b) and regulates TIMP (path e) expression in the granulosa and theca, potentially through paracrine mechanisms. The MMPs would facilitate remodeling of the thecal ECM and expansion of the basement membrane as the granulosa cells proliferate (path c). In addition to directly controlling ECM remodeling, the MMPs may act to regulate the availability of growth factors, cytokines, *etc*. For example, MMPs could cleave IGF binding proteins (IGFBP; path d) releasing IGFs to further enhance follicular growth. The TIMPs may provide control for the extent of MMP action (path f). However, ovarian TIMPs may be multifunctional, controlling follicular proteolysis (path f) as well as stimulating cellular proliferation, differentiation, neovascularization, or steroidogenesis during follicular growth (path g).

factors in cellular proliferation, differentiation, neovascularization, and/or steroidogenesis during follicular growth. Such a concept has led Garcia *et al*. (142) to propose that the MMP system may regulate normal follicular maturation and

atresia to achieve the appropriate number of ovulatory follicles.

C. Corpus luteum

The CL is formed by extensive cellular reorganization and neovascularization of the postovulatory follicle. During this reorganization, the antral cavity of the ruptured follicle is infiltrated by thecal cells, granulosa cells, fibroblasts, and blood vessels (155–157). Species differences exist in the degree of reorganization and intermixing of the theca and granulosa cell layers during luteal formation. In many nonprimate mammals, luteal formation is characterized by extensive cellular migration and reorganization of both the theca and granulosa cell layers, which results in a complete intermixing of the two cell types throughout the CL. This intermixing of the different follicular cells during luteal formation is less marked in many primates than in other species. This results in the formation of CL with a distinct granulosa-lutein and theca-lutein layer (158). In conjunction with these cellular changes, there are significant changes in the ECM of the forming CL. For example, the type of collagen present in the basement membrane of the follicle (type IV) is replaced by a fibrillar (type I) collagen that comprises a major component of the CL (159). This collagen component comprises up to one sixth of the luteal weight in the mature bovine CL (159), reflecting the importance of the ECM in the overall structure of the CL.

In addition to the structural alterations that take place during early luteal formation, functional changes occur throughout the lifespan of the CL. As the CL forms, progesterone production dramatically increases and becomes maximal during the midluteal period. This is accompanied by a period in which negligible changes take place in the luteal connective tissue matrix. At the end of the lifespan of the CL, two phases of luteolysis occur. The first phase is a functional luteolysis that is characterized by a rapid decline in progesterone production and secretion. The second phase is a slower, prolonged structural luteolysis of the CL. This structural regression has been postulated to occur in part by an apoptotic mechanism (160–162) and in part by proteolysis and destruction of the luteal ECM by MMPs (163). This destruction of the luteal extracellular scaffolding may involve invading macrophages. Macrophages increase in number in the involuting CL, contain MMPs, and can stimulate MMP production in other tissues, thereby supporting an active role for macrophages in the process of luteal matrix degradation and structural regression (160, 164).

1. Metalloproteinases during the luteal period. The MMP system has been explored in all three phases of the luteal cycle: formation of the CL, the midluteal period, and luteal regression (Table 2). During luteal formation, many of the MMPs are elevated in the rat (165–167), bovine (168), and human (169). In the rat, the mRNA expression and activity for the gelatinases increases during luteal development after induction of pseudopregnancy (165) as well as during pregnancy (166, 167). Concomitant with the increase in gelatinase in whole ovarian extracts, *in situ* zymography has revealed gelatinolytic activity throughout the forming CL but with higher levels of expression in the peripheral regions of the forming CL (Fig. 5) where intermixing of the granulosa and theca are taking place (96). Interestingly, collagenolytic activity is elevated during luteal formation in ovarian extracts from pseudopregnant rats without concomitant changes in MMP-13 mRNA expression (165), implying that other collagenolytic enzymes may be involved in the structural remodeling of the postovulatory follicle. During the midluteal period of pseudopregnancy in the rat, progesterone production is maximal, whereas mRNA expression patterns are at basal levels and unchanged for the gelatinases, MMP-13, and MT1-MMP (165, 167). At the end of pseudopregnancy, however, there is a marked induction of MMP-13 mRNA, but surprisingly no change in the mRNA expression of MMP-2, MMP-9, or MT1-MMP (165, 167) or gelatinolytic activity (165) as the CL begins to undergo regression. In contrast to these studies, when luteolysis is induced in the rat by PRL (170), GnRH (171), or GH (163), there is an increase in MMP-2 mRNA and activity. Similarly, induction of luteolysis with either GnRH (171) or a $PGF_{2\alpha}$ analog, cloprostenol (167), stimulated the expression of MT1-MMP mRNA in rat ovaries. The differences in MMP expression patterns may reflect disparities in experimental treatments or research models. Irrespective, these findings suggest a role for collagenase, the gelatinases, and the MT-MMPs in the structural remodeling that occurs during luteal regression.

In the mouse, ovarian expression of MMP-2 and MMP-10 was examined throughout pregnancy. Using slot blot analysis of whole ovarian extracts, Waterhouse *et al*. (172) reported that the temporal pattern of MMP-2 mRNA showed a marginal increase during gestation but a striking increase in the expression of MMP-10 mRNA at term. The increase in MMP-10 may reflect a role for this MMP in either luteal regression or the subsequent wave of follicular development.

In the bovine, preovulatory granulosa cells undergoing luteinization *in vitro* expressed gelatinolytic activity, but this activity decreased over a 4-d culture period (173). Goldberg *et al*. (168) examined the expression of gelatinolytic activity by zymography in CL isolated throughout the cycle. Activity for both MMP-2 and MMP-9 was present in luteal extracts. However, only MMP-9 activity was elevated during the early part of the cycle, whereas MMP-2 activity was unchanged during the luteal period (168). In contrast to these findings, Zhang *et al*. (174) reported an increase in MMP-2 activity in CL collected between d 4 and 10 of the cycle. Further evidence that the bovine CL produces gelatinases is apparent from *in vitro* studies in which cells isolated from 4-d-old CL exhibited zymographic evidence for both MMP-2 and MMP-9 activity (175). When these cells were cultured for up to 4 d, LH had no effect on gelatinolytic activity. MMP-9 activity declined with time in culture, whereas MMP-2 activity was unchanged (175). Recently, the luteal expression patterns for the MT1-MMP during the estrous cycle were reported (174). The cellular localization was dependent upon the age of the CL, with MT1-MMP protein present in endothelial cells and large luteal cells during the early (d 4) and midluteal (d 10) periods but a predominate fibroblastic expression pattern in the late CL (d 16). By Northern blot analysis, the levels of mRNA for MT1-MMP were unchanged across the cycle; however, at the protein level the active form

of MT1-MMP was lower in the CL from the early stage of the cycle compared with the mid- and late CL, suggesting a possible role in the activation of MMP-2 or the structural luteolysis of the CL (174). The other MMPs have received limited attention in the bovine CL.

In the porcine, mRNA expression for MMP-1, MMP-2, and MMP-9 was low during the early luteal period (d 6–8 of the estrous cycle), was significantly increased for all three MMPs during the midluteal period (d 9–11), and was highest in CL collected during the late luteal period (d 13–15) (176). The release of MMPs from steroidogenic large luteal cells *in vitro* corresponded with the changes in gene expression for the MMPs during the luteal period. For example, luteal cells from regressing CL released significantly more active MMPs than cells obtained from CL at the early luteal period. Pitzel *et al*. (176) further explored the regulation of MMPs in large luteal cells collected during the midluteal period by culturing these cells in the presence of $PGF_{2\alpha}$ or TNF-α. Uterine $PGF_{2\alpha}$ is well documented to induce luteolysis in the pig, and this action is enhanced by TNF-α (177). Both of these treatments stimulated an increase in mRNA and activity for MMP-1, MMP-2, and MMP-9 in large luteal cells and in conditioned medium, respectively. An interpretation of these findings is that both $PGF_{2\alpha}$ and TNF-α may play a role in regulating the MMP system during structural luteolysis and that the MMP system has a crucial role during luteolysis (176).

In the ovine, expression of the mRNA for MMP-2 was increased during the midluteal period (d 10 post estrus), MMP-14 mRNA was moderately elevated during early luteal development (d 4), whereas MMP-9 mRNA was undetectable by Northern blot analysis (178). Although MMP-9 mRNA was not observed, activity for both MMP-2 and MMP-9 was present in extracts of CL collected on d 2, 4, 10, and 15 post estrus by gel zymography. The activity patterns for both MMP-2 and MMP-9 were unchanged across the luteal phase, with the exception of an increase in MMP-2 activity on d 4 and 10 in samples treated to remove endogenous MMP inhibitors (178). Localization of MMP protein revealed that MMP-2 and MMP-9 were localized predominately to large luteal cells, whereas MMP-14 was present in cells other than large luteal cells. MMP-2 protein was also found in endothelial cells (178). To understand the role of the MMP system in ovine luteal regression, functional luteolysis has been induced by $PGF_{2\alpha}$, and expression and activity of the MMPs and TIMPs has been investigated (179, 180). Ricke *et al*. (179) demonstrated that mRNA expression for MMP-1, MMP-3, MMP-7, MMP-13, and MMP-14 were all transiently increased within 15–30 min after $PGF_{2\alpha}$ injection, whereas MMP-2 mRNA was unchanged and MMP-9 mRNA was undetectable. Although MMP-2 mRNA was unchanged and MMP-9 mRNA was not observed, activity for both of the gelatinases was present, and levels of active MMP-2 increased within 15 min of $PGF_{2\alpha}$ administration, which was reflected by an increase in pericellular activity as illustrated by *in situ* zymography (179). Similarly, Towle *et al*. (180) demonstrated the presence of MMP-2 and MMP-9 activity by gel zymography in isolated CL and showed that administration of $PGF_{2\alpha}$ resulted in a 60% increase in levels of active MMP-2. The findings that $PGF_{2\alpha}$ stimulates an increase in the mRNA and activity of the MMPs provide support for the role of the MMP system in ovine luteal regression. Of particular interest was the observation that the increase in MMP mRNA and MMP-2 activity occurred rapidly and concomitantly with the decrease in steroid production, implying that the induction of functional and structural luteolysis occurred simultaneously in the ovine CL. Similar findings of a rapid change in the TIMPs have also been observed (discussed in *Section II.C.2*; also see Ref. 180).

In the primate, MMP mRNA and protein patterns have been explored using real-time PCR and immunohistochemistry in the early, mid-, mid-late, late, and very late luteal stages (181). In the rhesus monkey, MMP-1 mRNA levels peaked in the early CL (more than 7-fold increase), and MMP-1 protein was detected in early steroidogenic cells (181). Treatment to induce premature luteolysis with the GnRH antagonist Antide resulted in a nearly 300-fold increase in the expression of MMP-1 mRNA, which appears to be regulated by progesterone (R. Stouffer, personal communication). Levels of MMP-2 mRNA were unchanged across the luteal period, with a trend toward an increase in the late CL. Localization of MMP-2 protein was prominent in the microvasculature of late, but not early CL. The expression of mRNA for MMP-9 was low in the early CL but increased 41-fold by in the very late stage CL. Similar to the changes in mRNA, MMP-9 protein was present in the early CL, and immunoreactive protein increased in later stage steroidogenic cells (181). These findings have led Young and colleagues (181) to propose that the expression of MMP-1 in the newly forming CL is associated with tissue remodeling during luteinization, whereas the expression of MMP-2 and MMP-9 in the late luteal stages may contribute to luteolysis.

In the human, the pattern of MMP expression is dependent on the stage of the menstrual cycle. During the early luteal period, the mRNA expression for MMP-1 and MMP-2 was unchanged, although there was an increase in MMP-9 activity (169). During the late luteal period, the activity of MMP-2 and MMP-9 was elevated, and administration of hCG to mimic luteal rescue *in vivo* was associated with a reduction in the mRNA expression and activity of MMP-2 (169). Localization of the mRNAs for the MMPs revealed that MMP-1 was present in stroma and connective tissue of the CL, whereas MMP-2 was observed associated with the thecalutein cells, the vasculature, and the surrounding connective tissue. The cellular distribution of MMP-9 mRNA was different from the other MMPs because it was not localized to the steroidogenic cells but rather was sparse and present in individual luteal cells (169).

Although there are species differences in the luteal expression patterns of the various MMPs, the emerging model is one in which the MMPs are elevated during extensive luteal ECM remodeling that occurs as the postovulatory follicle is transformed into the CL. After the CL is fully formed, steroidogenesis is maximal during the midluteal period, and MMP expression and activity are at basal levels. With the onset of structural regression, the MMPs are again called into action for the remodeling and removal of the CL. As noted above, there are differences in the specific MMP family members involved, as well as the pattern of MMP expression among species at the various stages of luteal development. These distinct patterns of MMP expression may reflect dif-

ferences in the intermixing of the theca and granulosa cells as the CL forms; differences in the makeup of the luteal ECM; physiological differences in the responsiveness of the CL to trophic factors, such as the influence of hCG in the primate *vs.* placental lactogen in the rodent; the absence or presence of progesterone receptors; the local action of growth factors and cytokines; as well as physiological differences in the models used to examine luteal function.

2. TIMPs during the luteal period. The initial cellular localization of TIMP-1 mRNA was described by Nomura *et al*. (182) in their 1989 survey on the developmental expression of TIMP-1 in various mouse tissues. These authors noted that TIMP-1 mRNA was highly abundant throughout the CL. Subsequently, the changes in the localization and expression patterns for the metalloproteinase inhibitors have been explored in numerous species throughout the luteal period (Table 2). In the rodent, expression patterns for TIMP-1 mRNA are elevated during early luteal formation in the rat (167, 183) and decline during the midluteal period of pregnancy or pseudopregnancy to remain low in the rat (167, 183) and mouse (172). During luteal regression, however, the expression of TIMP-1 mRNA increases in the rat (167, 183) and mouse (172). The expression of TIMP-2 mRNA in CL isolated throughout pregnancy in the rat increases at d 3 of pregnancy, declines between d 7 and 18, and then increases at 1 d postpartum, suggesting a role in luteal formation and regression (166). In contrast, levels of TIMP-2 mRNA were unchanged throughout pseudopregnancy in the rat (183). In the ovaries collected throughout the rat estrous cycle, the 3.5-kb transcript for TIMP-2 declined in whole ovarian extracts between metestrus and diestrus (44). In the mouse, a marginal increase in TIMP-2 mRNA was observed at the latter stages of pregnancy (172). For TIMP-3, expression of mRNA in extracts of rat ovaries collected throughout the estrous cycle decreased on the morning of estrus (44), although levels of mRNA increased during the mid- to latter stages of pseudopregnancy (183). Similarly, expression of mRNA for TIMP-3 was elevated in CL isolated during the first 9 d of pregnancy (166). For TIMP-4, neither the mRNA nor the protein changes significantly across the estrous cycle in the mouse (27) or the rat (26), although there was a trend toward higher levels of expression on the day of estrus.

To understand the significance of the quantitative changes in TIMP expression patterns in the rodent described above, the localization of TIMP mRNA within the CL has begun to be elucidated by *in situ* hybridization. TIMP-1 mRNA was first described as being abundantly distributed throughout the CL in the mouse by *in situ* hybridization (182). Further investigation into the localization of mRNA for TIMP-1 as well the other TIMPs in the rodent has revealed unique patterns of mRNA expression dependent on the inhibitor and the age of the CL (44). In ovaries collected during the rat estrous cycle, TIMP-1 mRNA was highly expressed throughout the newly forming CL with a band of expression encircling the CL. As the CL aged throughout the cycle, this encircling band of hybridization was lost, and the levels within the CL declined. TIMP-2 mRNA was expressed at low levels within the newly formed rat CL, but was very highly expressed in a band surrounding the CL. This high level of expression encircling the CL was lost as the CL aged, although the levels within the CL appeared to increase with age with areas of high expression in regressing CL. For TIMP-3, there was no mRNA pattern surrounding the rat CL as noted for TIMP-1 and TIMP-2. Instead, TIMP-3 mRNA was expressed in a punctate pattern throughout the CL that did not vary with the age of the CL (44), suggesting that it is expressed by a certain cell type. For TIMP-4, in the newly forming CL on the morning of estrus mRNA was at background levels but was observed in a punctate cell-specific manner in older CL from previous cycles (26). As the newly formed CL aged throughout the estrous cycle, TIMP-4 mRNA was present in punctate distribution within the CL; however, the pattern of localization did not correspond with localization of macrophages (26). Similar observations of a luteal distribution were observed in the mouse with the older CL exhibiting higher levels of TIMP-4 mRNA expression, although the pattern of expression was more uniform throughout the CL than in the rat (27). The observations of changes in the pattern of expression for the TIMPs suggest a role for these inhibitors in luteal physiology.

In the ovine, there was no statistical change in the levels of TIMP-1 mRNA across the luteal period ($P < 0.07$), although TIMP-1 levels were approximately 3-fold higher during the early luteal period (d 3) than during the midluteal period (d 10) (88). TIMP-2 mRNA expression was highest in ovine CL collected during the early luteal period (d 3–7) and declined throughout the cycle to the lowest levels during the late (d 16 of the estrous cycle) luteal phase (100). Induction of luteolysis by administration of $PGF_{2\alpha}$ resulted in a rapid decrease (within 1 h) in the levels of TIMP-1 mRNA (184) as well as TIMP-1 and TIMP-2 protein by Western blot analysis (180). Corresponding to the decline in TIMP-1, there was a loss of immunoreactive TIMP-1 protein in large luteal cells (184). However, levels of mRNA for TIMP-1, TIMP-2, and TIMP-3 were all elevated to various degrees within 2–4 h after $PGF_{2\alpha}$ administration (184). It has been postulated that the early induction of a loss of the TIMPs results in an imbalance in the MMP to TIMP ratio, allowing degradation of the luteal ECM (180, 184).

The cellular distribution of the TIMP-1 and TIMP-2 varies within the ovine CL (88, 100). TIMP-1 mRNA was sparse and present in specific cells along the infolding of the follicle wall during luteal development or randomly dispersed throughout the mature CL (88). In contrast to TIMP-1, TIMP-2 mRNA was highly abundant throughout the CL (100). Separation of ovine luteal cell populations by centrifugal elutriation demonstrated that the purified large luteal cells contained approximately 10-fold more TIMP-1 mRNA and 20-fold more TIMP-2 mRNA than the small luteal cells (88, 100).

Evidence that the TIMP mRNA is translated into protein has been forthcoming from immunohistochemistry and culture experiments. TIMP-1 is secreted from slices of ovine CL collected throughout the estrous cycle (153) and has been localized to ovine large luteal cells by immunostaining (91, 92, 185). Similarly, immunoreactive TIMP-2 has been found in large luteal cells (92) and secreted by cultured ovine luteal cells (100). Of particular interest is the finding that TIMP-1 is present in luteal cells containing TIMP-2 or oxytocin and that TIMP-1 was localized to secretory granules. These granules

were seen undergoing exocytosis during the late luteal period (92), implying that TIMP-1 was synthesized by large luteal cells and released during luteal regression.

In the bovine, granulosa cells undergoing luteinization *in vitro* contain TIMP-1 mRNA that markedly increased during 4 d in culture (173). These findings correspond with the reported increase of TIMP-1 mRNA during early luteal formation *in vivo* (98, 186). During the midluteal period, TIMP-1 mRNA expression declines from the high levels seen during luteal formation to remain low (98) before increasing during luteal regression (187). TIMP-2 mRNA was low during the early luteal phase (d 4), significantly increased between d 10 and 15 of the estrous cycle, and then declined at d 19 (98). While in the porcine, expression of TIMP-1 and TIMP-2 mRNA increased during formation of the CL compared with the late luteal phase (176). Isolation of large luteal cells revealed the presence of mRNA for both TIMP-1 and TIMP-2 (176).

In the rhesus monkey, the changes in the expression patterns for the TIMPs has been reported in CL from the early, mid-, mid-late, late, and very late luteal periods (181). TIMP-1 mRNA was highly expressed in the CL, but levels of mRNA declined approximately 20-fold at the very late luteal stage. The expression pattern of mRNA for TIMP-2 differed from that observed for TIMP-1 with the levels of TIMP-2 mRNA highest through the midluteal phase. Immunolocalization of TIMP-1 and TIMP-2 demonstrated the presence of both TIMP-1 and TIMP-2 protein in steroidogenic cells at all phases of the luteal cycle (181).

In the human, the mRNA expression of TIMP-1 was unchanged in CL collected throughout the menstrual cycle (169, 188), suggesting species differences between the human, rodent, and ruminants in the pattern of TIMP-1 mRNA expression. Of interest was the observation that TIMP-1 mRNA actually decreased in the CL from marmoset ovaries after induction of luteolysis with either $PGF_{2\alpha}$ or GnRH antagonist (97). TIMP-2 mRNA levels did not change in CL collected across the menstrual cycle (169); however, the pattern of localization was different from that found for TIMP-1. TIMP-1 mRNA and protein was highly expressed in the granulosa-lutein cells (188), whereas TIMP-2 mRNA was present in the theca-lutein cells and the connective tissue surrounding the steroidogenic cells of the CL (169).

As a model for luteal formation, human granulosa-lutein cells from *in vitro* fertilization programs have been placed in culture, and regulation of the MMP system has been explored. Granulosa-lutein cells placed in culture for 4–6 d produce both MMP-2 and MMP-9 protein, and the addition of hCG to the cultures resulted in a decrease in total gelatinolytic activity and an increase in TIMP-1 (73). Similarly, MMP-2 and MMP-9 activity increased in granulosa-lutein cells cultured up to 14 d, and the addition of hCG decreased MMP-2 activity (189). These findings led to the proposal that hCG may act to stabilize the luteal ECM during CL formation (73, 189). Further studies have reported that FSH and T_3 stimulate TIMP-1 protein production from cultured human granulosa cells (190). Additional experiments have demonstrated a differential regulation of the human MMP system. Addition of phorbol ester to granulosa-lutein cell cultures stimulated both MMP-9 and TIMP-1 secretion, whereas forskolin inhibited MMP-9 secretion while stimulating TIMP-1 secretion (43). The ability of forskolin, which mimics hCG action, to decrease MMP-9 and yet stimulate TIMP-1 is in concordance with previous findings (73) and supports the concept that hCG may stabilize the forming luteal ECM. Also of interest was the finding that a reduction in the MMP-9 to TIMP-1 ratio was associated with an increase in progesterone secretion, the mechanism of which is unknown (43).

The regulation of TIMP expression during luteal regression has been briefly explored in a limited number of species. In the ovine, levels of TIMP-1 protein secreted by cultured luteal slices from CL collected at d 10 after estrus were unchanged by addition of LH, FSH, or cholera toxin (153). Similarly, levels of TIMP-1 and TIMP-2 mRNA were unaffected by treatment of large porcine luteal cells with TNF-α or $PGF_{2\alpha}$ (176). To determine whether the induction of TIMP-1 and MMP-13 mRNA during the latter stages of pseudopregnancy in the rat is coupled with luteal regression, Liu *et al*. (167) either prolonged the luteal phase by performing hysterectomies or induced premature luteal regression by treatment with a $PGF_{2\alpha}$ analog, cloprostenol. In both experimental treatments, TIMP-1 and MMP-13 were induced only after the serum level of progesterone had decreased, suggesting that MMP-13 and TIMP-1 are induced by the physiological signals that initiate functional luteolysis. However, Towle *et al*. (180) reported that TIMP-1 and TIMP-2 protein decreased within 1 h after $PGF_{2\alpha}$-induced luteolysis in the sheep, a time period before or juxtaposed with the decline in progesterone.

It is readily apparent that the changes which occur in the expression and localization of the MMP system parallel the extensive ECM remodeling that occurs with luteal formation, luteal maintenance, and regression. A general model for the MMP system in luteal formation is one in which MMPs and TIMPs are elevated during the period of extensive connective tissue remodeling as the follicle is transformed into the CL. Luteotrophic signals would stimulate both MMPs and TIMPs. The stimulation of the MMPs would allow remodeling of the postovulatory follicle into the CL. The TIMPs would act to regulate the extent and location of MMP action as well as regulate luteal dynamics such as cell growth and steroidogenesis. However, studies of the functional significance of the MMP system in luteal physiology are lacking. The advent of specific MMP inhibitors and current gene deletion models will allow the precise role of the MMPs and TIMPs to be elucidated.

III. Uterus

A. The biology of the mammalian uterus

The female reproductive tract can be viewed as a dynamic, interrelated set of organ systems and associated specialized tissues that function in concert as a result of sequential, steroid-directed communication from the ovary. Although steroids impact the function of organ and tissue systems outside the reproductive tract, the steroid dependency of the adult uterus is uniquely directed in support of establishment and maintenance of pregnancy. The endometrial lining of the uterus is a highly vascularized tissue composed of special-

ized stromal and glandular elements that largely function independently from the muscular elements of the uterine corpus. The steroid-sensitive nature of the endometrium ultimately creates a specific tissue environment that provides for the complex physiological needs of the fetus. Additionally, in humans and certain other species, steroidal support of early pregnancy acts to control the highly invasive events required for implantation and placentation. Without steroid support, the endometrial lining of the uterus exhibits little morphological change and fails to demonstrate the requisite biochemical differentiation necessary for either establishment or maintenance of pregnancy. In contrast, a complex, precise, and highly interactive array of biological communication pathways involving the action of steroids in concert with local growth factors and cytokines is required to orchestrate the controlled growth and differentiation of the endometrial lining of the uterus. Each of these agents may affect the expression pattern as well as the activity of MMPs and TIMPs during the estrous and menstrual cycles or during pregnancy-related events. Studies in animals have shown that a considerable loss of collagen is associated with the changes in uterine size during the estrous cycle (191); however, rodents and domestic species exhibit much less uterine remodeling compared with the menstrual cycle of humans and most primates (192, 193). Among various mammalian species, the selective expression, activation, or inhibition of specific members of the MMP system allows for uterine restructuring while maintaining tissue stability during periods of estrogen-stimulated growth, in response to the invasive processes required for the establishment of pregnancy, and in association with parturition and postpartum remodeling. As will be discussed below (*Sections III.B–D*), the MMP family is intimately involved in uterine biology; however, we are just beginning to understand the complex regulatory systems that mediate the expression and action of these multifaceted enzymes during critical reproductive events.

B. The cycling uterus

1. MMP expression patterns in the cycling uterus. Changes in uterine mass and wet weight in rodents, associated with cyclic exposure to ovarian steroids, were linked to collagen breakdown more than 30 yr ago (194, 195). Originally identified during postpartum uterine involution, MMP-7 (Pump-1, matrilysin) was among the first MMP proteins linked to matrix remodeling in the rodent uterus (196), whereas MMP-13 is now known to be a primary source of the collagenase activity first associated with uterine involution (194, 197). Although studies in these animals have primarily focused on MMP expression during pregnancy, expression of MMP-3, MMP-9, and MMP-13 has also been demonstrated in stromal cells isolated from the nonpregnant rat endometrium (198), while epithelial cell specific MMP-7 has been demonstrated in the intact, nonpregnant rat uterus (175). It is important to note that mice with null mutations in specific MMP genes do not necessarily suffer the predicted consequences to reproductive success. For example, mice lacking MMP-7 expression proceed normally through the estrous cycle and appear to have normal fertility (199). Interestingly, MMP-3 and MMP-10, which are expressed only at low levels in the uterus of normal mice, are increased in cycling mice lacking expression of MMP-7 (200). Compared with the limited degree of tissue remodeling during the reproductive cycle of rodents, episodes of bleeding, tissue loss, and repair exhibited by menstruating species represent a unique adult phenotype, requiring a development-like process of tissue regeneration. In humans, reepithelialization of the luminal surface is one of the first steps involved in repair of the endometrium after menstruation. The expression of MMP-7 mRNA has been identified in epithelial cells during menstruation, early repair events, and focally among glandular structures as they grow and elongate distally from the basalis region in response to estradiol (201, 202). Although both MMP-7 and MMP-9 mRNA have been localized to endometrial epithelium (203), unlike the expression of MMP-7, which is exclusively produced by epithelial cells, MMP-9 expression also occurs among stromal, immune, and vascular cells (204–212). In addition to MMP-9, other MMPs, including MMP-8 and MT1-MMP, have been reported among resident endometrial immune cell populations, including neutrophils, eosinophils, and macrophages (204, 213, 214). In contrast to a rather limited expression of MMPs by endometrial epithelial and immune cells, *in situ* hybridization analysis reveals that stromal cells express numerous MMP mRNAs including MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-11, and MT1-MMP during the proliferative and menstrual phases of the human cycle (209, 215). As shown in Table 3, the MMPs show a variable expression pattern in the human endometrium during the menstrual cycle, which largely reflects a cell-type specific expression in response to cyclic

TABLE 3. Summary of the expression patterns of the MMP system in the human endometrium throughout the menstrual cycle

Proliferative		Secretory		Late secretory		Menstrual	
MMP-1	+	MMP-1	−	MMP-1	−	MMP-1	+
MMP-2	++	MMP-2	++	MMP-2	++	MMP-2	++
MMP-3	+	MMP-3	−	MMP-3	−	MMP-3	++
MMP-7	++	MMP-7	−	MMP-7	+	MMP-7	+++
MMP-9	−	MMP-9	−	MMP-9	−	MMP-9	+
MMP-10	−	MMP-10	−	MMP-10	+	MMP-10	++
MMP-11	++	MMP-11	−	MMP-11	+	MMP-11	+++
TIMP-1	+	TIMP-1	+	TIMP-1	++	TIMP-1	+++
TIMP-2	+	TIMP-2	+	TIMP-2	+	TIMP-2	++
TIMP-3	+/−	TIMP-3	+	TIMP-3	++	TIMP-3	+

Relative levels of mRNA are expressed as − (absent), + (focal), ++ (moderate), +++ (intense). Detailed information can be found in Refs. 201, 209, 257, 309, 312, 314, 315, 320, 350, 367, and 390.

changes in circulating steroid hormone levels (209, 216). In general, the expression of MMPs in humans and most other primate species appears to be similar and indicates that the expression of these enzymes reflects the direct and indirect influence of steroid hormones across the menstrual cycle (217, 218).

2. TIMP expression patterns in the cycling uterus. Maintaining homeostasis of the ECM during numerous reproductive processes requiring tissue turnover in the ovary and the uterus requires a balanced expression of MMP and TIMP expression. Reflecting the absence of extensive tissue remodeling across the relatively short estrous cycle, the coexpression of MMPs and TIMPs is not as robust in the rodent uterus compared with the human or primate menstrual cycle. During normal reproductive processes, only a modest expression of TIMP-1, TIMP-2, and TIMP-3 has been reported in the uterus of cycling rodents, in association with estrus (41) and with the decidual response in preparation for pregnancy (219, 220). In the absence of pregnancy, the expressions of TIMP-1, TIMP-2, and TIMP-3 are each found in rodents during artificial induction of decidualization (198). In contrast to limited TIMP expression in rodents, Rodgers *et al*. (209) demonstrated specific expression of TIMP-1 mRNA in both epithelial and stromal cells, detectable across each phase of the human menstrual cycle. In this *in situ* hybridization study, TIMP-1 mRNA was detected in the epithelium at approximately equal levels across the menstrual cycle, although TIMP-1 expression progressively increased in the stromal compartment (209). Compared with a relatively low pattern of TIMP-1 mRNA reported by these investigators (209), Zhang and Salamonsen (221) recently reported significant levels of immunoreactive TIMP-1 and TIMP-3 protein in all tissue compartments of the human endometrium throughout the cycle, with the most intense staining localized to luminal epithelium. Similar to TIMP studies in rodents, *in vitro* decidualization of human stromal cells is also associated with an increase in immunostaining for TIMP isotypes, although immunoreactive TIMP-1, TIMP-2, or TIMP-3 protein was not observed among resident immune cells (221). At this juncture, less is known regarding the expression of TIMP-4 in the rodent, primate, or human reproductive tissues, but a recent study demonstrated the presence of TIMP-4 in the decidua of term pregnancies in women (222). Similar to MMP expression discussed earlier, TIMP expression appears to increase significantly during endometrial breakdown and bleeding, leading to the detection of TIMP-1 in uterine flushings at the end of the menstrual cycle (223). In nonhuman primates, the expression of TIMP-1 is also highest during menstruation but exhibits an interesting shift to the stratum basalis after the breakdown and bleeding events in the upper functionalis (202). At least two studies indicate that TIMP-1 and TIMP-2 are expressed in small arteriolar and capillary vascular tissues in the secretory endometrium, suggesting that TIMPs may be involved in stabilization of uterine vasculature during the reproductive cycle and pregnancy (209, 211). Although the role of TIMP expression during endometrial vascularization remains to be fully elucidated, TIMP-1 and TIMP-2 have each been recently reported to have antiangiogenic activity that may be related to an inhibition of vascular endothelial growth factor (VEGF) expression (224, 225).

3. Regulation of the MMP system in the cycling uterus. Carefully controlled regulation of endometrial MMP and TIMP expression is critical for normal tissue remodeling during periods of growth, cyclic tissue breakdown, or in association with the establishment of pregnancy. Unfortunately, the mechanisms by which limited, largely focal patterns of MMP and TIMP expression are achieved across the menstrual cycle have yet to be fully elucidated. Although the role of estrogen in the induction of cyclic endometrial growth is well established, the relationship of estrogen to the specific expression of members of the MMP family remains poorly understood. During periods of increasing estrogen exposure, the ligand-bound estrogen receptor complex can increase expression of the transcription factors fos and jun (226, 227) that bind the activator protein-1 (AP-1) element. Because the promoters of most MMP genes contain AP-1 elements (228), estrogen induction of these transcription factors may provide a potential mechanism for MMP regulation by this steroid. In addition to steroidal activation, the AP-1 element also plays a crucial role in the *trans*-activation of MMP promoters by agents such as phorbol esters and proinflammatory cytokines, including IL-1 and TNF-α (229). Supporting an important role of the AP-1 element in MMP regulation, mutations within this element result in reduced MMP expression (230). Additionally, most MMP promoters contain a polyoma virus enhancer A3 (PEA-3) element, which binds the Ets family of transcription factors (231). This element also plays an important role in MMP gene regulation by both growth factors and cytokines, and binding sites for the Ets proteins can act synergistically with AP-1 proteins to activate MMP gene transcription (reviewed in Ref. 229). Under the influence of progesterone, a number of cellular and molecular mechanisms may serve to limit MMP expression. For example, the progesterone receptor, but not estrogen or glucocorticoid receptor, has been reported to induce AP-1 activation in the absence of ligand (232). In this study, stimulation of AP-1 activity could be reversed with the addition of progesterone, suggesting a mechanism for progesterone-mediated suppression of MMP gene transcription during the reproductive cycle. Progesterone can inhibit the estrogen-induced expression of c-*fos* mRNA (233), and c-*jun* and c-*fos* are down-regulated in human endometrium during pregnancy (234). In response to progesterone, retinoic acid, the active form of vitamin A, is synthesized in the human and rodent endometrium during stromal cell decidualization (235, 236) and can act as a potent MMP inhibitor *in vitro* (237). In a variety of tissues and cell types, the liganded retinoic acid receptor has been demonstrated to suppress MMP expression via sequestration of fos and jun proteins (229). Although little is known regarding the interaction of either the estrogen or progesterone receptors with PEA-3 elements, androgens have been found to inhibit multiple MMPs via interaction with Ets-related proteins (238), and the retinoic acid receptor can repress PEA-3-dependent v-*src* induction of MMP-1 gene transcription (239). Among the numerous MMPs expressed in the endometrium, MMP-2 has neither an AP-1 site nor a PEA-3 site and appears to be constitutively expressed in the uterus

(209). As noted earlier in this review, MMP-2 can be activated by a novel mechanism involving MT-MMPs and TIMP-2. The physiological significance of constituitive expression of MMP-2 in the human endometrium remains speculative, but it further indicates the potential for interaction among MMPs and TIMPs.

The mechanisms by which steroids influence the MMP system in uterine tissues appears to involve cell-specific changes in local cytokine expression and action in addition to any direct or indirect modulation of gene transcription by steroid-receptor mechanisms. Although the regulation of MMP and TIMP genes in the uterus likely reflects the nature of the specific promoters of each individual family member, there may be unique control mechanisms specific to the steroid-dependent nature of the adult uterus. As noted above, during endometrial differentiation in preparation for pregnancy, the local tissue environment of decidualization is largely inhibitory to the expression of MMPs, whereas the expression of most TIMPs increases (15, 235). In general, regulation of TIMP gene expression in adult tissues is not as well understood as that for MMP genes, but similar to that of most of the MMPs, TIMP expression is stimulated by factors such as phorbol esters and proinflammatory cytokines (235). The promoters of TIMP-1, TIMP-2, and TIMP-3 have each been characterized in human tissue and provide insight into their regulation. The TIMP-1 promoter does not contain a TATA box, but multiple other elements have been described including a TPA-responsive element, AP-1, SP-1, and Ets sites (240–242). The expression of TIMP-2 in the endometrium is largely constitutive, and the TIMP-2 promoter does not contain a TPA response element but rather contains both a TATA box and an AP-1 element (243, 244). The expression of TIMP-3 appears to be more highly regulated than TIMP-2 during development (245), and it is intensely expressed in the placenta (246). The promoter of TIMP-3 does not contain a TATA box but does contain multiple SP-1 sites (245). Little is currently known regarding the regulation of TIMP-4 mRNA, but this protein appears to be most abundantly expressed in heart tissues (28) and in intrauterine tissues during pregnancy (222).

The relationship of TIMP expression to the expression of MMPs is key to the balance of MMP activity during periods of growth, at menstruation, or in association with pregnancy-related ECM remodeling. Although the specific regulation of individual MMP and TIMP genes may be quite complex, the changing patterns of MMP and TIMP expression across each phase of the menstrual cycle largely reflects steroid-driven tissue changes necessary to prepare the endometrium for the establishment of pregnancy (247, 248). During endometrial growth, *in vitro* studies suggest that estrogen may not directly affect cell type-specific expression of MMPs (202, 249, 250), although many growth factors, chemokines, and cytokines expressed during estrogen-induced growth are potent modulators of MMP family members (193). For example, Salamonsen and co-workers (208) demonstrated that isolated endometrial stromal cells cultured in the absence of steroids, but in the presence of both IL-1α and TNF-α, express MMP-1, MMP-3, and MMP-9 in a concentration-dependent manner. As opposed to the apparent lack of estrogen action *in vitro*, addition of progesterone to cultures of isolated endometrial stromal cells acts to block IL-1α induction of MMP-1 (251) and MMP-3 (252, 253). Although TIMP levels generally fluctuate less during the human menstrual cycle compared with those of the MMPs (209, 216), the balance between MMPs and TIMPs shifts most dramatically during the secretory phase of the cycle, reflecting the influence of both progesterone and locally produced cytokines and growth factors. For example, TGF-β has been shown to mediate suppression of endometrial MMP-7 by epithelial cells in response to progesterone (254), but also can induce TIMP-1 and TIMP-3 mRNA in stromal cells (207). As noted previously, other locally produced factors, such as retinoic acid, may also act in concert with progesterone to regulate the ratio of MMPs and TIMPs during various reproductive processes (253). Additionally, both MMPs and TIMPs may act to regulate their own expression as well as other processes important to uterine biology (193). For example, recent studies indicate that TIMP-1 plays a role in regulating cell proliferation and survival (reviewed in Ref. 30), whereas TIMP-2 can inhibit phosphorylation of the EGF receptor, preventing its activation (255). As we uncover more information about the regulation of TIMP expression and action in the uterus, it is likely that an important role beyond regulation of MMP action will emerge.

C. Menstruation

1. MMP expression patterns during menstruation. The menstrual cycle of humans and primates is usually divided into two distinct phases, proliferative and secretory, followed by either the successful establishment of pregnancy or the destructive events of menstruation. Although the breakdown of the endometrium has historically been viewed to result from vasospasm of spiral arteries, evidence is accumulating that suggests that MMP expression by endometrial cells as well as migratory cells of vascular origin may also play an important role in the biological events required for menstruation (201, 209, 216, 256–258). The adult endometrium is frequently viewed to consist of three morphologically and functionally distinct layers: the stratum basalis, which lies adjacent to the myometrium; the stratum spongiosum or intermediate layer; and the stratum compactum. It is from the stratum basalis that the surface of the endometrium regenerates after each episode of cyclic tissue shedding, whereas the two uppermost layers, referred to jointly as the stratum functionalis, undergo coordinated histological and cytological changes throughout the menstrual cycle (259). It has been known for some time that the primate and human endometria contain reticular fibers of type I and type II collagen that undergo focal breakdown and lysis after a loss of progesterone support at menstruation (195, 260, 261). Not surprisingly, the withdrawal of steroid support is associated with the highest levels of endometrial MMP expression either *in vivo* or *in vitro* (201, 202, 209, 216, 217, 262–264). Immunohistochemical studies indicate that several MMP proteins, including MMP-1, MMP-2, MMP-3, and MMP-9, localize to endometrial endothelial cells during menstruation (211). Expression of MMP-1 mRNA occurs broadly among stromal fibroblasts, arterioles, and small vessels near the periphery of shed endometrial tissue, whereas MMP-3, MMP-10, and MMP-11 mRNA are observed only within the

fibroblast component (209). The epithelial-specific expression of MMP-7 mRNA is also very high during endometrial breakdown, appearing before the expression of MMPs within the stromal compartment (201). As noted earlier, the expression of MMP-7 appears limited to endometrial epithelial cells in normal tissue, although MMP-7 mRNA and protein have recently been reported in endothelial cells adjacent to MMP-7-positive tumors (265). Membrane-bound MT1-MMP and MT2-MMP mRNA and protein have also been detected in menstrual endometrium and in numerous cells of hematopoietic origin present within the endometrium during tissue breakdown (214). Although cellular expression and localization are valuable to aid our understanding of the role of MMPs in menstruation, it is equally important to determine the activity of these enzymes to appreciate their physiological significance. To this end, zymographic studies indicate that the activity of MMP-1, MMP-3, MMP-7, and MMP-9 appears to be most intense during the menstrual phase of the cycle (266). Among these MMPs, it is important to note that pro-MMP-9 can be activated by MMP-3, which is capable of self-activation (267). The activities of other MMPs within menstrual endometrium have yet to be reported, although several MMPs have recently been implicated in breakthrough bleeding associated with the use of contraceptives (204, 210, 268) as well as excess menstrual bleeding (237). For example, MMP-1, present in myometrial mast cells, is more highly expressed in women with contraceptive-related breakthrough bleeding than women who do not bleed (269).

2. TIMP expression patterns during menstruation. Although the expression of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 each appears to increase within the decidual tissue of pregnancy, the initial process of decidualization can be observed immediately before menstruation in humans. An initial examination of endometrial MMP and TIMP mRNA expression patterns at different times of the nonpregnant menstrual cycle found the highest expression of TIMP-1 during menstruation in concert with falling serum levels of progesterone and increased expression of numerous MMPs (209). In this study, several endometrial cell types expressed TIMP-1 mRNA by *in situ* hybridization with only subtle changes in expression across the other phases of the menstrual cycle. Nevertheless, the increase in TIMP-1 mRNA expression at menses and the detection of TIMP-1 in small arteriolar and capillary vascular tissue in the secretory endometrium suggested an important role in control of blood vessel stability to limit bleeding. More recently, Freitas *et al*. (211) have extended this work by demonstrating an intense expression of TIMP-1 and TIMP-2 protein in endometrial vasculature during menstruation. In both pre- and postmenopausal women, TIMP-1 has been detected in endometrial flushings associated with normal bleeding as well as in the presence of adenocarcinoma (223). Using specific antibodies directed against TIMP-1, TIMP-2, and TIMP-3, Salamonsen and colleagues (221, 270) found only TIMP-1 and TIMP-2 to be elevated in women at the time of menstruation in response to falling progesterone levels. In the primate, *Macaca mulatta*, TIMP-1 was also found to increase at menstruation in response to progesterone withdrawal, whereas TIMP-2 was not detected (202). The expression of TIMP-1 mRNA appears to shift from the functionalis to the basalis zone of the primate endometrium in the absence of steroids (202), perhaps reflecting endometrial preparation for pregnancy whether or not tissue breakdown and menstruation occurs.

3. Regulation of the MMP system during menstruation. During growth of the endometrium, only moderate and focal patterns of MMP and TIMP expression are noted, compared with high levels of both MMPs and TIMPs during the tissue breakdown associated with menstruating species. Recently, researchers have suggested that constriction of the spiral arteries induces a greater degree of hypoxia in the upper regions of the endometrium, which creates a hypoxic insult and subsequent MMP expression and tissue breakdown (202, 258). However, Zhang and Salamonsen (271) demonstrated that MMP expression by isolated stromal cells is decreased after culture under hypoxic conditions, suggesting that regulation of MMP expression during menstruation may be more complex. Using endometrial explants, Marbaix *et al*. (272) have demonstrated that endometrial breakdown after steroid withdrawal can be completely and reversibly blocked by specific inhibitors of MMPs, but not by inhibitors of cysteine and serine proteinases. In the face of declining steroid support at the end of the menstrual cycle, regional MMP expression can be observed in both epithelial and stromal cells (201, 209), and expression of endometrial MMPs occurs *in vitro* after progesterone withdrawal (216, 263, 272, 273). In isolated stromal cells, MMP-1 mRNA expression is significantly up-regulated in a model of menstruation after 4 d of exposure to the antiprogestin mifepristone (RU-486; Ref. 263). A more detailed survey, conducted by the laboratory of Salamonsen *et al*. (216), demonstrated that secretion of MMP-1, MMP-2, MMP-3, and MMP-9 by isolated stromal cells was increased on withdrawal of progesterone, although no effect was found on the expression of TIMP-1 and TIMP-2 mRNA or protein. Although steroid withdrawal is an important element of initiation of menstruation and MMP expression, this study concluded that due to the focal nature of menstruation-associated MMP expression, progesterone withdrawal is unlikely to be the only regulatory factor (216). For example, TNF-α may play a key role in induction of apoptosis in endometrial epithelium at the onset of menstruation (247, 274), and IL-1α and IL-1β have been proposed to play equally important roles via MMP regulation (206, 252, 263, 275, 276). It has been shown that IL-1 is abundantly produced during the normal menstrual cycle (277) and plays a pivotal role in stimulating expression of MMP-1 and MMP-3 (206, 263, 275, 276). Recently, Tabibzadeh and coworkers have identified a protein, endometrial bleeding-associated factor, also called lefty (278), that is transiently expressed before and during menstruation (279). Endometrial bleeding-associated factor, a member of the TGF-β superfamily, appears to act as a natural inhibitor to TGF-β (279) and is inappropriately expressed in women with dysfunctional bleeding. As noted previously, members of the TGF-β family appear to be important mediators of MMP and TIMP expression in response to progesterone during the secretory phase of the menstrual cycle (235, 254). Therefore, the existence of a natural inhibitor of TGF-β in the human endome-

trium may be necessary to block endogenous TGF-β action, allowing for a rapid expression of multiple MMPs during menstruation.

Although menstruation is generally viewed as a time of tissue destruction, endometrial repair begins within 24 h of initiation of tissue breakdown and is an important component of limiting tissue damage. Complex mechanisms, mediated by steroids and local tissue-specific factors, act in concert to promote the selective expression of members of the MMP family during periods of tissue repair and remodeling (280–282). During this transition period into the growth phase, growth factors associated with estrogen action such as EGF, IGF, VEGF, and basic fibroblast growth factor have been shown to be involved in menstrual repair (283–286). For example, during estrogen-stimulated growth, VEGF can be colocalized with MMPs in newly formed endometrial capillaries (211), and VEGF is known to stimulate MMP expression in vascular smooth muscle cells (287). Recently, Nayak *et al*. (264) demonstrated that progesterone withdrawal stimulates VEGF receptor type 2 in primate endometrial stromal cells that correlated with zone-specific MMP expression during menstruation. Additionally, many aspects of the extensive tissue remodeling occurring within the cycling endometrium are reminiscent of inflammatory processes, and not surprisingly, proinflammatory cytokines have been associated with the regulation of endometrial MMP expression *in vitro* (193, 247, 274). Although endometrial stromal and epithelial cells can produce proinflammatory cytokines (213, 252, 288–291), a dramatic influx of numerous types of lymphomyeloid cells into the endometrium before menstruation may co-mediate the expression and activation of MMPs during menstruation (213, 292, 293). For example, *in vitro* studies suggest that normal endometrial stromal cells release latent MMPs that are activated only when cells are cocultured with endometrial neutrophils (294). The mechanisms by which cells of hematopoetic origin can impact endometrial MMP and TIMP expression at menstruation likely involves the action of proinflammatory cytokines as noted above. Clinical studies have shown that elevated MMP expression during dysfunctional uterine bleeding and excessive menstrual bleeding are associated with increased levels of TNF-α and IL-6 (237). However, *in vitro* studies have shown that continued progesterone support, such as occurs *in vivo* during early pregnancy, may act to prevent the action of proinflammatory cytokines (237, 252). In menstruating species, the elevated progesterone production by the ovary during early pregnancy may prevent MMP-mediated tissue loss, protecting the fragile endometrial environment of early implantation and placentation (221, 252, 276). As shown in Fig. 8, maternal MMP expression must be limited throughout pregnancy to prevent early pregnancy loss as well as premature delivery.

D. Pregnancy

1. MMP expression patterns during pregnancy. Ovarian steroids are critical for directing a species-specific pattern of uterine growth and functional differentiation of the maternal endometrium before establishment of pregnancy (295–297). Given a receptive endometrium, blastocyst attachment, implanta-

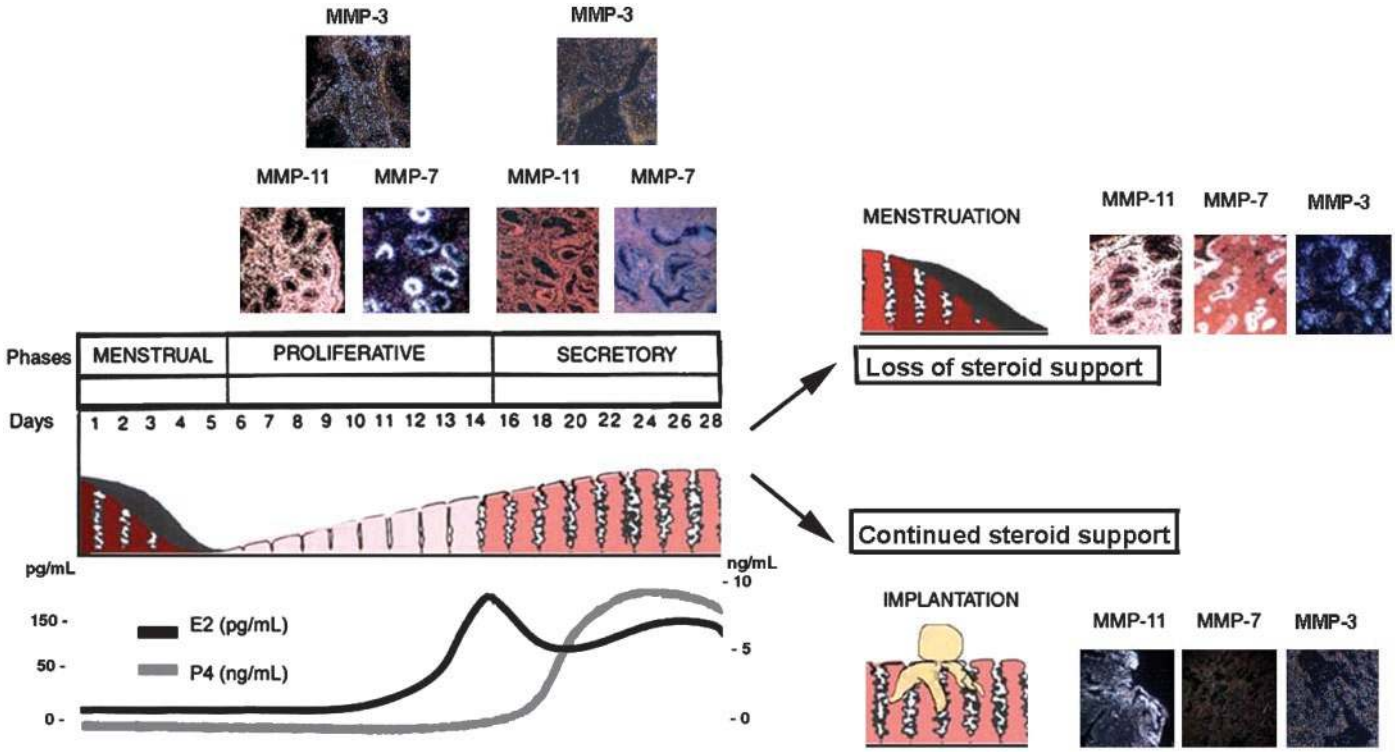

FIG. 8. Schematic representation of endometrial events during the menstrual cycle and potential outcomes. Adjacent photomicrographs depict *in situ* hybridization analysis of expression of MMP-11, MMP-7, and MMP-3 mRNA during the proliferative and secretory phases of the cycle as well as during menstruation or pregnancy establishment (original magnification of photomicrographs, ×200).

tion, and subsequent development of the placenta are among the most biologically complex processes within reproductive biology. Although the establishment of the maternal-fetal relationship of pregnancy requires focal MMP expression, largely by invasive cells of fetal origin, communication pathways involving both fetal tissue and the maternal endometrium must participate to control the expression and action of members of the MMP family. To investigate MMP expression patterns during pregnancy, numerous rodent models and, to a lesser extent sheep models, have been used as experimental systems because specimens of early human pregnancy are difficult to obtain. In rodent and ovine studies, early pregnancy is associated with the expression of mRNA and positive immunoreactivity for multiple MMPs, including MMP-2, MMP-3, MMP-7, MMP-9, MMP-11, MMP-13, and MT1-MMP (172, 220, 298–303). These animal studies have largely been in agreement that MMP-2 and MMP-9 represent the most important MMPs expressed by invading fetal cells during establishment of pregnancy, although some differences in the expression pattern of these MMPs have been reported by different laboratories. For example, in an initial report, Northern blot analysis of pregnant murine uteri demonstrated that MMP-2 mRNA expression was high on d 3–5, whereas MMP-9 mRNA expression occurred later (300). Rechtman *et al*. (301) also reported MMP-2 levels peaked on d 3 of pregnancy in rats, whereas MMP-9 was found only on d 9. Another study reported these MMPs to be more broadly expressed on d 6–8 (220). Despite some variations in the timing of MMP-2 and MMP-9 expression in rodents, evidence indicates that these MMPs are critical to implantation. For example, neutralizing antibodies directed against MMP-9 protein will block ECM degradation by murine blastocyst outgrowths, providing evidence of a specific role for MMP-9 during establishment of pregnancy (304). In a molecular-based study, targeted deletion of the conserved DNA binding domain of the Ets transcription factor resulted in deficient expression of MMP-9 mRNA, growth retardation, and death of homozygous embryos (305). Similarly, embryos lacking JunB, a transcription factor for AP-1, also exhibit dysregulation of MMP-9, resulting in embryonic death due to a failure to establish an adequate maternal-fetal circulation (306). A key role for other specific MMPs in pregnancy establishment is less clear, although endometrial epithelial expression of MMP-7 appears to be important during implantation in rats (301). Although knockout mice generated to be deficient in MMP-3, MMP-7, MMP-9, or MT1-MMP are fertile (200), MMP-9 knockout mice have been found to have reduced reproductive capacity (307), raising the interesting possibility regarding the importance of individual MMP genes for normal reproduction.

Within invading cell types in humans, early studies indicated that cytotrophoblasts isolated from first-trimester human placenta degraded basement-membrane substrates *in vitro*, whereas little or no matrix degradation occurred in the presence of second- or third-trimester cytotrophoblast cells (308, 309). Furthermore, the invasive ability of cytotrophoblasts could be inhibited by bioactive agents, which specifically neutralize MMP activity, but not by antagonists of other proteases (309). Recent studies have expanded these observations and confirm that primary cultures of first-trimester human trophoblasts constitutively express both MMP-2 and MMP-9 on a gelatin matrix, with higher levels of MMP-9 expression and activity compared with cells from the third-trimester (310). As noted in other species, the expression and local activation of MMP-9 may be a critical component of human implantation and placentation. For example, *in vitro* studies demonstrate that blocking this MMP prevents invasion of first trimester cytotrophoblasts into the artificial matrix Matrigel (311). Other MMPs have also been associated with the invasive characteristics of human trophoblasts (Table 4), including MMP-3, MMP-11, MT1-MMP, and MT2-MMP (222, 312–322). The coexpression and subsequent interactions of multiple MMPs are likely required for the rapid turnover rate of ECM during early establishment of the maternal-fetal relationship. For example, MT1-MMP, an activator of MMP-2, and the precursor for collagen type IV, a substrate of MMP-2, have been colocalized within the same cell type during establishment of pregnancy (312, 313, 318, 323). In humans and other primates, obtaining appropriate samples of tissues during the earliest stages of pregnancy is difficult, and only limited studies have documented the expression pattern of MMPs by maternal cell types. Nev-

TABLE 4. Summary of the expression patterns of the MMP system in the human endometrium throughout normal gestation

Early pregnancy			Late pregnancy			Parturition			Involution	
	Decidua	Trophoblasts		Decidua	Trophoblasts		Decidua	Trophoblasts		
MMP-1	+	+	MMP-1	+	+	MMP-1	+	−	MMP-1	?
MMP-2	++	+	MMP-2	?	++	MMP-2	?	+	MMP-2	?
MMP-3	+	+	MMP-3	+	+	MMP-3	+	−	MMP-3	?
MMP-7	−	++	MMP-7	−	+	MMP-7	−	++	MMP-7	+++
MMP-9	+	++	MMP-9	+	++	MMP-9	++	++	MMP-9	?
MMP-10	?	−	MMP-10	?	?	MMP-10	?	?	MMP-10	?
MMP-11	+/−	+	MMP-11	?	+	MMP-11	?	?	MMP-11	?
MMP-13	?	++	MMP-13	?	?	MMP-13	?	?	MMP-13	++
MMP-14	+	+	MMP-14	?	?	MMP-14	?	?	MMP-14	?
MT1-MMP	+	++	MT1-MMP	?	?	MT1-MMP	?	?	MT1-MMP	?
MT2-MMP	−	+	MT2-MMP	?	?	MT2-MMP	?	?	MT2-MMP	?
TIMP-1	+	++	TIMP-1	++	++	TIMP-1	++	+	TIMP-1	++
TIMP-2	+	−	TIMP-2	++	++	TIMP-2	++	+	TIMP-2	++
TIMP-3	++	++	TIMP-3	++	++	TIMP-3	++	+	TIMP-3	++
TIMP-4	+	+	TIMP-4	++	++	TIMP-4	++	+	TIMP-4	?

Relative levels of protein or mRNA are expressed as − (absent), + (focal), ++ (moderate), +++ (intense), ? (unknown).

ertheless, investigators have identified MMP-1, MMP-2, MMP-7, MMP-9, MMP-11, MMP-13, and MT1-MMP within stroma, epithelium, or the mature decidua at the maternal-fetal interface during early pregnancy (312–315, 318, 321, 323–325). Recently, MMP-26 (also known as matrilysin-2), an MMP with structural similarities to MMP-7, has been found to be highly expressed in the human placenta (326). Although the placental cell type expressing this new MMP is not yet clear, studies thus far indicate that, like MMP-7, this enzyme may be specific to the epithelium (327).

At the end of gestation, declining levels of steroids of ovarian and placental origin play a species-specific role in initiation of preparation for delivery within the uterus and reproductive tract. Although a complex array of local signal pathways within the uterus and birth canal are equally critical to delivery of live offspring in mammals, the MMPs play an important role in fetal membrane breakdown as well as necessary changes in maternal tissues. In rodents, MMP-2 and MMP-9 appear to be involved in preparation for parturition because a marked reduction in uterine type IV collagen content on d 21 of pregnancy, the day of delivery in rats, is correlated with increased expression of these enzymes (328, 329). However, there are species differences in uterine collagen breakdown at the time of labor, and an increase in the expression of MMP-2 and MMP-9 does not appear to be a component of labor in sheep (302). Although much is still to be learned regarding the expression and regulation of MMPs in normal human parturition, studies of premature rupture of the membranes (PROM) and preterm delivery further illustrate the importance of understanding MMP action in the process of parturition. Clinically, complications resulting from preterm birth account for the majority of infant mortality, and PROM is associated with over one third of preterm deliveries (330, 331). In human studies, MMP-2 has been reported in amnion at term before labor (320), although increases in active MMP-9, but not active MMP-2, have been reported at spontaneous term parturition (332). Other investigators have reported that both MMP-2 and MMP-9 expression increase in fetal membranes during active labor, before delivery (333, 334), and MMP-9 mRNA and protein have been colocalized in amnion epithelium, macrophages, chorion laeve trophoblasts, and decidual cells after labor (334). Additionally, recent reports indicate that MMP-9 is involved in placental separation during and after the onset of normal labor (335) and is expressed in the myometrium at parturition (336). After parturition, the involuting uterus undergoes a rapid reduction in size that requires extensive remodeling of the ECM, and the initial scientific observations of MMP involvement in uterine biology were made during this reproductive interval. During involution, a tremendous loss of collagen occurs as the uterus returns to its prepregnancy state, which was first described in the rat (337) and is now known to occur in humans as well (161). Nevertheless, the role of MMPs in uterine involution is most well described in the rodent, and only limited studies have been performed in other species. Collagenase activity is high during uterine involution, whereas MMP-1 is undetectable in nonpregnant or prepartum pregnant rats. Liu *et al*. (338) created mice with a collagen mutation resulting in a resistance to collagenase degradation and a dramatic reduction in postpartum involution. In addition to MMP-1 expression, MMP-8, MMP-10, MMP-7, and MMP-13 are also abundantly expressed during early involution, but expression decreases to undetectable levels by postpartum d 4.5 (339–341). Other MMPs, including MMP-2, MMP-3, and MMP-11 are expressed at a relatively constant level throughout involution (339, 340). As noted earlier, in MMP-7 knockout mice, uterine involution proceeds normally, although a strong up-regulation of MMP-3 and MMP-10 mRNA was noted in these animals (340). Similarly, uterine involution was not impaired in MMP-3 knockout mice, but again, a compensatory increase in MMP-7 was noted (200).

2. TIMP expression patterns during pregnancy. When one considers the invasive events of early pregnancy, the fetal portion of the placenta behaves much like a tumor in many species. Cytotrophoblasts not only invade into the endometrial stroma and musculature adjacent to the basalis layer but also readily breach the matrix of the maternal vasculature. Because of the invasive nature of early pregnancy in a broad range of mammalian species including rodents and primates, specialized stromal cells of the maternal endometrium have adapted a highly differentiated phenotype, the decidua, which acts to control invasion. Before the initiation of pregnancy, increases in TIMP-1 expression have been demonstrated in the nonpregnant uterus of rodents (198, 200, 219, 220) and sheep (342) and appear to be a component of normal endometrial differentiation and decidualization. During early pregnancy, Reponen *et al*. (343) found TIMP-3 to be intensely expressed in maternal cells in the area surrounding the invading embryonic tissue, whereas no expression of TIMP-1 or TIMP-2 was observed in other areas of the decidual reaction. In mice, TIMP-1 and TIMP-2 are expressed in undifferentiated stroma toward the outside of the decidua, whereas TIMP-3 was reported in mature decidual cells specifically associated with trophoblast invasion (298). Expression of an appropriate MMP/TIMP ratio at the maternal-fetal interface appears to be important in controlling the invasive behavior of cytotrophoblasts as well as maintaining a stabile uterine environment throughout pregnancy. For example, mRNAs for TIMP-1, TIMP-2, and TIMP-3 are coexpressed with MMP-2 and MMP-9 in the uterus of mice in a manner that suggests an important role during establishment and maintenance of pregnancy (300). Although most studies have found no alteration in fertility after gene deletions or mutations of TIMP-1 and TIMP-2 compared with wild-type animals (344), Nothnick (345) reported a reduced reproductive lifespan in TIMP-1 knockout mice. As noted earlier for MMP knockout mice, animals with a disruption in TIMP genes may have subtle alterations in fertility that may have been previously overlooked.

Although a transient tissue limited to the local environment of pregnancy, the decidua provides endocrine and nutritional support at the maternal-fetal interface and limits the invasive behavior of cytotrophoblasts (346). In humans, expression of TIMP-1, TIMP-2, and TIMP-3 has been observed in isolated stromal and epithelial cells obtained from nonpregnant endometrium, although the most intense expression occurs in decidualized stromal cells associated with pregnancy establishment (209, 221). In first-trimester human

placental decidua, immunohistochemical localization of TIMP-2 has been reported in the cytotrophoblast columns (347) as well as in the decidua and walls of blood vessels (348), whereas TIMP-3 has been observed in the maternal decidua near the end of the invasive stage of early implantation (219, 349). The coexpression of MMPs and TIMPs by cytotrophoblasts may also serve to limit invasive behavior in humans because high levels of both protein and mRNA for MMP-9 and TIMP-3 are coexpressed after differentiation of these cells to the fully invasive phenotype (350). Although studies of early pregnancy in humans are difficult, investigators have localized TIMP-1, TIMP-2, TIMP-3, and TIMP-4 expression in cytotrophoblastic columns, endothelial and fibroblastic cells of villi, and decidual membrane during all three trimesters (Table 4), as well as within amniotic fluid before term (222, 315, 319, 320, 351, 352). As noted above, PROM and preterm birth are among the most common causes of fetal morbidity associated with pregnancy complications. The balance between MMPs and TIMPs, as well as the specific expression of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 proteins, changes dramatically at the end of normal pregnancy. Women with preterm labor who deliver early exhibit elevated MMP-9 and decreased TIMP-1, associated with shifting the equilibrium toward fetal membrane breakdown (353). In amniotic fluid, increased expression and activity of multiple MMPs coupled with reduced levels of TIMP-1 or TIMP-2 expression has been associated with PROM (354–356). Taken together, animal models and clinical studies suggest that the balance between MMP and TIMP expression may be critical to controlling the timing of normal labor and delivery, whereas alterations in these ratios lead to multiple pregnancy complications.

3. Regulation of the MMP system during pregnancy. Establishing and maintaining a viable pregnancy is a biologically complex process, and numerous cytokines are expressed by maternal and fetal tissues that act in concert with steroids to mediate the selective expression of members of the MMP family during the various stages of gestation. The communication processes used among the diverse cell types present within the uterus and endometrium throughout gestation are poorly understood and clearly vary among species (357). Among the cytokines that appear to impact the MMP family during pregnancy establishment, the IL-1 family may be the most well-characterized (358). Members of this proinflammatory cytokine family have been shown to induce MMP expression in numerous tissues (359), and local induction of MMP expression in response to proinflammatory cytokines may aid cytotrophoblast invasion within the endometrial stroma during establishment of pregnancy. In rodents and humans, IL-1α and IL-1β are detected in both cyto- and villous trophoblasts and have been reported to be produced by blastocysts *in vitro* (360–362). The production of IL-1α at the maternal-fetal interface has been suggested to be critical to successful implantation, and at least one study found that exogenous IL-1 receptor antagonist prevents pregnancy establishment in mice (363). Clinical studies suggest that women possessing an IL-1 receptor antagonist polymorphism are at much greater risk of recurrent pregnancy loss (364), further indicating that the IL family is critical to establishment and maintenance of pregnancy. However, in a study with mice, ip injection of an IL-1 receptor antibody did not appear to prevent embryo implantation (365), and knockout mice lacking the IL-1 receptor expression remain fertile (366). Nevertheless, IL-1 receptor type 1-deficient mice have been shown to have reduced litter sizes (365), suggesting a subtle alteration in the ability to establish or maintain pregnancy.

Although the changing pattern of ovarian steroids impacts the uterine expression and action of members of the MMP family during the estrous and menstrual cycles, progesterone clearly plays a critical role in the regulation of this system during the events of pregnancy. For example, even before establishment of pregnancy, the stimulation of MMP-3 expression in response to IL-1α can be blocked in human stromal cells by progesterone exposure either *in vivo* or *in vitro* (252, 253). In contrast, a lack of progesterone-mediated resistance to proinflammatory cytokine action is associated with high levels of MMP expression at ectopic sites of pregnancy (367, 368). Over 40 yr ago, transplantation of murine blastocysts to the kidney capsule demonstrated uncontrolled invasion and widespread kidney damage (369), indicating that the uterus creates a unique environment for the establishment of pregnancy. Studies to date indicate that a balanced synthesis of MMPs and TIMPs by both trophoblasts and decidual membranes is crucial for rapid matrix remodeling and controlled invasion during early pregnancy. Some investigators have suggested that the control of selective MMP expression during pregnancy requires steroid-mediated production of local factors at the maternal-fetal interface. As noted earlier, during the menstrual cycle, the ability of progesterone to suppress MMP-3 and MMP-7 and block proinflammatory cytokine action requires the secondary action of TGF-β (254). The expression of TGF-β can induce TIMP-1 production by endometrial stromal cells (207), and the production of TGF-β isotypes at the maternal-fetal interface can modulate MMP expression and action in concert with local expression of TIMP (370). Although human studies are difficult to approach *in vivo*, production of both TGF-β and TIMP-1 by maternal decidua can limit the invasive behavior of first trimester trophoblasts in an *in vitro* assay (346). Graham *et al.* (371) have also demonstrated that TGF-β can significantly up-regulate TIMP-1 and TIMP-2 mRNA expression and reduce plasminogen activator expression in normal first trimester trophoblasts.

In addition to maternal tissues, invasive cytotrophoblasts acquired from human pregnancy express multiple MMPs and cytokines, which can further act to regulate members of the MMP family. Higher levels of MMP-9 expression and activity are observed in first-trimester cells compared with cells acquired during the third trimester, perhaps reflecting the changing influence of increased local progesterone production as well as progesterone-mediated production of locally active factors as pregnancy proceeds (310, 311). *In vitro* studies have shown that treatments of human trophoblast cells with progesterone decreases steady state levels of MMP-9 mRNA and protein in a concentration-dependent manner that can be reversed by addition of onapristone, a progesterone receptor antagonist (372). However, the release of both MMP-2 and MMP-9 occurs from the basolateral sur-

face of syncytiotrophoblasts, indicating that local regulatory mechanisms may equally influence the release of these MMPs by trophoblasts during villous tissue remodeling (373). As noted during our discussion of MMP regulation during menstruation, proinflammatory cytokines, including TNF-α and IL-1α, can increase gelatinolytic activity of first trimester cytotrophoblast cells via an increase in MMP-9 (274). Such *in vitro* studies indicate that multiple pathways involving autocrine and paracrine signals may be critical for MMP-9 regulation during early pregnancy (374). For example, the production of IL-1 has been shown to regulate expression of leptin, a circulating hormone that controls food intake and appears to be an important signal indicating the adequacy of nutrition for pregnancy (375). Leptin is secreted by purified first trimester cytotrophoblast cells and increases the activity of MMP-9 and MMP-2 (274, 376, 377) and may act to convert trophoblasts to the invasive phenotype (375). Leptin knockout mice are sterile, but fertility is restored by exogenous leptin. Although the precise role of leptin in reproduction has not been fully explored, this protein has been suggested to act at a signal to activate the hypothalamic-pituitary-ovarian axis at puberty (378). Importantly, although the endometrium expresses the leptin receptor, this tissue does not produce the ligand (379), and progesterone acts to suppress leptin receptor function, thus altering endometrial sensitivity to this hormone (379).

The above studies indicate that a normal pregnancy requires appropriate expression and action of multiple members of the MMP family. During the establishment and maintenance of pregnancy, the direct or indirect actions of progesterone appear to be key to the regulation of both MMPs and TIMPs. Not surprisingly, insufficient levels of this steroid are associated with pregnancy failure in women (380) and progesterone receptor-null mice exhibit extensive reproductive tract abnormalities and infertility (107, 381). Progesterone performs a largely suppressive role on MMP expression and action during pregnancy establishment, which must be reversed at the end of pregnancy. Successful cervical ripening and parturition at the end of pregnancy require the inhibition of progesterone to allow normal delivery. Mifepristone, an antiprogestin, is known to induce cervical ripening and has been recently found to increase infiltration of immune cells as well as increase expression of MMP-1, MMP-8, and MMP-9 by cells of the cervix (382). In the absence of medical intervention, normal parturition is preceded by a decline in progesterone action that is associated with an influx of leukocytes. Not surprisingly, cervical ripening has been likened to an inflammatory-like process. The associated expression of numerous proinflammatory cytokines, including IL-1, IL-8, and TNF-α, has been found to increase the expression of cervical MMPs that facilitate dilatation preceding normal and preterm delivery. In particular, MMP-1, MMP-3, MMP-8, and MMP-9 and TIMP-1 all are increased during cervical ripening (383–385) and are critically important to ECM remodeling at this time. Unfortunately, the cellular and molecular mechanisms by which progesterone and local factors act to allow the selective expression and action of specific MMPs and TIMPs while limiting the actions of others at the maternal-fetal interface, during cervical ripening at parturition or in association with postpartum uterine involution remains unclear. Nevertheless, additional members of the MMP family will certainly be found to be involved during the complicated processes associated with pregnancy.

IV. Lessons from Genetic Modification of the MMP System

The use of genetic modification such as gene deletion (*i.e.*, knockouts) or gene overexpression (*i.e.*, transgenics) has allowed an investigation into the role of the MMP system in various aspects of physiology, including reproductive function. Sternlicht and Werb (8) have summarized the nonmalignant phenotypes associated with the genetic modifications of nine of the various MMPs (*e.g.*, MMP-1, 2, 3, 7, 9, 11, 12, and 14) along with TIMPs 1–3. For the most part, there have been no reports of major impacts on the reproductive axis in the initial characterization of these genetically modified animals. These reports must be examined, however, with the understanding that careful analysis of ovarian and uterine physiology has not always been performed. Deletion of MMP-9 or TIMP-1 was not associated with an obvious reproductive phenotype in the initial description of these mice (386, 387), but subsequent analysis has revealed changes in the reproductive axis (40, 41, 307, 345). In mice lacking MMP-9, the individual litters are smaller, and the percentage of infertile breeding pairs is increased (307). Similarly, TIMP-1-null females achieved pregnancy at a lower rate than wild-type mice (52 *vs.* 78%) and had significantly fewer pups per litter (345), although induced ovulation rates were the same between wild-type and knockout females (40, 42). Careful examination by Nothnick (41) and colleagues has revealed that disruption of the TIMP-1 gene product was associated with an altered estrous cycle characterized by alterations in uterine morphology, higher serum estradiol levels, and lower serum progesterone levels compared with their wild-type counterparts, resulting in a decrease in the length of the estrous period. Progesterone levels were also diminished in TIMP-1-deficient mice during early luteal formation, although the ovaries from the knockout mice weighed more than the wild-type littermates (42). Another confounding factor in trying to understand MMP action in ovarian and uterine physiology is that removal of one MMP or TIMP often results in compensation by other members of the MMP family. For example, deletion of MMP-7 results in a 10–12-fold increase in MMP-3 and MMP-10 during uterine involution (200). Taken together, studies using genetic modification of the MMP system suggest that compensatory expression of MMPs may prevent MMP-specific loss of fertility in experimental animal models. An additional complexity in understanding the deletion of specific MMPs on reproductive physiology is that in certain genotypes there are striking phenotypic changes resulting in early postnatal death, such as deletion of MT1-MMP (389). Nevertheless, the changes in steroidogenesis in the TIMP-1 knockout mice (41) and the suboptimal fertility of the MMP-9 and TIMP-1-deficient mice (307, 345) emphasizes the importance of individual MMP genes for normal reproduction. As a better understanding of MMP action and interactions emerges, a

careful reexamination of other MMP knockouts may reveal similar subtle reductions in fertility.

V. Summary

The extensive remodeling of the extracellular architecture of the ovary and uterus is critical to the overall physiology of these organs. This remodeling is accomplished, in part, by the MMP system that is regulated by the cyclic hormonal cues associated with each stage of the estrous or menstrual cycle. These hormonal signals modulate individual MMPs and TIMPs to control the type of matrix to be remodeled, the site-specific location, and extent of matrix degradation. However, a delicate balance must exist between the activity of the MMPs and their inhibitors to allow precise remodeling of the ECM while at the same time limiting the site and extent of proteolytic degradation. Situations in which this control has gone awry could lead to pathological conditions such as luteinized unruptured follicle syndrome, ovarian cysts, endometriosis, uterine fibroids, inappropriate implantation resulting in tubal pregnancy or spontaneous abortion, premature rupture of fetal membranes, or carcinoma of the ovary or uterus. As research efforts continue to explore the ovarian and uterine MMP system, a more thorough understanding of the role that MMPs and TIMPs play as regulators of growth, cellular differentiation, and specialized tissue function in all aspects of ovarian and uterine physiology will become apparent.

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