Matrix metalloproteinases and their specific tissue inhibitors in menstruation

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The timely breakdown of extracellular matrix is essential for menstruation. Matrix metalloproteinases, which are able to degrade virtually all components of the extracellular matrix, are spatiotemporally expressed in the cyclic endometrium. The expression of most matrix metalloproteinases is regulated transcriptionally and their proteolytic activities are precisely controlled. The balance between matrix metalloproteinases and their specific tissue inhibitors is believed to be crucial for menstruation. This review focuses on the roles of matrix metalloproteinases and their tissue inhibitors in the initiation of menstruation and on the regulation of matrix metalloproteinase expression and activation. For example, the function of matrix metalloproteinases and their tissue inhibitors in endometrial re-epithelialization and angiogenesis during endometrial regeneration, when cell migration is facilitated to ensure endometrial repair, is discussed. This and other processes, although not fully resolved, serve to illustrate the involvement of matrix metalloproteinases and their tissue inhibitors in the process of menstruation.

Matrix metalloproteinases and their specific tissue inhibitors

Matrix metalloproteinases (MMPs) form a family of homologous zinc-dependent enzymes collectively capable of degrading most proteins of the ECM at neutral pH. Several laboratories have identified MMP expression in the endometrium (Marbaix et al., 1992; Martelli et al., 1993; Rodgers et al., 1994). A marked increase in endometrial expression of several MMPs occurs just before and during menstruation (Hampton and Salamonsen, 1994; Rodgers et al., 1994). The specific spatial and temporal expression patterns of MMPs indicate their pivotal roles in the process of menstruation (Salamonsen and Woolley, 1996). As the activity of all MMPs can be inhibited by tissue inhibitors of metalloproteinase (TIMPs) in a 1:1 stoichiometry (Docherty et al., 1985) and since TIMPs are also distributed in the endometrium throughout the menstrual cycle (Rodgers et al., 1994; Freitas et al., 1999), the balance between MMPs and TIMPs is likely to be critical for menstruation (Hampton and Salamonsen, 1994).

Matrix metalloproteinases and their specific tissue inhibitors

MMPs form a multigene family of enzymes (Table 1) that share significant sequence arrangement and a common multidomain structure, but are glycosylated to different extents and at different sites. With the exception of membrane-type (MT)-MMPs, MMPs are extracellular enzymes that are secreted as inactive proenzymes (zymogens). These proenzymes consist of an N-terminal propeptide domain of about 80 amino acids followed by a catalytic domain of about 170 residues. With the exception of matrilysin (MMP-7) and MMP-26, all catalytic domains are bound covalently via a proline-rich linker of 10–70 residues to a C-terminal haemopexin-like domain of about 195 residues (Massova et al., 1998; Bode et al., 1999). The haemopexin-like domain may determine the substrate specificity of MMPs or play a functional role in their interaction with TIMPs (Massova et al., 1998). In addition, MMP-2 and MMP-9 contain three repeats of a fibronectin type II-like domain that are inserted within their catalytic domain. In MT-MMPs, the polypeptide chain possesses an additional 75–100 residues, which form a transmembrane helix and a small cytoplasmatic domain (Sato et al., 1996). Zymogens become activated upon limited hydrolysis,
<table>
<thead>
<tr>
<th>Subfamily</th>
<th>MMP</th>
<th>Other names</th>
<th>Molecular mass (Da)</th>
<th>Location of gene</th>
<th>Substrates</th>
<th>Accession number</th>
</tr>
</thead>
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<tr>
<td>Collagenases</td>
<td>MMP-1</td>
<td>Interstitial collagenase Fibroblast collagenase</td>
<td>54007</td>
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<td>Col I, II, III, VII and X; gelatins; entactin; aggrecan; link protein</td>
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<td></td>
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<td>Col I, II and III; aggrecan; link protein</td>
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<td></td>
<td>MMP-13</td>
<td>Collagenase 3</td>
<td>53819</td>
<td>Chromosome 11</td>
<td>Col I, II and III</td>
<td>P45452</td>
</tr>
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<td>Gelatinases</td>
<td>MMP-2</td>
<td>Gelatinase A 72 kDa Gelatinase</td>
<td>73882</td>
<td>Chromosome 16</td>
<td>Gelatins; col I, IV, V, VII, X and XI; fibronectin; laminin; aggrecan; elastin; large tenasin C; vitronectin, β-amyloid protein precursor</td>
<td>P08253</td>
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<tr>
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<td>MMP-9</td>
<td>Gelatinase B 92 kDa Gelatinase</td>
<td>78427</td>
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<td>Gelatins; col IV, V and XIV; aggrecan; elastin; entactin; vitronectin</td>
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<td>Stromelysins</td>
<td>MMP-3</td>
<td>Stromelysin-1 Transin-1</td>
<td>53977</td>
<td>Chromosome 11</td>
<td>Aggrecan; col III, IV, IX and X; gelatins; fibronectin; laminin; large tenasin-C; vitronectin; elastin; casein</td>
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<td>MMP-7</td>
<td>Matrilysin</td>
<td>29677</td>
<td>Chromosome 11</td>
<td>Aggrecan, IGFBP-1; fibronectin; laminin; gelatins; elastin; col IV; entactin; small tenasin C; vitronectin; casein</td>
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<td>MMP-10</td>
<td>Stromelysin-2 Transin-2</td>
<td>54151</td>
<td>Chromosome 11</td>
<td>Aggrecan; col II, IV and V; fibronectin; gelatins; weakly col III, IV and V; activate procollagenase</td>
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<td></td>
<td>MMP-11</td>
<td>Stromelysin-3</td>
<td>54595</td>
<td>Chromosome 22</td>
<td>Weak activity on fibronectin; laminin; col IV; aggrecan; gelatins; IGFBP-1; α1-proteinase inhibitor</td>
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<td>MMP-12</td>
<td>Metalloelastase</td>
<td>54001</td>
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<td>Elastin; fibronectin</td>
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<td>Membrane-type MMPs</td>
<td>MMP-14</td>
<td>MT1-MMP MMP-X1</td>
<td>65883</td>
<td>Chromosome 14</td>
<td>Col I, II and III; fibronectin; laminin-1; vitronectin; dermapan sulphate proteoglycan; activates proMMP-2, proMMP-13</td>
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<td></td>
<td>MMP-15</td>
<td>MT2-MMP</td>
<td>75807</td>
<td>Chromosome 16</td>
<td>Activates pro-MMP-2</td>
<td>P51511</td>
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<td></td>
<td>MMP-16</td>
<td>MT3-MMP</td>
<td>69521</td>
<td>Chromosome 8</td>
<td>Activates pro-MMP-2</td>
<td>P51512</td>
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<td>MMP-17</td>
<td>MT4-MMP</td>
<td>67123</td>
<td>Chromosome 12</td>
<td>FibrinQ9ULZ9</td>
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<td>MMP-24</td>
<td>MT5-MMP</td>
<td>73231</td>
<td>Chromosome 20</td>
<td>Activates proMMP-2</td>
<td>Q9Y5R2</td>
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<td>MMP-25</td>
<td>MT6-MMP</td>
<td>62554</td>
<td>Chromosome 16</td>
<td>Activates proMMP-2</td>
<td>Q9NPA2</td>
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<td>Others</td>
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<td>MMPase RASI</td>
<td>57357</td>
<td>Chromosome 12</td>
<td>Aggrecan</td>
<td>Q99542</td>
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<td>MMP-20</td>
<td>Enamelysin</td>
<td>54413</td>
<td>Chromosome 11</td>
<td>Amelogenin</td>
<td>O60882</td>
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<td>MMP-23</td>
<td>MIFR/FEMAL-YSIN</td>
<td>43934</td>
<td>Chromosome 1</td>
<td></td>
<td>Q9UBR9</td>
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<td></td>
<td>MMP-26</td>
<td>Matrilysin-2 Endometase</td>
<td>29708</td>
<td>Chromosome 11</td>
<td>Gelatin; β-casein; fibronectin</td>
<td>Q9NRE1</td>
</tr>
</tbody>
</table>

Information from Swiss-Prot database at www.expasy.ch/sprot/
Col: collagen; IGFBP-1: insulin-like growth factor binding protein 1; MIFR: metalloproteinase in the female reproductive tract; RASI: rheumatoid arthritis synovial inflammation.

*aCalculated from the number of amino acids.*
during which the propeptide is lost. Activation of the pro-
MMPs into active MMPs can be reproduced in vitro by
addition of different agents such as mercurial salts, plasmin
or trypsin. Although the physiological activation of the
different MMPs is poorly understood, plasmin (Eeckhout
and Vaes, 1977) and MMP-3 (Birkedal-Hansen et al.,
1993) have been shown to be potent activators of several
MMPs.

MMPs play a critical role in many physiological processes
such as wound healing, bone growth, nerve outgrowth,
ovulation, embryo implantation, embryonic development,
uterine involution and menstruation. Overexpression of
MMPs is also linked to pathological processes such as
tumour invasion and metastasis, arthritis and atherosclerosis.

TIMPs (Table 2) are the major endogenous inhibitors of
MMP activities in tissues (Nagase and Woessner, 1999).
Binding of TIMPs to the catalytic domain results in efficient
inhibition of the enzymatic activity of MMPs (Massova et
al., 1998). The TIMP family includes four proteins, TIMP-
1–4, which share some sequence identity (40, 30 and 37%
between TIMP-1 and TIMP-2, -3 and -4, respectively)
(Douglas et al., 1997). Although TIMPs do not seem to
differentiate much among the various MMPs, a certain
degree of specificity has been observed. For example,
TIMP-2 and -3, unlike TIMP-1, are effective inhibitors of
MT-MMPs, whereas TIMP-1 preferentially binds MMP-9
(Strongin et al., 1995).

TIMPs are multifunctional proteins. Besides inhibiting
MMP, TIMPs have other functions such as growth factor-
like and anti-angiogenic activity (Gomez et al., 1997).
TIMP-1 also stimulates fibroblasts to produce MMP-1 (Clark
et al., 1994). TIMP-1 and -2 bind not only to activated
gelatinases but also to proMMP-9 and -2, respectively
(Strongin et al., 1995). The complex between MT1-MMP
and TIMP-2 acts as a cell surface ‘receptor’ for proMMP-2
activation in vivo (Butler et al., 1998).

Respective roles of MMPs and TIMPs in the process
of menstruation

Role of MMPs and TIMPs in the onset of menstruation

Markee (1948) used intraocular endometrial transplants
in rhesus monkeys to determine that menstruation was
initiated by the rapid regression of endometrial thickness as
a result of fluid loss and tissue lysis, which were followed
by intense vasoconstriction of the spiral arterioles and
subsequent bleeding from focal points. Regression of the
endometrium was later observed in non-menstruating
mammals. The endometrial wet mass and collagen content
of cyclic rats decrease during metoestrus to 20% of their
value at pro-oestrus (Yochim and Blahna, 1976), indicating
that proteolysis of the ECM takes part in this regression
(Eeckhout, 1990). Even before bleeding commences, small
lesions in human endometrial luminal epithelium can be
seen under an electron microscope (Ludwig and Spornitz,
1991); these lesions contribute to the very rapid but
incomplete degeneration of the functional layer and
exposure of open capillary vessels. Widespread degeneration
of human endometrial stroma and basal lamina occurs at
the end of the luteal phase (Roberts et al., 1992). The classic
view that menstruation is a process generated primarily
by vascular events is thus challenged by the concept that
breakdown of the ECM in the functionalis initiates
menstruation.

A large number of studies strongly implicated MMPs and
TIMPs in menstruation. Studies using immunocytochemistry
(Martelli et al., 1993; Rodgers et al., 1993) or in situ
hybridization (Hampton and Salamonsen, 1994; Rodgers
et al., 1994) showed that endometrial cells produce
MMP-1, -2, -3, -7 and -9, and TIMP-1 and -2. Messenger
RNAs for proMMP-1 and -3 are detected in normal
endometrium only peri-menstrually and menstrually.
Therefore, it is likely that both MMP-1 and -3 have

Table 2. Human tissue inhibitors of metalloproteinases (TIMPs)

<table>
<thead>
<tr>
<th>TIMP</th>
<th>Other names</th>
<th>Molecular mass (Da)</th>
<th>Location of gene</th>
<th>Matrix metalloproteinase (MMP) substrate</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP-1</td>
<td>Metalloprotease inhibitor 1&lt;br&gt;Erytroid potentiating activity (EPA)&lt;br&gt;Fibroblast collagenase inhibitor&lt;br&gt;Collagenase inhibitor</td>
<td>23,171</td>
<td>Chromosome X</td>
<td>MMP-1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 16</td>
<td>P01033</td>
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<tr>
<td>TIMP-2</td>
<td>Metalloprotease inhibitor 2&lt;br&gt;CSC-21K</td>
<td>24,399</td>
<td>Chromosome 17</td>
<td>MMP-1, 2, 3, 7, 8, 9, 10, 13, 14, 15, 16, 19</td>
<td>P16035</td>
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<tr>
<td>TIMP-3</td>
<td>Metalloprotease inhibitor 3&lt;br&gt;MIG-5 protein</td>
<td>24,145</td>
<td>Chromosome 22</td>
<td>MMP-1, 2, 3, 7, 9, 13, 14, 15</td>
<td>P35625</td>
</tr>
<tr>
<td>TIMP-4</td>
<td>Metalloprotease inhibitor 4</td>
<td>25,502</td>
<td>Chromosome 3</td>
<td>MMP-1, 2, 3, 7, 9</td>
<td>Q99727</td>
</tr>
</tbody>
</table>

Information from Swiss-Prot database at www.expasy.ch/sprot/
*Calculated from the number of amino acids.
important roles in the tissue degradation that initiates menstruation (Hampton and Salamonsen, 1994). mRNAs encoding TIMP-1 and -2 are present throughout the cycle but increase to a lesser extent than do mRNAs encoding MMP on days 1 and 2 at menstruation (Hampton and Salamonsen, 1994). Given that the stability of mRNAs encoding MMPs and TIMPs is equivalent and that translation of the mRNAs results in the production of proteins in endometrial tissue, a major alteration in the balance between enzymes and their inhibitors is in favour of tissue degradation (Salamonsen and Woolley, 1996). Endometrial MMPs have different cellular origin. Observations in vivo and in vitro indicate that human endometrial stromal and epithelial cells, when cultured separately, can release several MMPs (Martelli et al., 1993). Most MMPs are produced by stromal cells at menstruation (Rawdanowicz et al., 1994; Rodgers et al., 1994). MMP-2 and -9 have a stromal expression throughout the menstrual cycle. MMP-9 is detected in the glandular epithelium but only during the secretory phase (days 19–20) (Freitas et al., 1999). An immunocytochemical study (Freitas et al., 1999) showed that MMP-2, -3 and -9 were also detected in endometrial vascular structures, such as pericytes and smooth muscle cells. In particular, MMP-3 was expressed in vessels during the pre-menstrual and menstrual phases. The abundant expression of MMP-3 in the vessels situated in the superficial layer of the endometrium during menses indicates that it initiates degradation of the vascular wall during menstrual breakdown. The occurrence of MMPs in both stromal and vascular compartments at the beginning of menstruation indicates that bleeding results from degradation of both stromal components and vascular walls (Freitas et al., 1999).

MMP-7 is also clearly related to menses as mRNA encoding MMP-7 has been localized to endometrial glandular and luminal epithelial cells, and reaches a peak concentration in the pre-menstrual and menstrual phases (Rodgers et al., 1993). However, the role of MMP-7 in the degradation of epithelial tissue remains to be investigated.

In addition to stromal cells, epithelial cells, endothelial cells and vascular smooth muscle cells (SMCs), the endometrium contains many different bone marrow-derived cells, including macrophages, T cells, B cells and granulocytes, which colonize the endometrium at each cycle. Leucocytes represent an increasing proportion of endometrial cells as the menstrual cycle progresses (Shi et al., 1995). Large granular lymphocytes (LGLs), T cells , B cells and macrophages also secrete MMP-2 and MMP-9 (Shi et al., 1995). LGLs release the highest gelatinolytic activity, followed by the macrophages, T cells, B cells and monocytes. Although gelatinolytic activity does not represent the total proteolytic activity of these cells, the contribution of these bone marrow-derived cells to the total proteolytic potential of the endometrium is quantitatively greater than the contribution of the epithelial and stromal cells after ovulation (Bischof, 1997).

The spatiotemporal expression and activation of MMPs in the endometrium is closely associated with the process of normal menstruation (Hampton and Salamonsen, 1994; Rodgers et al., 1994; Salamonsen and Woolley, 1996). The fact that synthetic MMP inhibitors completely inhibit the breakdown of menstrual tissue in vitro is taken as evidence for the instrumental role of MMPs in the onset of menstruation (Marbaix et al., 1996).

Norplant is an effective and widely used progesterin-only contraceptive, which has a high discontinuation rate owing to unpredictable uterine bleeding. Expression of MMP-9 (Vincent et al., 1999) and MMP-1 (Vincent et al., 2000) is significantly higher in endometrial tissues from users of Norplant as compared with non-users. Irregular bleeding in women treated with Norplant correlates with the appearance of MMP-1 and -3 and the increased production of MMP-2, the activation of proMMP-1, -2, -3 and -9 and the decreased secretion of TIMP-1 (Galant et al., 2000; Marbaix et al., 2000). These findings imply that irregular bleeding occurs when the concentration of active MMPs exceeds the inhibitory capacity of TIMP-1, so as to freely degrade the ECM.

Putative role of MMPs and TIMPs in endometrial regeneration

Endometrial regeneration usually begins by the second day after bleeding has started (Smith, 2001). Epithelial cell migration followed by replication characterizes the biodynamics of endometrial surface repair (Bullett et al., 1998). Bleeding stops when the surface has been completely re-epithelialized.

Heterogeneous expression of TIMP-1 and TIMP-2 occurs in arteriolar SMCs and in the stroma during menstruation, and staining occurs in non-necrotic tissue and is especially abundant in the arterioles, delimiting necrotic from non-necrotic areas. This expression pattern implies that TIMPs limit the action of MMPs in some areas, thus preserving the capacity of endometrial regeneration (Freitas et al., 1999).

The roles of MMPs and TIMPs during endometrial repair have not been elucidated in detail. Most of our understanding derives from studies carried out on wound healing in skin.

It is generally accepted that migration and remodelling events during skin repair require both the activity of MMPs and their counter-regulation by TIMPs. After injury, an elaborate chain of events is triggered to ensure that basal keratinocytes migrate to re-epithelialize the wound (Martin, 1997). Increased MMP and plasmin expression is critical for the leading keratinocytes to dissolve the fibrin barrier around them, thereby facilitating their locomotion. Once the wound has been covered, the keratinocytes stop proliferating and switch off their MMP secretion (Martin, 1997). A similar expression pattern of MMPs in migrating epithelium has been found regardless of wound types and species (Saarialho-Kere et al., 1994; Salo et al., 1994; Kahari and Saarialho-Kere, 1997; Madlener et al., 1998). Although endometrial regeneration differs from wound
healing because no scarring occurs, it is still reasonable to speculate that MMPs and TIMPs participate in the process of endometrial re-epithelialization.

Mechanisms of endometrial repair include angiogenesis, the process of new blood vessel formation from pre-existing blood vessels (Folkman, 1997). Angiogenesis consists of a sequence of events, including dissolution of the basement membrane underlying the endothelial layer, migration and proliferation of endothelial cells, formation of the vascular capillary, and formation of a new basement membrane (Sang, 1998). Angiogenesis can occur by sprouting or non-sprouting mechanisms. The pleiotropic effects of most MMPs and TIMPs in the angiogenic process have been reviewed in detail by Sang (1998). For example, MMP-2 and -9 activities are important in the early steps of endothelial capillary formation. MMP-1 activity is responsible for endothelial cell migration through the interstitial spaces. TIMP-1, -2, -3 and possibly -4 inhibit neovascularization through anti-MMP-dependent or -independent pathways. Fibrinolysis by the plasma membrane-anchored MT1-MMP in endothelial cells is critical for neovascularization (Hiraoka et al., 1998). Furthermore, mice made genetically deficient in MMP-2, although revealing no gross developmental abnormalities, have an impaired angiogenic response to tumour stimuli (Itoh et al., 1998). Targeted inactivation of MMP-9 causes a transient delay in bone development because of defective vascular invasion (Vu et al., 1998). Overexpression of the multipotent fibroblast growth factor 1 (FGF-1) stimulates endothelial cell migration by mediating MMP-1 gene transcription and collagenolytic activity (Partridge et al., 2000).

Angiogenesis is an essential component of endometrial renewal, because new blood vessel formation is required to supply oxygen and nutrients. Disruption of angiogenesis in mice results in endometrial atrophy (Klauber et al., 1997). Endometrial angiogenesis was characterized by non-sprouting mechanisms such as intussusception and elongation (Rogers and Gargett, 1999). In humans, growth of endometrial endothelial cells begins at menstruation and starts from the ruptured spiral arterioles and venules (Smith, 1998).

Vascular endothelial growth factor (VEGF) is the main angiogenic and permeability factor of the endometrium, and is involved in both physiological endometrial angiogenesis and endometriotic angiogenesis (Shifren et al., 1996). VEGF expression is controlled by ovarian steroids in proliferative and secretory endometrium (Shifren et al., 1996). During menstruation, local factors, such as hypoxia, can upregulate VEGF expression and this may play an important role in angiogenesis and endometrial repair after menstruation (Sharkey et al., 2000). Emerging evidence demonstrates that VEGF can induce MMP expression and inhibit TIMP expression in endothelial cells (Lamoreaux et al., 1998). VEGF can also stimulate MMP secretion from human aortic SMCs (Wang and Keiser, 1998). Both endothelial cell- and SMC-derived MMPs may facilitate degradation of vascular basement membrane, which is a crucial stage of angiogenesis. Upregulated SMC-derived MMPs may also promote SMC migration in a later stage of angiogenesis (Wang and Keiser, 1998). Thrombospondin type 1 (TSP1) is thought to function as an inhibitor of angiogenesis (Bein and Simons, 2000) and is expressed in endometrial stromal cells (Iruela-Arispe et al., 1996). TSP1 can prevent the activation of MMP-2 and -9 zymogens, which probably contributes to the anti-angiogenic activity of TSP1 (Bein and Simons, 2000). In this respect, it is perhaps significant that, in the endometrium, MMP-2, TIMP-1 and -2 are detectable in vessels throughout the menstrual cycle (Freitas et al., 1999). MMP-2 is expressed intensely in capillaries and SMCs at the end of the luteal phase, MMP-3 is expressed markedly in SMCs and in the basal portion of endothelial cells during the pre-menstrual and menstrual period. In contrast, MMP-9 is detected in spiral arteries during the secretory phase and in vascular structures during the midfollicular and menstrual phases.

Taken together, these observations indicate that MMPs and TIMPs actively take part in the vascular events accompanying endometrial regeneration. A delicate shift in the balance between MMPs and their inhibitors occurs probably within the capillaries or in their environment during endometrial angiogenesis.

**Regulation of MMP gene expression**

Acute progesterone withdrawal induces both secretion and activation of certain MMPs (Martelli et al., 1993; Hampton and Salamonsen, 1994; Rodgers et al., 1994; Lockwood et al., 1998). In contrast, physiological concentrations of progesterone almost totally abolish MMP-1 release, as well as MMP-2 and -9 activity, in explant cultures of human endometrium (Marbaix et al., 1992). Therefore, it may be hypothesized that the expression of several MMPs in the human endometrium is controlled ultimately by ovarian steroids. In parallel to endometrial tissue changes, the temporal and spatial expression pattern of MMPs indicates the involvement of autocrine–paracrine regulations. The only study in vivo of the effects of steroids on the expression of MMPs and TIMPs in the endometrium of a primate (Rudolph-Owen et al., 1998) showed that, although endometrial MMPs were upregulated by withdrawal of progesterone in ovariectomized monkeys, many of the MMP transcripts decreased either abruptly or gradually after menstruation in the absence of progesterone. However, MMP expression was confined to the upper functionalis zone during menstruation, but after menstrual breakdown was complete, and expression of MMP-7 and TIMP-1 shifted from the functionalis to the basalis zone in the absence of both oestradiol and progesterone. Therefore, it seems unlikely that progesterone is the only hormone involved in regulating the expression, localization and function of endometrial MMPs.

Cell–cell interaction has an important role in mediating aspects of steroid action during growth and differentiation in the endometrium (McClellan et al., 1986). After the
withdrawal of progesterone, there is a marked endometrial stromal compaction that increases cell–cell contacts. Regulation of MMP expression has been associated with changes both in the shape of cells and in cell contacts (Aggeler et al., 1984; James et al., 1993). Osteen et al. (1994) used isolated and recombined types of endometrial cell to demonstrate that isolated human endometrial epithelial cells require the presence of stromal cells to restore steroid suppression of MMP-7 secretion, confirming the necessity for stromal-derived factors as mediators in this suppression process. The number of lymphomyeloid cells (for example, eosinophils, macrophages and neutrophils) increases markedly in the endometrium during the pre-menstrual phase, and these cells synthesize a plethora of cytokines and proteases, including MPPs (Salamonson and Woolley, 1999). Mast cell activation, which results in the release of potent regulators, such as tumour necrosis factor α (TNF-α), interleukin 1 (IL-1), histamine, heparin, tryptase and chymase (Salamonsen and Lathbury, 2000), increases immediately before menstruation (Jezierska et al., 1995).

Expression of endometrial MMPs can be regulated by locally produced cytokines and growth factors or by products of migratory or resident cells (Bischof and Campana, 2000). Transforming growth factor β (TGF-β) is produced by endometrial stromal cells in response to progesterone, and this cytokine can suppress expression of epithelial MMP-7 independently of progesterone. Indeed, antibodies against TGF-β abolish progesterone-induced MMP-7 suppression in stromal–epithelial cell co-cultures (Bruner et al., 1995). IL-1α derived from the epithelium is the key paracrine inducer of MMP-1 in endometrial fibroblasts in the absence of ovarian steroids (Singer et al., 1997). IL-1 and TNF-α derived from human mast cells may stimulate production of stromal cell proMMP-1, -3 and, to a lesser extent, -2 (Zhang et al., 1998). Several recombinant cytokines can induce the selective expression of MMPs in cultured human endometrial fibroblasts: MMP-1 was induced by epidermal growth factor (EGF), platelet-derived growth factor-BB (PDGF-BB), TNF-α and IL-1α; mRNA encoding MMP-11 was induced concomitantly on treatment with insulin-like growth factor II (IGF-II) or IL-6 and, particularly, with EGF and PDGF-BB; and mRNA encoding MMP-9 was induced quickly by TNF-α, but disappeared within 12 h despite continuing stimulation (Singer et al., 1999).

Cultured human endometrial stromal cells mimic menstruation-related events with remarkable fidelity. For example, Lockwood et al. (1998) demonstrated changes in vitro in MMP-1 and -3 mRNA and protein and an absence of effect on MMP-2 and TIMP-1 expression consistent with the results of the in situ hybridization study of Rodger et al. (1994). These results are consistent with the notion that some MMPs (MMP-1, -3 and -9) are regulated whereas others are constitutively expressed (MMP-2). Indeed, MMPs can be divided into two groups according to their regulation of gene expression: most MMPs exhibit inducible expression and a small number are produced constitutively and are not inducible (Borden and Heller, 1997). The promoter regions of inducible MMP genes (MMP-1, -3, -7, -9, -10, -12 and -13; Fig. 1) have a remarkable conservation of important regulatory cis elements, such as activator protein 1 (AP-1) and polyomavirus enhancer element (PEA3) (Westermarck and Kahari, 1999). Most of these genes are not expressed in unstimulated cells, and their expression is induced markedly by growth factors, cytokines, chemical agents (for example, phorbol esters and actin stress fibre-disrupting drugs), physical stress, and oncogenic cellular transformation. In addition, the enhanced MMP gene expression may be downregulated by suppressive factors (for example, TGF-β, retinoic acid and glucocorticoids) (Nagase and Woessner, 1999). AP-1 is a homo- or heterodimeric DNA-binding protein composed of either two proteins of the Jun family or one protein of the Jun family and one protein of the Fos family (Westermarck and Kahari, 1999). In general, the AP-1 element seems to be important in initiating transcription of these genes, whereas PEA3 and possibly other sites contribute to the fine tuning mechanisms that modulate these genes (Matrisian, 1992). In contrast, MMP-2 is constitutively expressed. The promoter region of the MMP-2 gene (Fig. 1) contains an AP-2 binding site and two specificity protein 1 (SP-1) sites but lacks a TATA box and AP-1- and PEA-3-binding sequences (Huhtala et al., 1990). The MT1-MMP promoter shows similarity with that of MMP-2 (Lohi et al., 2000).

Progesterone clearly acts to suppress endometrial MMP expression, but the mechanism of its regulation on transcription of specific MMP genes is not yet understood. The fact that the promoter region of most MMPs (both inducible and non-inducible MPPs) lacks traditional progesterone responsive elements indicates that progesterone may regulate certain MMP genes via nonclassical DNA sequences (Huhtala et al., 1997). In addition, ligand-activated progesterone receptor (PR), like other activated nuclear receptors, such as glucocorticoid receptor (Yang-Yen et al., 1990), retinoic acid receptor (Schule et al., 1991) and thyroid receptor (Zhang et al., 1991), may decrease the amounts of transcription factors (such as fos–jun) by a direct protein–protein interaction, resulting in a decreased binding of the transcription factors on the AP-1 site of MMP promoters. When human PR (hPR) was transfected into hPR-devoid human endometrial (HEC-1-B) cells, AP-1 activity was stimulated by the mere presence of the receptor in the absence of progesterone or of any other stimulus, whereas addition of the ligand (progesterone) reversed the effect (Bamberger et al., 1996). Moreover, the influx of leucocytes (which are known to produce MMPs) into human endometrium coincides with progesterone withdrawal (Salamonson and Lathbury, 2000), and so the progesterone decline may also provide cell–cell communication among the various migratory and resident cells and further influence local expression of MMPs.
Control of MMP activation

Apart from MMP-11 and most MT-MMPs, which are activated intracellularly by the Golgi-associated proteinase furin (Pei and Weiss, 1995, 1996), most MMPs are secreted from cells as inactive zymogens. In all cases, activation requires the disruption of the Cys–Zn$^{2+}$ (cysteine switch) interaction, and the removal of the propeptide proceeds thereafter often in a stepwise manner (Nagase, 1997). Most pro-MMPs are likely to be activated by tissue proteinases or opportunistic bacterial proteinases in vivo (Nagase and Woessner, 1999). Studies in transgenic mice indicate that the urokinase plasminogen activator (uPA)–plasmin system is a key activator of pro-MMPs (Carmeliet et al., 1997). Studies in vitro using human endometrial stromal cells indicated that PA activity is regulated primarily by changes in the expression of plasminogen activator inhibitor 1 (PAI-1) (Lockwood et al., 1995; Schatz et al., 1995), which is different from the regulation of expression of MMP, brought about primarily by changes in transcription of MMP that are independent of TIMP expression (Lockwood et al., 1998). Progestosterone inhibits the secretion of PAs by cultured endometrial explants (Casslen et al., 1986) and stimulates the expression of PAI-1 by cultured endometrial stromal cells (Casslen et al., 1992), indicating a control of this system by sex steroids during the menstrual cycle and its release before menstruation (Marbaix et al., 1996).

Secreted pro-uPA binds to, and is activated at, specific cell membrane receptors, where it converts plasminogen to plasmin. Plasmin may degrade matrices that undergo rapid turnover but also may exert its prime impact on ECM proteolysis by activating MMP zymogen (Eeckhout and Vaes, 1977).

Both mast cell tryptase and chymase may play roles in MMPs activation at menstruation. MMP-3, which initiates a cascade of MMP activation (Birkedal-Hansen et al., 1993), is activated by tryptase in human endometrial stromal cells (Zhang et al., 1998). In addition, chymase can activate latent MMP-1 (Saarinen et al., 1994).

MMP-2 is an exception. In tumour cells, endometrial neutrophils and endometrial granular leukocytes, activation of proMMP-2 has been shown to take place on the cell surface. MT1-MMP has been postulated to activate proMMP-2 in endometrium (Zhang et al., 2000). This activation process requires both active membrane-type 1 (MT1-)MMP- and TIMP-2-bound MT1-MMP. The TIMP-2 in this complex binds proMMP-2 to generate a ternary complex, which is assumed to bring the zymogen close to the active MT1-MMP. ProMT1-MMP is activated intracellularly by furin. TIE: TGF-β inhibitory element; SBE: STAT binding element; SP-1: specificity protein 1 binding site; sil: silencer sequence.

Fig. 1. Human matrix metalloproteinase (MMP) activity is controlled by transcriptional regulation, pro-enzyme activation and inhibition by specific tissue inhibitors (TIMPs). With respect to gene expression, MMP-1 and -2 represent examples of an inducible and an uninducible MMP, respectively. Human MMP-1 promoter region contains binding sites (activator protein 1 binding site (AP-1) and polyomavirus enhancer A binding protein 3 responsive element (PEA3)) that are responsive elements for several growth factors and cytokines. ProMMP-1 (like most MMPs) is activated extracellularly in a stepwise manner. Human MMP-2 promoter region lacks AP-1- and PEA3-binding sequences. ProMMP-2 is activated on the cell surface. This activation process requires both active membrane-type 1 (MT1)-MMP- and TIMP-2-bound MT1-MMP. The TIMP-2 in this complex binds proMMP-2 to generate a ternary complex, which is assumed to bring the zymogen close to the active MT1-MMP. ProMT1-MMP is activated intracellularly by furin. TIE: TGF-β inhibitory element; SBE: STAT binding element; SP-1: specificity protein 1 binding site; sil: silencer sequence.
whereas soluble plasmin degrades it (Mazzieri et al., 1997). In addition, MMP-2 can be activated through auto-lysis (Atkinson et al., 1995).

Active MMPs are inhibited by α2-macroglobulin or by TIMPs, which are both present in human endometrium throughout the menstrual cycle, and protect the endometrium from excessive tissue degradation.

**Conclusion**

Matrix metalloproteinases have diverse substrate specificity, ranging from multiple ECM components to growth factors, cytokines and other proteinases. Recent studies have increased our understanding of the significance of MMPs and TIMPs in the process of menstruation (Fig. 2). It is becoming clear that MMPs play a key role in the cyclical breakdown of ECM that ultimately leads to menstruation. Although circulating concentrations of progesterone affect the expression pattern of several endometrial MMPs, local factors can enhance or override this regulation. Although the cellular and molecular processes of endometrial repair are still unclear, MMPs cleave structural substances and fulfill additional regulatory roles (such as the processing of inactive MMP and cytokine precursors) and TIMPs exhibit multiple biological functions and, therefore, are likely to play a highly complex and tightly regulated role in endometrial repair. Some intriguing issues remain to be investigated. For example, which MMPs are the key enzymes in the initiation of menstruation; what are the respective contributions of the various MMPs in the process of endometrial repair; and how does progesterone influence leucocyte migration and activation?

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