Membrane-type matrix metalloproteinase-1 (MT-MMP-1) has been proposed to play a critical role in regulating the expression of tissue-invasive phenotypes in normal and neoplastic cells by directly or indirectly mediating the activation of progelatinase A. To begin characterizing MT-MMP-1 structure-function relationships, transmembrane-deletion mutants were constructed, and the processing of the zymogens as well as the catalytic activity of the mature proteinases was analyzed. We now demonstrate that pro-MT-MMP-1 mutants are efficiently processed to active proteinases following post-translational endoproteolysis immediately downstream of an Arg	extsuperscript{108}-Arg-Lys-Arg basic motif by a proprotein convertase-dependent pathway. The secreted form of active MT-MMP-1 not only displays an N terminus identical with that described for the processed wild-type enzyme at Tyr	extsuperscript{112} (Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grants, G. A., and Goldberg, G. I. (1995) J. Biol. Chem. 270, 5331-5338), but also directly mediates progelatinase A activation via a two-step proteolytic cascade indistinguishable from that observed with intact cells. Furthermore, although the only function previously ascribed to MT-MMP-1 is its ability to act as a progelatinase A activator, purified transmembrane deletion mutants also expressed proteolytic activities against a wide range of extracellular matrix molecules. Given recent reports that MT-MMP-1 ectodomains may undergo intercellular transfer in vivo (Okada, A., Bellocq, J.-P., Rouyer, N., Chenard, M.-P., Rio, M.-C., Chambon, P., and Basset, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2730-2734), our data suggest that soluble forms of the proteinase confer recipient cells with the ability to not only process progelatinase A, but also directly degrade extracellular matrix components.

Members of the matrix metalloproteinase (MMP)	extsuperscript{1} gene family have been implicated in the physiologic as well as pathologic remodeling of the extracellular matrix (ECM) in events ranging from organogenesis to tumor metastasis (1-3). Very recently, three new members of this family were discovered by screening cDNA libraries for homologs to conserved regions of the known MMP genes and named the membrane-type matrix metalloproteinases-1, -2, and -3 (MT-MMP-1, -2, and -3; Refs. 4-6). Based on their predicted amino acid sequences, each of the MT-MMPs, like almost all previously characterized MMPs, contains (i) a candidate leader sequence, (ii) a propeptide region which includes a highly conserved PRGCGXD sequence that helps stabilize the MMP zymogen in a catalytically inactive state, (iii) a zinc-binding catalytic domain, and (iv) a hemopexin-like domain near their respective C termini (4-7). In addition, in a pattern similar to that described for stromelysin-3, each of the MT-MMPs contains a short amino acid insert sandwiched between their pro- and catalytic domains that encodes a potential recognition motif for members of the proprotein convertase family (4-8).

Although little is known with regard to the potential functions of the MT-MMPs, most attention has focused on the ability of MT-MMP-1 as well as MT-MMP-3 to induce the processing of the MMP zymogen, progelatinase A, to its activated form (i.e. [Tyr	extsuperscript{111}]gelatinase A) via a [Leu	extsuperscript{38}]gelatinase A intermediate (4, 6, 10). Given the ability of activated gelatinase A to cleave a wide range of ECM substrates (including native types I, IV, V, VII, and XI collagen, denatured collagens, elastin, proteoglycans, laminin, and fibronectin) as well as the association of gelatinase A activation with the expression of tissue-invasive phenotypes (1-3, 11-15), MT-MMPs have been dubbed as possible “master switches” that control ECM remodeling (16). Nonetheless, the processes that regulate the activation of the MT-MMP zymogens themselves to mature forms remain undefined as does the mechanism by which MT-MMPs mediate gelatinase A activation (4-6, 9, 10). In large part, further progress in characterizing MT-MMP activities has been hindered by the technical problems associated with isolating and purifying membrane-associated molecules. Given that similar difficulties with other transmembrane enzymes have been negotiacted by generating TM-deleted soluble mutants (17-19), we noted that, with the exception of the extended C-terminal domain, the modular organization of the MT-MMPs is identical with that of the secreted MMPs (7). Hence, two

\[\text{MT-MMP-1}_{\text{1-508}}\text{TM, transmembrane; MDCK, Madin-Darby canine kidney cells; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.}\]

\[\text{a}1\text{b}1\text{c}1\text{d}1\text{e}1\text{f}1\text{g}1\text{h}1\text{i}1\text{j}1\text{k}1\text{l}1\text{m}1\text{n}1\text{o}1\text{p}1\text{q}1\text{r}1\text{s}1\text{t}1\text{u}1\text{v}1\text{w}1\text{x}1\text{y}1\text{z}1\]
TM-deletion mutants of MT-MMP-1 were constructed by either truncating the molecule (i) immediately upstream of the start site of the TM domain (i.e., MT-MMP-1-D105A) or (ii) at the conserved cysteinyI residue found at, or near, the terminus of all hemopexin domain-containing MMPs (i.e., MT-MMP-1-D508 or MT-MMP-1-D508). Utilizing these constructs, we now demonstrate that TM-deleted MT-MMP-1 (ΔMT-MMP-1) mutants undergo efficient post-translational endoproteolysis by Arg111-Tyr112 by a proprotin convertase-dependent pathway to generate fully active proteinases. Furthermore, the purified ΔMT-MMP-1 mutants not only activate recombinant progelatinase A directly via a two-step activation cascade identical with that described for the membrane-associated enzyme, but they also express hertofore unsuspected ECM-degrading activities.

EXPERIMENTAL PROCEDURES

Cell Culture—COS-7 cells and MDCK cells (both obtained from ATCC) were maintained in Dulbecco's modified Eagle's medium (Life Technologies Inc.) containing 10% fetal bovine serum (HyClone) and 4 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin as described (8, 20). Cells were transfected with purified plasmid DNA by LipofectAMINE treatment (Life Technologies Inc.). MDCK clones stably expressing MT-MMP-1-D108 were established by transfection and subsequently selected with G418 (Life Technologies, Inc.) as described previously (8, 20).

Plasmids—Following screening of an HT-1080 cDNA library constructed in the pBK-CMV vector with a 0.45-kilobase canine MT-MMP-1 cDNA isolated from an MDCK cDNA library, a human clone was obtained and sequenced. The predicted amino acid sequence of full-length HT-1080 MT-MMP-1 was identical with that described by Okada et al. (13). To generate pro-MT-MMP-1-D105A and pro-MT-MMP-1-D508, a 5′ primer (ACCATGCTTCCGCGCCCAAGACCCCGCTG) was paired with the 3′ primers, TTCAGCTACCCCGCCCCCGCC or TTCAGAAAGAAGACTCCGGGAGC, respectively, in separate PCR reactions to generate cDNA fragments with the intended truncations at the C terminus (i.e., immediately prior to the TM domain as defined by Cao et al. (9) at Ser508 or at the conserved Cys residue at position 508 (6, 7); see Fig. 1A). The PCR fragments were then cloned into pcR3-Uni vector (Invitrogen) and characterized by sequencing as described (8). Expression vectors for α1-proteinase inhibitor (α1PI), the Pittsburgh mutant of α1PI (α1PI-P), and a TM-deletion mutant of furin were provided by A. Hemerttulla and R. Kaufman (University of Michigan).

Mutagenesis—Sequential PCR-based mutagenesis was performed as described previously, and the mutagenized fragments were cloned into pcR3-Uni vector and characterized by sequencing (8). The mutagenic primers used are as follows: ATCAAGGCCAATGTTGCGAGCTGATTGCAGCC for R108A, GCCATATTCGAGAGGCCCGGCAGTGGCCAAATGCGTTACACAATGTTCAGCG for K110A, AATTGGGTAGGAAAGCTGTTCACTCGGT for R112A, and GTGCCAGAGAGCTTGGCTGGCCAGGCCTGTT for Y112F (bold nucleotides indicate the altered codons). MT-MMP-1-D105A expression constructs were as well as those harvesting the above modifications (1 μg each) were transiently transfected into COS-7 cells (2 × 10⁶/ml) by LipofectAMINE treatment, and, after a 24-h incubation, an aliquot of the cell-free supernatant (0.02 ml) was analyzed by Western blotting as described below.

Protein Purification—MDCK cells stably expressing MT-MMP-1-D108 were incubated in Opti-MEM (Life Technologies Inc.) supplemented with the synthetic MMP inhibitor, BB-94 (0.5 μM, British Biotechnology, Ref. 21), to trap the active form of the proteinase as a reversible enzyme-inhibitor complex (20). After 48 h, ~2 liters of conditioned media were collected, dialyzed against buffer A (50 mM Tris, pH 7.5, 5 mM CaCl2), and then loaded onto a Q-Sepharose column (1.5 × 10 cm). Bound material was eluted with a NaCl gradient (0 to 1 M), and fractions containing MT-MMP-1-D108 (identified by Western blotting) were combined and dialyzed against buffer A. A heparin-Sepharose column (1 × 10 cm) was then loaded with the dialyzed materials and developed with a NaCl gradient (0 to 1 M). Positive fractions were pooled and passed through a gelatin-Sepharose column (1 × 5 cm) followed by gel filtration on Ultrogel A4 (1 × 150 cm) in the presence of BB-94 to regenerate the active proteinase. In selected experiments, MT-MMP-1-D108 was purified from a batch culture of transiently transfected MDCK cells as described above.

3D. Pei and S. J. Weiss, unpublished observation.

FIG. 1. Expression and characterization of ΔMT-MMP-1. A, domain alignments of MT-MMP-1 and the ΔMT-MMP-1 mutants. Wild-type MT-MPP-1 contains 582 amino acids arranged as a series of pre- (shaded box), pro-, catalytic, hemopexin (bounded by a pair of highly conserved cysteinyl residues indicated as C), TM (shaded in black and marked TM), and cytosolic (residues 564 to 582) domains. MT-MMP-1-D105A (lane 3), or wild-type MT-MMP-1 (lane 4) expression vectors were analyzed by immunoblotting with MT-MMP-1-specific polyclonal antiserum. The dark and clear arrowsheads indicate the positions of the putative pro- and processed forms of ΔMT-MMP-1. B, Western blot analysis of ΔMT-MMP-1-transfected cells. Serum-free conditioned medium from COS-7 cells transiently transfected with control (lane 1), MT-MMP-1-D105A (lane 2), MT-MMP-1-D508 (lane 3), or MT-MMP-1-D108 (lane 4) expression vectors were depleted of endogenous progelatinase A by gelatin affinity chromatography and analyzed by zymography. In lane 5, the MT-MMP-1-D108 zymogram was developed in the presence of 10 μM BB-94. Identical results were obtained with β-casein or κ-casein zymograms (D. Pei and S. J. Weiss, unpublished observation).

SDS-PAGE, Western Blots, Zymography, and N-terminal Sequencing—Basic protocols for these techniques have been described (8, 20). The MT-MMP antisera were raised in New Zealand rabbit using a bacterially generated recombinant fusion protein between glutathione transferase and MT-MMP-1 (10). Enzymic Reactions—Enzymic assays of MT-MMP-1 and MT-MMP-1-D108 were performed in buffer A supplemented with 150 mM NaCl at 37°C unless noted otherwise. Matrix substrates (devoid of contaminating gelatinase A activity as determined by gelatin zymography; data not shown) were obtained from Collaborative Research (type I, IV, and V collagen, fibronectin, laminin, vitronectin, and dermatan sulfate proteoglycan). Processing of pro-MT-MMP-1-D108 by purified soluble furin (specific activity 500 units/μg Ref. 18) was performed as described (8). Recombinant gelatinase A (purified as described in Ref. 22) was a gift from R. Fridman (Wayne State University, Detroit, MI), recombinant human TIMP-2 was supplied by Argen, and soluble human furin was provided by R. Fuller, University of Michigan.

RESULTS

Expression of ΔMT-MMP-1 Mutants and Detection of Enzymic Activity—COS-7 cells transiently transfected with either MT-MMP-1-D105A or MT-MMP-1-D508 cDNA each secreted a pair of major and minor products that were specifically recognized by polyclonal antibodies to a truncated form of the bacterially expressed protein (Fig. 1B). While the molecular mass of the minor secreted proteins (~64 kDa for MT-MMP-1-D108 and ~60 kDa for MT-MMP-1-D508, lanes 2 and 3, respectively) were consistent with those of the predicted proforms of the metalloproteinases, the major soluble species detected with either TMDelletion mutant was a fragment ~10 kDa smaller in size. The generation of the major and minor forms was not specific to COS-7 cells since a similar profile was generated with trans-
Fig. 2. Purification of MT-MMP-1<sub>B</sub>. A and B, fractionation of MT-MMP-1<sub>B</sub> on Q-Sepharose and heparin-Sepharose, respectively. Conditioned media from MDCK cells stably transfected with MT-MMP-1<sub>B</sub> were loaded onto a Q-Sepharose column and eluted with a NaCl gradient. The protein content of each fraction was monitored at A<sub>280</sub> (dark squares) while MT-MMP-1<sub>B</sub> content in each fraction was monitored by immunoblotting and reported as the percent recovered relative to the fraction containing the highest concentration of MT-MMP-1<sub>B</sub> (open squares). Fractions 9–16 were combined, dialyzed against buffer A, loaded onto a heparin-Sepharose column, and eluted with a NaCl gradient. C, characterization of the isolated MT-MMP-1<sub>B</sub> products. Conditioned media (lane 1), a pool of fractions 9–16 eluted from Q-Sepharose (lane 2), flow-through of fractions 9–16 that did not bind to heparin-Sepharose (lane 3), pool of fractions 10–15 eluted from heparin-Sepharose column (lane 4), and final purified form of MT-MMP-1<sub>B</sub> (4.5 pmol; lane 5) were separated by SDS-PAGE and visualized by Coomassie staining. In lanes 6 and 7, purified MT-MMP-1<sub>B</sub> (1.2 pmol) was analyzed by immunoblotting and gelatin zymography, respectively. D, the N terminus of MT-MMP-1<sub>B</sub>, as determined after 10 cycles of sequencing (indicated by bold letters). The open box represents the MT-MMP-1<sub>B</sub> open reading frame with the amino acid sequence of MT-MMP-1 from Pro<sup>21</sup> to Glu<sup>123</sup>.

As expected, when cells were transiently transfected with wild-type MT-MMP-1, soluble forms of the enzyme were not detected in the conditioned media (Fig. 1B).

In intact cell systems, MMPs can be recovered in conditioned medium as a mixture of zymogens, processed active enzymes, zymogen-inhibitor complexes, or enzyme-inhibitor complexes (1–3). In the case of almost all MMP family members, many of these forms can be detected following SDS-PAGE in substrate-impregnated gels (1–3). Thus, serum-free conditioned media from control, MT-MMP-1<sub>A</sub>, MT-MMP-1<sub>B</sub>, or MT-MMP-1<sub>B</sub> transfected cells were depleted of endogenous gelatinases by gelatin affinity chromatography (ΔMT-MMP-1 does not bind to gelatin; see below) and electrophoresed in gelatin-containing gels. Proteinases were then allowed to renature following the removal of SDS and then incubated overnight at 37°C. As shown in Fig. 1C, supernatants recovered from MT-MMP-1<sub>A</sub>- or MT-MMP-1<sub>B</sub>-transfected cells each revealed the presence of a single band of gelatinolytic activity whose relative mobility matched that of the major form of ΔMT-MMP-1 detected by Western blotting. Significantly, identical results were obtained whenzymograms were performed with either β-casein- or κ-elastin-impregnated gels as well (data not shown). Regardless of substrate used, the band of proteolytic activity attributed to either ΔMT-MMP-1 mutant was completely inhibited whenzymograms were performed in the presence of the MMP-specific inhibitor, BB-94 (Fig. 1C).

Purification of ΔMT-MMP-1 and Characterization of Zymogen Processing—Because both MT-MMP-1<sub>A</sub> and MT-MMP-1<sub>B</sub> appeared to undergo a similar, if not identical, process-
Transmembrane-deletion Mutants of MT-MMP-1

Given that COS are known to express only two members of the proprotein convertase family that recognize RXKR motifs (i.e. furin and PACE4; Ref. 24), cells were co-transfected with MT-MMP-1B and the Pittsburgh mutant of αPI (αPIp1pit), a reactive site variant that inhibits furin (but not PACE4) activity in situ (25, 26). Under these conditions, αPIp1pit completely blocked MT-MMP-1B processing while wild-type αPI exerted no inhibitory effect (Fig. 3C). MT-MMP-1B processing was similarly inhibited by αPIp1pit in MDCK cells (data not shown).

These results (together with the demonstration that soluble furin processes pro-MT-MMP-1 to its active form under cell-free conditions; see below) indicate that pro-MT-MMP-1 maturation is regulated by a furin-dependent pathway in an intact cell system. Activation of Recombinant Progelatinase A by Purified Pro-MT-MMP-1—Current evidence indicates that MT-MMP-1-expressing cells initiate progelatinase A activation via a two-step process that involves an initial cleavage of the Asn37Leu bond followed by an autocatalytic conversion of the Leu38 intermediate into a 62-kDa active enzyme with an N-terminal Tyr81 residue (10, 27). Nonetheless, the ability of MT-MMP to directly cleave progelatinase A is unclear, and it has been postulated that additional intermediates may be involved (9, 10).

Thus, purified active MT-MMP-1B was incubated with recombinant progelatinase A and processing monitored by gelatin zymography and N-terminal sequence analysis. Following a 2-h incubation at 37 °C, MT-MMP-1B initially cleaved progelatinase A (which migrates as a ~68-kDa species) into a ~64-kDa fragment (Fig. 4A). N-terminal sequence analysis of the ~64-kDa gelatinase A fragment yielded the Leu38 form of the enzyme (Fig. 4A). Consequently, the 64-kDa form of the enzyme underwent further processing to a 62-kDa product whose N terminus confirmed the generation of [Tyr81]gelatinase A (Fig. 4A). As expected, the ability of MT-MMP-1B (as well as MT-MMP-1A; data not shown) to activate progelatinase A was completely blocked by the addition of the MMP inhibitor, TIMP-2 (Fig. 4A) or BB-94 (data not shown). Interestingly, while earlier studies have demonstrated that membrane-asso-
ciated forms of MT-MMP-1 can also process progelatinase A into a 42-kDa active species (10, 27, 28), this product was not detected under the standard conditions employed. However, when either MT-MMP-1A or MT-MMP-1B were incubated with progelatinase A in a low ionic strength buffer identical to that used previously (10, 27, 28), MT-MMP-1A/B-dependent progelatinase A activation was significantly accelerated and the 42-kDa form of gelatinase generated (Fig. 4A, lanes 8–10).

The ability of mature MT-MMP-1A/B to mediate progelatinase A activation is consistent with a model wherein active MT-MMP-1A/B directly cleaves the gelatinase zymogen, but the data do not rule out the possibility that the MT-MMP-1 zymogen only induces progelatinase A to undergo autocatalytic processing to its active form. Hence, purified pro-MT-MMP-1 was isolated (i.e. from cells co-transfected with MT-MMP-1A and αPIp1pit), and its ability to mediate progelatinase A activation was examined. As shown in Fig. 4B, pro-MT-MMP-1B was unable to stimulate progelatinase A activation. However, when pro-MT-MMP-1B was processed to its active form ex situ with a TM-deleted soluble form of furin (Fig. 4B, lane 2) and then incubated with progelatinase A, the gelatinase zymogen was readily activated (lane 5). Thus, only the processed active form
of ∆MT-MMP-1 is able to mediate progelatinase A activation.

ECM-degrading Activity of ∆MT-MMP-1—While previous attention has focused solely on the role of MT-MMP-1 in progelatinase A activation (4, 9, 10, 13, 27), the ability of ∆MT-MMP-1 to degrade gelatin, β-casein, or κ-elastin following zymography suggested that the proteinase might express activity against a wider range of targets. Thus, purified MT-MMP-1∆AB was incubated with either basement membrane- or interstitial-associated ECM molecules, and proteolysis was assessed in the absence or presence of BB-94. As shown in Fig. 5, while MT-MMP-1∆AB did not degrade native type I, IV, or V collagens, the enzyme readily proteolyzed gelatin as well as fibronectin, the B chain of laminin, vitronectin, and dermatan sulfate proteoglycan (5 μg; lane 19) were incubated alone or with 45 μM MT-MMP-1∆AB (lanes 2, 4, and 6, respectively) at 25°C for 16 h. Type I gelatin (3 μg; lane 7), fibronectin (4 μg; lane 10), laminin (4 μg; lane 13), vitronectin (4 μg; lane 16), or dermatan sulfate proteoglycan (5 μg; lane 19) were incubated alone, with MT-MMP-1∆AB (45 μM; lanes 8, 11, 14, 17, and 20, respectively) or with MT-MMP-1∆AB and 1 μM BB-94 (lanes 9, 12, 15, 18, and 21, respectively) at 37°C in a final volume of 0.025 ml for 16 h. Reaction mixtures were separated by SDS-PAGE and Coomassie-stained. The arrowhead indicates the position of the laminin B chain, while the hatch marks at the margin of lanes 16 and 19 indicate the positions of the 97-, 69-, 45-, 32-, and 28-kDa molecular mass markers, respectively. Dermatan sulfate proteoglycan is recorded as DSPG.

DISCUSSION

Sequence alignments of the 13 human MMPs that have been characterized to date indicate that amino acids 1–508 of the 538-residue-long extracellular domain of MT-MMP-1 contain all of the major structural elements of the secreted members of this gene family (i.e. a propeptide and catalytic domain as well as a hemopexin-like region that is bounded by a pair of highly conserved cysteinyI residues; Refs. 4–7). Given that the C termini of virtually all secreted MMPs end at, or extend no more than 8 amino acids beyond, the final cysteinyI residue in the hemopexin domain (7), we reasoned that TM-deletion mutants of MT-MMP-1 that retained this modular organization would encode functional proteinases. Indeed, as demonstrated, regardless of whether soluble mutants of MT-MMP-1 were truncated either at the edge of the TM domain or at the end of the hemopexin domain, the expressed proteins displayed similar, if not identical, activities as assessed by zymography, progelatinase A processing, or substrate specificity.

By itself, our work does not rule out the possibility that the TM or cytosolic domains of MT-MMP-1 convey additional structural information to the processed proteinase (i.e. beyond acting as a membrane anchor). However, while our work was in progress, Cao et al. (9) reported that an MT-MMP-1 chimera generated by exchanging the TM and cytosolic domains of the metalloproteinase with those of the IL-2 receptor functioned normally in terms of its ability to mediate progelatinase A activation (9). Although this result is consistent with our conclusion that the extracellular domain of MT-MMP-1 confers the proteinase with its distinct characteristics, these authors also reported that a TM-deletion mutant encoding residues 1–535 of MT-MMP-1 was unable to process progelatinase A (9). In comparing our experimental approaches, it is important to note that the soluble MT-MMP-1 generated in their study was not isolated nor were its interactions with progelatinase A examined directly (9). Instead, Cao et al. (9) judged their TM-deletion mutant to be inactive on the basis of its inability to process endogenously derived progelatinase A secreted by COS-1 cells in a transient transfection assay system (9). Under these conditions, however, attempts to assess the activity of secreted MT-MMP-1 would be complicated by the presence of cell-derived TIMPs which can interfere with progelatinase A processing by either inhibiting MT-MMP-1 activity directly, or, in the case of TIMP-2, by binding to the C-terminal domain of the gelatinasezymogen (4, 27, 28). Indeed, when endogenous levels of TIMP are overwhelmed by co-transfecting COS cells with MT-MMP-1∆AB and progelatinase A, gelatinase activation can be readily detected in the intact cell system as well as our purified system. Thus, while anchoring a proteinase to the cell membrane might be predicted to more effectively shield an active proteinase from soluble inhibitors (and to perhaps provide a surface more conducive for accelerating processing events) (e.g. Refs. 29–31), our data demonstrate that the TM-deletion mutants retain the key functional properties of the wild-type enzyme.

As a consequence of our attempts to characterize the activity of ∆MT-MMP-1, we also discovered that the TM-deletion mutants are capable of undergoing rapid processing to their mature forms. This finding is noteworthy since, as a general rule, MMPs are synthesized and secreted as inactivezymogens (1–3, 7). However, we recently reported that in a fashion similar to that observed for the ∆MT-MMP-1 mutants, prostromelysin-3 is secreted as an active enzyme following its intracellular processing within the constitutive secretory pathway (8). In this case, activation was dependent upon a decapeptide insert that is sandwiched between the pro- and catalytic domains of stromelysin-3 and encrypted with an extended furin recognition motif (i.e. RXRXXR). At the time that these earlier studies were completed, stromelysin-3 was the only member of the MMPs family known to contain this recognition sequence. However, with the recent cloning of MT-MMP-1, -2, and -3, it is clear that all three of these enzymes contain homologous inserts which include an array of basic residues (i.e. RRKR) that match the recognition motif of the proprotein convertases (i.e. RX(K,R)-R; Refs. 4–6). Consistent with this prediction, (i) the N terminus of ∆MT-MMP-1 was located at Tyr112 on the C-terminal side of the Arg-Arg-Lys-Arg motif, (ii) ∆MT-MMP-1 processing could be inhibited by either inserting point mutations in the RRKR motif or by co-transfecting cells with the furin-specific inhibitor, a5P110L, and (iii) the ∆MT-MMP-1zymogen could be processed to its active form ex situ by soluble furin. While we have not yet identified the intracellular/extracellular compartments in which the ∆MT-MMP-1zymogen undergoes processing in the intact cell, furin is a membrane-associateendopeptidase that not only cycles between the trans-

*Although stromelysin-3 and MT-MMP-1 both contain proprotein convertase recognition motifs, comparisons of their genomic organization and chromosomal localization indicate that the two metalloproteinases are not closely related and belong to separate branches of the phylogenetic tree (D. Pei and S.J. Weiss, unpublished observation).
Golgi network and the cell surface, but also undergoes processing to a soluble form that accumulates extracellularly (32–34). Indeed, the possibility that ΔMT-MMP-1 may undergo extracellular processing is further supported by our results with the TM-deleted form of soluble furin. Nonetheless, in spite of the fact that furin is the most credible MT-MMP-1 activator identified to date, caution should be exercised in terms of extrapolating processing pathways that are operative for ΔMT-MMP-1 to the wild-type enzyme. Indeed, in contrast to the results obtained with ΔMT-MMP-1, we and others have found that COS cells transfected with wild-type MT-MMP-1 route most of the enzyme to the cell surface as the unprocessedzymogen rather than the mature enzyme (4, 9, 31).αβ Utilizing chimeric constructs between stromelysin-3 and wild-type MT-MMP-1, it appears that while the furin recognition motif in either of the secreted metalloproteinases can be processed effectively, the TM domain of MT-MMP-1 appears to “shield” the recipient proteinase from undergoing rapid intracellular processing.αβ The mechanisms responsible for controlling the intracellular and extracellular processing of wild-type MT-MMP-1 require further analysis, but the fact that the active form of the full-length (10) and mutant enzyme display an identical N terminal directly downstream of the proprotein convertase-recognition motif strongly suggests a role for furin or, perhaps, a related proprotein convertase (e.g., PC6; Refs. 24 and 35) inzymogen activation.

In the presence of purified active ΔMT-MMP-1 (but not itszymogen), progelatinase A was processed to its mature form (i.e., [Tyr81]gelatinase) via the formation of the [Leu38] intermediate. This two-step, TIMP-2-sensitive activation cascade is identical with that previously established for crude preparations of plasma membrane-associated MT-MMP-1 (27) and allows us to conclude that ΔMT-MMP-1 can initiate the processing event independently of additional co-factors or substrates. Interestingly, the ability of ΔMT-MMP-1 to directly activateprogelatinase A under cell-free conditions contrasts with a recent report by Strongin et al. (10) wherein an MT-MMP-1-TIMP-1 complex (rather than MT-MMP-1 alone) was proposed to function as the membrane-associated activator of the gelatinzymogen. We were unable to reproduce this finding with MT-MMP-1ΔAAβ but cannot rule out the possibility that TIMP-2 plays a more complex role in the membrane surface. However, an interpretation of the data presented by Strongin et al. (10) is complicated by the fact that even in the apparent absence of TIMP-2, MT-MMP-1 continued to process progelatinase A to the [Leu38]gelatinase intermediate, but not the final mature form. Thus, it remains possible that TIMP-2 exerts its stimulatory effect by accelerating the inter- or intramolecular autocatalytic conversion of [Leu38]gelatinase to [Tyr81]gelatinase on the cell surface (31) rather than by stimulating MT-MMP-1 activity directly.

To date, the only function ascribed to MT-MMP-1 has been its ability to activateprogelatinase A (4, 9, 10, 13, 27, 28). However, we have demonstrated that purified ΔMT-MMP-1ΔAAβ can also degrade a number of extracellular matrix components. These data indicate that the ability of ΔMT-MMP-1-transfected cells to express a heightened invasive potential may not necessarily be linked to progelatinase A activation alone (4). Although our studies have employed a soluble form of MT-MMP-1, we believe that the modular organization of MT-MMP-1 strengthens the likelihood that the membrane-tethered form displays a similar substrate specificity. Furthermore, the potential physiologic relevance of ΔMT-MMP-1 mutants have been heightened by recent findings which suggest that soluble forms of MT-MMP-1 may be generated in vivo (13). Thus, while the MT-MMP-1 antigen has been immunodetected on the surface of cancer cells in vivo (4), RNA in situ hybridization studies have more recently demonstrated that ΔMT-MMP-1 transcripts are confined to the surrounding stromal cells (13). Should MT-MMP-1 undergo solubilization and intercellular transfer in situ (4, 13), tumor cells could potentially use the stroma-derived enzyme to assemble a multicatalytic complex on their surface that would not only arm them with the ability to catalyzeprogelatinase A activation, but also to express an additional repertoire of proteolytic activities. Additional studies will be required to directly compare the soluble and membrane-anchored forms of MT-MMP-1, but the established catalytic activity of the TM-deletion mutants should provide a useful tool for characterizing the enzymic properties of this new family of membrane-anchored MMPs.

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