Here we report the cloning and initial biochemical characterization of the mouse metalloprotease/disintegrin/cysteine-rich (MDC) protein meltrin \( \beta \) and the analysis of the mRNA expression of four MDC genes (meltrin \( \alpha \), meltrin \( \beta \), mdc9, and mdc15) in bone cells, including osteoclasts and osteoblasts. Like most other MDC proteins, the predicted meltrin \( \beta \) protein consists of a signal sequence, prodomain, metalloprotease domain with a predicted catalytic site, disintegrin domain, cysteine-rich region, epidermal growth factor repeat, transmembrane domain, and cytoplasmic domain with putative signaling motifs, such as potential SH3 ligand domains. Northern blot analysis indicates that meltrin \( \beta \) is widely expressed, with the highest expression in bone, heart, and lung. RNase protection studies revealed expression of all four MDC genes analyzed here in osteoblasts, whereas only mdc9 and mdc15 mRNAs were detectable in osteoclast-like cells generated in vitro. Treatment of primary osteoblasts with 10 nM calcitriol increased meltrin \( \beta \) expression more than 3-fold, and both meltrin \( \alpha \) and meltrin \( \beta \) expression is apparently regulated in a differentiation-associated manner in a mouse osteoblastic cell line, MC3T3E1. Collectively, these results suggest that meltrin \( \alpha \) and meltrin \( \beta \) may play a role in osteoblast differentiation and/or function but are not likely to be involved in osteoclast fusion.

Proteins containing a metalloprotease domain, a disintegrin domain, and a cysteine-rich region (MDC) proteins, also referred to as ADAMs (1) are a family of membrane-anchored glycoproteins that are related to soluble snake venom metalloproteases and integrin ligands. MDC proteins have been implicated in a variety of important cellular processes, including sperm-egg and muscle cell membrane binding and fusion (2–6); in neuronal induction, lateral inhibition, and neuronal outgrowth in Drosophila (7–9); and in the release of the membrane-anchored cytokine tumor necrosis factor (10, 11). The finding that the tumor necrosis factor \( \alpha \) convertase is an MDC protein (10, 11) and that Drosophila Kuzbanian may be involved in the intracellular processing Notch (9, 12) raises the intriguing possibility that other membrane-anchored proteins are also processed and/or released by metalloprotease-disintegrin proteins (13). Because several MDC proteins contain potential cytoplasmic signaling motifs, including SH3 ligand domains (1, 14–16), MDC proteins may also play a role in signaling events, or they may be regulated through interactions with cytoplasmic proteins. At present, over 20 genes encoding MDC proteins have been identified in different species, including Caenorhabditis elegans (17), Drosophila melanogaster (7, 8), Xenopus laevis (18, 19), mice (1, 6, 14, 20, 21), guinea pigs (1, 4, 21, 22), and humans (10, 11, 14, 15, 23). Although the function of many of these proteins remains to be determined, it is clear that different MDC proteins are capable of performing a variety of important tasks.

To date, MDC proteins have been implicated in two distinct membrane binding and fusion events. Fertilin, which functions in sperm-egg binding and fusion, is a heterodimeric complex of two MDC proteins, fertilin \( \alpha \) and fertilin \( \beta \). A monomolecular antibody against fertilin \( \beta \) blocks sperm-egg fusion (2), as do peptides corresponding to the predicted integrin binding sequence of fertilin (5, 24–26). On the egg, an \( \alpha_{6}\beta_{4} \) integrin has been identified as a receptor for sperm, and thus as a candidate receptor for fertilin (25). In C2C12 mouse muscle cells, an MDC protein called meltrin \( \alpha \) has been found to play a role in the membrane fusion event that gives rise to multinucleated myotubes (6). The involvement of MDC proteins in sperm-egg fusion and in muscle cell fusion suggests that MDC proteins are closely linked to the process of membrane fusion, either by mediating a prerequisite binding or signaling step, or by directly triggering the membrane fusion event via a predicted fusion peptide (27).

In this study, we report the cDNA cloning, sequencing, and initial biochemical characterization of the mouse MDC protein meltrin \( \beta \). Because the highest expression of meltrin \( \beta \) in adult mice was previously reported in bone (6), we further investigated the expression of meltrin \( \beta \) and of the three other metalloprotease-disintegrins, meltrin \( \alpha \), mdc9, and mdc15 (margetigin), in osteoblasts and osteoclasts. Our results demonstrate
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All four MDC genes are expressed in osteoblasts and that the expression of meltrin β is inducible by 1α,25-dihydroxyvitamin D3 (calcitriol). Furthermore, expression of meltrin α and meltrin β appears to be regulated during osteoblast differentiation. Neither meltrin α, which has been implicated in muscle fusion, nor meltrin β is detectable in in vitro-generated osteoclast-like cells (OCLs), which arise from the fusion of mononuclear precursors of the monocyte/macrophage/osteoclast lineage. Therefore, we conclude that Meltrin α and Meltrin β may not play a role in osteoclast fusion but instead are more likely involved in osteoblast differentiation and/or osteoblast activity in bone.

Materials and Methods

cDNA Cloning of Meltrin α and Meltrin β—mRNA from ~20% fused mouse C2C12 cells (28) was used to synthesize a cDNA library, utilizing the Stratagene ZAP cDNA library synthesis kit (according to the manufacturer’s instructions; see also Ref. 29). The unamplified cDNA library was screened under high stringency conditions with a [α-32P]dCTP-labeled cDNA probe for meltrin β, which was generated as follows: nested reverse transcription-PCR was performed on C2C12 cDNA (see above) with the following primers designed from the published partial meltrin β sequence: primer 1 (sense primer), GTGGAGA; primer 2 (sense primer), GTTGAGGAG; primer 2 (sense primer), GTGACCTCCC CGAGTTTGCTG; and primer 3 (antisense primer), CAGTTTCCAT AGGTGTCACC. Primers 1 and 3 were used in a primary reaction, which was diluted 1:400 and used as template for a secondary reaction with primers 2 and 3, performed in the presence of [α-32P]dCTP. Using this probe, 20 positive cDNA clones were identified from 106 plaques screened. A PCR analysis labeled cDNA probe for sequence and an apparently full-length open reading frame encoding a antisense primer (TTCAGAGCCA CGTTGGAGGC), designed from the sequence (6), was carried out to find the clone with the longest 5’ extension. The longest clone thus identified was subjected to in vitro excision, sequenced completely on both strands using a primer walk approach, and found to have a cDNA insert of 6413 base pairs. The initial methionine residue of the deduced meltrin β protein sequence is followed by a hydrophobic predicted signal sequence and an apparently full-length open reading frame encoding a protein with 920 amino acid residues. Analysis of the mouse meltrin β cDNA and translated protein sequences was performed using MacVector sequence analysis software (Kodak Scientific Imaging Systems), and the alignment and hydrophobicity plot was generated using DNASTAR software (DNalign and Protein sequence). Using the same approach, meltrin α cDNA was cloned from the library as a template to make cRNA for RNase protection studies (see below). The following PCR primers were used in a nested reaction to generate the [α-32P]dCTP labeled cDNA probe, which corresponds to nucleotides 298–500 of the meltrin α sequence cited in Ref. 6 (GenBank accession no. D50411): primer 1 (sense primer), ATACGAGAC GC CCGGCG; primer 2 (sense primer), CGTGCAGCCC GAGGGGATGTGGAGA; primer 2 (sense primer), GTGACCTCCC CGAGTTTGCTG; and primer 3 (antisense primer), ACACGCGCGCTGAGGAGG. Designers 1 and 3 were used in a primary reaction, which was diluted 1:400 and used as template for a secondary reaction with primers 2 and 3, performed in the presence of [α-32P]dCTP. Using this probe, 20 positive cDNA clones were identified from 106 plaques screened. A PCR analysis labeled cDNA probe for sequence and a apparently full-length open reading frame encoding a protein with 920 amino acid residues. Analysis of the mouse meltrin β cDNA and translated protein sequences was performed using MacVector sequence analysis software (Kodak Scientific Imaging Systems), and the alignment and hydrophobicity plot was generated using DNASTAR software (DNalign and Protein sequence). Using the same approach, meltrin α cDNA was cloned from the library as a template to make cRNA for RNase protection studies (see below). The following PCR primers were used in a nested reaction to generate the [α-32P]dCTP labeled cDNA probe, which corresponds to nucleotides 298–500 of the meltrin α sequence cited in Ref. 6 (GenBank accession no. D50411): primer 1 (sense primer), ATACGAGAC GC CCGGCG; primer 2 (sense primer), CGTGCAGCCC GAGGGGATGTGGAGA; primer 2 (sense primer), GTGACCTCCC CGAGTTTGCTG; and primer 3 (antisense primer), ACACGCGCGCTGAGGAGG. Designers 1 and 3 were used in a primary reaction, which was diluted 1:400 and used as template for a secondary reaction with primers 2 and 3, performed in the presence of [α-32P]dCTP. The resulting labeled cDNA probe was used to isolate two cDNA clones for meltrin α out of 106 plaques screened. One of the cDNA clones contained an open reading frame sequence (6), which was sequenced and the calcitonin receptor (36). In this coculture system, formation of authentic osteoclasts, such as the ability to resorb bone, and the expression of lineage-specific markers, including tartrate-resistant acid phosphatase and the calcitonin receptor, was detected. In this coculture system, formation of OCLs is totally dependent on the presence of calcitriol. In every preparation, more than 80% of the OCL-enriched population was positively stained for tartrate-resistant acid phosphatase.

Expression of Meltrin β in COS-7 Cells—A pcDNA3 expression vector (Invitrogen) for meltrin β was constructed by subcloning an EcoRI/HindIII fragment of the full-length open reading frame. The EcoRI site is part of the 5’ linker sequence in a ZAP library, and the HindIII site is at nucleotide 2883, just downstream of the meltrin β stop codon at nucleotide 2808. The meltrin β pcDNA3 expression vector, or an identical control vector without an insert, were used to transfect COS-7 cells using LipofectAMINE reagent as a delivery vehicle. A mixture of pcDNA3 expression vector and pcDNA3 vector lacking an insert. The affinity-purified antibodies were used as a control for some Western blots. The affinity-purified IgG, the control IgG, and the complete antisera and preimmune antisera were tested by Western blot analysis on COS-7 cells expressing meltrin β protein and pcDNA3 vector, or pcDNA3 vector lacking an insert. The affinity-purified and control antibodies were also used on a lysate of C2C12 mouse myoblasts that included about 20% fused multinucleated cells. The Western blot of a primary osteoblast (POB) lysate was probed with immune or preimmune serum at a 1:500 dilution, which produced a stronger signal than the affinity-purified antibodies under the conditions used here. All cells

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RESULTS

cDNA Cloning and Sequence of Meltrin β—A cDNA clone coding for full-length mouse Meltrin β was first isolated from a mouse C2C12 muscle cell library, using a PCR-generated cDNA fragment as a probe (see "Materials and Methods") and then sequenced using a primer walk approach. This clone had an insert of 6413 base pairs, with an open reading frame of 2760 base pairs, 35 base pairs of 5′ untranslated sequence, and 3618 base pairs of 3′ untranslated sequence. The meltrin β protein sequence, as deduced from the open reading frame, predicts a protein of 920 amino acid residues with four sites of N-linked glycosylation. The sequence of Meltrin β contains all protein modules that are characteristic of members of the MDC protein family: a signal sequence is followed by a prodomain, a metzincin protease domain with a predicted catalytic site consensus sequence (HEIGHNFGMSHD), a disintegrin domain, a cysteine-rich region, an epidermal growth factor-like repeat, a transmembrane domain, and a cytoplasmic tail. Like several other MDC proteins, such as MDC9 (14), meltrin α (6), MDC15 (15, 16), and the tumor necrosis factor α convertase (10, 11), the cysteine-rich tail harbors potential signaling motifs that include proline-rich predicted SH3 ligand sequences. The sequence of Meltrin β is shown in Fig. 1A in an alignment with the two most closely related MDC proteins presently known, meltrin α and ADAM 13. The hydrophilicity plot of Meltrin β (Fig. 1B) predicts an N-terminal hydrophobic signal sequence and a transmembrane domain between amino acid residues 707 and 725. After removal of the predicted signal sequence, Meltrin β has a deduced molecular mass of 99.23 kDa.

Analysis of the Tissue Distribution of Meltrin α, Meltrin β, mdc9, and mdc15 and of the Expression in Bone Cell Cultures in Vivo—By Northern blot analysis, meltrin β mRNA expression can be detected in all adult mouse tissues examined, with the highest expression in heart and lung, followed by brain and spleen and then by relatively low expression in liver, skeletal muscle, kidney, and testis (Fig. 2). Analysis by RNase protection assays revealed that meltrin α mRNA is also expressed in various tissues, including brain, heart, kidney, liver, lung, and skeletal muscle (see Fig. 4; some data not shown). Because the highest expression of meltrin α and meltrin β in adult mouse tissues was observed in bone (see Fig. 4; some data not shown), confirming the data from a previous report (6), we attempted to determine which cell types in bone express the mRNA coding for these proteins by RNase protection analysis. For comparison, we also examined mRNA levels of two other MDC genes, mdc9 and mdc15, which are both widely expressed. As primary cell materials, we utilized POBs isolated from newborn mouse calvariae and OCLs generated in vitro by cocultures of POBs and bone marrow cells. First, to investigate whether expression

Fig. 2. Northern blot analysis of meltrin β expression in adult mouse tissues. A mouse tissue Northern blot (CLONTECH) was probed with α-32P-labeled meltrin β cDNA, or as a control with β-actin cDNA, under high stringency conditions. The size of the major meltrin β mRNA was found to be approximately 6.5 kilobases, indicating that the meltrin β cDNA clone of 6.4 kilobases is likely to be full-length. The highest expression level of meltrin β mRNA was observed in heart and lung, followed by brain, spleen, testis, kidney, skeletal muscle, and liver. A band of just under 9 kilobases was also detected in heart and liver, which may be a splice variant of meltrin β.

Fig. 1. Sequence alignment of mouse meltrin α, X. laevis ADAM 13, and mouse meltrin β and hydrophilicity plot of meltrin β. A, a mouse meltrin β cDNA was cloned and sequenced as described under "Materials and Methods," and the deduced protein sequence was aligned with the published protein sequences of the two most closely related MDC proteins, mouse meltrin α (6) and X. laevis ADAM 13 (18). The alignment was generated using the Megalign module of the DNASTAR sequence analysis software. Amino acid residues present in at least two of the three proteins are boxed. The sequence similarity was found to be 41% between mouse meltrin β and X. laevis ADAM 13, 40% between mouse meltrin α and ADAM 13 (values calculated with DNASTAR Megalign software, using a PAM 250 residue weight table). The predicted signal sequence cleavage site of meltrin β is indicated by an arrowhead, and the predicted boundaries of the different protein domains are indicated and labeled. The metzincin protease catalytic site consensus sequence is surrounded by a box with a hatched outline, and predicted N-linked glycosylation sites are marked by an asterisk. B, the hydrophilicity plot of meltrin β was generated using the Protean module of the DNASTAR sequence analysis software.
of meltrin α and meltrin β is associated with osteoclast differentiation, we examined the expression in cocultures in the absence or presence of calcitriol, which is a strong inducer of osteoclast differentiation (36). As shown in Fig. 3A, an osteoclast-specific marker, the calcitonin receptor, was only detectable in cocultures treated with calcitriol and in purified OCLs, thus verifying the integrity and fidelity of the mRNA samples. Interestingly, mRNA expression of meltrin β, and to a lesser extent of meltrin α, in cocultures was up-regulated by treatment with calcitriol. However, neither meltrin α nor meltrin β was detectable in purified mature OCLs. When we analyzed mRNA from POBs cultured alone in the absence or presence of calcitriol, we found abundant expression of both meltrin α and meltrin β in the POB cultures and a 3.6-fold induction in the steady state level of meltrin β mRNA by calcitriol (Fig. 3A and Table I). Consistent with the absence of meltrin α and meltrin β messages in purified OCLs, a macrophage cell line, IC21, also lacked the expression of meltrin α and meltrin β. These results suggest that meltrin α and meltrin β are not appreciably expressed in the monocyte/macrophage/osteoclast lineage and that their expression is restricted to cells of mesenchymal origin such as osteoblasts and myoblasts. The fact that the level of meltrin α and meltrin β expression in cultured POBs is much higher than that in the whole bone (Fig. 4) also supports the idea that the osteoblast is the major cell type expressing these two MDC genes. However, because heterogeneous primary cultures were used in this study, we cannot exclude the possibility that meltrin α and meltrin β are expressed in early osteoclast precursors and other hematopoietic cells. Taken together with the calcitriol effects on POBs, these results indicate a correlation between meltrin α and meltrin β expression and osteoblast differentiation.

To further address this issue, we analyzed the temporal profile of meltrin α and meltrin β expression in long term cultures of a mouse calvariae-derived osteoblastic cell line, MC3T3E1. It has been shown that this cell line is able to progressively differentiate into mature osteoblast-like cells that produce mineralized matrix in vitro. As shown in Fig. 4, MC3T3E1 cells expressed a fairly high level of meltrin α and meltrin β mRNA at the proliferative stage (day 1). Immediately after the cells became confluent (day 4), the expression of the two genes was down-regulated, and from this point, a relatively low level of expression was maintained throughout the remaining culture period (days 4, 8, and 21). Interestingly, meltrin α expression showed a slight but reproducible increase at later stages (days 8 and 21). Thus, both meltrin α and meltrin β are apparently predominantly expressed in cells of the osteoblast lineage in bone, and the expression of these genes in the osteoblast-like cell line MC3T3 E1 is dependent on the stage of differentiation.

Similar analysis of mdc9 and mdc15 revealed expression in both of the bone cell types analyzed, i.e. in osteoblasts and osteoclasts (Fig. 3, B and C). These results are consistent with previous findings that these two genes are ubiquitously expressed (14, 15). Interestingly, we found that the steady state levels of mdc9 and mdc15 mRNA in cocultures were slightly but consistently up-regulated by treatment with calcitriol, whereas the same treatment had no effect on POB cells (Fig. 3 and Table I). These results suggest that calcitriol increased expression of mdc9 and mdc15 in hematopoietic cells derived from bone marrow, most likely in cells of the osteoclast lineage, although the increased expression may also be due to nonosteoclast cells present in the primary cell cocultures containing
various other cells that may respond to vitamin D treatment. Because the level of expression in purified mature OCLs was relatively lower than that observed in cocultures (Fig. 3, B and C), one interpretation is that the up-regulation of mdc9 and mdc15 mRNA expression by calcitriol correlates with an early stage of osteoclast differentiation.

Western Blot Analysis of Meltrin β—One of the questions raised by the domain structure of MDC proteins is how the domains are processed to yield a functional protein. To assess any potential proteolytic processing of Meltrin β, we raised antibodies in rabbits against GST fusion proteins with different parts of the Meltrin β cytoplasmic tail (see “Materials and Methods”). Probing Western blots of reduced and alkylated extracts from COS-7 cells transfected with meltrin β with affinity-purified anti-Meltrin β cytoplasmic tail antibodies (see “Materials and Methods”) revealed bands of 115, 87, 42, and 35 kDa (Fig. 5, lane 1). These bands appear to be specific for Meltrin β, as they are not recognized by control antibodies depleted of Meltrin β reactive IgG (Fig. 5, lane 2) and are not seen when COS-7 cells that have been transfected with the expression vector alone are probed with the affinity-purified antibodies (Fig. 5, lane 3). On a Western blot of extracts from primary osteoblasts probed with the Meltrin β antiserum, bands of 115, 87, and 42 kDa and some minor bands close to 35 kDa are visible (Fig. 5, lane 4); these bands are not recognized by preimmune serum (Fig. 5, lane 5). This band pattern closely resembles the pattern observed in COS-7 cells expressing Meltrin β (Fig. 5, lane 1) When an extract of ~20% fused C2C12 mouse myoblasts was probed with the affinity-purified Meltrin β IgG, a band of 115 kDa and weaker bands of 110 and 42 kDa were visible (Fig. 5, lane 6); these bands were not present in an identical sample probed with the control IgG (Fig. 5, lane 7). These results suggest that a majority of Meltrin β is present in a form that most likely includes the prodomain, although POB cells apparently contain a significant amount of an 87-kDa Meltrin β band that presumably lacks the prodomain. The faster migrating bands likely correspond to processed forms of the protein in all three cell types examined here, although these bands could conceivably also arise due to cross-reactivity of the antibodies with other proteins, including related MDC proteins.

![Western blot analysis of meltrin β](image)

**DISCUSSION**

Proteins containing a metalloprotease domain, a disintegrin domain, and a cysteine-rich region (MDC proteins) have been implicated in a variety of important cellular processes, including neurogenesis (7, 8), protein ectodomain shedding (10, 11), and cell-cell binding and fusion (2, 4, 6). According to a previous report, an MDC protein termed meltrin α (also referred to as ADAM 12 (1)) has been shown to play a critical role in C2C12 myoblast fusion (6). Furthermore, both meltrin α and a second MDC protein of unknown function termed meltrin β were shown to be most abundantly expressed in adult bone, and in neonatal muscle (6), which was confirmed in the present study. Because bone also contains fusing cells, i.e. multinucleated osteoclasts, these observations raised the possibility that meltrin α and/or meltrin β may be expressed in cells of the osteoclast lineage and may therefore be involved in the fusion process of these cells. However, our results indicate that in bone, both meltrin α and meltrin β are primarily expressed in cells of the osteoclast lineage but cannot be detected in mature osteoclasts by the relatively sensitive method of RNase protection. We also found that meltrin α and meltrin β are widely expressed in other tissues at various levels, implying that both meltrins may exert divergent functions in different types of cells, including myoblasts and osteoclasts.

In this study, the full-length cDNA of mouse meltrin β has been cloned and sequenced. Of the presently known MDC proteins, X. laevis ADAM 13 (18) and Meltrin α (6) are most closely related to Meltrin β. X. laevis ADAM 13 is localized to somatic mesoderm and cranial neural crest cells from gastrulation through neurulation and tail bud stages and has therefore been suggested to function in neural crest adhesion and migration and in myoblast differentiation (18). From the sequence similarity between Meltrin β and X. laevis ADAM 13 (41.9%) it can not be judged whether these two proteins are orthologues, and further study will be necessary to compare the developmental expression of Meltrin β to that of ADAM 13. A PCR sequence tag generated from X. laevis was found to be 74% identical to Meltrin α, making it unlikely that Meltrin α and ADAM 13 are orthologues (18). Because a similarly high level of sequence conservation has also been observed between mouse and X. laevis Mdc9 (75% sequence similarity) and between human...
MDC11 and X. laevis Mdc11 (72% sequence similarity), other potential orthologues may be equally well conserved. For this reason, Meltrin β was considered to likely be a novel metalloprotease-disintegrin protein and was therefore assigned the number 19 in the ADAM numbering system (see Ref. 1).

To obtain some insight into the potential functions of meltrin α, meltrin β, mdc9, and mdc15 in bone, we analyzed the expression levels of these four MDC genes in bone cells. The full-length sequences of mouse Mdc9, human MDC9 (14), mouse Meltrin α (6), and human MDC15 (15) have been previously reported. Because mouse bone cells were used here, mouse Mdc15 cDNA was used for RNase protection experiments. Mouse Mdc15 is 79.7% identical to human MDC15 at the amino acid level, is widely expressed, and, interestingly, contains the sequence TDD instead of RGD in the predicted integrin binding sequence. All four MDC proteins analyzed here contain a metalloprotease domain with a predicted catalytic site, a disintegrin domain, and cytoplasmic domains with predicted signaling motifs, including SH3 ligand domains. Other features of potential functional significance shared by the four MDC proteins include a predicted subtilisin type protein convertase cleavage site (RXKR/RKR) between the prodomain and the metalloprotease domain (38, 39) and an odd-numbered cysteine in the prodomain that most likely is involved in a cysteine switch regulation of the protease (40, 41).

Our results demonstrate that in bone, the major cell type expressing meltrin α and meltrin β is the osteoblast. It is now well established that osteoblasts originate from mesenchymal stem cells, which also give rise to myocytes, chondrocytes, and adipocytes. For example, several clonal cell lines of mesenchymal origin have been reported to be able to differentiate into two or more distinct cell types under appropriate conditions (42, 43). Although it is yet to be determined whether or not meltrin α and meltrin β are expressed in chondrocytes and adipocytes, the relatively restricted pattern of expression in myoblasts and osteoblasts implies that they may play key roles in differentiation and/or functions of mesenchymal cells in connective tissues. Further evidence for potential functions of the meltrins in bone can be derived from our findings that the expression of meltrin α and meltrin β is regulated during osteoblast differentiation in vitro and that expression of meltrin β in primary osteoblasts is inducible by calcitriol, an active metabolite of vitamin D3. Osteoblasts are known to express receptors for vitamin D, and calcitriol has been shown to have various direct effects on both primary osteoblasts and osteoblastic cell lines (44). Vitamin D is one of the major regulators of bone and mineral metabolism, as evidenced by the fact that lack of vitamin D action causes clinical disorders such as osteomalacia and rickets due to a mineralization defect (reviewed in Ref. 44). Collectively, our results suggest a link between osteoblast differentiation and the expression of meltrin α and meltrin β. It should, however, be noted that the cells used in this study, mouse calvarial cells and MC3T3E1 cells, may not represent normal osteoblast populations in every aspect. For example, calcitriol has inhibitory effects on osteocalcin expression in mouse calvarial cells, as opposed to stimulatory effects observed in rat and human osteoblasts (45). Furthermore, it is known that in vitro differentiation of MC3T3E1 cells may differ from primary osteoblasts because they express very little, if any, of the transcription factor Msx-2 during the proliferative stage (46).

Based on what is known about other metalloprotease-disintegrin proteins, we predict that the meltrins may function in cell adhesion and/or signaling between osteoblasts or may be involved in protein ectodomain cleavage or shedding of osteoblast proteins that may require processing for activity (13). Preliminary studies with MC3T3E1 cells that were stably transfected with meltrin β failed to reveal any changes in the expression of osteoblastic genes, such as type 1 collagen and osteocalcin, when compared with vector-transfected control cells (data not shown). Clearly, further study, such as the analysis of the function of individual protein domains of the meltrins, and targeted gene knockout experiments will be necessary to clarify the physiological role of meltrin α and meltrin β in osteoblasts.

Osteoclasts, another major type of bone cells, are terminally differentiated, multinucleated bone-resorbing cells that originate in multipotent hematopoietic stem cells and belong to the monocyte/macrophage lineage (reviewed in Refs. 36 and 47). RNase protection assays suggest that osteoclasts express only mdc9 and mdc15, but not meltrin α or meltrin β, whereas osteoblasts express all four MDC genes. This would argue against a role of meltrin α and meltrin β in osteoclast fusion, although an expression in fusing osteoclasts that is downregulated immediately after fusion cannot be ruled out with the assay used here. Although the level of mdc9 and mdc15 expression observed in purified mature OCLs was considerably lower than that found in osteoblasts and cocultures, it seems unlikely that the expression of meltrin α and meltrin β observed here is due to contamination with other cells because we were not able to detect meltrin α and meltrin β expression in the OCL RNA samples, even after longer exposure (data not shown). Thus, our results suggest that mdc9 and mdc15 are expressed in cells of the osteoclast lineage, although their expression in osteoclast precursors remains to be verified by single-cell level analyses.

For an initial biochemical characterization of Meltrin β, antibodies against the cytoplasmic tail were raised and used in a Western blot analysis of COS-7 cell expressing Meltrin β, of mouse C2C12 cells, and of mouse POB cells. In all three cases, the predominant form of Meltrin β has a molecular mass of 115 kDa, which is ~15 kDa larger than the predicted molecular mass of 99.72 kDa of Meltrin β after removal of the signal sequence. This band most likely corresponds to a glycosylated form of Meltrin β, in which the prodomain and metalloprotease domain are membrane-anchored. A band of 85 kDa, which is relatively more abundant in POB cells, probably corresponds to a form of Meltrin β in which the prodomain has been removed but the metalloprotease domain is still attached. The less prominent bands of 42 and 35 kDa most likely represent shorter proteolytically processed forms of Meltrin β. Although the functional significance of these processed forms remains to be determined, for fertilin β and for Meltrin α, there is evidence that removal of the metalloprotease domain may regulate a role in membrane binding and/or fusion (3, 6, 13).

In summary, we have cloned the full-length cDNA of mouse meltrin β and demonstrated that meltrin β and three other members of the MDC gene family, meltrin α, mdc9, and mdc15, are expressed in bone cells. The finding that meltrin α and meltrin β are highly expressed in osteoblasts and are regulated by vitamin D and during osteoblast differentiation suggests that these two proteins may play a role in bone formation. Our results further show that meltrin α, which was previously implicated in muscle fusion, is apparently not expressed in mature osteoclasts. Further study will be necessary to dissect the role of the metalloprotease domain, the disintegrin domain, and the cytoplasmic signaling motifs of these MDC gene products in bone metabolism.

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4 L. Lum and C. Blobel, manuscript in preparation.
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