Biochemical and Biophysical Research Communications **261,** 163–168 (1999)
Article ID bbrc.1999.1008, available online at http://www.idealibrary.com on IDEAL®

Identification and Localization of *MEP1A-like* Sequences (*MEP1AL1-4*) in the Human Genome[1]

Weiping Jiang*,[2] and Barbara G. Beatty†
**Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033; and †Ontario Cancer Institute/Princess Margaret Hospital, Toronto, Ontario M5G 2M9, Canada*

Received June 11, 1999

The human *MEP1A* gene encodes the meprin α subunit that consists of a protease domain conserved in the astacin family of metalloendopeptidases and several C-terminal interaction domains present in other proteins. Using the α subunit cDNA, we identified two clones from a human P1-derived artificial chromosome (PAC) library. Fluorescence *in situ* hybridization (FISH) mapped both PACs (1e12, 65a14) to chromosome 6p21, confirming the *MEP1A* location. FISH also mapped PAC 65a14 to chromosome 13cen, and to chromosome 9 in three different regions, 9p12-13, 9q21, and 9q22. Southern blot analysis showed that sequences of PAC 65a14 and *MEP1A* were similar in the 3′ end but different in the 5′ end, revealing for the first time that the human genome may encode multiple interaction domains highly similar to those of the meprin α subunit. The symbols of *MEP1AL1, MEP1AL2, MEP1AL3,* and *MEP1AL4* have been designated for *MEP1A-like* sequences on 9p12-13, 9q21, 9q22, and 13cen, respectively.

The astacin family of metalloendopeptidases contains a homologous protease domain and is proposed to function in digestion, bone/cartilage formation, hatching, and dorsal/ventral patterning (1–4). Meprins are secreted and brush border membrane proteases capable of cleaving a variety of peptides and proteins such as hormones, growth factors, extracellular matrix proteins, and secreted protein kinases, resulting in activation or inactivation of these molecules (5–13). In addition, meprins have been implicated in several physiological and pathological processes such as embryonic development, acute renal failure, proinflammatory and profibrogenic state of the kidney, immune defense of the intestine, and intestinal tumor progression (14–20).

Meprins are oligomeric glycoproteins composed of two types of subunits, α and β. Like the majority of the astacin family members, the deduced amino acid sequences of the two subunits contain additional domains flanking the protease domain. The predicted protein structure of the α subunit, as illustrated in Fig. 2A, consists of the following domains: S (N-terminal signal peptide), P (prosequence), Protease (astacin-like), MAM (meprin, A-5 protein, receptor protein-tyrosine phosphatase μ), MATH (meprin and TRAF homology), AM (after MATH), I (inserted), EGF (epidermal growth factor-like), TM (transmembrane) and C (cytosolic). The domain structure of the β subunit is similar to the α subunit except that the I domain is absent in the β subunit. The C-terminal domains of the meprin α subunit (MAM to I) are known to have following functions. The I domain is responsible for C-terminal proteolytic processing of the α subunit in the endoplasmic reticulum (ER), leading to the secretion of the α subunit if not associated with the β subunit at the cell surface (21, 22). The MAM domain is necessary for correct folding and transport through the secretory pathway, the MATH domain is required for folding of an activable zymogen, and the AM domain is important for efficient secretion and activity against proteins (23). The functions of the MAM and MATH domains in the meprin α subunit are consistent with the proposed roles of "adhesion" or "interaction" for

[1] The symbols of *MEP1AL1*, *MEP1AL2*, *MEP1AL3,* and *MEP1AL4* for *MEP1A-like* sequences on 9p12-13, 9q21, 9q22, and 13cen, respectively, have been approved by the HUGO/GDB Nomenclature Committee. The GDB Accession IDs are 5885989 for *MEP1AL1,* 5885990 for *MEP1AL2*, 5885991 for *MEP1AL3,* and 5885992 for *MEP1AL4.*

[2] To whom correspondence should be addressed at Department of Biochemistry and Molecular Biology H171, Pennsylvania State University College of Medicine, Hershey, PA 17033. Fax: (717) 531-7072. E-mail: wjiang@psu.edu.

Abbreviations used: PAC, P1-derived artificial chromosome; FISH, fluorescence *in situ* hybridization; TRAF, tumor necrosis factor receptor-associated factor; EGF, epidermal growth factor-like; TM, transmembrane; ER, endoplasmic reticulum; DAPI, 4,6-diamidino-2-phenylindole-dihydrochloride; FITC, fluorescein isothiocyanate; PCR, polymerase chain reaction; NIGMS, National Institute of General Medical Sciences; YAC, yeast artificial chromosome; CEPH, Centre d'Etude du Polymorphism Humain; MHC, major histocompatibility complex.

0006-291X/99 $30.00

these domains (24–27). The MAM domains are present in several other cell surface proteins and the MATH domains are also found in intracellular proteins. The amino acid identities between the meprin subunits and these other proteins are approximately 30% for both the MAM and MATH domains.

The cDNA sequences of the meprin subunits have been determined from mouse, rat, and human (28–33). The same subunits from mouse, rat, and human are 75% to 90% identical and the different subunits from the same species are approximately 50% identical in their deduced amino acid sequences. The mouse genes encoding the two subunits, *Mep1a* and *Mep1b*, are present in single copies on mouse chromosomes 17 and 18, respectively (32, 34, 35). The human β gene, *MEP1B*, is present as a single copy on human chromosome 18q12.2-q12.3 (18, 36). The human α subunit gene, *MEP1A*, is on 6p21 (37). However, data available on the copy number of the *MEP1A* gene seem contradictory. On one hand, multiple copies of *MEP1A* were suggested from the observation that the human genomic DNA hybridized to a mouse cDNA probe more strongly than the mouse genomic DNA (34). On the other hand, a single copy of *MEP1A* was indicated by Southern analysis of a human colon carcinoma cell line (Caco-2) using a smaller probe (31).

To determine whether there are one or more copies of the *MEP1A* gene, fluorescence *in situ* hybridization (FISH) was performed on human metaphase chromosomes using two clones that were isolated from a human P1-derived artificial chromosome (PAC) library (38). Using this technique, the *MEP1A* location was confirmed and additional sequences were identified and localized. The relationship of these sequences to *MEP1A* was determined.

MATERIAL AND METHODS

Screening of the PAC library and FISH. The total human PAC library (38) was screened separately with a PCR fragment derived from the human *MEP1A* cDNA (probe c in Fig. 2A) and a full length mouse meprin α cDNA (28) under the conditions described below for Southern blot analysis. The resulting PAC clones were mapped by FISH to normal human lymphocyte chromosomes counterstained with propidium iodide and 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI; 39). Biotinylated probes were detected with avidin-fluorescein isothiocyanate (FITC). Separate images of DAPI banded chromosomes (40) and of FITC targeted chromosomes were captured by a thermoelectrically cooled charged coupled camera (Photometrics, Tucson, AZ), and merged using image analysis software and pseudo colored blue (DAPI) and yellow (FITC) described by Boyle *et al.* (41). The regional assignments were determined by the analyses of 20 well-spread metaphases. The band assignment was determined by measuring the fractional chromosome length and by analyzing the band pattern generated by the DAPI counterstained image.

Southern blot analysis. Genomic clones were cleaved with EcoRI and BamHI and subjected to agarose gel electrophoresis. DNA samples were transferred from gels to nylon membranes by a downward procedure (42). The resulting blots were hybridized with probes using the conditions described previously (34). The probes were generated by either restriction enzyme digestion or polymerase chain reaction (PCR) amplification of the *MEP1A* cDNA.

RESULTS AND DISCUSSION

Localization of PAC clones in the human genome. Two clones were isolated from the PAC library. PAC 1e12 was obtained with a human *MEP1A* cDNA corresponding to the protease domain (probe c in Fig. 2A). PAC 65a14 was obtained with the full length mouse meprin α cDNA (28). The *MEP1A* gene was clearly mapped to chromosome 6p21 by FISH using both PAC clones (Fig. 1). This is the same location as previously determined by both linkage analysis and overlapping yeast artificial chromosome (YAC) clones (37).

Unexpectedly, PAC 65a14 also hybridized to 13cen and three regions, 9p12-13, 9q21, and 9q22, on chromosome 9 (Fig. 1, bottom). The exact location of the signals on chromosome 9 was difficult to determine due to their multiplicity and large size, suggesting the presence of multiple copies of PAC 65a14 sequences in these regions. This is also supported by much stronger signals on chromosome 9 than those on 6p21. The hybridization pattern of chromosome 9 by PAC 65a14 indicates that evolution of this chromosome has resulted in specific regions of similar sequences and highly repetitive sequences within certain regions. The former has been observed before, for example, the telomeric regions of 9p, 9p11-13, and 9q13 were all detected by FISH using a single radiation hybrid probe that was positive only for markers in telomeric regions by PCR (43).

Relationships of the various clones derived from the MEP1A gene and MEP1A-like sequences. PAC 65a14 was further characterized by Southern analysis using the cDNA probes corresponding to different regions of the *MEP1A* gene (Fig. 2A). To represent the 3′ end of the *MEP1A* gene, probe b (nt 1749–2517) was synthesized by PCR to contain the sequences from the I domain to the 3′ untranslated region. To represent the 5′ end of the gene, probe c (nt 6–641) was generated by PCR to correspond to a part of prosequence and almost entire protease domain. A restriction fragment ending at the HindIII site, probe d (nt 1–1263), extended probe c into the MATH domain.

PAC 65a14 contains sequences that are highly similar but not identical to the *MEP1A* gene in the 3′ end (Fig. 2B). YACs 939e12 (1450 kb) and 638f8 (310 kb) were obtained from PCR screening of the CEPH MEGA YAC library using a pair of primers within the I domain (37). YAC 638f8 differed from 939e12 in restriction fragment pattern as revealed by Southern analysis with a full-length α cDNA (data not shown). YAC 939e12 was known to contain both *MEP1A* and D6S452 loci and was recently mapped to 6p12-21 by FISH (37, 44). It produced distinct EcoRI and BamHI

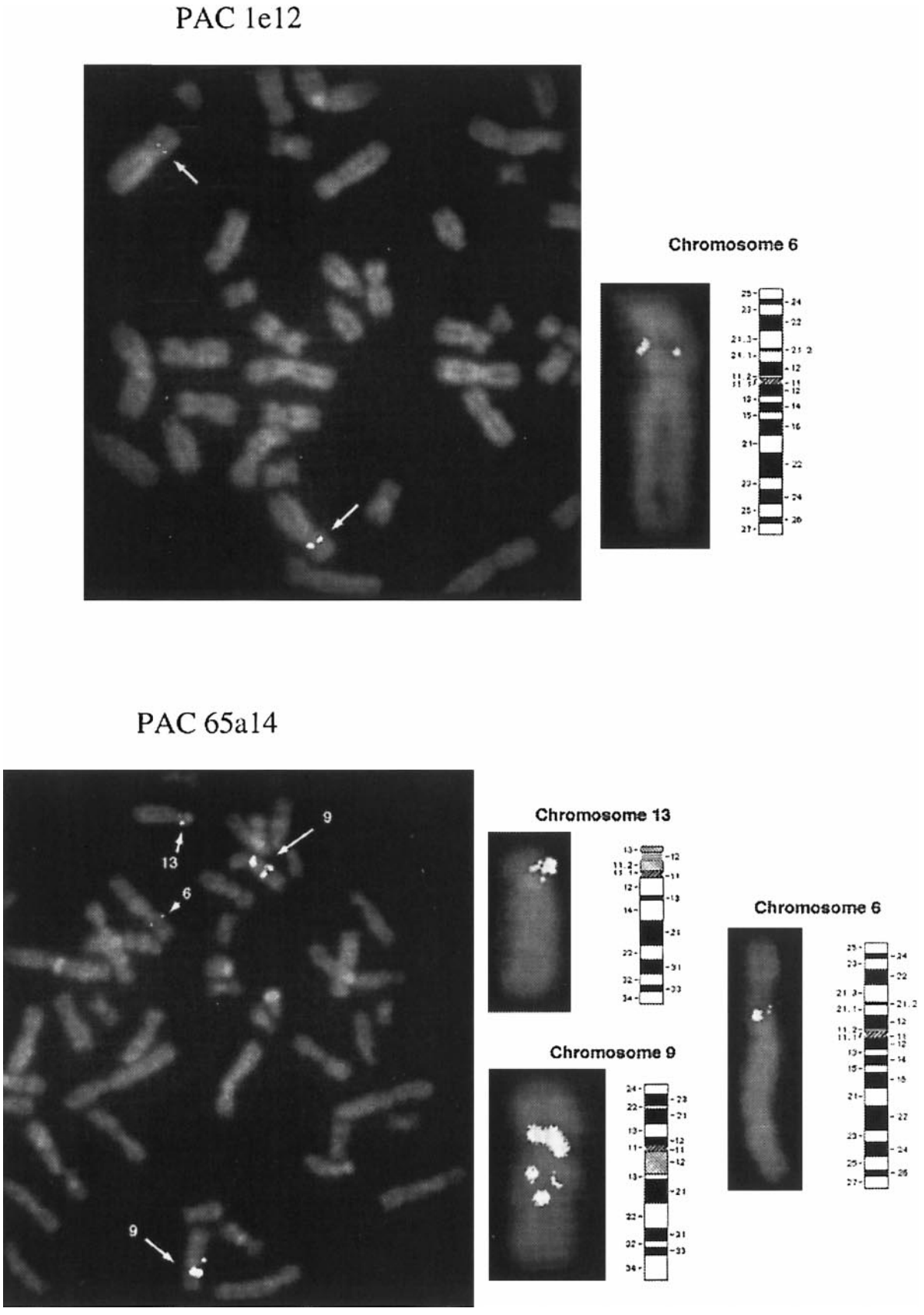

FIG. 1. Localization of *MEP1A-like* sequences in the human genome. Specific hybridization of P1-derived artificial chromosome clones, PAC 1e12, to 6p21 (top), and PAC 65a14 to 6p21, 13cen, 9p12-13, 9q21, and 9q22 (bottom) is indicated by arrows. Ideograms of the individual chromosomes together with hybridization signals are also shown. Positive hybridization signals for PAC 65a14 on chromosome 9 and on 6p21 were noted in more than 90% of the cells; the signals on 6p21 were weaker than those on chromosome 9. Signals at 9p22 were less frequent than the signals at the other two locations on chromosome 9. The signals on chromosome 13cen were less frequent, weak and usually visualized on only one allele.

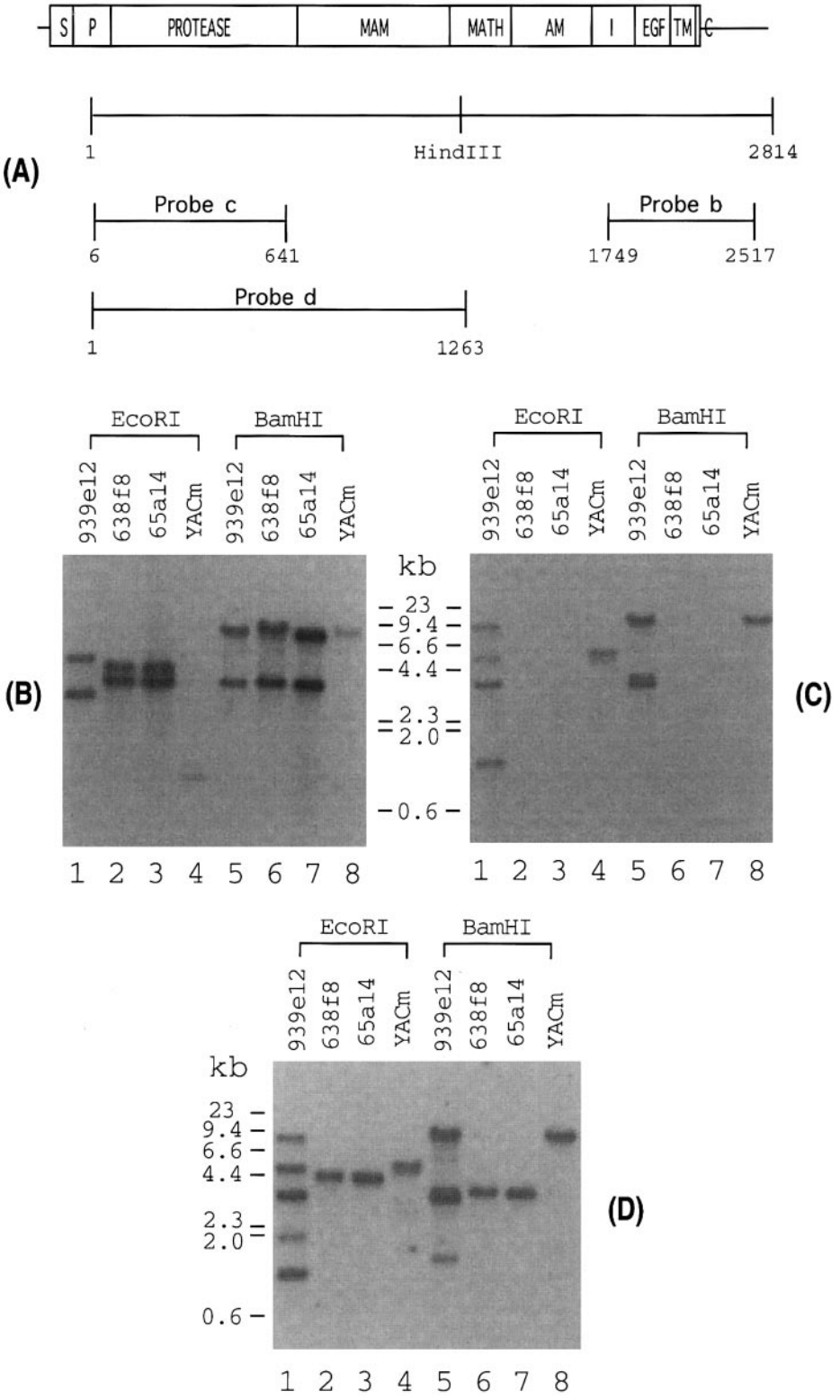

FIG. 2. Southern blot analyses of *MEP1A*-related sequences. (A) The predicted protein domain structure, cDNA and probes of the human meprin α subunit. The boxes represent domains and horizontal lines indicate 5′ and 3′ untranslated regions. See text for the description of the protein domains. The nucleotide numbering (1–2814) is according to Dumermuth *et al.* (31), and the HindIII site is indicated in the *MEP1A* cDNA. The probes (b, c and d) were used in the Southern blot analyses. (B) Probe b (nt 1749–2517) detected fragments from all four genomic clones, human YACs 939e12 (lanes 1 and 5) and 638f8 (lanes 2 and 6), PAC 65a14 (lanes 3 and 7), and a mouse YAC (YACm, lanes 4 and 8) that were cleaved with either EcoRI (lanes 1–4) or BamHI (lanes 5–8). (C) Probe c (nt 6–641) detected fragments from YAC 939e12 and the mouse YAC (lanes 1, 4, 5 and 8) and no fragments from YAC 638f8 and PAC 65a14 (lanes 2, 3, 6 and 7). (D) The blot used in (C) was re-hybridized (without being striped) with probe d (nt 1–1263), which detected additional fragments from YAC 939e12 (lanes 1 and 5) and fragments from YAC 638f8 and PAC 65a14 (lanes 2, 3, 6 and 7).

fragments that hybridized to probe b (lanes 1 and 5). YAC 638f8 and PAC 65a14 contained similar EcoRI (lanes 2 and 3) and BamHI fragments (lanes 6 and 7). Both YAC 638f8/PAC 65a14 and YAC 939e12 contained similar BamHI (lanes 5-7), but different EcoRI fragments (lanes 1-3), demonstrating their sequence similarities and differences. In addition, PAC 65a14 and YAC 638f8 hybridized more strongly than YAC 939e12 to probe b that was derived from the *MEP1A* gene, indicating that they contain multiple copies of similar sequences. This is consistent with the FISH results (Fig. 1, bottom). Weak signals were also detected from a mouse YAC clone that contain the mouse meprin α gene (lanes 4 and 8; Jiang, unpublished work).

In contrast, PAC 65a14 contains sequences that differ from the 5′ end of the *MEP1A* gene including the protease domain (Fig. 2C). Using the 5′ end probe c, no signals were detected from PAC 65a14 (lanes 3 and 7) or YAC 638f8 (lanes 2 and 6) whereas signals were clearly visible from YAC 939e12 (lanes 1 and 5). Since signals were not detected from 638f8, the larger YAC clone, it is unlikely that lack of signals from PAC 65a14 was due to truncation of the 5′ end regions that correspond to probe c. Sequence differences between PAC 65a14/YAC 638f8 and *MEP1A* in the 5′ end should be more than 20% because signals were detected from the mouse YAC clone with the human probe under the conditions used (lanes 4 and 8, the nucleotide differences between the mouse and human α cDNAs corresponding to probe c (nt 6-641) are 20%). These sequence differences were further indicated by detection of signals only on 6p21, but not on chromosomes 9 and 13, by FISH using PAC 1e12 which was obtained using probe c (Fig. 1, top, and Fig. 2A). PAC 1e12 did not contain the 3′ end of the *MEP1A* gene as revealed by PCR using the primers corresponding to the 3′ untranslated region (data not shown).

To more clearly define the region of sequence similarity between YAC 638f8/PAC 65a14 and *MEP1A*, the blot used in Fig. 2C was re-hybridized with probe d that extended probe c to include the MAM domain (Fig. 2D). This probe detected fragments from YAC 638f8 and PAC 65a14, and additional fragments from YAC 939e12 (lanes 1–3, 5–7). Therefore, PAC 65a14 and YAC 638f8 both contain sequences that are highly similar to those of the *MEP1A* gene encoding the C-terminal "interactions" or "adhesion" domains of the meprin α subunit.

It is likely that YAC 638f8 and PAC 65a14 were derived from the same chromosome (9 or 13, but not 6) because they yielded similar restriction fragments and signal intensities with all probes used. In addition, there is another YAC clone, 878e3, which was thought to be chimeric and mapped by FISH to 6p22-23, 9p12-13, and 9q21 (44). This YAC was also positive by PCR using the primers within the I domain of the meprin α subunit (37). Southern analysis showed that it contained *MEP1A-like* sequences similar to those of 638f8 (data not shown). Therefore, it is possible that YAC 878e3 is not a chimeric clone as originally suggested (44).

Multiple copies of highly similar sequences in the human genome. Recently, a region in the major histocompatibility complex (MHC) has been identified to present in three copies on human chromosomes 1, 6, and 9 (46). The *MEP1A* gene is closely linked to the MHC loci in both the mouse and human genome (37, 47). Chromosome 1 did not show positive hybridization signals to PAC 65A14, by FISH. However, PCR screening of NIGMS human/rodent somatic cell hybrid mapping panel #2, version 2 (NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ) indicated that chromosome 1 also contained *MEP1A-like* sequences. With the primers corresponding to both 5′ and 3′ ends of the *MEP1A* gene, the PCR signals were detected from the somatic cell hybrids that contained chromosomes 1 and 6, respectively, and the signals from chromosome 1 were weaker than those from chromosome 6. In conjunction with the FISH results, the PCR results suggest that chromosome 1 contains *MEP1A-like* sequences that are less similar than those on chromosome 9 and *MEP1A* on chromosome 6. It is therefore possible that the *MEP1A* gene and/or *MEP1A-like* sequences are part of, or are at the centromeric boundary of the triplicated region containing the MHC loci.

The mouse genome also contains the syntenic regions on chromosomes 1, 2/4, and 17 that correspond to the triplicated regions on human chromosomes 1, 9, and 6, respectively (46, 48). The *Mep1a* gene has been recently mapped to chromosome 17C1-D1 by FISH using a genomic DNA probe corresponding to the protease domain of the mouse meprin α subunit (35, 49). Therefore, it is possible that *Mep1a*-related sequences are present in the mouse genome.

In summary, our FISH results indicate that the *MEP1A* gene is present as a single copy on 6p21 and that *MEP1A-like* sequences are present in human chromosomes 9 and 13. The symbols of *MEP1AL1*, *MEP1AL2*, *MEP1AL3*, and *MEP1AL4* have been designated for *MEP1A-like* sequences on 9p12-13, 9q21, 9q22, and 13cen, respectively. *MEP1AL1-4* could potentially encode novel proteins containing domains highly similar to those of the meprin subunits including MAM, MATH, AM and EGF-like domains involved in interaction or adhesion. Alternatively, they could represent pseudo genes of *MEP1A* which do not possess a catalytic (protease) domain. Work is in process to distinguish between these possibilities. Identification of *MEP1A-like* sequences in the human genome provides new information on evolution of the *MEP1A* gene, and indicates that multiple copies of highly sim-

ilar sequences may be more common to the human genome than previously realized. In this regard, the identification of *MEP1A-like* sequences illustrate the challenges we face in mapping, sequencing, and functional analysis of these sequences in the human genome because of their large sizes and high sequence similarities.

ACKNOWLEDGMENTS

This work is aided by Grant IRG-196A from the American Cancer Society (W.J.) and the Canadian Genome Analysis and Technology Program (B.G.B.). We thank Dr. Judith Bond for her critical reading of the manuscript. We thank Dr. Erwin Sterchi of University of Bern for providing a *MEP1A* cDNA clone (pPPHα22.4). We thank Dr. Dwijendra Gupta for his help, and Bettina Noel, Brian Le, Zong Mei Zhang, Teresa Scheidl, and Jo-Anne Herbrick for their excellent technical assistance during the course of this work.

REFERENCES

1. Dumermuth, E., Sterchi, E. E., Jiang, W., Wolz, R. L., Bond, J. S., Flannery, A. V., and Beynon, R. J. (1991) *J. Biol. Chem.* **266,** 21381–21385.
2. Bond, J. S., and Beynon, R. J. (1995) *Protein Sci.* **4,** 1247–1261.
3. Stöcker, W., Grams, F., Baumann, U., Reinemer, P., Gomis-Ruth, F. X., McKay, D. B., and Bode, W. (1995) *Protein Sci.* **4,** 823–840.
4. Jiang, W. and Bond, J. S. (1996) *in* Zinc Metalloproteases in Health and Disease (Hooper, N. M., Ed.), pp. 131–151, Taylor & Francis, Bristol, PA.
5. Barnes, K., Ingram, J., and Kenny, A. J. (1989) *Biochem. J.* **264,** 335–346.
6. Beynon, R. J., Oliver, S., and Robertson, D. H. L. (1996) *Biochem. J.* **315,** 461–466.
7. Kounnas, M. Z., Wolz, R. L., Gorbea, C. M., and Bond, J. S. (1991) *J. Biol. Chem.* **266,** 17350–17357.
8. Sterchi, E. E., Naim, H. Y., Lentze, M. J., Huri, H.-P. and Fransen, J. A. M. (1988) *Arch. Biochem. Biophys.* **265,** 10–118.
9. Chestukhin, A., Muradov, K., Litovchick, L., and Shaltiel, S. (1996) *J. Biol. Chem.* **271,** 30272–30280.
10. Choudry, Y., and Kenny, A. J. (1991) *Biochem. J.* **280,** 57–60.
11. Kaushal, G. P., Walker, P. D., and Shah, S. V. (1994) *J. Cell Biol.* **126,** 1319–1327.
12. Wolz, R. L., Harris, R. B., and Bond, J. S. (1991) *Biochemistry* **30,** 8488–8493.
13. Yamaguchi, T., Fukase, M., Sugimoto, T., Kido, H., and Chihara, K. (1994) *Biol. Chem. Hoppe-Seyler* **375,** 821–824.
14. Bankus, J. M., and Bond, J. S. (1996) *Arch. Biochem. Biophys.* **331,** 87–94.
15. Ricardo, S. D., Bond, J. S., Johnson, G. D., Kaspar, J., and Diamond, J. R. (1996) *Am. J. Physiol.* **270,** F669–676.
16. Spencer-Dene, B., Thorogood, P., Nair, S., Kenny, J., Harris, M., and Henderson, B. (1994) *Development* **120,** 3213–3226.
17. Trachtman, H., Valderrama, E., Dietrich, J. M., and Bond, J. S. (1995) *Biochem. Biophys. Res. Commun.* **208,** 498–505.
18. Dietrich, J. M., Jiang, W., and Bond, J. S. (1996) *J. Biol. Chem.* **271,** 2271–2278.
19. Matters, G. L., and Bond, J. S. (1999) *APMIS* **107,** 19–27.
20. Lottaz, D., Maurer, C. A., Hahn, D., Büchler, M. W., and Sterchi, E. E. (1999) *Cancer Res.* **59,** 1127–1133.
21. Marchand, P., Tang, J., Johnson, G. D., and Bond, J. S. (1995) *J. Biol. Chem.* **269,** 15388–15393.
22. Tang, J., and Bond, J. S. (1998) *Arch. Biochem. Biophys.* **349,** 192–200.
23. Tsukuba, T., and Bond, J. S. (1998) *J. Biol. Chem.* **273,** 35260–35267.
24. Beckman, G., and Bork, P. (1993) *Trends Biochem. Sci.* **18,** 40–41.
25. Uren, A. G., and Vaux, D. L. (1996) *Trends Biochem. Sci.* **21,** 244–245.
26. Zondag, G. C. M., Koningstein, G. M., Jiang, Y.-P., Sap, J., Moolenaar, W. H., and Gebbink, M. F. B. G. (1995) *J. Biol. Chem.* **270,** 14247–14250.
27. Cheng, G., Cleary, A. M., Ye, Z. S., Hong, D. I., Lederman, S., and Baltimore, D. (1995) *Science* **267,** 1494–1498.
28. Jiang, W., Gorbea, C. M., Flannery, A. V., Beynon, R. J., Grant, G. A., and Bond, J. S. (1992) *J. Biol. Chem.* **267,** 9185–9193.
29. Johnson, G. D. and Hersh, L. B. (1992) *J. Biol. Chem.* **267,** 13505–13512.
30. Corbeil, D., Gaudoux, F., Wainwright, S., Ingram, J., Kenny, A. J., Boileau, G., and Crine, P. (1992) *FEBS Lett.* **309,** 203–208.
31. Dumermuth, E., Eldering, J. A., Grünberg, H., Jiang, W., and Sterchi, E. E. (1993) *FEBS Lett.* **335,** 367–375.
32. Gorbea, C. M., Marchand, P., Jiang, W., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Bond, J. S. (1993) *J. Biol. Chem.* **268,** 21035–21043.
33. Eldering, J. A., Grünberg, J., Hahn, D., Croes, H. J., Fransen, J. A., and Sterchi, E. E. (1997) *Eur. J. Biochem.* **247,** 920–932.
34. Jiang, W., Sadler, P. M., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., and Bond, J. S. (1993) *J. Biol. Chem.* **268,** 10380–10385.
35. Jiang, W., and Beatty, G. G. (1997) *Cytogenet. Cell Genet.* **76,** 206–207.
36. Bond, J. S., Rojas, K., Overhauser, J., Zoghbi, H. Y., and Jiang, W. (1995) *Genomics* **25,** 300–303.
37. Jiang, W., Dewald, G., Brundage, E., Mücher, G., Schildhaus, H.-U., Zerres, K., and Bond, J. S. (1995) *Biochem. Biophys. Res. Commun.* **216,** 630–635.
38. Ioannou, P. A., Amemiya, C. T., Garnes, J., Kroisel, P. M., Shizuya, H., Batzer, M. A., and de Jong, P. J. (1994) *Nature Genet.* **6,** 84–89.
39. Lichter, P., Tang, C. J., Call, K., Hermanson, G., Evans, G. A., Housman, D., and Ward, D. C. (1990) *Science* **247,** 64–69.
40. Heng, H., and Tsui, L.-C. (1993) *Chromosoma* **102,** 325–332.
41. Boyle, A. L., Feltquite, D. M., Dracopoli, N. C., Housman, D. E., and Ward, D. C. (1992) *Genomics* **12,** 106–115.
42. Ausubel, F. A., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1996) Current Protocols in Molecular Biology, John Wiley & Sons, New York.
43. Bouzyk, M., Bryant, S. P., Schmitt, K., Goodfellow, P. N., Ekong, R., and Spurr, N. K. (1996) *Genomics* **34,** 187–192.
44. Bray-Ward, P., Menninger, J., Lieman, J., Desai, T., Mokady, N., Banks, A., and Ward, D. C. (1996) *Genomics* **32,** 1–14.
45. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22,** 4673–4680.
46. Katsanis, N., Fitzgibbon, J., and Fisher, E. M. (1996) *Genomics* **35,** 101–108.
47. Bond, J. S., Beynon, R. J., Reckelhoff, J. F., and David, C. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5542–5545.
48. Debry, R. W., and Seldin, M. F. (1996) *Genomics* **33,** 337–351.
49. Jiang, W., and Flannery, A. V. (1997) *Gene* **189,** 65–71.