The human megakaryocyte potentiating factor (hMPF) has been previously purified from a culture supernatant of human pancreatic cancer cells HPC-Y5 (Yamaguchi, N., Hattori, K., Oh-edu, M., Kojima, T., Imai, N., and Ochi, N. (1994) J. Biol. Chem. 269, 805–808). We have now isolated hMPF cDNA from a HPC-Y5 cDNA library using polymerase chain reaction and plaque hybridization methods. The hMPF cDNA encodes a polypeptide consisting of 622 amino acids, including a signal peptide of 33 amino acids, and with a deduced molecular mass of 68 kDa, although HPC-Y5 cells secrete a 33-kDa form of hMPF. Human MPF does not show any significant homology with other previously described sequences. The cDNA was expressed in COS-7 and Chinese hamster ovary (CHO) cells, and megakaryocyte potentiating activity was detected in their culture supernatant. The COS-7 cells secreted only a 33-kDa recombinant hMPF, whereas an additional 30-kDa form was detected in the culture medium of CHO cells. The 33-kDa rhMPF purified from CHO cells showed megakaryocyte potentiating activity, but not the purified 30-kDa rhMPF. The difference in structure and activity between the 33- and 30-kDa forms of hMPF was ascribed to the existence of the 33-kDa form of the C-terminal 25 amino acid residues.

Megakaryocytes originate from pluripotent hematopoietic stem cells through a complex process involving commitment of the pluripotent hematopoietic progenitor to megakaryocytic precursor cells and their mitotic amplification. The regulatory system governing megakaryocytogenesis and platelet production is thought to take place in at least two stages, 1) during proliferation and differentiation of megakaryocytic progenitor cells, leading to the production of megakaryocytes, and 2) during maturation of megakaryocytes which leads to the production of platelets. Megakaryocyte proliferation is thought to be dependent on an essential megakaryocyte colony-stimulating factor, and this proliferation can be potentiated in vitro by ancillary megakaryocyte potentiators (Meg-POT)1 (1–3), which also stimulate the maturation of the megakaryocytes. We have recently identified, in the culture supernatant of the human pancreatic cancer cells HPC-Y5, a novel megakaryocyte potentiating factor (hMPF), which stimulates the megakaryocyte colony forming activity of murine interleukin-3 in mouse bone marrow cell culture (4). The factor was found to consist of a single polypeptide of about 32 kDa with at least one N-linked sugar chain. In this paper, we describe the molecular cloning and characterization of the cDNA encoding human MPF.

MATERIALS AND METHODS

**Protein and Amino Acid Sequence Determination**—Human MPF was purified from HPC-Y5 culture supernatant as described previously (4) and partially digested with endoproteinase Glu-C (Boehringer-Mannheim) in the presence of 10 mM 2-mercaptoethanol and 2 M urea at 37 °C for 18 h. Peptide fragments were then separated on a Vydac C18 reverse-phase column, and subjected, with purified MPF, to sequence analysis in an Applied Biosystems model 473A or 476A protein sequencer.

**cDNA Library (I) and Polymerase Chain Reaction (PCR)**—Total RNA was prepared from HPC-Y5 cells (5), and poly(A)+ RNA was further purified by oligo(dT)-cellulose chromatography. Complementary DNA was synthesized by oligo(dT) priming and inserted into pZAP phage vector (Stratagene). After one round of amplification of the resulting cDNA library (106 independent clones), the DNA was extracted, and an aliquot (500 ng) was subjected to PCR in the presence of 50 pmol of each forward and backward primers and 1.25 units of Taq DNA polymerase (Perkin-Elmer). The DNA was denatured at 95 °C for 7 min, and PCR was carried out for 40 cycles as follows: annealing at 60 °C for 1 min, extension at 72 °C for 2 min, and denaturation at 94 °C for 1 min. An aliquot (1/20) of the PCR products was subjected to a second PCR with inner primers. The PCR products were separated on polyacrylamide gel, and the desired DNA fragment was excised, purified, and subcloned into a pSP73 vector (Promega) for sequencing.

**cDNA Library (II) and Screening**—A second HPC-Y5 cDNA library was used for isolating a full-length cDNA. Complementary DNA was synthesized as above (6), inserted into the UniZAP XR directional cloning vector (Stratagene), and packaged into a phage using Gigapack Gold (Stratagene), thus yielding 1.8 × 106 independent clones.

In order to isolate a full-length hMPF cDNA, approximately 1.7 × 106 clones were screened with Q197A (7), a 197-base pair MPF cDNA fragment generated by PCR. This fragment was labeled by 32P using a Random Primer Labeling kit (TAKARA) in the presence of Q5 and AA primers instead of the kit’s random primers, and used as hybridization probe. Filters (Hybond N*, Amersham) were hybridized with the 32P-labeled Q197A probe. Isolated clones were subjected to in vivo excision of pBluescript SK(−) phagemid (Stratagene), and plasmid DNA was prepared by the standard method.

**DNA Sequencing**—DNA sequences were determined using Seque-
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nase version 2.0 (U. S. Biochemical Corp.) and appropriate synthetic oligonucleotide primers. Antibody Preparation — The XhoI fragment of MPF cDNA (nucleotides 2106–2109) was excised from pKPO27, and inserted into the StuI site of a PMAL–C vector (Clontech, Palo Alto, Calif.), in which hMPF is expressed as a fusion protein with maltose-binding protein. This fusion protein was purified according to the manufacturer's protocol, and used for immunization. Rabbits or BALB/c mice were immunized five times at 2-week intervals with rhMPF emulsified with complete Freund’s adjuvant. Rabbit anti-MPF polyclonal antibody was collected 10 days after the final injection. Mouse splenocytes were isolated 9 days after the final injection, and fused to P3U1 mouse myeloma cells to establish hybridomas.

Expression of MPF cDNA in COS-7 Cells — Plasmid pRVK207f, harboring the MPF cDNA downstream of the human elongation factor 1α (8) promoter, was constructed from pKPO27 and HEF-12 h-gly (9), which includes the simian virus 40 (SV40) origin site. The plasmid (10 μg) was introduced by electroporation (1900 V, 25 microfarads, Bio-Rad Gene Pulser) into COS-7 cells suspended in phosphate-buffered saline at a density of 1 × 10⁶ cells/ml. Transformants were cultured in Dulbecco’s minimum essential medium containing 1% fetal calf serum (FCS) at 37°C under 5% CO₂ for 72 h.

Establishment of rhMPF-producing CHO Cell line (FKL5f) — Plasmid pEFDKPO27f, harboring the mouse dihydrofolate reductase (DHFR) cDNA downstream of the SV40 early promoter and hMPF cDNA downstream of the elongation factor 1α promoter, was constructed from DHFR-ΔE-RVh (9), pCDMS (10), and pKPO27. Dihydrofolate reductase-deficient CHO cells (DBK-11) were cultured in α-minimum essential medium, supplemented with 10% FCS, and transfected with pEFDKPO27f. Transfected cells were selected on G418 (Sigma) for 2 weeks. Supernatant was repeatedly aspirated and fresh medium was added every 2 days. When the density of transfected cells reached 1 x 10⁶ cells/ml, the concentrated supernatant was harvested and stored at 4°C for 1 week and then cleared by centrifugation at 10,000 × g for 30 min. The supernatant was applied to an S-Sepharose column (5 × 12 cm, Pharmacia) equilibrated with 20 mM sodium acetate buffer (pH 5.0) containing 0.01% Tween 20. The eluate was desalted by repeated concentration and dilutions, the resulting concentrate centrifuged (10,000 × g, 30 min), and the supernatant was applied to an S-Sepharose Fast Flow column (5 × 12 cm, Pharmacia) equilibrated with 20 mM sodium acetate buffer (pH 5.0) containing 0.01% Tween 20. The column was washed with the same buffer, and proteins were eluted with 2 M KC₁ in 50 mM Tris-HCl (pH 9.0) containing 0.1% Tween 20. The eluate was desalted by repeated concentration and dilutions, the resulting concentrate centrifuged (10,000 × g, 30 min), and the supernatant was applied to an S-Sepharose Fast Flow column (5 × 12 cm, Pharmacia) equilibrated with 20 mM sodium acetate buffer (pH 5.0) containing 0.01% Tween 20. The column was washed with the same buffer, and proteins were eluted stepwise with a NaCl gradient (0.1, 0.2, 0.3, 0.5, and 1.0 M) in equilibration buffer. The fraction containing MPF was applied to a reverse-phase HPLC column (C4, 20 × 250 mm, Vydac) equilibrated with 24% acetonitrile in 0.1% trifluoroacetic acid. The column was washed with the same buffer, and proteins were eluted with a 24–48% linear gradient of acetonitrile in 0.1% trifluoroacetic acid for 48 min at a flow rate of 2 ml/min. MPF fractions were pooled and applied to a TSKgel G3000SW column (21.5 × 600 mm, Tosoh) equilibrated with 40% acetonitrile in 0.1% trifluoroacetic acid, and the column was developed with the same solvent at a flow rate of 3 ml/min. The MPF fraction was applied to a reverse-phase HPLC column (C4, 20 × 250 mm, Vydac) at a flow rate of 1 ml/min, and MPF was eluted with a linear gradient of acetonitrile as described above.

For 30-kDa MPF purification, the cell culture supernatant was filtered through a 5-μm membrane (Amicon, Inc.) to remove cellular debris. The filtrate was then applied to a Blue Sepharose Fast Flow column (5 × 20 cm, Pharmacia) equilibrated with 50 mM Tris-HCl (pH 8.0) containing 0.2 mM NaCl and 0.01% Tween 20. The column was washed with the same buffer, and proteins were eluted with 2 mM KCl in 50 mM Tris-HCl (pH 9.0) containing 0.01% Tween 20. The eluate was desalted by repeated concentration and dilutions, the resulting concentrate centrifuged (10,000 × g, 30 min), and the supernatant was applied to an S-Sepharose Fast Flow column (5 × 12 cm, Pharmacia) equilibrated with 20 mM sodium acetate buffer (pH 5.0) containing 0.01% Tween 20. The column was washed with the same buffer, and proteins were eluted stepwise with a NaCl gradient (0.1, 0.2, 0.3, 0.5, and 1.0 M) in equilibration buffer. The fraction containing MPF was applied to a reverse-phase HPLC column (C4, 20 × 250 mm, Vydac) equilibrated with 24% acetonitrile in 0.1% trifluoroacetic acid. The column was washed with the same solvent, and proteins were eluted with a 24–48% linear gradient of acetonitrile in 0.1% trifluoroacetic acid for 48 min at a flow rate of 2 ml/min. MPF fractions were pooled and applied to a TSKgel G3000SW column (21.5 × 600 mm, Tosoh) equilibrated with 40% acetonitrile in 0.1% trifluoroacetic acid, and the column was developed with the same solvent at a flow rate of 3 ml/min. The MPF fraction was applied to a reverse-phase HPLC column (10 × 250 mm, Vydac) at a flow rate of 1 ml/min, and MPF was eluted with a linear gradient of acetonitrile as described above.

Structure analysis showed that the first ATG conforms well to the underlined amino acid sequences shown above. The expected size (84 base pairs) was obtained from the HPC-Y5 cDNA library after 2 rounds of PCR amplification, as described in Fig. 1A, first in the presence of T7 promoter sequence and N1 primer, then in the presence of the inner primers K4S and K4A. The sequence of this fragment shallowed in pSP73 vector was 5'-CTCTTACAGAGCAGCTGCGCTGTCTGGCTACCGGCAGTCTCGA'–3' (not including the primers). The deduced amino acid sequence encoded by this PCR-amplified fragment and that of the peptide obtained by endopeptidase Glu-C digestion were found to be identical. Using four synthetic primers on the basis of this sequence information, a 197-base pair fragment Q197A was generated by repeated PCR amplification, as shown in Fig. 1A. Its nucleotide sequence was found to encode the amino acid sequence of a fragment of MPF, demonstrating that Q197A is a fragment of MPF cDNA.

Twenty-three positive clones were isolated from the cDNA library II (1.7 × 10⁸) by plaque hybridization using the Q197A fragment as a probe. One of them, clone pKPO27, was subjected to further analysis, since it contained the longest insert. Structure of MPF cDNA — Fig. 2 shows the entire nucleotide sequence of the cDNA insert of pKPO27. To confirm this sequence, another positive clone pKPO32 was analyzed, and no difference was found in the overlapping cDNA regions of pKPO27 and pKPO32. The cDNA contains a single long open reading frame of 1869 nucleotides, with a potential polyadenylation sequence AGTAAA just upstream of the poly(A) tail. Structure analysis showed that the first ATG conforms well to the Kozak consensus sequence for an initiator methionine, and that the N-terminal amino acid of MPF, Leu, which is followed by Ala, Gly, Gin, and Thr (4), was located at nucleotides 191–193. This cDNA encodes a 622-amino acid long precursor, whose N-terminal 33 hydrophobic amino acids correspond to a peptide signal sequence. The remaining portion of this precursor has a deduced molecular mass of 66.8 kDa, which is different from the molecular size of natural MPF, i.e., 32 kDa. However, when an in vitro translation reaction was performed on RNA templates, transcribed in vitro from a vector harboring MPF cDNA downstream of the T7 promoter, the molecular weight of the in vitro translation product was equivalent to that predicted from the nucleotide sequence of the MPF cDNA.
**A**

FIG. 1. A, strategy for PCR. The cDNA insertion is represented by a rectangle. T3 and T7, promoters for T3 and T7 polymerases, respectively. ZAP phage sequence is shown as solid bars. Primers used for amplification are denoted by arrows. Four inner oligonucleotide primers (KS1, KS2, KA1, and KA2) derived from this DNA fragment were then synthesized and two sets of primers, T7 promoter sequence primer/KS1 and T3 promoter sequence primer/KA1, were used to carry out PCR amplification using HPC-Y5 cDNA library as template. Aliquots of the PCR products were subjected to further amplification in the presence of either T7/KS2 or T3/KA2 primers. The PCR products (200 and 240 base pairs) were gel-purified, and their sequences were determined by direct sequencing. One more set of primers, sense primer QS and antisense primer AA, were synthesized based on this sequence information, and used for the final PCR amplification. B, amino acid sequences and corresponding oligonucleotide sequences used as primers for PCR. Numbering of sequences 1-6 was taken from that of the corresponding amino acids in Fig. 2. The primers contain all the possible combinations of nucleotide sequences that correspond to a given amino acid. Code for nucleotides is as follow: R, either A or G; Y, either C or T; N, A, G, C, or T.

**B**

Sequence 1

Primer N1

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Sequence 2

Primer K4S

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Sequence 3

Primer K4A

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Sequence 4

Primer KS1

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Sequence 5

Primer KS2

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Sequence 6

Primer QS

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Sequence 7

Primer AA

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(data not shown). A dibasic proteolytic cleavage site RXRXRR and four potential N-glycosylation sites were found in the MPF precursor amino acid sequence. No significant homology to any sequences in GenBank and EMBL was revealed by comparison of nucleotide and predicted amino acid sequences.

**COS Cell Expression of MPF cDNA**—To further verify the identity of the MPF cDNA, an expression plasmid pRVKPO-27K was constructed and transfected into COS-7 cells, and the culture supernatant was subjected to megakaryocyte colony-forming assay. The assay was performed in the presence of anti-mouse IL-6 receptor antibody, since the culture supernatant of COS cells contains IL-6. The culture supernatant of cDNA-transfected COS cells, but not that of cells transfected with the vector alone, potentiated megakaryocyte-colony formation. Western blotting analysis was performed on the aforementioned supernatants using an antiserum raised against
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GGCACTCGCTGGTACCAAGTGCAGGAGACCACATGGCTGCCCACACACAGACCTGAGATG

rMPF expressed in E. coli. In the case of cDNA-transfected cells, a band corresponding to MPF protein was detected at approximately 33 kDa as shown in Fig. 3. Since the 55-kDa band, detected in both cases, is also present in FCS, it was presumably not related to rMPF (data not shown).

CHO Cell Expression of MPF cDNA—After transfection of pEFDKPO27f and establishment of FLK5F stable transformants resistant to 10 mM methotrexate, whose culture medium exhibited the highest concentration of MPF (5 mg/liter) were selected. The molecular species of the MPF protein produced in these cells were examined by Western blotting, and two bands of different molecular size, 33 and 30 kDa, were detected in the culture supernatant. In contrast, HPC-Y5 cells and cDNA-transfected COS cells expressed only the 33-kDa form.

Purification and Characterization of rMPF Produced in CHO Cells—Each of the two purified forms appeared as a single band under both nonreducing and reducing SDS-PAGE conditions (Fig. 4). To clarify the difference in their molecular weight, their N- and C-terminal amino acid sequences were analyzed. Both forms had the same N-terminal amino acids, i.e., Ser1, Leu4, and Gly6. The C-terminal sequences were identified by analyzing the C-terminal fragments obtained from the 33- and 30-kDa MPF after cyanogen bromide cleavage and endoproteinase Asp-N
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**Fig. 3.** Detection of MPF in a culture supernatant of transfected COS cells. The culture supernatant of cDNA-transfected COS cells was concentrated by Centriprep-10 (Amicon), electrophoresed on a 12% gel (120 V, 2 h), and transferred on a nitrocellulose membrane. MPF was detected with rabbit anti-MPF antiserum and alkaline phosphatase-conjugated antibody to rabbit IgG (Cappel). Lane 1, culture supernatant of mock transfected COS cells; lane 2, cDNA transfected COS cells.

**Fig. 4.** SDS-PAGE analysis of the purified recombinant MPF. The two MPF forms (100 ng) were electrophoresed on a 12% gel (120 V, 2 h) and stained with Coomassie Brilliant Blue R-250. Apparent molecular weight was determined by using a molecular weight standard mixture (Bio-Rad). Lane 1, 33-kDa MPF; lane 2, 30-kDa MPF.

digestion. The amino acid sequence of the C-terminal peptide derived from the 30-kDa MPF was determined as Met-Asp-Ala-Leu-Arg-Gly-Leu-Leu-Pro-Val-Leu-Gly-Gln-Pro-Ile-Ile-Arg261.

The C-terminal peptide obtained from the 33-kDa MPF by cyanogen bromide cleavage was further digested with endoproteinase Asp-N, since this peptide was too long to determine the complete amino acid sequence. The peaks obtained by reverse phase HPLC were analyzed in a protein sequencer, and their amino acid sequences, Met-Asp-Ala-Leu-Arg-Gly-Leu-Leu-Pro-Val-Leu-Gly-Gln-Pro-Ile-Ile-Arg-Ser-Ile-Pro-Glu-Arg286, were identified. These results demonstrated that the 30-kDa rMPF was generated by truncation of the C-terminal 25 amino acid residues from the 33-kDa rMPF.

**Activity of Recombinant MPFs**—The Meg-POT activity of the rMPFs was determined by megakaryocyte colony-forming assay, in comparison with native MPF derived from HPC-Y5 as a positive control. The 33-kDa rMPF potentiated megakaryocyte colony formation in the presence of mouse IL-3 in a dose-dependent manner, as did native MPF (Fig. 5). On the other hand, as shown in Fig. 6, the 30-kDa rMPF showed no Meg-POT activity in the dose range of 0.156 to 40 ng/ml.

Using a sandwich enzyme-linked immunosorbent assay, as shown in Fig. 7, the antigenicity of 33-kDa rMPF was detected with the prepared anti-MPF monoclonal and polyclonal antibodies, whereas the 30-kDa rMPF displayed only a weak antigenicity, approximately 1/50 of the 33-kDa rMPF, for any antibody. Thus, deletion of the C-terminal 25 amino acids from the 33-kDa rMPF decreased the antigenicity of the protein for these antibodies.

**MPF mRNA Expression in Tissues**—To obtain further insight into MPF function, the expression of MPF mRNA was examined by Northern blot analysis using labeled MPF cDNA as a probe. MPF mRNA was detected in HPC-Y5 cells, with an approximate molecular size of 2.4 kilobases and a low expression level, which was in agreement with the low content of MPF in the culture supernatant of HPC-Y5 cells. We also examined the expression of the MPF gene in various human tissues, and the 2.4-kilobase band was detected only in lung. With a long exposure, MPF mRNA could be detected in heart, placenta, and

**Fig. 5.** Megakaryocyte potentiating activities of natural and recombinant MPFs. Bone marrow cells (2 x 10⁵) were cultured as described under "Materials and Methods." Megakaryocyte colonies were scored using dehydrated cultures stained for acetylcholinesterase. Each point represents the mean of duplicate cultures. Similar results were obtained with repeated experiments, and a typical pattern is shown here. O, natural MPF; ●, 33-kDa rMPF; ○, rhIL-6; ■, mouse IL-3 alone.

**Fig. 6.** Megakaryocyte potentiating activities of 30- and 33-kDa rMPFs. The Meg-POT activities of two molecular species of MPF were determined as described under "Materials and Methods." Each value represents the mean of duplicate cultures. Similar results were obtained with repeated experiments, and a typical pattern is shown here. With the prepared anti-MPF monoclonal and polyclonal antibodies, whereas the 30-kDa rMPF displayed only a weak antigenicity, approximately 1/50 of the 33-kDa rMPF, for any antibody. Thus, deletion of the C-terminal 25 amino acids from the 33-kDa rMPF decreased the antigenicity of the protein for these antibodies.

**MPF mRNA Expression in Tissues**—To obtain further insight into MPF function, the expression of MPF mRNA was examined by Northern blot analysis using labeled MPF cDNA as a probe. MPF mRNA was detected in HPC-Y5 cells, with an approximate molecular size of 2.4 kilobases and a low expression level, which was in agreement with the low content of MPF in the culture supernatant of HPC-Y5 cells. We also examined the expression of the MPF gene in various human tissues, and the 2.4-kilobase band was detected only in lung. With a long exposure, MPF mRNA could be detected in heart, placenta, and...
kidney in lower level than in lung, but not in any other tissues so far examined, i.e. brain, liver, skeletal muscle, or pancreas (Fig. 8).

**DISCUSSION**

We have isolated a cDNA for hMPF, which exhibits a Meg-POT activity in the presence of murine IL-3 in a colony-forming assay with mouse marrow assay cells, by means of PCR and plaque hybridization methods. By structural analysis of the cDNA, the primary translation product of MPF was shown to consist of 622 amino acid residues. The sequence of the mature MPF protein (33 kDa) starts at Ser and ends at Arg in complete agreement with that of natural MPF (4), and the C-terminal Arg residue of the mature 33-kDa MPF is followed by a 336-amino acid polypeptide. Several polypeptide hormones are synthesized as large precursor proteins, such as human pro-parathyroid hormone related protein (12), porcine proendothelin-1 (13), human pro-transforming growth factor β1 (14), mouse pro-nerve growth factor (15), and human pro-platelet derived growth factor (16). In each of these cases, active polypeptide sequences are usually bound by pairs of basic amino acid residues such as Arg-Arg or Lys-Arg, and it is at these sites that proteolytic processing occurs. Watanabe et al. (17, 18) have demonstrated that the sequence Arg-X-Arg/Lys-Arg is a signal for precursor cleavage catalyzed with furin, a mammalian homologue of the yeast precursor-processing nuclease, Kex2, within the constitutive secretory pathway in nonendocrine cells. The sequence Arg-Pro-Arg-Phe-Arg-Arg deduced from the MPF cDNA matches this cleavage signal model. The C-terminal Arg residues of the 33- and 30-kDa rMPFs, Arg and Arg, are located 9 and 34 amino acid residues upstream of this cleavage signal, respectively. Moreover, the hydrophobicity profile predicts that the signal is located in a hydrophilic region between Trp and Glu. This suggests that the MPF precursor would fold and then, be successively processed with furin-like and trypsin-like proteases. A similar processing has been found in leukocyte-derived natural interferon-α (19) and interferon-γ (20, 21). Although cleavage of the precursor gives rise to the mature MPF, it is not known if other peptides released from the C-terminal region of the MPF precursor are also biologically active.

The 33-kDa rMPF displayed a Meg-POT activity, whereas the 30-kDa rMPF, which was generated by truncation of the C-terminal 25 amino acid residues from the 33-kDa rMPF, possessed no Meg-POT activity equivalent to that of natural MPF and IL-6. Furthermore, the 30-kDa rMPF was not antigenic for any of the antibodies raised against the MPF precursor protein produced in E. coli, although the 30- and 33-kDa forms of rMPF are detectable in Western blotting analysis with these antibodies. Therefore, the C-terminal 25 amino acid residues are not epitopes. Circular dichroism (CD) analysis showed no influence of C-terminal truncation on the secondary structure, that is, there are no significant differences between the CD spectra of the 33- and 30-kDa rMPFs. However, Hattori et al. (22) have previously shown that monoclonal antibodies can detect subtle conformational changes in local areas within a protein molecule during denaturation, by determining affinity changes. Shortle and Meeker (23) have also found that removal of the C-terminal 13 amino acids of staphylococcal nuclease is sufficient to destabilize the protein native state or stabilize it in the denatured state. Therefore, loss of the C-terminal 25 amino acids might give rise to a local conformational change, altering the antigenicity of MPF.

Slodowski et al. (24) have previously examined the biological function of the C-terminal 20 amino acid residues of IFN-γ. The antiviral activities of the INF-γ analogue proteins lacking 14 (C-14) or more amino acids from the C-terminal are less than 2% of the native protein activity. In contrast, a variant, shortened by 11 only amino acids (C-11), showed a higher biological activity than the wild type INF-γ. CD analysis showed no influence of these C-terminal truncations on the secondary structure. In the case of 33-kDa MPF, however, removal of the Arg-Arg region leads to a drastic loss in biological activity and antigenicity, while truncation at Arg, excising the 33-kDa rMPF from its precursor, may result in a significant increase in activity. The loss of biological activity of the 33-kDa rMPF due to the absence of its C-terminal peptide may be linked to the delicate regulation of megakaryocytogenesis in vivo. It remains to be determined whether MPF exists in vivo as a precursor of the shorter species, 33- and 30-kDa MPF.

MPF was originally isolated from a pancreatic cancer cell line, HPC-Y5, however, Northern blot analysis shows that the MPF gene is expressed in the lung, not in the pancreas. Since the structure of the lung consist of alveoli and abundant capillaries, and is fraught with the danger of bleeding, this would suggest that MPF might play a role in the growth of

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megakaryocytes and/or the regulation of platelet production. But since MPF is weakly expressed in heart, placenta, and kidney, MPF may exhibit other biological activities.

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