Cloning, Sequencing, and Expression of Human Macrophage Stimulating Protein (MSP, MST1) Confirms MSP as a Member of the Family of Kringle Proteins and Locates the MSP Gene on Chromosome 3*

(Received for publication, January 29, 1993, and in revised form, March 29, 1993)

Teizo Yoshimura‡‡, Naoya Yuhki‡, Ming-Hai Wang‡, Alison Skeel‡, and Edward J. Leonard‡

From the ‡Immunopathology Section, Laboratory of Immunobiology, and the ‡Genetics Section, Laboratory of Viral Carcinogenesis, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

A human hepatoma (HepG2) cell line library was screened with an oligonucleotide probe for macrophage stimulating protein (MSP) to clone an MSP cDNA. Deduced sequences of isolated clones were compared with peptide fragment sequences of MSP. MSP cDNA encoded most of the known sequence of MSP except for a small segment of the 5' end of the open reading frame. Consequently, a hybrid 2300-base pair cDNA that encoded the complete MSP amino acid sequence was constructed from 2 clones. Culture fluid from COS-7 cells transfected with this full-length MSP cDNA had MSP biological activity, and the expressed MSP was detected by immunoprecipitation with antibody against native MSP. The deduced amino acid sequence of MSP includes 4 kringle domains, which have been found in hepatocyte growth factor and several proteins of the blood coagulation system. Among them, MSP has the highest sequence similarity to hepatocyte growth factor (45% identity). The MSP cDNA hybridized strongly to mRNA from liver, and to a lesser extent to mRNA from kidney and pancreas, suggesting that a cell type in the liver is the source of MSP. Several cloned and sequenced MSP cDNAs had insertions or deletions, suggesting that alternatively spliced MSP mRNAs may occur. This was reflected in Northern blots probed with an individual MSP cDNA, which showed more than one mRNA species. Furthermore, although the gene coding for MSP is on chromosome 3, the sequence of one of the cDNAs was identical with a unique sequence in chromosome 1, indicating that there may be a family of MSP genes, located on chromosomes 3 and 1.

Macrophage stimulating protein (MSP) is the name originally given to a human serum protein that makes resident peritoneal mouse macrophages capable of responding to the chemotaxant C5a (1, 2). We recently purified MSP to homogeneity by immunoaffinity column and high performance liquid chromatography-ion exchange chromatography (3). By SDS-polyacrylamide gel electrophoresis, the molecular mass of MSP was about 70 kDa; under reducing conditions, MSP comprised α and β chains of about 47 and 22 kDa. Two partial sequences from a lysylendopeptidase digest of the MSP α chain had a highly conserved motif (NYCRNPD) found in the triple disulfide loop structure (kringle) of a family of proteins that includes prothrombin (4), plasminogen (5), and hepatocyte growth factor/scatter factor (6, 7). In analogy to prothrombin or plasminogen, we postulated that MSP circulates in the blood as pro-MSP, which becomes activated when an Arg-Val bond is cleaved by a serine protease to make separate α and β chains characteristic of this family.

We now report the cloning of MSP cDNA from mRNA of a human hepatoma cell line, HepG2. The probe for the initial screening of the cDNA library was an oligonucleotide mixture based on the sequence of an MSP peptide (BU-11, Ref. 3) that included the conserved kringle motif. Because of alternative splicing that resulted in insertions or deletions, it was necessary to isolate and sequence several cDNAs to deduce the complete open reading frame for MSP. A hybrid cDNA that coded for complete MSP was then constructed and used for transfection of COS-7 cells. Immunoprecipitation of the COS-7 cell product revealed protein bands of the expected mass, and the secreted MSP was biologically active. Sequence and restriction site analyses show that the MSP gene is located on chromosome 3.

MATERIALS AND METHODS

Reagents—DMEM, L-glutamine, and gentamycin were from Quality Biological, Inc., Gaithersburg, MD. Cystine-free RPMI 1640 was from Advanced Biotechnologies, Inc., Silver Spring, MD. FCS was from HyClone Laboratories. Protein G-Sepharose was from Pharmacia LKB Biotechnology Inc. γ-[32P]ATP and L-[35S]cysteine were from Amersham. Restriction enzymes and reagents for cDNA preparation were from Bethesda Research Laboratories. DNA sequencing reagents were from United States Biochemical Corp. α-[32P]dATP were from Du Pont-New England Nuclear. AZAP II vector was from Stratagene. Oligonucleotides were from Operon Technologies, Inc., Alameda, CA.

Reagents for preparation of ElgMC3b included sheep blood, anticoagulated with acid/citrate/dextrose; veronal-buffered saline with 0.1% gelatin (VBS-gel): 0.14 M NaCl, 0.1 M veronal, pH 7.4, 1 mM MgCl2, 0.15 mM CaCl2; rabbit IgM anti-Forsmann antibody, kindly supplied by Dr. Tibor Boros; C5-deficient AKR mouse serum, stored at -80 °C; Dulbecco’s modified Eagle’s medium (DMEM); and ammonium chloride lysis buffer (0.16 M NH4Cl, 0.01 M KHCO3, 0.001 M MgCl2, 1.0 mM CaCl2; rabbit IgM anti-Forsmann antibody, kindly supplied by Dr. Tibor Boros; C5-deficient AKR mouse serum, stored at -80 °C; Dulbecco’s modified Eagle’s medium (DMEM); and ammonium chloride lysis buffer (0.16 M NH4Cl, 0.01 M KHCO3, 0.001 M MgCl2, 1.0 mM CaCl2).
cells were washed and resuspended in 10 ml of VBS-gel and then stored at 4 °C in 4 ml of VBS-gel. An oligodeoxynucleotide probe was prepared from a portion of human FCS, and total RNA was isolated by a guanidinium isothiocyanate method; poly(A) RNA was isolated by oligo(dT)-cellulose chromatography (8). cDNAs were synthesized by random priming or oligo(dT) priming, then ligated to the XZAP II vector (9) to prepare libraries. An oligodeoxynucleotide probe was prepared from a portion of human MSP amino acid sequence [DDNYCRNPDG] previously reported (10).

Approximately 5 x 10^8 recombinant phages from the cDNA library constructed by random priming were screened by high density plaque hybridization with the 32P-labeled oligonucleotide probe. Hybridization to nitrocellulose filters was carried out overnight at 45 °C in a solution containing 6 x standard saline citrate (SSC), 5 x Denhardt's solution, 0.05% sodium pyrophosphate, 1% SDS, 100 µg/ml heat-denatured, sheared, salmon sperm DNA, and 1 x 10^6 dpm/ml probe. Filters were washed three times with 6 x SSC, 0.1% SDS at 40 °C for 30 min and were dried and exposed overnight to XAR-5 films (Kodak) with the intensifying screen, MIF d 50, -80 °C. Phage plaques exhibited within XZAP II recombinants were rescued with helper phage (9). cDNA inserts were subcloned into M13mp18, and single strands were sequenced by the primer extension method (10).

Southern Blot Analysis—Southern blot analysis was performed as described (8) in a 1% agarose gel with 10 µg of EcoRI-cleaved DNA per lane. Filters were hybridized at 40 °C overnight in 30% formamide, 5 x SSC, 5 x Denhardt’s solution, 100 µg/ml sheared-denatured salmon sperm DNA, 1% SDS, and 1 x 10^6 dpm/ml probe. Filters were washed twice with 2 x SSC, 1% SDS at 50 °C for 5 min and 0.3 x SSC, 1% SDS at 50 °C for 30 min prior to autoradiographic exposure.

Northern Blot Analysis—Northern blot analysis of poly(A) RNA was done by the glyoxal-dimethyl sulfoxide method in a 1.2% agarose gel with MSP9 cDNA probe (8). Hybridization was as described for Southern blot analysis except that the temperature was 50 °C. The filters were washed in 2 x SSC twice at room temperature, then once in 0.1 x SSC at 65 °C. A human multiple tissue Northern blot was purchased from Clontech.

Expression of MSP in COS-7 Cells—MSP cDNA, MSP9.13B, was excised from pBluescript by EcoRI digestion, then ligated into an expression vector, pCAGGS (11), which was a generous gift from Dr. J. Miyazaki, Tokyo University, Japan. Transfection of COS-7 cells was carried out with N-[1-(2,3-dideoxyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (Boehringer Mannheim) according to instructions of the supplier. Briefly, COS-7 cells were cultured on 100-mm tissue culture dishes in 10 ml of DMEM containing 10% FCS. Cells were washed twice with PBS containing 2% FCS and then starved in serum-free medium for 24 h. When cells became about 80% confluent, medium was aspirated, and 15 ml of fresh medium containing complexes of DNA and N-[1-(2,3-dideoxyloxy)propyl]-N,N,N-trimethylammonium methylsulfate was added (10 µg of DNA and 70 µl of N-[1-(2,3-dideoxyloxy)propyl]-N,N,N-trimethylammonium methylsulfate per plate). After overnight culture, medium was aspirated, and 30 ml of fresh medium was added. Two to four days later, medium was collected and frozen. For metabolic labeling of human MSP, transfection was carried out in a 35-mm-diameter well (CLUSTER, Costar, Cambridge, MA) in 6 ml of medium. The cells were then incubated in 1 ml of cysteine-free RPMI 1640 supplemented with 2 nm glutamine, 10% FCS dialyzed against phosphate-buffered saline, 250 µl of I(-)[35]S]cysteine for 24 h.

Immunoprecipitation and Gel Electrophoresis—One-mi samples from the metabolic labeling experiment were diluted with an equal volume of 0.1 M Tris-HCl, pH 8.6, 0.15 M NaCl, 0.5% Tween 20, 0.1% bovine serum albumin (wash buffer), and incubated with an approximately 20 µl packed volume of protein G-Sepharose for 1 h at 4 °C. After a brief centrifugation in a Microfuge, supernatants were mixed with 20 µg of normal rabbit IgG and 20 µl of protein G-Sepharose. After incubation for another hour at 4 °C, supernatants were obtained by centrifugation. Meanwhile, 20-µg quantities of normal rabbit IgG and polyclonal anti-human MSP (3) were precipitated with 20 µl of protein G-Sepharose by incubation in wash buffer without bovine serum albumin (1 h at 4 °C). IgG-coupled protein G-Sepharose was washed with wash buffer three times, then incubated with pretreated samples (1 ml) for 1 h at 4 °C. Three washes with wash buffer and two washes with wash buffer without bovine serum albumin, phosphate buffer (all double-strength sample buffer), 10% glycerol, 6% SDS, 10% 2-mercaptoethanol), and applied to a 12.5% polyacrylamide gel. After electrophoresis at 13 mA for 4 h, the gel was stained with Coomassie Blue, treated with Enlightening (Du Pont-New England Nuclear), dried, and exposed to Kodak XR film at -80 °C.

Detection of Protein-bound Carbohydrate—SDS-polyacrylamide gel electrophoresis of pure natural MSP on a Pharmaacia-LKB PhastGel apparatus was followed by transfer to nitrocellulose. Protein-bound carbohydrate was detected with a GlycoTrack kit (Oxford GlycoSystems, Rosedale, NY) according to the directions of the manufacturer. Carbohydrate is oxidized with periodate, and the aldehyde groups formed are reacted with biotin hydrazide. Bound biotin is detected with a streptavidin-alanine phosphatase reagent.

Measurement of ElgMC3B Binding and Phagocytosis by Resident Mouse Peritoneal Macrophages—For phagocytosis experiments (9), 0.5-ml aliquots of mouse resident peritoneal cells at a concentration of 1 x 10^6 cells/ml of DMEM without FCS (obtained as described for the MSP bioassay) were added to 24-well polystyrene plates (Costar). Macrophages comprised about 70% of total peritoneal cells. After 1 h at 37 °C, the plates were washed twice with DMEM, and then 0.5 ml of ElgMC3B was added, followed by 0.1 ml of DMEM with different concentrations of MSP. The ratio of erythrocytes to peritoneal cells was 50:1. After incubation for specified intervals, nonadherent ElgMC3B were washed out of the wells, and ammonium...
chloride buffer was added to lyse adherent erythrocytes without affecting ingested erythrocytes. This buffer was removed after 2.5 min, and the monolayers were stained with Diff-Quik. The bottoms purified MSP.

The cleavage site between the 

FIG. 2. Nucleotide and deduced amino acids sequence of human MSP. The arrows indicate a possible signal peptide cleavage site (1) and the cleavage site between the a and b chains (2). Underlined segments show sequences obtained for peptides of a lysylpeptidase digest of human MSP (3). These sequence data are available from EMBL/GenBank/DDBJ under accession number L11924.
Cloning of Human Macrophage Stimulating Protein

RESULTS

cDNA Cloning—Based on the findings that partial amino acid sequences obtained from purified MSP were similar to those of hepatocyte growth factor/scatter factor (HGF/SF) and that preliminary Northern blot analysis with an oligonucleotide probe showed a faint band at about 4 kb, we expected that the size of the mRNA for MSP might be large. Therefore, we constructed a cDNA library by random priming. About 5 x 10^6 phages were screened for MSP with an oligonucleotide probe shown under “Materials and Methods.” After the first screening, two weakly positive spots were found on the film. In a second screening of these two clones, a single isolated plaque was picked from each plate. Phagemids that contained cDNAs were rescued and DNA sequencing was performed. One of two clones, designated MSP6, encoded a fragment of the α chain of MSP (BU-11) that we previously reported (3).

To clone a full-length cDNA, we constructed a second cDNA library by oligo(dT) priming. Using MSP6 as a probe, we screened approximately 2 x 10^6 clones, and 85 cDNA clones were isolated, sized on agarose gels, and sequenced. One of the clones, MSP9, was found to encode most of the amino acid sequences obtained from a partial digested purified MSP reported previously. The matching sequences were in 9 peptides of the partial digest and included a total of 74 residues from the α chain and 65 residues from the β chain. However, MSP9 lacked the 5′ end of the open reading frame (Fig. 1). Several other clones had the 5′ end of the open reading frame, but had either a deletion or insertion which resulted in the creation of a termination codon. Three examples are shown in Fig. 1.

We then constructed a hybrid cDNA clone, MSP9.13B, using MSP13B to supply the 5′ segment that was missing from MSP9 and substituting the MluI fragment of MSP9 for MluI fragment of MSP13 (Fig. 1). The substitution replaced the region of MSP13B that contained a deletion with the corresponding intact region of MSP9 and was designed to complete the open reading frame for MSP. The nucleotide and the translated amino acid sequences, starting with the putative initiator methionine, is shown in Fig. 2. The open reading frame codes for 711 amino acids, with a total mass of 80 kDa. There is a series of leucines near the N terminus, which probably represents part of the signal peptide for this secreted protein. The location of the signal peptide cleavage site will remain in doubt until the blocked N terminus (3) of pure MSP is identified. A cleavage site between residues 18 and 19, making a secreted protein with a mass of 78 kDa, would fit with the frequencies of the last 6 residues in signal peptides described by Heijne (13), except for proline in position −2, which did not occur in Heijne’s series of proteins. As established by our previous work, MSP belongs to the family of kringle proteins. The sequence shows that MSP has 4 kringles in the α chain, and, like other members of the family, an R-V bond is the junction between α and β chains, which are joined by an interchain disulfide (6). Predicted masses of the α and β chains of secreted MSP are 53 and 25 kDa, respectively. Asparagines at positions 72 and 296 in the α chain and position 615 in the β chain are within consensus sequences for N-linked carbohydrate. Protein-bound carbohydrate in natural MSP was found in both chains (Fig. 3). Among the proteins in the kringle family, the translated sequence of the MSP9.13B cDNA is most similar to HGF/SF, as shown in Fig. 4 (45% amino acid sequence identity, and an additional 17% conservative substitutions (14)).

Fig. 3 shows the hybridization of MSP9 cDNA to multiple sizes of mRNA derived from HepG2 cells. This is consistent with the fact that different lengths of cDNAs were cloned (Fig. 1) and appear to reflect alternative splicing of the MSP gene (13). To study MSP gene expression, hybridization of MSP9 to mRNAs from different human tissues was examined. As shown in Fig. 5B, liver expressed the highest level of MSP mRNA. The hybridization pattern was exactly the same as for HepG2 cells, suggesting that the same alternative splicing occurs in normal liver cells. In addition to liver, weak hybridization was seen to mRNAs from kidney and pancreas.

Expression of MSP in COS-7 Cells—To confirm that the hybrid cDNA, MSP9.13B, codes for biologically active MSP, we transfected COS-7 cells with MSP9.13B, which was ligated into the EcoRI site of the expression vector, pCAGGS, containing the β-actin promoter (11). The metabolically labeled MSP9.13B product expressed in COS-7 cells was immunoprecipitated with an antibody against natural MSP (3). After radioautography, three bands were observed in the lane (Fig. 6, lane 4) where the supernatant of pCAGGS.MSP9.13B-transfected cells was treated with anti-MSP IgG. The 63-kDa and 30-kDa bands migrated to exactly the same places where purified natural MSP migrated on the same gel (data not shown). The 80-kDa band probably corresponds to uncleaved pro-MSP (see introduction). No specific bands were observed in the other lanes. These results suggest that the transfected COS-7 cells secreted pro-MSP, and that some of the molecules were cleaved by a serine protease from the cells or FCS to form the α and β chains of active MSP.

Culture fluids from MSP9.13B-transfected COS-7 cells were also tested for MSP biological activity and for MSP protein by sandwich ELISA. The concentration of MSP in culture fluids from two transfected preparations of COS-7 cells ranged from 16 to 45 ng/ml. As shown in Fig. 7, culture fluid from the transfected cells stimulated mouse peritoneal macrophages to phagocytize ElgMC3b. The magnitude of the response shown in Fig. 7 corresponds to a concentration of 15 ng/ml MSP, as determined in the same assay from a dose-response curve with pure MSP isolated from human serum. The result indicates that MSP9.13B encodes biologically active MSP.

Hybridization of Human MSP cDNA to Genomic DNAs of Different Species—As shown above, our assay system uses mouse peritoneal cells to detect human MSP activity, with an ED50 of about 10⁻¹⁰ M. This suggests that mouse and human MSP are similar. Therefore, we examined the hybridization of human MSP cDNA to genomic DNAs of other
species. As expected, human MSP cDNA hybridized to mouse genomic DNA fragments (Fig. 8). Human MSP cDNA also hybridized to rabbit, guinea pig, mouse, and rat DNAs, but definite hybridization was not seen to chicken DNA.

**DISCUSSION**

To select a cell line suitable for cloning an MSP cDNA, we developed a sandwich ELISA to assay culture fluids from different human cell lines for secreted MSP. Among the cell lines tested (B-cells, glioma, bronchus, liver, osteosarcoma, fibroblast), only culture fluid from the human hepatoma line HepG2 reacted in the ELISA. Screening the HepG2 library with a probe designed from the partial sequence of pure MSP exons, separated by 17 introns, yielded many cDNAs, none of which had the complete coding sequence of MSP. After establishing overlapping sequences of 2 clones, we made a hybrid cDNA that had the complete MSP coding sequence. Transfection of COS-7 cells with this cDNA resulted in secretion of MSP, assessed by three independent assays: immunoprecipitation, MSP sandwich ELISA, and MSP bioassay.

We made a computer search for sequences similar to the cDNA for MSP and its translated amino acid sequence (14). The search revealed that Han et al. (15), by screening a human genomic DNA library with a probe coding for the second kringle domain in bovine prothrombin, identified a gene coding for a 4-kringle protein that has considerable sequence similarity to hepatocyte growth factor. The gene comprises 18 exons, separated by 17 introns. The sequence of the 18 exons is identical with the sequence of the MSP cDNA. This work...
and our own complement each other, since our data assign a function to the product of the gene described by Han et al. (15), and their work describes the sequence of the MSP gene. The gene described by Han et al. (15), which our work establishes as the gene for MSP, is located on chromosome 3. The evidence can be summarized as follows. The human genomic probe, H3H2, which identifies a locus present on both chromosomes 1 (DNF15S1) and 3p (DNF15S2) corresponds to nucleotides 918–2868 of the MSP genomic DNA sequence reported by Han et al. (15). Carritt et al. (16) have shown that H3H2 hybridizes to three HindIII restriction fragments of human DNA, 2.0, 3.8, and 8.0 kb in length. Analysis of human-rodent chromosome hybrids assigned the 2.0-kb fragment to chromosome 3; the larger fragments were from a corresponding part of chromosome 1 (which has a different location of one of the HindIII sites). Since the H3H2 hybridization locus on the MSP gene is the 2.0-kb HindIII fragment, it follows that the MSP gene is on chromosome 3.

Confirmatory evidence comes from sequencing data of Welch et al. (17), summarized in Fig. 9. Whereas the sequence of the MSP gene upstream from nucleotide 4928 is common to sequences of both chromosomes 1 and 3, identity with chromosome 1 abruptly ceases downstream from this position, which rules out a chromosome 1 location for the MSP gene and confirms assignment of the MSP gene to chromosome 3. As noted under “Results” and Fig. 1, we isolated many MSP cDNA clones that had either deletions or insertions, which resulted in shifts in the reading frame and the creation of stop codons. MSP15A is of particular interest, because comparison with the data of Welch et al. (17) reveals two pieces of evidence that assign this clone to chromosome 1, not chromosome 3. First, there is a single base pair substitution at nucleotide 787 of MSP cDNA 15A that creates an EcoRI
restriction site as shown in Fig. 1 (located in the 7th exon at nucleotide 2150 in the MSP gene sequence of Han et al. (15)). This site is absent in the H3H2 region of chromosome 3, but is present in the H3H2 region of chromosome 1 (Ref. 17 and Fig. 2). Second, as shown in Fig. 9, the sequence of the 3’ end of MSP15A matches the corresponding sequence of chromosome 1, not chromosome 3. In the view of Welch et al. (17), position 4928 in Fig. 9 is an “ancient junction” where about 20 kb of chromosome 3 sequence to the left of this point was transposed to chromosome 1. This accounts for sequence identity between the two chromosomes to the left of position 4928. Thus, it appears that the 3’ end of MSP cDNA 15A represents ancient chromosome 1 sequence, and the 5’ end is from transposed and altered chromosome 3. MSP15A is representative of several cDNA clones with similar 3’ sequences. The cloning of these cDNAs from the HepG2 cDNA library raises the possibility that there may be a family of MSP genes, with representatives on chromosomes 3 and 1.

In the normal human population, the alleles on chromosome 3p at the locus defined by the H3H2 probe are frequently heterozygous. The difference in the two alleles can be detected by size differences (2.3 versus 2.0 kb) in genomic HindIII restriction fragments to which H3H2 hybridizes in a Southern blot. This is also the location of the MSP gene, and H3H2-defined heterozygosity of the gene will depend on the presence or absence of a HindIII restriction site at position 917 of the Han et al. (15) MSP gene. Since this site is located within the first MSP intron, the heterozygosity should not affect the MSP coding sequence.

It is now well established that there is a loss of one or the other member of the allelic pair defined by H3H2 on chromosome 3p in hereditary kidney cancer (18) and small cell lung cancer cells (19). Therefore, these tumors are deficient in one of the alleles coding for MSP. According to the theory proposed by Knudson (20), one mechanism leading to development of cancer requires inactivation of both alleles of a cancer suppressor gene. Thus, the 3p deletions in cancer cells represent inactivation of the remaining cancer suppressor gene allele, alteration of the first member of the pair having occurred earlier. Since the 3p deletion is very large, involving many genes, it is statistically unlikely that the MSP gene is the cancer suppressor gene. If it were, the Knudson theory predicts absent MSP expression in the tumor. We found MSP mRNA in Northern blots of normal kidney. If expression is by the renal cell type that is tumorigenic, the finding of a normal MSP gene sequence in the corresponding tumor cells would rule out MSP as the cancer suppressor gene.

We have described three in vitro effects of MSP on resident peritoneal macrophages of the mouse: 1) stimulation of chemotactic responsiveness to C5a (1, 2); 2) stimulation of uptake of C3bi-coated erythrocytes via the CR1 receptor of resident macrophages (3); 3) appearance of long cytoplasmic processes and pinocytic vesicles when macrophages are plated in polystyrene tissue culture wells (1). These three effects have a motility response in common: the first is characterized by translational movement, the second by membrane internalization, the third by plasma membrane and cytoplasmic protein realignment. Among the members of the kringle protein family, the MSP sequence is most closely related to HGF/SF. In addition to its mitogenic activity for hepatocytes, HGF/SF causes disruption of epithelial cell junctions and an increase in cellular motility. Thus, both MSP and HGF/SF, in contrast to their relatives with proteolytic activity, have a role in cell motility. The range of target cells and the biochemical mechanism of action remain to be determined.

Acknowledgments—We thank Mark Gunnell at the Biomedical Supercomputer Center of the Frederick Cancer Research and Development Center for searching the sequence data bank and doing the sequence between MSP and HGF/SF. We also thank Drs. William Modi, Michael Lerman, and Berton Zbar for helpful discussions.

REFERENCES
1. Leonard, E. J., and Skeel, A. (1976) Exp. Cell Res. 102, 434-438
2. Leonard, E. J., and Skeel, A. (1978) Exp. Cell Res. 114, 117-126
3. Skeel, A., Yoshimura, T., Showalter, S., Tanaka, S., Appella, E., and Leonard, E. (1991) J. Exp. Med. 173, 1227-1234
4. Park, C. H., and Tulinsky, A. (1986) Biochemistry 25, 3977-3982
5. Magnusson, S., Petersen, T. E., Sottrup-Jensen, L., and Claeyss, H. (1975) in Proteases and Biological Control (Reich, E., Rifkin, D. B., and Shaw,
Cloning of Human Macrophage Stimulating Protein

1. Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimomura, M., Sugimura, A., Tashiro, K., and Shimizu, S. (1989) *Nature* **342**, 440-443
2. Stoker, M., Gherardi, E., Perryman, M., and Gray, J. (1987) *Nature* **327**, 239-242
3. Maniatis, T., Fritsch, E. J., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
4. Short, J. M., Fernandez, J. M., Sorge, J. A., and Huse, W. D. (1988) *Nucleic Acids Res.* **16**, 75-83
5. Sanger, F., Nicklen, S., and Coulson, A. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463
6. Yoshimura, T., Yuhki, N., Moore, S. K., Appella, E., Lerman, M. I., and Leonard, E. J. (1989) *FEBS Lett.* **244**, 487-493
7. Leonard, E. J., Sylvester, I., and Yoshimura, T. (1991) in *Current Protocols in Immunology* (Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M., and Strober, W., eds) Wiley Interscience, New York
8. Maniatis, T., Fritsch, E. J., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
9. Short, J. M., Fernandez, J. M., Sorge, J. A., and Huse, W. D. (1988) *Nucleic Acids Res.* **16**, 75-83
10. Sanger, F., Nicklen, S., and Coulson, A. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463
11. Yoshimura, T., Yuhki, N., Moore, S. K., Appella, E., Lerman, M. I., and Leonard, E. J. (1989) *FEBS Lett.* **244**, 487-493
12. Leonard, E. J., Sylvester, I., and Yoshimura, T. (1991) in *Current Protocols in Immunology* (Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M., and Strober, W., eds) Wiley Interscience, New York
13. Andres, A., Gallego, M. E., and Nadal-Ginard, B. (1987) *Annu. Rev. Cell Biol.* **3**, 271-292
14. Devereux, J., Haeberli, P., and Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387-395
15. Han, S., Stuart, L. A., and Degen, S. J. F. (1991) *Biochemistry* **30**, 9768-9780
16. Carritt, B., Welch, H. M., and Parry-Jones, N. J. (1986) *Am. J. Hum. Genet.* **38**, 425-436
17. Welch, H. M., Darby, J. K., Pilz, A. J., Ko, C. M., and Carritt, B. (1989) *Genomics* **5**, 423-430
18. Zbar, B., Brauch, H., Talmadge, C., and Linehan, M. (1987) *Nature* **327**, 721-724
19. Brauch, H., Johnson, B., Hovis, J., Yano, T., Gauder, A., Pettenzlilii, O. S., Graziano, S., Sorenson, G. D., Poiesz, B., Minna, J., Linehan, M., and Zbar, B. (1987) *N. Engl. J. Med.* **317**, 1109-1113
20. Knudson, A. G., Jr. (1986) *Annu. Rev. Genet.* **20**, 231-251