Identification and Characterization of a Novel Tyrosine Kinase from Megakaryocytes*

(Received for publication, June 16, 1993, and in revised form, September 13, 1993)

Brian D. Bennett†, Sally Cowley†, Shuxian Jiang, Roanna London, Bijia Deng, Jadwiga Grabarek, Jerome E. Groopman, David V. Goeddel†, and Hava Avraham†

From the Division of Hematology/Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02215 and the Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080

Protein-tyrosine kinases play pivotal roles in cell signal transduction. We have isolated a cDNA clone encoding a novel human intracytoplasmic tyrosine kinase, termed matk (megakaryocyte-associated tyrosine kinase). Expression of matk mRNA was predominantly found in cells of megakaryocytic lineage. The matk cDNA clone encodes a polypeptide of 527 amino acids and has closest sequence similarity to the csk tyrosine kinase. Sequence comparisons also indicate that matk contains src homology region 2 and 3 domains but lacks the NH2-terminal myristylation signal, the negative regulatory tyrosine (Tyr-527), and the autophosphorylation site (Tyr-416) corresponding to those found in src. Antibodies raised against the NH2-terminus of matk immunoprecipitated a 60-kDa protein from the CMK human megakaryocyte cell line. Expression of matk mRNA was up-regulated in megakaryocytic cells induced to differentiate by the phorbol ester. Based on its restriction in expression and its modulation during in vitro differentiation, it is likely that matk participates in signal transduction during megakaryocytosis.

Protein-tyrosine kinases (PTKs)1 play major roles in signal transduction pathways and include several oncoproteins and growth factor receptors (1, 2). The conservation of many tyrosine kinase sequences among organisms as divergent as invertebrates and mammals emphasizes the importance of these signaling molecules. Many PTKs span the cell membrane and function as receptor molecules for polypeptide ligands, while others are intracytoplasmic. Intracytoplasmic PTKs with homology to src have been described, which participate in signaling in normal and neoplastic blood cells (3–14). Although many intracytoplasmic PTKs are ubiquitously expressed in a variety of cell types, several are relatively restricted in expression and mediate transduction of signals specific to cells of one lineage. Examples of restricted PTKs include lek (8) and ikb (11), which mediate signal transduction in association with unique T cell-specific surface receptors and adhesion structures.

The most extensively characterized cytoplasmic PTKs belong to the c-src family. These intracellular PTKs are myristylated on glycine at position 2, which localizes them to the inner plasma membrane (15). Familial members usually contain SH2 and SH3 domains, which are important for regulating enzyme activity. src family members also contain tyrosine residues at positions corresponding to amino acids 416 and 527 of c-src. Tyrosine 416 is an autophosphorylation site, and phosphorylation of tyrosine 527 results in attenuation of enzyme activity (16).

Cak, a PTK initially purified from rat brain (15) and subsequently cloned from rat, human, and chicken sources (17–19), does not appear to be a member of the c-src gene family (19). Cak lacks an autophosphorylation site within its kinase domain and is further distinguished from the src family of kinases by the lack of a carboxyl terminus equivalent of Tyr-527. Cak is the enzyme responsible for phosphorylation of Tyr-527 of c-src (16).

Relatively little is known about the repertoire of signal transduction molecules in human megakaryocytes (20, 21). Three transmembrane PTKs, the c-Kit protooncogene product and two fibroblast growth factor receptors (bg and bkb), have been identified in human megakaryocytes (22–25). We have identified and characterized a novel intracytoplasmic kinase with some homology to csk, which is predominantly expressed in cells of megakaryocytic lineage. Given its homology to PTKs, we have termed this new gene megakaryocyte-associated tyrosine kinase (matk). Based on its molecular structure, its general restriction to cells of this lineage, and its increased expression during differentiation, it is likely that matk participates in signal transduction in human megakaryocytes.

**MATERIALS AND METHODS**

**Cells**—Human bone marrow was obtained by aspiration from the iliac crest of normal donors who gave informed consent in a protocol approved by the New England Deaconess Hospital Institutional Review Board. The marrow was aspirated into preservative-free heparin (Sigma) and separated by centrifugation through Ficoll-Hypaque (Pharmacia LKB Biotechnology Inc.) at 1,200 X g at room temperature for 30 min. After two washes with sterile 1 X phosphate-buffered saline (PBS), the cells were resuspended in RPMI 1640 medium with 7.5% platelet poor plasma, penicillin/streptomycin (P/S), and l-glutamine, seeded onto T-75 tissue culture flasks (Corning, Corning, NY), and incubated at 37 °C in 5% CO2. Human marrow megakaryocytes were isolated by a method employing immunomagnetic beads using anti-human glycoprotein GpIIb monoclonal antibody as de-
scribed previously (26). All of the isolated cells were recognizable as megakaryocytes by morphology and/or specific immunofluorescence using antiplatelet antibodies to the surface structures GPlb/GPlla and GPlb. CD34 bearing marrow progenitor cells were purified from hemiparized bone marrow aspirates using immunomagnetic beads coated with an anti-CD34 monoclonal antibody as described (27).

The human tissue Northern blot was obtained from Clontech (Palo Alto, CA). Additional permanent human megakaryocytic cell lines studied were generous gifts to our laboratory. DANI cells were from Dr. S. Greenberg, Brigham and Women's Hospital, Boston, MA, MEG-01 and Mo7E cells were from Drs. J. Hoxie, University of Pennsylvania, Philadelphia, and erythroid-megakaryocytic HEL cells and CHRF cells were from Dr. L. Zon, Children's Hospital, Boston, MA. Each cell line was cultured as previously described (30). All other permanent cell lines were obtained from the American Type Tissue Culture Collection and maintained in limited culture according to the specifications in the catalog.

In some experiments, megakaryocytic cells were induced to differentiate by treatment with phorbol 12-myristate 13-acetate (PMA) (Sigma). PMA was dissolved in dimethyl sulfoxide and stored at –20 °C until use when it was diluted in RPMI 1640 medium and used at 10 ng/ml.

**DNA Amplification and Cloning**—Total RNA was prepared by a standard protocol of lysis in guanidinium isothiocyanate followed by cesium chloride gradient centrifugation (31). Protein-tyrosine kinase sequences were amplified with degenerate oligonucleotide primers as described by Wilks (32).

PCR products of the amplified tyrosine kinase domains were purified from the agarose gel, digested with EcoRI and BamHI, ligated into pUC19, and transformed into Escherichia coli DH5α. Sequencing was carried out by the dideoxynucleotide chain termination cycle sequencing method (32) using a Sequenase kit (US Biochemical Corp.). Sequences were compared with known sequences in GenBank and EMBL data bases using the Autosearch computer program to identify novel clones. The 160-base pair PCR product from clone number D4 was radio labeled using the random priming protocol (31) and used as a probe to screen an oligo(dT)-primed cDNA library in λgt11 prepared from CMK cells. Positive clones were isolated, plaque-purified, cDNA inserts excised, subcloned into Bluescript-SK (Stratagene), and sequenced on both strands.

**Northern Blot Analysis**—Poly(A) RNA was isolated directly from whole CMK cells from the cell line. A poly(dT)-cellulose column kit (Invitrogen, San Diego, CA) according to the manufacturer’s instructions. 2 μg was denatured and loaded onto a denaturing 1% agarose gel. Following electrophoresis, the RNA was vacuum blotted (Pharmacia LKB Biotechnology Inc.) onto a nylon filter (Nytran, Schleicher and Schuell) electrophoresis, the RNA was vacuum blotted (Pharmacia LKB Biotechnology Inc.) and the filter was probed with a full-length matk cDNA, which was labeled by random priming as described above. Hybridization was carried out as described previously (31) at 42 °C in buffer containing 0.1 × sodium chloride/sodium citrate (SSC) and 6× Denhardt’s solution, 0.1% SDS, 1% bovine serum albumin as the standard. The filter was washed twice with 2× SSC and 0.1% SDS, then twice with 0.1% SDS, 0.1× SSC, 2× SSC, and 0.1× SSC, at 65 °C.

**Protein Analysis**—Metabolic labeling, immunoprecipitation, and immunolocalization assays were performed in CMK cells as described previously (34–38). For immunoblot analysis, total lysates were prepared, separated by SDS-10% PAGE, transferred to nitrocellulose membrane (Amersham International), and detected by autoradiography for 16 h at –70 °C with an intensifying screen.

**Cloning and Regulation of matk**

The human tissue Northern blot was obtained from Clontech (Palo Alto, CA).

**PCR Biots**—cDNA was prepared from several cell lines and amplified by PCR using specific matk primers. First-strand DNA was synthesized at 37 °C for 1 h in a volume of 10 μl containing 4.5 μl of total RNA (4.5 μg) in diethyl pyrocarbonate-dH₂O, 50 mM Tris-

HCl, pH 8.3, 75 μM KCl, 10 μM dithiothreitol, 3 mM MgCl₂, and 50 μg/ml actinomycin D, 20 units RNAsin (Promega Corp.), 1.0 mM dNTPs (Pharmacia), 1 μg/μl oligo(dT)₁₆, and 0.2 units of Moloney murine leukemia virus reverse transcriptase (200 units/μl) (Boehringer Mannheim). The first-strand CDNA mix was combined with 90 μl of PCR mix that contained both matk primers at 1 μM, 0.2 mM dNTPs, 1.0 mM Tris-Cl (pH 8.3), 2.5 units Taq polymerase (Perkin-Elmer Cetus Instruments). The mixture was then subjected to PCR amplification using the Perkin-Elmer: Cetus thermal cycler set for 40 cycles as follows: denature 94 °C, 1 min; primer anneal 55 °C, 2 min; primer extension 72 °C, 3 min. Each 1-ml ramp times were used between these temperatures. The sequence of the upstream primer was 5′-GCC GCG CGA GGC TCT CTT GCT T-3′ (position 265–285, Fig. 1). The nucleotide sequence of the downstream primer was 5′-TGC GAG CAC ACC CGC CCC AAG-3′ (position 430–450, Fig. 1). The PCR products were electrophoresed on a 2% agarose gel, denatured, neutralized, transferred to filters, and vacuum blotted. The filters were baked at 80 °C for 2 h and then photolyticized according to the manufacturer’s instructions. The probe used was the full-length matk cDNA, which was labeled by random priming as described above. Hybridization was carried out as described previously (31) at 42 °C in buffer containing 0.1 × sodium chloride/sodium citrate (SSC) and 6× Denhardt’s solution, 0.1% SDS, 0.1× SSC, 2× SSC, and 0.1× SSC, at 62 °C and then subjected to autoradiography.

**In Vitro Transcription and Translation**—2 pmol of template DNA (pBluescript-SK containing the entire coding region of matk) was linearized by digestion with SphI. Transcription was performed at 37 °C for 1 h in a volume of 2 μl containing 0.5 μg of bovine serum albumin, 0.25 mM each dNTP, 0.5 mM N7RNA cap (New England Biolabs, Beverly, MA), 2.5 units of RNasin (Promega Corp.), 3 units of T3 RNA polymerase (Pharmacia), and the template DNA. RNA was subsequently purified by addition of 1 μg of RNase-free DNase (Promega Corp.) for 15 min at 37 °C then phenol/chloroform extraction and ethanol precipitation. Translation was performed using the rabbit reticulocyte lysate system (Promega Corp.) according to the manufacturer’s instructions. [35S]methionine (350 μCi) was used to label the translation product, which was then mixed with SDS sample buffer containing β-mercaptoethanol, boiled for 5 min on a 105 °C water bath, and analyzed on a 10% SDS-polyacrylamide gel. For the kinase assay, the in vitro translated products were added to 40 μl of kinase buffer (20 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM NaVO₄, 4 μl of 0.03% enolase, and 5 μCi of [γ-32P]ATP (3000 Ci/mmol). The mixtures were incubated for 25 min at room temperature and then at 57 °C. Proteins were fractionated by SDS/10% polyacrylamide gel electrophoresis, and detected by autoradiography for 16 h at –70 °C with an intensifying screen.

**Protein Analysis**—Metabolic labeling, immunoprecipitation, and immunolocalization assays were performed in CMK cells as described previously (34–38). For immunoblot analysis, total lysates were prepared, separated by SDS-10% PAGE, and transferred to nitrocellulose membrane (Amersham International) and detected by autoradiography for 16 h at –70 °C with an intensifying screen.

**Cloning and Regulation of matk**

For metabolic labeling, 106 cells were labeled with 100 μCi of [35S] methionine in 1 ml of Dulbecco’s modified Eagle’s medium minus methionine (Amersham Corp.) for 16 h. Immunoprecipitation of matk protein from labeled cells with antiserum against mouse immunoglobulin G (IgG) was performed as described before (36). Portions of lysates containing 10 μg of matk were boiled and fractionated on 10% polyacrylamide-SDS gels (35) and transferred to Immobilon polyvinylidene difluoride (Millipore Corp., Bedford, MA) filters. Protein blots were treated with specific antibody against keratin 8 (see below). Primary binding of the matk-specific antibodies was detected using anti-IgG second antibodies conjugated to horseradish peroxidase and subsequent chemiluminescence detection ECL Western blotting system (Amersham International). Lysates were prepared from CMK cells by sonication in lysis buffer for 10 s each and centrifuged at 25,000 × g for 10 min at 4 °C. The supernatant was collected and the pellets were resuspended in 1 ml of sonicated buffer and added to an equal volume
Cloning and Regulation of matk

of 2 × SDS sample buffer. The supernatant obtained above (after the first sonication) was again centrifuged at 100,000 × g for 40 min at 4°C. The supernatant (cytosolic fraction) was removed and added to an equal volume of 2 × concentrated sample buffer. The remaining pellet (membrane fraction) was washed and dissolved in sonication buffer and SDS sample buffer as described above. Protein samples were analyzed by electrophoresis on 10% polyacrylamide gels, according to the Laemmli method (35). The proteins were transferred from the gels onto a 0.45-μm polyvinylidene difluoride membrane for subsequent immunoblot analysis. Primary binding of the matk-specific antibodies was detected using anti-IgG second antibodies conjugated to horseradish peroxidase.

For immunohistochemical localization of matk protein, CMK cells were grown on cover slips to approximately 50% confluency and were washed with PBS (pH 7.4) after removing the medium. The cells were prefixed for 1 min at 37°C in 1% paraformaldehyde containing 0.075% Triton X-100, rinsed with PBS, and then fixed for 10 min with 4% paraformaldehyde. After the fixation step, cells were rinsed in PBS, quenched in PBS with 0.1 mM glycine for 5 min, treated with PBS containing 0.1% Triton X-100, quenched in PBS with 0.1% Triton X-100, and finally rinsed again in PBS. For antibody staining, the cells were first blocked with a blocking solution (3% bovine serum albumin in PBS) and incubated for 1 h at 37°C. The cells were then incubated for 1 h at 37°C with matk antisera (1:100 dilution) or with preimmune rabbit serum (1:100) (see below). After the incubation with the primary antibody, the cells were washed in PBS containing 3% bovine serum albumin and 0.1% Tween 20 and incubated for 1 h at 37°C in fluorescein-conjugated donkey anti-rabbit IgGs (Jackson Immunoresearch, Maine) diluted 1:1000 in blocking solution. The coverslips were washed in PBS and a drop of 1 mg/ml p-phenylenediamine in a mixture of PBS (pH 8.0), and glyceral was added to each coverslip before mounting on glass slides and sealing with clear nail polish. All glass slides were examined with a Zeiss Axiohot microscope.

Antibodies—The peptide SALD03 corresponding to amino acid residues 2–16 of the matk protein was synthesized. Coupling of the peptide to carrier protein and immunization was performed as described (39). Rabbit antibodies against this peptide were raised, and sera were titered against peptide antigen by ELISA (40). The sera exhibiting the highest titer (1:27,000) were used in subsequent experiments.

RESULTS

Identification and Localization of Full-length matk cDNA—To identify tyrosine kinases in human megakaryocytes, we adopted the method of Wilks (32) that uses PCR primers based on conserved sequences of PTKs. RNA from the human megakaryocytic CMK cell line was used as a template to

FIG. 1. Nucleotide and deduced amino acid sequence of two overlapping matk cDNA clones representing the full-length cDNA. Nucleotide numbers are shown on left. The putative initiation codon at nucleotide position 263 is shown in bold type.
Cloning and Regulation of \textit{mtk}

\textit{mtk} contains several structural motifs common to many 
\textit{src}-related PTKs. A unique domain is found at the NH\textsubscript{2} 
terminus of \textit{mtk} (amino acids 1–50). This region is the most

divergent among various cytoplasmic PTKs and may be involved
in cellular localization and/or interaction with other

cellular proteins. SH3 and SH2 domains are found at amino

acids 54–105 and 120–212, respectively (Fig. 2). These do-

mains are thought to be important for regulating the enzy-

matic activity of intracellular PTKs, the

\textit{SH3} domain through

association with the cytoskeleton or membrane proteins (41),

and the \textit{SH2} domain through its role as a phosphotyrosine

binding site (42). The carboxyl part of \textit{mtk} (amino acid 235–

527) consists primarily of the catalytic domain (amino acids

235–478), including the ATP binding site (amino acids 242–

262). It also contains the highly conserved PTK sequence

motifs HRDLAARN (HRDLRAAN) in \textit{src} family proteins

and PVKWTAPE (amino acids 350–357 and 387–394, respec-

tively), which have been implicated in tyrosine phosphoryla-

tion specificity (43).

\textit{mtk} shares the highest degree of amino acid identity with

the human \textit{csk} (14) and \textit{c-src} (44) gene products. The overall

amino acid identity of \textit{mtk} with \textit{csk} and \textit{c-src} is 50 and 35%.
respectively (Fig. 2). The regions of highest homology are found in the catalytic domain (54% with csk and 44% with c-src) and the SH2 domain (55% with csk and 41% with c-src).

Although matk shares many common features with src, there are significant differences in other key motifs. Like csk, matk is missing a putative myristylation signal (glycine at position 2 and lysine or arginine at position 7), which is present in src family PTKs and is required for membrane localization (15). Similarly, matk lacks the autophosphorylation site corresponding to Tyr-416 of src as well as the negative regulatory tyrosine residue corresponding to src Tyr-527. These are conserved amino acids in most src family members but not in matk and csk. Taken together, these results indicate that matk is a member of the csk family.

Matk Is Highly Restricted in Tissue Expression—An extensive survey of permanent human cell lines and primary tissues was performed by Northern blot analysis. These experiments revealed that matk RNA (2.3 kilobases) is abundantly expressed in human megakaryocytic cell lines (Fig. 3A). No expression of matk was detected by Northern blot in primary tissues of various origins (heart, placenta, lung, liver, skeletal muscle, kidney, and pancreas), with the exception of a 2.3-kilobase message in adult brain (Fig. 3B). Using PCR techniques, expression of matk was found in primary bone marrow megakaryocytes, blood platelets, and in marrow CD34+ progenitor cells (Table I). matk expression was also detected by PCR in the K562 pluripotent hematopoietic cell line, the primitive PLB leukemic line, and the MCF-7 breast cancer line but not by Northern blot. No expression of matk was seen in other hematopoietic cells including T cells, B cells, monocytes, or mast cells (Table I).

Regulation of matk Expression during PMA-induced Differentiation—To determine whether matk expression may be regulated during megakaryocytosis, we have performed kinetic analysis of matk expression in CMK cells induced to differentiate in vitro by the phorbol ester PMA for 3, 6, or 24 h. This induction results in increased DNA content (polyploidy) and increased expression of surface platelet glycoproteins GpIb and GpIIb/GpIIIa. Northern blot analysis indicated 3–8-fold up-regulation of matk expression after 6 h of stimulation with PMA followed by down-regulation of matk expression after 24 h (Fig. 4A).

![Fig. 3. matk expression. Panel A, expression of matk by Northern blot analysis in human megakaryocytic cell lines. Panel B, expression of matk by Northern blot analysis in human tissues. In both cases, poly(A)+ RNA (2 μg) was extracted from human cell lines or tissues, electrophoresed in a denatured 1% agarose-formaldehyde gel, and transferred to a nitrocellulose filter as described under "Materials and Methods." The filters were hybridized with 32P-labeled matk cDNA, followed by hybridization with β-actin as the control for uniform RNA loading. Skel. Masc., skeletal muscle.](image)

![Fig. 4. Expression of matk in human megakaryocytic cell lines induced to differentiate by the phorbol diester. A, poly(A)+ RNA (2 μg), extracted from CMK cells with or without PMA treatment for 3, 6, and 24 h, was electrophoresed in a denatured 1% agarose-formaldehyde gel and transferred to nitrocellulose filters. Hybridization was performed with 32P-labeled matk cDNA, followed by hybridization with 32P-actin as the control. The exposure time used for all lanes with both probes was 10 h. B, poly(A)+ RNA (2 μg), extracted from human cell lines with or without PMA treatment for 6 h, was electrophoresed in a denatured 1% agarose-formaldehyde gel and transferred to nitrocellulose filters. The filters were hybridized with 32P-labeled matk cDNA, followed by hybridization with β-actin as the control for uniform RNA loading. The matk transcript is 2.3 kilobases. The exposure time used was equivalent for all lanes with both probes for 6 h.](image)
Cloning and Regulation of matk

expression in CMK, 2B, and CHRF cells approximately 3–8-fold during 6 h of PMA induction (Fig. 4B). These results indicate up-regulation of matk expression during PMA-induced differentiation in megakaryocytic cells.

Detection of matk Protein, Kinase Activity, and Subcellular Localization—The expression of the matk gene product was investigated using an antisera prepared in rabbits against the unique amino-terminal region (see "Materials and Methods"). The specificity of this antisera was examined by immunoprecipitating in vitro translated matk protein labeled with [35S]methionine (Fig. 5A). In vitro translated matk protein exhibiting a molecular mass of about 60 kDa was specifically precipitated using this antisera. Following addition of [γ-32P]ATP to the in vitro translated product, phosphorylation was detected (Fig. 5B).

We subsequently used this rabbit antisera for the detection of matk protein in vivo by immunoprecipitation. The CMK cell line was metabolically labeled with [35S]methionine, and extracts were immunoprecipitated with anti-matk antisera. A major protein species of 60 kDa was detected in CMK cells (Fig. 5C) as well as in other human megakaryocytic cell lines such as CMK-6 and Meg-01 (data not shown).

mtk protein was localized in the cytoplasm of CMK cells by Western blot analysis of the nuclear, membrane, and cytoplasmic fractions (Fig. 5D). This localization of mtk was confirmed by immunofluorescence analysis to be associated with the cytoplasm and not with the plasma membrane or nucleus (data not shown).

DISCUSSION

The phosphorylation of tyrosine residues in specific protein substrates by PTKs is a central cell signaling event modulating growth and differentiation. Characterization of lineage-restricted PTKs should therefore provide insights into unique pathways of cell proliferation in normal and neoplastic states.

The technique of PCR using degenerate primers allowed us to identify in human megakaryocytic cells a novel intracytoplasmic tyrosine kinase, which we have termed matk. matk is expressed predominantly in the megakaryocyte cell lineage.

Sequence analysis of matk demonstrates homology to csk, suggesting that matk belongs to this subfamily of cytoplasmic tyrosine kinases. The features of the csk subfamily include the lack of myristylation signals, the lack of carboxyl-terminal regulatory phosphorylation sites, and the presence of SH2 and SH3 domains. The SH2 domain (amino acids 121–207 in matk) is believed to interact with phosphotyrosines in protein substrates (2, 45, 46) and may modulate the enzymatic activity of src proteins. The SH3 domain (amino acids 54–105 in matk) can bind to proline-rich domains, and it may interact with the cytoskeleton (2, 38, 47). The putative amino terminus of matk shares limited homology with other intracytoplasmic PTKs, including those of the csk family. This region of the protein could be important in unique signal functions of matk or its association with specific cell structures in the megakaryocyte.

matk protein is located in the cytoplasm of megakaryocytic cells as determined by immunoblot analysis of subcellular fractions and by immunofluorescence staining. This result is consistent with the predicted lack of a myristylation signal. Several PTKs that are located in the cytosolic fraction have been reported including csk (17), c-fps (48), and FAK (49).

The phosphorylation of Tyr-527 of c-src by csk results in attenuation of c-src kinase activity (16). Similarly, csk phosphorylates the equivalents of Tyr-527 in other src-family PTKs, with resultant down-regulation of their activities in vitro. This phosphorylation of c-src has not yet been demonstrated in vivo. In yeast, phosphorylation of Tyr-527 of co-expressed c-src by csk eliminated c-src-mediated growth inhibition (46). It will thus be of interest to elucidate the role of matk in the phosphorylation of src family tyrosine kinases in megakaryocytes. Demonstration of substrate specificity similar to csk will be pursued in future studies using purified matk protein.

Expression of the matk gene appears highly restricted, with abundant expression observed by Northern blot only in cells of the megakaryocyte lineage. An extensive tissue survey demonstrates expression of matk in brain adult tissue. Within the hematopoietic system, expression of matk was uniform and high in megakaryocytic cells with low level expression detected only by PCR in the cell lines K562 and PLB and in narrow CD34 bearing progenitor cells. Because megakaryocyte precursors bear the CD34 surface structure (50), further work is required to determine if matk is present in progenitor cells committed to this lineage or in multipotential hematopoietic progenitors.

The homology to other intracytoplasmic PTKs and the restriction in tissue expression suggest that matk may function in signal transduction pathways important in megakaryocytic growth and/or differentiation. Our initial examination of matk expression during PMA treatment of a number of megakaryocyte cell lines revealed its up-regulation during cellular differentiation. Future studies will aim to better understand the role of matk in megakaryocyte signal transduction, particularly that mediated by cytokines and adhesive interactions that may modulate megakaryocytopenesis.

Acknowledgments—We thank Dr. John Burnier for synthesis of peptide SALD03, Greg Bennett for raising the rabbit anti-SALD03 polyclonal antibody, and Patricia Gryniewicz for preparation of the manuscript.
REFERENCES

1. Ullrich, A., and Schlessinger, J. (1990) Cell 6, 203-212
2. Pawson, T., and Gish, G. D. (1992) Cell 71, 359-369
3. Dynowski, S. M., Niederhuber, J. E., and Desiderio, S. V. (1990) Science 247, 332-336
4. Eisenman, E. and Bolen, J. B. (1990) Cancer Cells (Cold Spring Harbor) 2, 303-310

22. 20.
23. 18.
16.
19.
17.
1074 Cloning and Regulation

Anderson, D. M., Lyman, S. D., Baird, A., Wignall, J. M., Eisenman, J., Fitch, C., March, C. J., Rosewell, H. S., Gimpel, S. P., Cosman, D., and Williams, D. E. (1990) Cell 63, 235-245

25. Bokfai, A., Han, Z. C., and Fuhrmann, (1992) Blood 80, 1905-1913
26. Tanaka, H., Yahida, Y., Ranelo, T., and Mutsumoto, N. (1989) Br. J. Haematol. 73, 19-24
27. Avraham, H., Scadden, D. T., Chi, S., Wang, Z., Brodie, V., Zsebo, K., and Groopman, J. E. (1992) Blood 80, 1679-1684
28. Sato, T., Fuse, A., Eguchi, M., Hayashi, Y., Sugita, K., Nakazawa, S., Minato, T., Shima, Y., Komori, I., Sunami, S., Okinoto, Y., and Nakajima, H. (1987) Exp. Hematol. (N.Y.) 15, 495-502
29. Komatsu, N., Suda, T., Miyoi, M., Tokuyama, N., Sakata, Y., Okada, M., Nishida, T., Hirai, Y., Sato, T., Fuse, A., and Miura, Y. (1989) Blood 74, 42-48
30. Avraham, H., Vannier, E., Cowley, S., Jiang, S., Chi, S., Dinarello, C. A., Zsebo, K. M., and Groopman, J. E. (1992) Blood 79, 365-371
31. Manista, T., Fritsch, E. F., and Sambrook, J. (1992) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
32. Wilks, A. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1653-1657
33. Avraham, H. A., Vannier, E., Chi, S. Y., Dinarello, C. A., and Groopman, J. E. (1992) Int. J. Cell Cloning 10, 70-75
34. Furth, M. E., Airdrich, T. H., and Cordon-Cardo, C. (1987) Oncogene 1, 47-58
35. Lasenbli, U. K. (1970) Nature 227, 680-685
36. Yarden, Y., Kuang, W., Wang-Feng, T., Coussens, L., Munemitsu, S., Dull, R. J., Chen, E., Schlessinger, J., Frankcy, U., and Ulrich, A. (1987) EMBO J. 6, 3341-3361
37. Konopka, J. B., and Witte, O. N. (1986) Mol. Cell. Biol. 6, 3116-3123
38. Konopka, J. B., Davis, R. L., Watanabe, S. M., Pontecellci, A. S., Schiff-Maker, L., Rosenburg, N., and Witte, O. N. (1984) J. Biol. Chem. 261, 233-222
39. Dynowski, S. M., Zwolfo, P., Zeller, K., Kuhajda, F. P., and Desiderio, S. V. (1992) J. Biol. Chem. 267, 4815-4823
40. Bennett, B. D., Bennett, G. L., Vitadello, B. V., Jewett, R. S., Burnier, J., Henzel, W., and Low, D. G. (1991) J. Biol. Chem. 266, 23060-23067
41. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Greziana, A., Kaeplein, R., and Schiold, S. (1991) Cell 64, 291-302
42. Matsuda, M., Mayer, B. J., Fukui, Y., and Hanafusa, H. (1990) Science 248, 137-1569
43. Han, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42-52
44. Takeya, T., and Hanafusa, H. (1986) Cell 42, 581-590
45. Waksman, G., Kominos, D., Robertson, S. C., Fann, N., Baltimore, D., Birge, R. B., Cowburn, D., Hanafusa, H., Mayer, B. I., Oderduy, M., Resh, M. D., Rice, C. B., Silverman, L., and Kuriyan, J. (1992) Nature 353, 464-465
46. Taylor S. J., and Shalloway, D. (1993) Current Opin. Genet. & Dev. 3, 26-34
47. Yu, H., Rosen, M. K., Shin, T. B., Seidel-Dugan, C., Bruegg, J. S., and Schreiber, S. L. (1992) Science 258, 1665-1668
48. Young, J. C., and Martin, G. S. (1984) J. Biol. Chem. 52, 913-919
49. Schwartz, M. D., Boguniewicz, M., Cohn, B. S., Vines, R. R., Reynolds, A. B., and Parsons, J. T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5192-5196
50. Deblin, N., Issaad, C., Maes, J. M., Guichard, J., Katz, A., Breton-Gorius, J., and Vainchenker, W. (1992) Blood 80, 3022-3035