Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8325–8329, December 1987
Biochemistry

Isolation and sequence of a cDNA corresponding to a *src*-related gene expressed in murine hemopoietic cells

(protein-tyrosine kinase/lineage commitment/hemopoietic cell differentiation)

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Communicated by Donald Metcalf, August 14, 1987

ABSTRACT **We have isolated a murine cDNA that shares extensive homology with genes encoding the *src* (Rous sarcoma virus oncogene)-related family of protein-tyrosine kinases. The cDNA includes an open reading frame of 1509 base pairs, and conceptual translation predicts a protein of 56 kDa. Blot-hybridization analysis indicates that this *src*-related gene is expressed in normal macrophages and in cell lines representing both the myeloid and lymphoid B-cell lineages and, accordingly, is designated "*bmk*" (B cell/myeloid kinase). In addition, bmk mRNA levels increase following the induced differentiation of the murine myelomonocytic leukemic cell line WEHI-3B.**

Tyrosine kinase activity is an intrinsic property of proteins encoded by a number of retroviral oncogenes (1). In addition, the receptors for a number of polypeptide growth factors have been shown to phosphorylate proteins on tyrosine residues (2), and this kinase activity is stimulated upon binding of the corresponding ligand (3–5). These observations implicate tyrosine kinases in signal transduction within normal cells and suggest that deregulation of their activity might represent one step in neoplastic transformation.

The c-*src* gene, the cellular homologue of the transforming gene of the Rous sarcoma virus (v-*src*) (1, 6), encodes one member of a family of protein-tyrosine kinases. These kinases have been conserved in widely divergent species (7–9), suggesting that they play an important role in some aspect of cellular function. Individual members of the *src*-related family share a highly conserved carboxyl-terminal region, which contains the catalytic domain for protein-tyrosine kinase activity (2). However, these molecules differ considerably in their amino-terminal domains, and it has been speculated that this region determines the specificity of their interaction with substrates or receptors or both (10, 11).

While the biological function of each *src*-related gene product is unknown, it is interesting that the genes encoding these proteins appear to show some specificity in their pattern of expression (12–14). High levels of $pp60^{c\text{-}src}$ kinase activity have been found in platelets (15) and postmitotic neurons (16); since these cell types have little or no proliferative potential, it is conceivable that $pp60^{c\text{-}src}$ plays a role in some aspect of end-cell function rather than a direct involvement in growth regulation.

Previous reports have demonstrated distinct tyrosine-specific protein-tyrosine kinase activity in membrane preparations of B and T lymphocytes (17, 18). The isolation of a cDNA encoding a lymphocyte-specific protein-tyrosine kinase (lck, originally designated lsk or tck) related to the product of c-*src* has recently been reported, and expression of the corresponding gene appeared to be confined to thymocytes and T lymphocytes (12, 19).

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Our interest in hemopoietic cells and the reports of hemopoietic protein-tyrosine kinase activity encouraged us to screen various cDNA libraries for *src*-related sequences. We report here the isolation and complete nucleotide sequence† of a murine cDNA that shares extensive homology with members of the *src*-related family of protein-tyrosine kinases. Transcripts corresponding to this cDNA are found predominantly in myeloid cells as well as lymphoid cells of the B lineage; thus, we term this gene *bmk* (B cell/myeloid kinase). The complementary pattern of expression of *bmk* and *lck* in lymphoid cells may reflect a functional separation between T and B cells.

MATERIALS AND METHODS

cDNA Library Screening. A murine macrophage cDNA library (Clontech no. ML1005), prepared with mRNA isolated from pleural cavity macrophages and cloned in phage λgt11, was probed with the 800-base-pair (bp) *Pvu* II–*Pvu* II fragment of v-*src* encompassing the conserved tyrosine kinase domain (20). Screening was performed by the method of Benton and Davis (21). Hybridization was carried out at 50°C in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), and the filters were washed at 54°C in 2× SSC/0.1% $NaDodSO_4$.

DNA Sequencing. Sequencing of both strands of DNA was performed on overlapping restriction fragments cloned into pGEM-3 (Promega Biotec, Madison, WI) by using the dideoxy chain-termination method (22) as modified for double-stranded DNA (23).

Filter Hybridization Analysis. DNA and RNA preparation, restriction enzyme analysis, and RNA and Southern blotting procedures were performed as described (24). DNA probes were labeled by nick-translation or by random primer extension. For *bmk* and *lck* expression studies, 5′ fragments (a 313-bp *Eco*RI–*Sph* I and a 261-bp *Eco*RI–*Bgl* II, respectively) corresponding to the unique amino termini of the gene products were used as probes to avoid cross-hybridization with the related transcript.

Cell Culture. Cell lines were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum supplemented with 1 μM asparagine for murine PU 5.1.8 cells and with 50 μM 2-mercaptoethanol for lymphoid cells. Murine W279.1, TT28 ING, and AVRij RNAs were a generous gift from Jerry Adams (Walter and Eliza Hall, Institute of Medical Research). Bone marrow macrophages were prepared by the method of Tushinski *et al.* (25) as modified by Vairo and Hamilton (26).

Abbreviations: Ab-MuLV, Abelson murine leukemia virus; bmk, B-cell/myeloid kinase.

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†The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03023).

RESULTS

To isolate *src*-related genes expressed in the murine hemopoietic system, a macrophage cDNA library was screened with the 800-bp *Pvu* II fragment of v-*src* as a probe. Of the 500,000 recombinants screened, 500 hybridizing clones were obtained, and 50 of these were plaque-purified for further analysis. Fig. 1 shows the nucleotide sequence of the 2-kilobase (kb) insert of one of these clones. The cDNA contains an open reading frame encoding a protein of 503 amino acid residues (M_r, 56,500). The methionine codon at position 178 within the DNA sequence is flanked by nucleotides that conform to the criteria established for efficient initiation of translation of eukaryotic mRNAs (27). An in-frame termination codon is found 93 bp upstream from this putative initiator methionine, strengthening the assignment of this AUG as the translation start site. A stretch of 34 adenosine residues at the 3′ terminus of the clone is preceded by a eukaryotic polyadenylylation signal, AATAAA; thus, the cDNA contains an essentially full-length representation of the corresponding mRNA.

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GAATTCCGGAAGGGGGCTCTGACCCGTTCCGAGCGCCAACGCAGCCTCTGTAGCCCGCAAGTCTTCGTCGCTTGCTCCG
                                                                              79
GGCTCTGAAGTCCGGGGCCACCAGGGGCCGCAGCGCTGGGGGGTCGGTCTAGCTGCGAGGATCCGGGCTGCCCACGAAG
                                                                             158
                    Met Gly Cys Val Lys Ser Arg Phe Leu Arg Asp Gly Ser Lys Ala
CGAAGGGCGGGCGCCCAGG ATG GGA TGC GTG AAG TCC AGG TTC CTC CGA GAT GGA AGC AAG GCC
                                                                             222
Ser Lys Thr Glu Pro Ser Ala Asn Gln Lys Gly Pro Val Tyr Val Pro Asp Pro Thr Ser
TCA AAA ACA GAG CCA AGT GCC AAT CAG AAG GGC CCT GTG TAT GTG CCG GAT CCC ACG TCC
                                                                             282
Ser Ser Lys Leu Gly Pro Asn Asn Ser Asn Ser Met Pro Pro Gly Phe Val Glu Gly Ser
TCC AGC AAG CTG GGA CCA AAC AAC AGC AAC AGC ATG CCC CCA GGG TTT GTG GAG GGC TCT
                                                                             342
Glu Asp Thr Ile Val Val Ala Leu Tyr Asp Tyr Glu Ala Ile His Arg Glu Asp Leu Ser
GAG GAT ACC ATT GTG GTC GCA CTG TAC GAC TAT GAG GCT ATT CAC CGT GAA GAC CTC AGC
                                                                             402
Phe Gln Lys Gly Asp Gln Met Val Val Leu Glu Glu Ala Gly Glu Trp Trp Lys Ala Arg
TTC CAG AAG GGA GAC CAG ATG GTG GTT CTG GAG GAG GCT GGG GAG TGG TGG AAG GCA CGG
                                                                             462
Ser Leu Ala Thr Lys Lys Glu Gly Tyr Ile Pro Ser Asn Tyr Val Ala Arg Val Asn Ser
TCC CTG GCT ACC AAG AAG GAA GGC TAC ATC CCA AGC AAC TAT GTG GCT CGA GTT AAC TCT
                                                                             522
Leu Glu Thr Glu Glu Trp Phe Phe Lys Gly Ile Ser Arg Lys Asp Ala Glu Arg His Leu
TTG GAG ACA GAA GAG TGG TTC TTC AAG GGG ATC AGC CGG AAG GAT GCA GAG CGC CAC CTC
                                                                             582
Leu Ala Pro Gly Asn Met Leu Gly Ser Phe Met Ile Arg Asp Ser Glu Thr Thr Lys Gly
CTG GCT CCA GGC AAC ATG CTG GGC TCC TTC ATG ATC CGG GAC AGT GAG ACC ACC AAA GGG
                                                                             642
Ser Tyr Ser Leu Ser Val Arg Asp Phe Asp Pro Gln His Gly Asp Thr Val Lys His Tyr
AGC TAC TCG TTG TCT GTT CGA GAC TTT GAC CCC CAG CAC GGA GAC ACC GTG AAG CAC TAT
                                                                             702
Lys Ile Arg Thr Leu Asp Ser Gly Gly Phe Tyr Ile Ser Pro Arg Ser Thr Phe Ser Ser
AAG ATC CGG ACG CTG GAC AGT GGA GGC TTC TAC ATC TCT CCA AGG AGC ACC TTC AGC AGC
                                                                             762
Leu Gln Glu Leu Val Leu His Tyr Lys Lys Gly Lys Asp Gly Leu Cys Gln Lys Leu Ser
CTG CAG GAA CTC GTG CTC CAC TAC AAG AAG GGG AAG GAT GGG CTC TGC CAG AAG CTG TCA
                                                                             822
Val Pro Cys Val Ser Pro Lys Pro Gln Lys Pro Trp Glu Lys Asp Ala Trp Glu Ile Pro
GTG CCC TGT GTG TCT CCC AAA CCC CAG AAG CCA TGG GAG AAA GAT GCT TGG GAG ATT CCT
                                                                             882
Arg Glu Ser Leu Gln Met Glu Lys Lys Leu Gly Ala Gly Gln Phe Gly Glu Val Trp Met
CGA GAA TCC CTC CAG ATG GAG AAG AAA CTT GGA GCT GGG CAG TTT GGA GAA GTG TGG ATG
                                                                             942
Ala Thr Tyr Asn Lys His Thr Lys Val Ala Val Lys Thr Met Lys Pro Gly Ser Met Ser
GCC ACC TAC AAC AAG CAC ACC AAA GTG GCG GTG AAG ACA ATG AAG CCA GGG AGC ATG TCC
                                                                            1002
Val Glu Ala Phe Leu Ala Glu Ala Asn Leu Met Lys Ser Leu Gln His Asp Lys Leu Val
GTG GAG GCC TTC CTG GCT GAG GCC AAC CTG ATG AAG TCG CTG CAG CAT GAC AAA CTG GTG
                                                                            1062
Lys Leu His Ala Val Val Ser Gln Glu Pro Ile Phe Ile Val Thr Glu Phe Met Ala Lys
AAG CTA CAC GCT GTG GTC TCT CAG GAG CCC ATC TTT ATT GTC ACG GAG TTC ATG GCC AAA
                                                                            1122
Gly Ser Leu Leu Asp Phe Leu Lys Ser Glu Glu Gly Ser Lys Gln Pro Leu Pro Lys Leu
GGA AGC CTG CTG GAC TTT CTC AAG AGT GAA GAA GGC AGC AAG CAG CCA CTG CCA AAA CTC
                                                                            1182
Ile Asp Phe Ser Ala Gln Ile Ser Glu Gly Met Ala Phe Ile Glu Gln Arg Asn Tyr Ile
ATT GAC TTC TCA GCC CAG ATC TCA GAA GGC ATG GCC TTC ATT GAG CAG AGG AAC TAC ATC
                                                                            1242
His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val Ser Ala Ser Leu Val Cys Lys Ile Ala
CAC CGA GAC CTG AGG GCT GCC AAC ATC TTA GTC TCT GCA TCA CTG GTG TGT AAG ATT GCT
                                                                            1302
Asp Phe Gly Leu Ala Arg Ile Ile Glu Asp Asn Glu Tyr Thr Ala Arg Glu Gly Ala Lys
GAC TTT GGA CTG GCA CGA ATC ATC GAG GAC AAT GAG TAC ACA GCT CGG GAA GGA GCC AAG
                                                                            1362
Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ile Asn Phe Gly Ser Phe Thr Ile Lys Ser
TTC CCC ATC AAG TGG ACA GCT CCT GAA GCC ATC AAC TTT GGT TCC TTC ACC ATC AAG TCA
                                                                            1422
Asp Val Trp Ser Phe Gly Ile Leu Leu Met Glu Ile Val Thr Tyr Gly Arg Ile Pro Tyr
GAT GTC TGG TCC TTT GGT ATC CTG CTG ATG GAA ATT GTC ACC TAT GGC CGG ATC CCT TAC
                                                                            1482
Pro Gly Met Ser Asn Pro Glu Val Ile Arg Ala Leu Glu His Gly Tyr Arg Met Pro Arg
CCA GGT ATG TCA AAC CCA GAG GTG ATT CGG GCA CTA GAG CAT GGG TAC CGT ATG CCT CGA
                                                                            1542
Pro Asp Asn Cys Pro Glu Glu Leu Tyr Asn Ile Met Ile Arg Cys Trp Lys Asn Arg Pro
CCA GAT AAC TGT CCA GAA GAG CTC TAC AAT ATC ATG ATC CGC TGC TGG AAG AAC CGC CCC
                                                                            1602
Glu Glu Arg Pro Thr Phe Glu Tyr Ile Gln Ser Val Leu Asp Asp Phe Tyr Thr Ala Thr
GAG GAA CGG CCC ACC TTT GAA TAC ATC CAG AGT GTG CTG GAT GAC TTC TAC ACG GCC ACT
                                                                            1662
Glu Ser Gln Tyr Gln Gln Gln Pro ***
GAG AGC CAG TAT CAG CAG CAG CCT TGA CAGCAGTAAGGACATGAGCAGAGCCAGAAGCCCCATCAGTGCC
                                                                            1732
TTGACACGCCCAACTTGCTGGGCCCACTCTCAGACACCACACCACACACACTGCAGCTGTTGAGTGGGTGGGAGGACTT
                                                                            1811
CACAATCTCTTTCTGACTCTAGTCATCTGCAATCCGCCACTCTCAGGGCCTCCAAGTTGGTATGTCTCATTTGCCTGGA
                                                                            1890
ATGACTGAATTCAATCTATAGCTGTGATTTAAGTGGAAACTGTTAGAATAGTATTTAAATAAAAGATATGAATGTCAAA
                                                                            1969
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
                                                                            2002
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FIG. 1. Nucleotide sequence of the cDNA encoding murine bmk and the deduced amino acid sequence. An in-frame termination codon, TGA, in the 5′ untranslated region is underlined, as is the presumed polyadenylylation signal (AATAAA) in the 3′ untranslated region.

Comparison of the murine *bmk*-encoded sequence with other published sequences revealed extensive homology to members of the *src*-related family of tyrosine kinases. Fig. 2 shows an alignment of the deduced amino acid sequences of murine bmk, human LYN (14), murine lck (12, 19), and chicken c-src (28). Overall, bmk is 70% homologous with human LYN, 65% with murine lck, and 57% with chicken c-src, with the majority of the differences located in the amino termini of these molecules. The carboxyl-terminal domain of bmk is highly homologous to other tyrosine kinases and contains sequence motifs implicated in nucleotide binding [Gly-Xaa-Gly-Xaa-Xaa-Gly, residues 246–251 (where Xaa = other amino acid) and lysine residue 267] (29, 30) and substrates for autophosphorylation (Asp-Asn-Gln-Tyr-Thr-Ala-Arg-Glu, residues 385–392) (2). The tyrosine residue at position 499 in bmk is in an analogous position to tyrosine-527 in chicken c-src, a residue implicated in the regulation of $pp60^{c\text{-}src}$ kinase activity (31, 32).

Expression of *bmk*. To determine the sites of expression of *bmk*, polyadenylylated RNA was isolated from a number of murine tissues, and analyzed by blot hybridization. Since at least one other member of the *src*-related family of tyrosine kinases, lck, is encoded by a message indistinguishable in size from that of bmk (12, 19), we eliminated any potential for cross-hybridization by utilizing a bmk-specific probe corresponding to the unique sequences at the amino terminus. bmk mRNA was present predominantly in bone marrow and spleen, with lower levels of the transcript found in lung, liver, and thymus (Fig. 3). We are uncertain of the nature of the two minor bands present in bone marrow; they are presumably related to the 2.2-kb bmk transcript since they share homology with the amino-terminal portion of bmk. It will be necessary to isolate cDNAs corresponding to these mRNAs to establish their structure and possible significance.

As bone marrow and spleen are both hemopoietic organs, it was of interest to investigate further the expression of *bmk* in cells of hemopoietic origin and to compare its pattern of expression with that of the T-cell-specific *lck* gene. To this end, polyadenylylated RNA from a variety of spontaneously arising and Abelson murine leukemia virus (Ab-MuLV)-transformed myeloid and lymphoid cell lines was examined for expression of *bmk* and *lck* with probes specific for the nonconserved portions of each transcript.

Two myeloid cell lines, murine RAW 8 and PU 5.1.8 (33) expressed *bmk* gene products at a similar level to murine bone marrow macrophages (Fig. 4*A*). Interestingly, while we failed to detect lck transcripts in bone marrow macrophages or RAW 8 cells, PU 5.1.8 cells expressed lck mRNA at levels similar to that observed in three Ab-MuLV-transformed murine T-cell lines (RK4, RK7, and RK11) (34) (Fig. 4*B*). Within the B-cell lineage the surface Ig^+ B-cell W279.1 coexpressed bmk and lck mRNAs, while two pre-B-cell lines, TT28 Ing and AVRij, expressed only low levels of bmk mRNA.

We also analyzed the mRNA from several immature Ab-MuLV virus transformed-thymocytes for expression of *bmk* and *lck*. These murine cell lines, which display rearrangements of both the T-cell receptor and the immunoglobulin genes, cannot be assigned unambiguously to either the T- or B-cell lineage (34). Three of these four cell lines (MK 121.3, RB 26.3, and RB 32.1) expressed lck mRNA, though at much lower levels than RK4, RK7, and RK11, which express T-cell characteristics exclusively. MK 121.3 and RB 26.3 also express bmk mRNA, while one cell line, MK 1-3, which lacked lck transcripts, expressed bmk mRNA at levels comparable with the myeloid cells examined. Neither lck nor bmk transcripts were detected in the mRNA of murine NIH 3T3 fibroblasts.

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bmk     MGCVKSR---FLRDGSKASKTEPSANQKGPVYVPDPTSSSKLGPNNSNSMPPG--F-VEG    54
LYN     ...I..KGKDS.S.DGVDL..Q.VR.TERTI..R....NKQQR.VPESQLL..QR.QTKD    60
lck     ....C.SNPEDDWMENIDVCENCHYPIVPLDSKISLPIRNGSEVRDPLVTYE.SLPPA--    58
c-src   ..SS..K-PKDPSQRRRSLEPPD.THHG.FPASQT.NKTAAPDTHRTP.RSF.TVATEPK    59

bmk     SED--T--------------------IVVALYDYEAIHREDLSFQKGDQMVVLEEA-GEWW    92
LYN     P.EQGD---------------------.....P.DG..PD....K..EK.K....H-....   100
lck     .PLQDN--------------------L.I..HS..PS.DG..G.E..E.LRI..QS-....    98
c-src   LFGGFNTSDTVTSPQRAGALAGGVTTF.......SRTET....K..ERLQIVNNTE.D..   119

bmk     KARSLATKKEGYIPSNYVARVNSLETEEWFFKGISRKDAERHLLAPGNMLGSFMIRDSET   152
LYN     ..K..L.....F.......KL.T.........D.T......Q......SA.A.L..E...   160
lck     ..Q..T.GQ..F..F.F..KA....P.P....NL.......Q......TH...L..E..S   158
c-src   L.H..T.GQT.........PSD.IQA...Y.GK.T.RES..L..N.E.PR.T.LV.E...   179

bmk     TKGSYSLSVRDFDPQHGDTVKHYKIRTLDSGGFYISPRSTFSSLQELVLHYKKGKDGLCQ   212
LYN     L...F.........V...VI......S..N..Y.....I..PCISDMIK..Q.QA....R   220
lck     .A..F........QNQ.EV.......N..N........I..PG.HD..R..TNAS....T   218
c-src   ...A.C...S...NAK.LN.......K........TS.TQ.....Q..AY.S.HA....H   239

bmk     KLSVPCVSPKPQKP-WEKDAWEIPRESLQMEKKLGAGQFGEVWMATYNKHTKVAVKTMKP   271
LYN     R.EKA.I.......-.D..........IKLV.R.........GY..NS.......L..    279
lck     ...R..QTQ.....-.WE.E..V...T.KLVER.........GY..G.......SL.Q    277
c-src   R.TNV.PTS...TQGLA..........RL.V...Q.C.....G.W.GT.R..I..L..    299

bmk     GSMSVEAFLAEANLMKSLQHDKLVKLHAVVS-QEPIFIVTEFMAKGSLLDFLKSEEGSKQ   330
LYN     .T...Q...E......T.......R.Y...TRE...Y.I..Y.............D..G.V   339
lck     ....PVP.........Q...PR..R.Y...T-....Y.I..Y.EN...V....TPS.I.L   336
c-src   .N..P....Q..QV..K.R.E...Q.Y....-E...Y....Y.S.........G.M.KYL   358

bmk     PLPKLIDFSAQISEGMAFIEQRNYIHRDLRAANILVSASLVCKIADFGLARIIEDNEYTA   390
LYN     L...........A....Y..RK...........V...E..M.........V..........   399
lck     NVN..L.MA...A.......EQ.............DT.S.........L..........   396
c-src   R..Q.V.MA...AS...YV.RM..V...........GEN....V.......L..........   418

bmk     REGAKFPIKWTAPEAINFGSFTIKSDVWSFGILLMEIVTYGRIPYPGMSNPEVIRALEHG   450
LYN     ..................C.............Y......K.....RT.AD.MT..SQ.     459
lck     ..............Y.T.............T....H........T.....QN..R.      456
c-src   .Q............ALY.R.............T.LT.K..V.....V.R..LDQV.R.    478

                                                    *
bmk     YRMPRPDNCPEELYNIMIRCWKNRPEERPTFEYIQSVLDDFYTATESQYQQQP          503
LYN     .....VE...D...D..KM...EKA......D.L............G......          512
lck     ...V..........HL.ML...E...D....D.LR......F....G...P..          509
c-src   ....C.PE...S.HDL.CQ..RRD.........L.AF.E.YF.S..P...PGENL         533
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FIG. 2. Comparison of the *bmk*-encoded product with other related protein-tyrosine kinases. The amino acid residues of bmk, LYN, lck, and chicken c-src are given in the single-letter amino acid code, with dots signifying identity with the bmk protein. Numbering of amino acids begins with the initiator codon for all sequences. Dashes indicate gaps introduced for alignment. Boxed residues indicate regions implicated in nucleotide binding [GXGXXG (X = other), bmk residues 246–251] and autophosphorylation in c-src (DNEYTARE, bmk residues 385–392). The carboxyl-terminal tyrosine residue implicated in the regulation of chicken c-src kinase activity is marked with an asterisk. The sequences were aligned pairwise by using the Needleman–Wunsch algorithm.

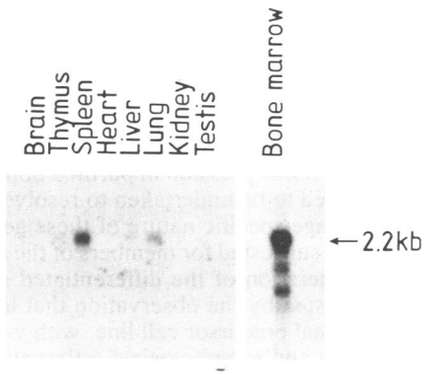

FIG. 3. Blot-hybridization analysis of poly(A)$^+$ RNA (1 μg) isolated from a number of murine tissues. To avoid cross-hybridization to any lck transcripts, a 5′ bmk mRNA-specific fragment (nucleotides 1–313 of the cDNA) was nick-translated and used as a probe.

Previous studies have shown that c-src message levels and/or pp60$^{c\text{-}src}$ kinase activity increase during cellular differentiation in a number of systems (35–37). Therefore, we were interested in examining *bmk* expression during myeloid differentiation. Since it is difficult to isolate sufficient numbers of defined normal myeloid progenitor cells, we utilized the murine myelomonocytic cell line WEHI-3B that undergoes morphological differentiation similar to that seen in cultures of purified colony-forming cells (38). Although we were unable to detect bmk transcripts in undifferentiated WEHI-3B cells, bmk mRNA was present in the population of essentially mature monocytes resulting from the induced differentiation of this cell line with granulocyte-colony stimulating factor and actinomycin D (39) (Fig. 5). Thus, the changes in *bmk* expression observed in this system may reflect similar changes in *bmk* expression in normal myeloid cell development.

DISCUSSION

We have molecularly cloned and sequenced a cDNA (designated bmk) whose corresponding mRNA is expressed

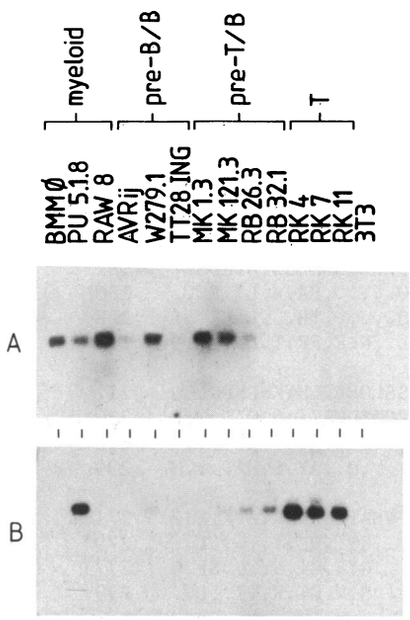

FIG. 4. Blot-hybridization analysis of poly(A)$^+$ RNA (1 μg) from myeloid and lymphoid cell lines. Blots were hybridized with 5′ bmk (*A*) or lck cDNA-specific probes (*B*). Exposure times were 5 days and 16 hr, respectively. BMMϕ, bone marrow macrophage.

predominantly, perhaps exclusively, in cells of hemopoietic origin. The deduced amino acid sequence of bmk shares extensive homology with members of the *src*-related family of protein-tyrosine kinases, in particular with the products of the *LYN* and *lck* genes. Despite the homology to human *LYN*, we do not believe that *bmk* represents the murine version of this locus. Southern blots of *Bam*HI-digested murine genomic DNA revealed bands of 11, 6.5, 2.5, and 1.5 kb (data not shown), while those previously reported for *LYN* are 30, 14, 8, and 5 kb (14). Moreover, recent studies comparing the human and chicken c-*src* genes show that the conservation between species was >90% (11). Since the amino-terminal domain of the *bmk* gene product is quite different from the corresponding regions in products of other *src*-related genes, it is likely that *bmk* represents a distinct locus in the murine genome.

Members of the src-family of protein-tyrosine kinases possess several strongly conserved features that are also

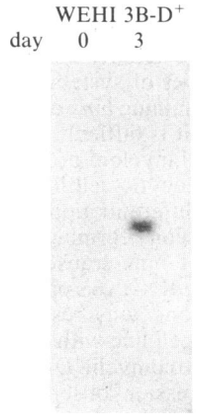

FIG. 5. Blot-hybridization analysis of poly(A)$^+$ RNA (1 μg) from WEHI-3B cells before and after induced differentiation with granulocyte colony-stimulating factor and actinomycin D. After 3 days, ≈90% of the cells were monocytes as judged by morphology after cytospin centrifugation.

present in the predicted translation product of *bmk*. The glycine residue at position 2, the amino-terminal residue in mature src proteins, is known to be myristylated in c-*src*, v-*src*, and *lck* gene products (40–42). This modification is required for the plasma membrane association and transformation potential of v-*src* (42, 43). Another conserved feature between *src*-related molecules is a carboxyl-terminal tyrosine residue, located at amino acid position 499 in bmk, which is analogous to tyrosine residue 527 in chicken c-src. Phosphorylation at this site has been implicated in the negative regulation of c-src tyrosine kinase activity (31, 32), and mutations that change this residue to phenylalanine result in a 5- to 10-fold increase in pp60$^{c\text{-}src}$ kinase activity and the generation of a transforming protein (44–46).

In the murine tissues surveyed in our study, expression of *bmk* gene products was highest in organs of active hemopoiesis. While we cannot rule out transcription in nonhemopoietic cells, our failure to detect bmk transcripts in cultured fibroblasts may indicate that the low levels of bmk mRNA in lung and liver are the result of resident macrophages or blood contamination of these tissues.

Our analysis of a number of hemopoietic cell lines has revealed a complex pattern of expression for *bmk* and the related gene *lck*. Previous reports have indicated that lck mRNA is found exclusively in lymphoid cells, predominantly those of the T-cell lineage (12, 19). Similarly, we find high levels of lck mRNA expression in three Ab-MuLV-transformed T-cell lines. By contrast, we find that *bmk* expression is associated with cells of the myeloid and B lineages. There are, however, a number of deviations from this general pattern of expression. The high level of lck mRNA found in the macrophage-like cell line PU 5.1.8 is interesting given previous assertions that expression of *lck* is confined to lymphoid cells. It is unknown whether this observation diminishes the case for the specific association of *lck* with lymphoid cells *in vivo* or rather reflects some unusual characteristic of this established cell line. In addition, the B-cell line W279.1 also coexpresses *bmk* and *lck*; thus expression of these genes is not mutually exclusive.

Intriguingly, coexpression of *bmk* and *lck* also occurs in a group of Ab-MuLV-induced thymic transformants that appear to be lymphoid but uncommitted to either the T or B lineage. While two of these cell lines, MK 1.3 and RB 32.1, express only bmk and lck mRNA, respectively, two others, MK 121.3 and RB 26.3, express both messages. The exclusive expression of *bmk* in MK 1.3 is consistent with a B-cell differentiation potential for this cell line and is interesting in light of its surface expression of the B-cell marker B220 (34). Conversely, RB 32.1 lacks the B220 marker, and expression of *lck* in this cell line may suggest a more close association with the T-cell lineage. However, the presence of lck transcripts in RB 26.3 (bmk$^+$, B220$^+$) and the absence of the B220 marker on MK 121.3 (bmk$^+$, lck$^+$) complicates any potential correlation between *bmk*, *lck*, and B220 marker expression. This in turn raises doubts about the utility of bmk and lck mRNA as markers of lineage commitment in these immature Ab-MuLV-transformed cell lines. Clearly a comprehensive analysis of *bmk* and *lck* expression in purified populations of normal cells will need to be undertaken to resolve questions concerning the lineage-specific nature of these genes.

One possible role suggested for members of the *src*-related family is in the generation of the differentiated state. This hypothesis is suggested by the observation that infection of PC12 cells, a neuronal precursor cell line, with v-*src* results in neurite extension and morphological differentiation (47). For hemopoietic cells, it has been shown that differentiation of two myeloid cell lines HL-60 and U937 (35, 36) is associated with elevated levels of pp60$^{c\text{-}src}$ tyrosine kinase activity. Moreover, infection of the murine myelomonocytic cell line M1 with a recombinant retrovirus encoding polyoma

middle tumor (T) antigen leads to the generation of cells expressing the differentiation marker Mac-1 (35). Since middle T antigen is known to specifically activate $pp60^{c\text{-}src}$ kinase (48), it is possible that induction of *src*-related kinase may represent a key event in myeloid differentiation. Our finding that expression of bmk mRNA accompanies the differentiation of murine WEHI-3B cells to monocytes is consistent with a role for *bmk* in either the induction or maintenance of differentiation. Alternatively, *bmk* expression may play no causal role in the differentiation process but could be an inevitable consequence of cells acquiring differentiated characteristics. Indeed the high levels of $pp60^{c\text{-}src}$ found in platelets and postmitotic neurons may suggest an involvement for *src*-related kinases in some aspect of end-cell function. Understanding the role that *src*-related molecules play in hemopoiesis will ultimately require the elucidation of the changes that result from the induction of their protein-tyrosine kinase activity *in vivo*. Such an analysis may be facilitated by the generation of modified *src* genes that, upon introduction into hemopoietic cells, result in constitutive tyrosine kinase activity.

Since the completion of this work, two reports describing the isolation of a human cDNA corresponding to a new member (HCK) of the *SRC*-related family of protein-tyrosine kinases have been published (49, 50). Comparison of the HCK protein sequence with that of bmk showed 86% homology and, together with the similar pattern of gene expression, strongly suggests that *bmk* is the murine homologue of human *HCK*. To avoid confusion, we propose in the future to adapt the term *HCK* for both the murine and human loci.

The authors are indebted to Ed Stanley, Richard Lang, and Tony Burgess for their valuable contributions to this manuscript. We are grateful to Tom Gonda for the WEHI-3B RNA blot, and to Jerry Adams for gifts of various RNAs. We would also like to thank John Hamilton and Gino Vairo for their help in culturing macrophage cells, Rob Maxwell and Tony Kyne for computer analyses, and Jean Kingett and Pat Collier for help in the preparation of this manuscript.

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