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Identification of a Human Gene (*HCK*) That Encodes a Protein-Tyrosine Kinase and Is Expressed in Hemopoietic Cells

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We have isolated cDNAs representing a previously unrecognized human gene that apparently encodes a protein-tyrosine kinase. We have designated the gene as *HCK* (hemopoietic cell kinase) because its expression is prominent in the lymphoid and myeloid lineages of hemopoiesis. Expression in granulocytic and monocytic leukemia cells increases after the cells have been induced to differentiate. The 57-kilodalton protein encoded by *HCK* resembles the product of the proto-oncogene c-*src* and is therefore likely to be a peripheral membrane protein. *HCK* is located on human chromosome 20 at bands q11-12, a region that is affected by interstitial deletions in some acute myeloid leukemias and myeloproliferative disorders. Our findings add to the diversity of protein-tyrosine kinases that may serve specialized functions in hemopoietic cells, and they raise the possibility that damage to *HCK* may contribute to the pathogenesis of some human leukemias.

Protein-tyrosine kinases occupy prominent positions in pathways that govern the phenotypes of vertebrate cells (13). The existence of these enzymes was discovered through the study of the retroviral oncogene v-*src* (3, 14, 29) and its cellular progenitor c-*src* (2, 18, 39), but the number of protein-tyrosine kinases has since grown to 20 or more. Close to half of the protein-tyrosine kinases are transmembrane receptors for polypeptide hormones, such as epidermal growth factor, insulin, and platelet-derived growth factor. The remainder are peripheral membrane proteins, generally situated at the cytoplasmic surface of the plasma membrane, without known physiological functions, and exemplified by the products of c-*src* and v-*src* ($pp60^{c\text{-}src}$ and $pp60^{v\text{-}src}$, respectively).

During a search for a cDNA clone representing human *SRC*, we encountered a previously unrecognized gene that apparently encodes a protein-tyrosine kinase similar to $pp60^{c\text{-}src}$. (We have followed the international convention of capitalizing and italicizing designations for human genes and have otherwise adhered to convention in the designation of mouse genes, retroviral oncogenes, and their proto-oncogenes.) Ziegler et al. have found the same gene by a different route (63). Expression of this gene may be limited to certain hemopoietic cells and is especially prominent in cells of myeloid lineage, particularly mature granulocytes and monocytes. We have therefore designated the gene as *HCK* (pronounced "hick") for hemopoietic cell kinase. We describe here the nucleotide sequence of a cDNA clone that represents most or all of the mRNA for *HCK*, the location of *HCK* on human chromosome 20, the deduced amino acid sequence of the protein encoded by *HCK*, and the distribution of RNA transcribed from *HCK* among various hemopoietic cells. Our findings add to the growing diversity of protein-tyrosine kinases that may play specialized roles in the development or function of hemopoietic cells, and they raise the possibility that damage to *HCK* may contribute to the pathogenesis of certain human hematological malignancies.

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MATERIALS AND METHODS

Cell culture. Human foreskin fibroblasts, the human neuroblastoma cell line Kelly, and the human retinoblastoma cell line Y79 were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and 0.15% (wt/vol) $NaHCO_3$. All other cell lines were maintained in RPMI 1640 supplemented with 10 or 20% heat-inactivated fetal calf serum, 0.15% (wt/vol) $NaHCO_3$, 2 mM L-glutamine, and 12 mM pyruvic acid. All cell lines were propagated in the presence of penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml) and maintained at 37°C with 5% CO_2. We used established human leukemia and lymphoma cell lines that exhibit stable phenotypic markers representative of the original tumors and that provide a spectrum of lymphoid, myeloid, and erythroid cell types at various stages of hemopoiesis. A summary of the phenotypes has been published (36). Cell lines used included the B-lymphocyte line IM-9 (9), the B-lymphoblast lines Daudi (21) and Raji (44), the immature T-cell line MOLT-4 (37), the immature myeloid line KG-1 (22), the myeloblast line ML-1 (35), the promyelocytic cell line HL-60 (4), the monocytic cell line U-937 (54), and the line K-562 derived from a chronic myelogenous leukemia (30).

To induce differentiation, cells were washed, suspended in fresh RPMI 1640 containing 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) (20 ng/ml) or dimethyl sulfoxide (1.25, vol/vol), and incubated for 3 days. The ability of ML-1, HL-60, and U-937 cells to differentiate has been evaluated in detail previously (12, 38, 46). We used light microscopy for routine assessments. Whereas untreated ML-1, HL-60, and U-937 cells grew as suspensions of single cells, 24 h after induction, 80% of the treated cells were attached to the substrate, many in small clumps. The cells could not be detached from the substrate by vigorous shaking but could be detached by treatment with trypsin-EDTA for 10 min. After 48 h of continuous treatment with the differentiating agent, more than 95% of the cells were adherent.

Isolation of clones. We used two cDNA libraries, prepared with RNA from a human placenta and human retinas (gen-

erous gifts from William Rutter and Jeremy Nathans, respectively). Both libraries were constructed as *Eco*RI fragments inserted into the bacteriophage vector λgt10. The cDNA libraries (5×10^5 plaques each) were screened at reduced hybridization stringency (0.45 M NaCl, 40% formamide, 41°C) with a ^{32}P-labeled 800-base-pair fragment of DNA encompassing the highly conserved tyrosine-kinase domain of v-*src*, nick translated to a specific activity of 2×10^8 cpm/μg of DNA. Details of the probe and conditions for hybridization have been reported previously (31, 40).

Nucleotide sequence analysis. Restriction enzyme fragments derived from the cDNA clones were transferred into the polylinker regions of the M13 phage vector mp18 or mp19 (34). The nucleotide sequence was determined by the chain termination method as described previously (49), modified to accommodate the use of [^{35}S]dATP (1). All sequences were confirmed either by analysis of cDNA strands or by repeated sequencing of overlapping regions of the same strand.

Polyadenylated and total RNAs were prepared as described previously (40, 59), electrophoresed in 1% agarose gels containing 2.2 M formaldehyde, blotted onto nitrocellulose filters, and hybridized under stringent conditions (40, 57). The DNA probes were labeled with ^{32}P by nick translation to a specific activity of 2×10^8 cpm/μg of DNA.

Spot-blot chromosome analysis. Chromosome suspensions prepared from cell lines GM 130 and GM 131 were sorted onto nitrocellulose filter paper with a triple laser custom FACS IV chromosome sorter (26a) and then analyzed by molecular hybridization as described previously (27, 28).

Hybridization in situ to chromosomes. Human metaphase cells prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes were fixed on slides and hybridized with a ^{3}H-labeled plasmid subclone of λa2/1a. Radiolabeled probe was prepared by nick translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.3×10^8 dpm/μg. In situ hybridization was performed as previously described (25). Metaphase cells were hybridized at 4.0 and 8.0 ng of probe per ml of hybridization mixture. Autoradiographs were exposed for 11 days.

RESULTS

Isolation and sequencing of cDNA clones for the *HCK* gene. We used an 800-base-pair DNA probe that encompasses the protein kinase domain of v-*src* to screen cDNA libraries representing the polyadenylated RNAs of human placenta and retinas. Six identical clones with inserts of 2.1 kilobase pairs were isolated from the placental library, and one clone with an insert of 1.6 kilobase pairs was isolated from the retinal library. One of the placental clones (designated λa2/1a) and the retinal isolate were subcloned into M13 phage and then sequenced. The placental clone included all of the sequence found in the retinal clone and is diagrammed in Fig. 1a.

The nucleotide sequence of the insert in the placental clone is 2,021 residues in length (Fig. 1b). It includes the canonical signal for polyadenylation (AATAAA) at residues 1988 to 1993 and terminates in a stretch of poly(A) of about 25 residues in length. We therefore conclude that the clone represents the entire 3′ domain of *HCK* mRNA.

A large open reading frame extends from a methionine codon at residues 169 to 171 to an opal termination codon (TGA) at residues 1684 to 1696, encoding a 57,305-dalton protein containing 505 amino acids. The initial methionine codon is flanked by nucleotides that generally favor the initiation of translation (23, 24). Moreover, eight termination codons occur upstream of the methionine codon in the same reading frame. We therefore conclude that although the cDNA may not extend to the 5′ end of the *HCK* mRNA, the complete coding domain of the gene is nevertheless represented.

Seven methionine codons are located upstream of the large open reading frame in another reading frame (Fig. 2). None of these codons is flanked by nucleotide sequences that favor the initiation of translation (23, 24), and the open reading frames that follow are closed by termination codons within 150 to 210 nucleotides. We therefore conclude (but cannot prove) that none of these reading frames is likely to be used for translation.

The 5′ untranslated region of the nucleotide sequence contains one other notable feature, a threefold repetition of the nine-nucleotide motif GGAAGATGA situated between residues 10 and 39. Two of the units are arranged exactly in tandem, whereas the third is separated from the other two by three nucleotides. A repetition of this sort is unlikely to occur by chance, but we have no other indication of its significance.

Product of the human *HCK* gene. Analysis of the amino acid sequence encoded by *HCK* revealed that the gene product belongs to the family of protein-tyrosine kinases (Fig. 2). Among the known members of this family, the proteins encoded by the recently described human *LYN* gene (62) and the mouse *lck* gene (32, 60) are most closely related to the product of *HCK* (see reference 63 for a detailed comparison of the proteins encoded by the *HCK* and *LYN* genes). Other genes closely related to *HCK* include the retroviral oncogene v-*yes* (20), chicken c-*src* (55), and human *SYN/SLK* (50; data not shown). Table 1 summarizes these similarities, which are most pronounced within the domain containing the enzymatic activity of tyrosine kinases (13).

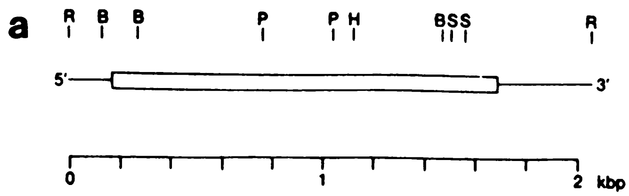
The proteins encoded by v-*src* and c-*src* possess structural features that provide further reference points for the topography of protein-tyrosine kinases.

(i) A tyrosine at residue 416 of the viral and cellular *src* proteins is a substrate for phosphorylation (53), and the amino acid sequence immediately upstream of this residue has helped engender a general definition of tyrosine phosphorylation sites (13). The *HCK*-encoded protein contains an analogous tyrosine at residue 390 (Fig. 2), and the amino acid sequence immediately upstream conforms to the previously described consensus.

(ii) Phosphorylation of tyrosine at residue 527 of pp60$^{c\text{-}src}$ appears to be an important means by which the enzymatic activity of the protein is regulated (5). Deletion of this tyrosine from pp60$^{v\text{-}src}$ augments the constitutive activity of the protein and may account for the transforming potential and tumorigenicity of the viral protein (6, 15, 61). The *HCK*-encoded protein contains an analogous tyrosine at residue 501 (Fig. 2).

(iii) The amino acids at residues 274 to 279 of pp60$^{v\text{-}src}$ (after the motif GXGXXG) help define the ATP-binding site of the protein (13), and the lysine at residue 295 interacts directly with ATP (17). Comparable features are present at residues 248 to 253 and 269 of the *HCK*-encoded protein (Fig. 2).

(iv) The proteins encoded by c-*src* and *HCK* share five of the first seven amino acids at their amino termini (Fig. 2). Residues 2 through 7 of this sequence are a major part of the signal that directs attachment of the *src* protein to mem-



b CGGACGCACGGAAGATGAGGAAGATGATCAGGAGGATGATGAAGGTGAAGAGGGAGATGAAGACGATGACGACGATGGCTCTGAGGCGACCTCAGCGGCTGCCGAGCTGGGGGGGGCT 119

1 10
Met Gly Cys Met Lys Ser Lys Phe Leu Gln Val Gly Gly Asn Thr Phe Ser
CAAGCTGCGAGGATCCGGGCTGCCCCGCAGACGAGGAGCGGGGGCCAGG ATG GGC TGC ATG AAG TCC AAG TTC CTC CAG GTC GGA GGC AAT ACA TTC TCA 219

20 30 40
Lys Thr Glu Thr Ser Ala Ser Pro His Cys Pro Val Tyr Val Pro Asp Pro Thr Ser Thr Ile Lys Pro Gly Pro Asn Ser His Asn Ser
AAA ACT GAA ACC AGC GCC AGC CCA CAC TGT CCT GTG TAC GTG CCC GAT CCC ACA TCC ACC ATC AAG CCG GGG CCT AAT AGC CAC AAC AGC 309

50 60 70
Asn Thr Pro Gly Ile Arg Glu Ala Gly Ser Glu Asp Ile Ile Val Val Ala Leu Tyr Asp Tyr Glu Ala Ile His His Glu Asp Leu Ser
AAC ACA CCA GGA ATC AGG GAG GCA GGC TCT GAG GAC ATC ATC GTG GTT GCC CTG TAT GAT TAC GAG GCC ATT CAC CAC GAA GAC CTC AGC 399

80 90 100
Phe Gln Lys Gly Asp Gln Met Val Val Leu Glu Glu Ser Gly Glu Trp Trp Lys Ala Arg Ser Leu Ala Thr Arg Lys Glu Gly Tyr Ile
TTC CAG AAG GGG GAC CAG ATG GTG GTC CTA GAG GAA TCC GGG GAG TGG TGG AAG GCT CGA TCC CTG GCC ACC CGG AAG GAG GGC TAC ATC 489

110 120 130
Pro Ser Asn Tyr Val Ala Arg Val Asp Ser Leu Glu Thr Glu Glu Trp Phe Phe Lys Gly Ile Ser Arg Lys Asp Ala Glu Arg Gln Leu
CCA AGC AAC TAT GTC GCC CGC GTT GAC TCT CTG GAG ACA GAG GAG TGG TTT TTC AAG GGC ATC AGC CGG AAG GAC GCA GAG CGC CAA CTG 579

140 150 160
Leu Ala Pro Gly Asn Met Leu Gly Ser Phe Met Ile Arg Asp Ser Glu Thr Thr Lys Gly Ser Tyr Ser Leu Ser Val Arg Asp Tyr Asp
CTG GCT CCC GGC AAC ATG CTG GGC TCC TTC ATG ATC CGG GAT AGC GAG ACC ACT AAA GGA AGC TAC TCT TTG TCC GTG CGA GAC TAC GAC 669

170 180 190
Pro Arg Gln Gly Asp Thr Val Lys His Tyr Lys Ile Arg Thr Leu Asp Asn Gly Gly Phe Tyr Ile Ser Pro Arg Ser Thr Phe Ser Thr
CCT CGG CAG GGA GAT ACC GTG AAA CAT TAC AAG ATC CGG ACC CTG GAC AAC GGG GGC TTC TAC ATA TCC CCC CGA AGC ACC TTC AGC ACT 759

200 210 220
Leu Gln Glu Leu Val Asp His Tyr Lys Lys Gly Asn Asp Gly Leu Cys Gln Lys Leu Ser Val Pro Cys Met Ser Ser Lys Pro Gln Lys
CTG CAG GAG CTG GTG GAC CAC TAC AAG AAG GGG AAC GAC GGG CTC TGC CAG AAA CTG TCG GTG CCC TGC ATG TCT TCC AAG CCC CAG AAG 849

230 240 250
Pro Trp Glu Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Lys Leu Glu Lys Lys Leu Gly Ala Gly Gln Phe Gly Glu Val Trp Met
CCT TGG GAG AAA GAT GCC TGG GAG ATC CCT CGG GAA TCC CTC AAG CTG GAG AAG AAA CTT GGA GCT GGG CAG TTT GGG GAA GTC TGG ATG 939

260 270 280
Ala Thr Tyr Asn Lys His Thr Lys Val Ala Val Lys Thr Met Lys Pro Gly Ser Met Ser Val Glu Ala Phe Leu Ala Glu Ala Asn Val
GCC ACC TAC AAC AAG CAC ACC AAG GTG GCA GTG AAG ACG ATG AAG CCA GGG AGC ATG TCG GTG GAG GCC TTC CTG GCA GAG GCC AAC GTG 1029

290 300 310
Met Lys Thr Leu Gln His Asp Lys Leu Val Lys Leu His Ala Val Val Thr Lys Glu Pro Ile Tyr Ile Ile Thr Glu Phe Met Ala Lys
ATG AAA ACT CTG CAG CAT GAC AAG CTG GTC AAA CTT CAT GCG GTG GTC ACC AAG GAG CCC ATC TAC ATC ATC ACG GAG TTC ATG GCC AAA 1119

320 330 340
Gly Ser Leu Leu Asp Phe Leu Lys Ser Asp Glu Gly Ser Lys Gln Pro Leu Pro Lys Leu Ile Asp Phe Ser Ala Gln Ile Ala Glu Gly
GGA AGC TTG CTG GAC TTT CTG AAA AGT GAT GAG GGC AGC AAG CAG CCA TTG CCA AAA CTC ATT GAC TTC TCA GCC CAG ATT GCA GAA GGC 1209

350 360 370
Met Ala Phe Ile Glu Gln Arg Asn Tyr Ile His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val Ser Ala Ser Leu Val Cys Lys Ile Ala
ATG GCC TTC ATC GAG CAG AGG AAC TAC ATC CAC CGA GAC CTC CGA GCT GCC AAC ATC TTG GTC TCT GCA TCC CTG GTG TGT AAG ATT GCT 1299

380 390 400
Asp Phe Gly Leu Ala Arg Val Ile Glu Asp Asn Glu Tyr Thr Ala Arg Glu Gly Ala Lys Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala
GAC TTT GGC CTG GCC CGG GTC ATT GAG GAC AAC GAG TAC ACG GCT CGG GAA GGG GCC AAG TTC CCC ATC AAG TGG ACA GCT CCT GAA GCC 1389

410 420 430
Ile Asn Phe Gly Ser Phe Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Met Glu Ile Val Thr Tyr Gly Arg Ile Pro Tyr
ATC AAC TTT GGC TCC TTC ACC ATC AAG TCA GAC GTC TGG TCC TTT GGT ATC CTG CTG ATG GAG ATC GTC ACC TAC GGC CGG ATC CCT TAC 1479

440 450 460
Pro Gly Met Ser Asn Pro Glu Val Ile Arg Ala Leu Glu Arg Gly Tyr Arg Met Pro Arg Pro Glu Asn Cys Pro Glu Glu Leu Tyr Asn
CCA GGG ATG TCA AAC CCT GAA GTG ATC CGA GCT CTG GAG CGT GGA TAC CGG ATG CCT CGC CCA GAG AAC TGC CCA GAG GAG CTC TAC AAC 1569

470 480 490
Ile Met Met Arg Cys Trp Lys Asn Arg Pro Glu Glu Arg Pro Thr Phe Glu Tyr Ile Gln Ser Val Leu Asp Asp Phe Tyr Thr Ala Thr
ATC ATG ATG CGC TGC TGG AAA AAC CGT CCG GAG GAG CGG CCG ACC TTC GAA TAC ATC CAG AGT GTG CTG GAT GAC TTC TAC ACG GCC ACA 1659

500 505
Glu Ser Gln Tyr Gln Gln Gln Pro OP
GAG AGC CAG TAC CAA CAG CAG CCA TGA TAGGGACGACCAGGGCAGGGCAGGGGGTCCCAGGTGGTGGCTCGAAGGTGGCTCCAGCACCATCCGCCAGGGCCCACACCCCC 1770

TTCCTACTCCCAGACACCCACCCTCGCTTCAGCCACAGTTTCCTCATCTGTCCAGTGGGTAGGTTGGACTGGAAAATCTCTTTTTGACTCTTGCAATCCACAATCTGACATTCTCAGGAA 1890

GCCCCCAAGTTGATATTTCTATTTCCTGGAATGGTTGGATTTTAGTTACAGCTGTGATTTGGAAGGGAAACTTTCAAAATAGTGAAATGAATATTTAAATAAAAGATATAAATGCAAGTC 2010

TTACCAAAAAAAAAAAAAA

FIG. 1. cDNA clone for *HCK*. (a) Topography of cDNA clone λa2/1a, prepared with human placental RNA, and representing mRNA of the gene *HCK*. Symbols for restriction sites: B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; and S, *Sst*I. The open box delineates the coding domain of *HCK*. kbp, Kilobase pairs. (b) Complete nucleotide sequence of the cDNA clone diagrammed in panel a. Nucleotides are numbered at the right of the figure, whereas the amino acid residues are above the sequence. Numbering of the amino acid sequence begins at the postulated initiator methionine. The putative signal for polyadenylation (AATAAA) is underlined.

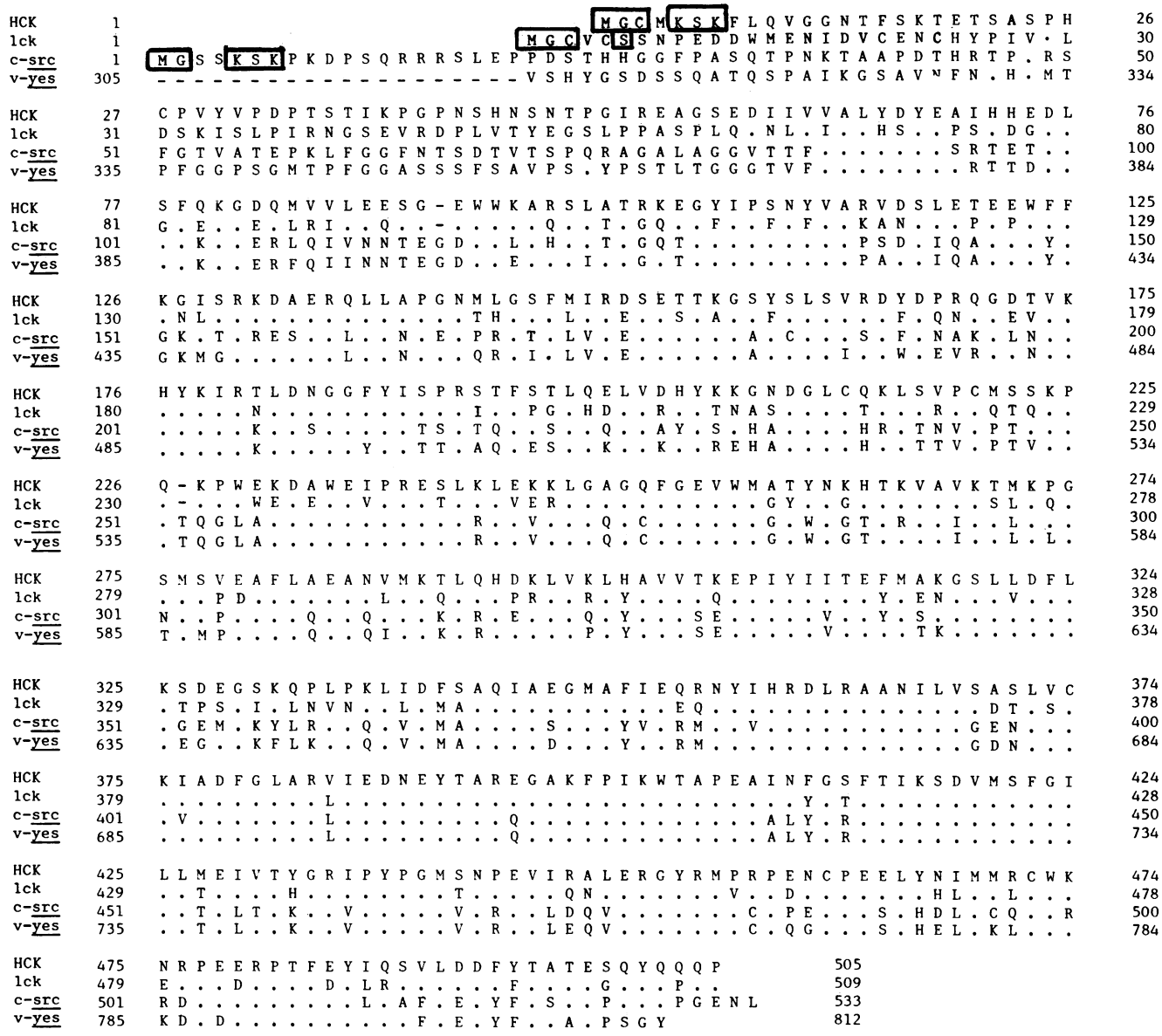


FIG. 2. Protein encoded by *HCK* related to other protein-tyrosine kinases. The amino acid sequences of the proteins encoded by the human *HCK*, mouse *lck*, chicken c-*src*, and the retroviral gene v-*yes* are given in the single letter code. Dots signify identity with the amino acid residues found in the *HCK* protein. Dashes indicate gaps that have been introduced for alignment. Numbering of the amino acid sequences begins with the initiator codons for *HCK*, *lck*, chicken c-*src*, and the *gag* component of the chimeric gene *gag-yes* in the Y73 avian sarcoma virus. The sequences are aligned for a best fit without recourse to special algorithms. Boxed residues dramatize shared features at the amino termini.

branes (7, 41, 42), and the glycine at residue 2 is essential for posttranslational myristylation of the protein (16). By analogy, we anticipate that the *HCK*-encoded protein is bound to the plasma membrane and that myristylation is required for this binding.

Expression of *HCK* in hemopoietic cells. We first sought, but failed to find, RNA transcribed from *HCK* in human placentas and human foreskin fibroblasts. Because of difficulties encountered in obtaining other samples of normal human tissues, we then turned to a series of cell lines derived from human neuroblastomas and leukemias, the latter representing various stages in the development of hemopoietic cells.

Hybridization with a probe for *HCK* detected no RNA in the neuroblastoma cells (data not shown), but a 2.2-kilobase polyadenylated RNA was detected in several of the leukemia cell lines (Fig. 3). The RNA was most abundant in cells representing the lineages that give rise to either granulocytes (ML-1 and HL-60) or monocytes (U-937) (Fig. 3; Table 2). The quantities of *HCK* RNA in all of these cell lines appeared to be roughly the same. *HCK* RNA was also found in the B-cell line IM-9. By contrast, we failed to detect *HCK* expression in T cells, in a line of apparently bipotential precursors to granulocytes and monocytes (KG-1), and in precursors to erythroid cells (K-562 cells).

We next examined the possibility that expression of *HCK* might be regulated in concert with differentiation. TPA was used to induce the differentiation of ML-1, HL-60, and

TABLE 1. Similarities among protein-tyrosine kinases

Gene	Source	% Identical amino acids in *HCK*-encoded protein	
		Overall	Kinase domain[a]
LYN	Human	67	83
lck	Mouse	63	77
SYN/SLK	Human	57	68
v-*yes*	Avian sarcoma virus Y73	56	71
c-*src*	Chicken	54	68

[a] Residues 230 to 505 in the *HCK*-encoded protein.

U-937 cells into macrophages (38, 46). After differentiation, the steady-state levels of *HCK* RNA had increased approximately twofold in U-937 cells and approximately fivefold in ML-1 and HL-60 cells (Fig. 4; Table 2). Differentiation of HL-60 cells to granulocytes in response to dimethyl sulfoxide was also accompanied by a sevenfold increase in *HCK* RNA (Fig. 3; Table 2).

Location of *HCK* on bands q11-12 of human chromosome 20. DNA derived from human chromosomes fractionated by dual laser sorting was analyzed for the presence of *HCK*. Results of two complete spot-blot filter panels assigned the *HCK* gene uniquely to chromosome 20 (data not shown). To further localize the *HCK* gene on human chromosome 20, we performed in situ hybridizations with normal human metaphase cells. This resulted in specific labeling of the proximal region of the long arm of chromosome 20. Of 200 metaphase cells examined from this hybridization, 62 (31%) were labeled on region q1, on bands q11-12 of one or both chromosome 20 homologs ($P < 0.0005$). The distribution of labeled sites on chromosome 20 is illustrated in Fig. 5. A total of 103 grains were observed on this chromosome; of these, 77 (75%) were clustered at bands q11-12 and represented 19% (77/406) of all labeled sites. The largest cluster of grains was observed at 20q11. In situ hybridization experiments were repeated twice and gave similar results. Thus, these results indicate that the *HCK* gene is localized on the proximal long arm of human chromosome 20 in the q11-12 region.

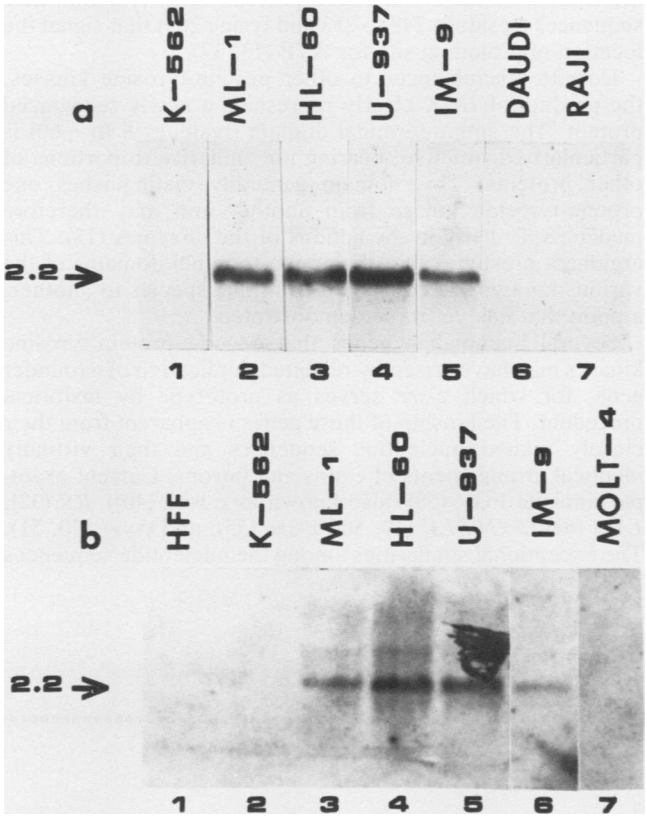


FIG. 3. RNA transcribed from *HCK* in human cells. RNAs were fractionated by electrophoresis and hybridized with a ^{32}P-labeled probe for *HCK*. Electrophoretic mobilities were calibrated with RNAs (10^3 kilobases). The 2.2-kilobase RNA detected is indicated by 2.2 and the arrow to the left of the gels. (a) Polyadenylated RNAs (8 to 10 μg per lane) from the indicated cell lines. (b) Total RNA (ca. 30 μg per lane) from human foreskin fibroblasts (HFF, lane 1) and the indicated cell lines.

DISCUSSION

Identification of *HCK*. We have identified a previously unrecognized member of the gene family that encodes protein-tyrosine kinases. The identification occurred by happenstance during a search for cDNA clones representing the kinase gene *SRC*. We have designated the gene as *HCK* (hemopoietic cell kinase) for the cells in which expression of the gene seems most pronounced. Ziegler et al. have also uncovered *HCK* by screening a cDNA library prepared with RNA from peripheral blood leukocytes (63).

The largest cDNA for *HCK* that we have isolated is virtually the same length as the mRNA for the gene and clearly includes the 3′ terminus of the RNA. By contrast, we cannot be certain that the cDNA extends to the 5′ terminus of the mRNA. Three findings suggest that we have nevertheless identified the entire coding domain of *HCK*. First,

TABLE 2. Expression of *HCK* in hemopoiectic cells

Cell line	Treatment	Phenotype	Relative abundance of HCK RNA[a]
KG-1		Early myeloid precursor	
ML-1		Myeloblast	1
ML-1	TPA	Macrophage	4
HL-60		Promyelocyte	1
HL-60	TPA	Macrophage	5.2
HL-60	Dimethyl sulfoxide	Granulocyte	7
U-937		Monocyte	1
U-937	TPA	Macrophage	1.9
Daudi		B lymphoblast (surface Ig[b])	
Raji		B lymphoblast (surface Ig)	
IM-9		B lymphoblast (secretes Ig)	0.5–0.75
MOLT-4		Immature T cell	
K-562		Erythroid precursor	

[a] Quantitation was obtained by scanning autoradiograms with a densitometer. The values obtained with the ML-1 cell line were arbitrarily given the value of 1.00.
[b] Ig, Immunoglobulin.

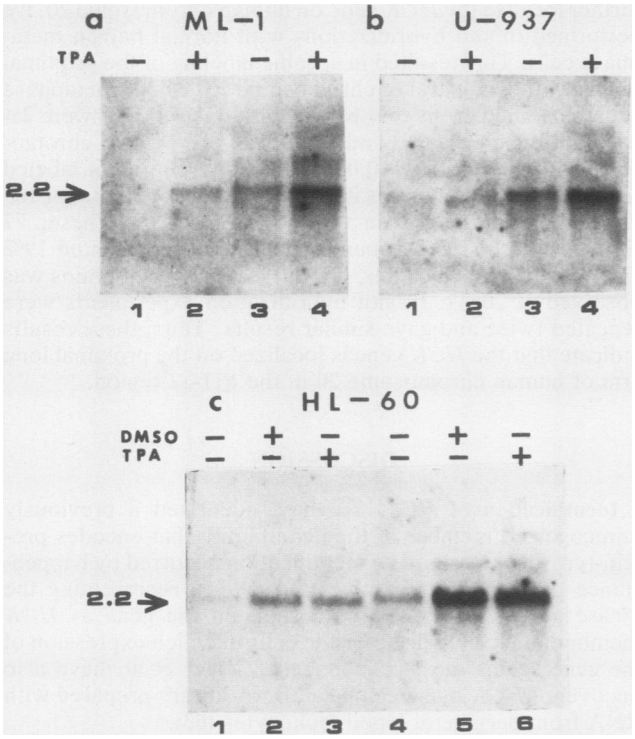


FIG. 4. Effect of myeloid differentiation on expression of *HCK*. Total cellular RNAs from the lines ML-1 (a), U-937 (b), and HL-60 (c) were analyzed as for Fig. 3. In panels a and b, 5 μg of RNA (lanes 1 and 2) and 25 μg of RNA (lanes 3 and 4) from untreated cells (lanes 1 and 3) and cells treated with TPA as described in Materials and Methods (lanes 2 and 4). In panel c, 5 μg of RNA (lanes 1 to 3) and 25 μg of RNA (lanes 4 to 6) from untreated cells (lanes 1 and 4), cells treated with dimethyl sulfoxide (DMSO) (lanes 2 and 5), and cells treated with TPA (lanes 3 and 6). The 2.2-kilobase RNA detected is indicated by 2.2 and the arrow to the left of the gels.

eight termination codons lie upstream of, and in frame with, the methionine codon that we have designated as the site for the initiation of translation. Second, the candidate initiation codon is flanked by nucleotide sequences that conform to the consensus proposed by Kozak (23, 24). Third, the amino terminus of the postulated *HCK*-encoded protein is similar (five of seven amino acid residues) to that of chicken c-*src*-encoded protein in a domain that is required to myristylate *src* protein and attach it to membranes (7, 41, 42).

Chromosomal location of *HCK*. We have mapped *HCK* to band q11-12 of human chromosome 20. This location is provocative in two regards.

First, the closely related gene *SRC* is on chromosome 20 at band q13.3 (25). Although *HCK* apparently belongs to a family of genes that arose by repeated duplication (see below), the molecular (as opposed to cytogenetic) distance between *SRC* and *HCK* seems too great for one gene to have arisen from the other by tandem duplication within the chromosome.

Second, abnormalities of the long arm of chromosome 20 have been observed in a number of patients with hematologic malignant diseases. Although translocations involving 20q have been reported, a more common abnormality is a deletion of the long arm of this chromosome. Such a deletion has been noted in patients with acute myeloid leukemia or a myelodysplastic syndrome, as well as in patients with myeloproliferative disorders (8, 45, 56). In the past, this abnormality has been interpreted as a terminal deletion with a breakpoint in band q11; however, recent data indicate that these deletions are interstitial with a proximal breakpoint in band q11 (either band q11.1 or q11.2) and a distal breakpoint in band q13.3 (26). Although this deletion does not include the *SRC* locus (25), it may encompass part or all of *HCK*. The chromosomal location of *HCK* therefore raises the possibility that damage to this gene may be involved in the pathogenesis of some human malignant hematological diseases.

Protein-tyrosine kinase encoded by *HCK*. The protein encoded by *HCK* includes a large domain (residues 230 to 505) whose amino acid sequence is characteristic of the enzymatically active portions of protein kinases (13). Other features of the protein affirm that it is a kinase and that its amino acid substrate is likely to be tyrosine: (i) a close resemblance to proteins that are demonstrably tyrosine-specific kinases, including the products of *lck* (32), v-*yes* (20), and c-*src* (55); (ii) short stretches of amino acids that serve as signatures of protein-tyrosine kinases, including the sequences KWTAPE at residues 401 to 406 and DVMSFGILL at residues 418 to 426 (13); (iii) tyrosine residues at positions 390 and 501 that typify protein-tyrosine kinases and are themselves subject to phosphorylation (5, 13, 53); (iv) an amino-terminal sequence similar to that required for the myristylation and membrane attachment of the *src*-encoded protein; and (v) amino acid sequences (residues 248 to 253 and lysine 269) that signal the location of a binding site for ATP (13, 17).

Despite resemblances to other protein-tyrosine kinases, the product of *HCK* clearly represents a newly recognized protein. The amino-terminal domain (residues 8 to ~60) is particularly distinctive, bearing no similarity to portions of other proteins. This domain generally distinguishes one protein-tyrosine kinase from another and may therefore mediate specificity in the actions of the enzymes (13). This argument presumes that the amino-terminal domains of the various kinases are conserved from one species to another, a point that has yet to be demonstrated.

Several mammalian genes that encode protein-tyrosine kinases may have arisen by repeated duplication of a founder gene, for which c-*src* serves as prototype by historical precedent. The kinship of these genes is apparent from their closely related nucleotide sequences and their virtually identical arrangements of exons and introns. Current examples include FGR (58) (also known as c-*src*2 [40]), *lck* (32), *LYN* (62), *SYN/SLK* (19, 50), c-*src* (55), and c-*yes* (20, 51). The exceptional similarities among the nucleotide sequences

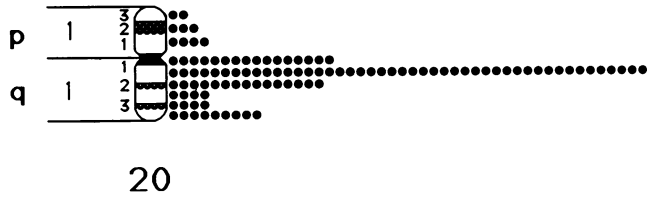


FIG. 5. Localization of *HCK* by hybridization in situ to metaphase chromosomes. Distribution of labeled sites on chromosome 20 in 200 normal metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes that were hybridized with a radiolabeled *HCK* probe. The labeled sites observed in this hybridization were clustered at bands 20q11-12; the largest cluster of grains was located at 20q11.

of *HCK*, *LYN*, c-*src*, *SYN/SLK*, and v-*yes* raise the possibility that *HCK* also arose by gene duplication. The topography of mouse *hck* supports this possibility (63).

Expression of *HCK* in hemopoietic cells. Although *HCK* was discovered from cDNA clones prepared with placental RNA, we first detected appreciable expression of the gene during a survey of cell lines representing various stages in the development of hemopoietic cells. Expression was evident in B lymphocytes and in cells from the intermediate stages of granulocytic and monocytic lineages and increased substantially when the myeloid cells were induced to differentiate to more mature phenotypes. By contrast, expression of the gene was undetectable in immature B cells, limited samplings of the T-cell and erythroid lineages, the early stages of myeloid development, human fibroblasts, and human placentas.

These findings are provocative in at least two regards. They raise the possibility that the function of *HCK* has been specialized for the hemopoietic cells in which it is expressed, and they recall the fact that *HCK* is closely related to *lck*, another kinase gene whose expression may also be restricted to hemopoietic cells. However, there are caveats to these arguments. We have yet to perform a comprehensive survey of normal cells and tissues for expression of *HCK*, so expression of the gene may be less specific than is presently perceived. Most of the available data have been obtained with leukemia cells, which may not faithfully represent their normal counterparts in hemopoiesis. We discount these caveats because Ziegler et al. (63) report expression of *HCK* in normal lymphoid cells and granulocytes and have failed to find expression of mouse *hck* in a variety of other normal cells and tissues.

Physiological function of *HCK*. The protein-tyrosine kinases to which the product of *HCK* is related are generally peripheral membrane proteins, located at the cytoplasmic surface of the plasma membrane and on other cytoplasmic membranes (13). Myristylation of the amino terminus of these proteins is apparently required for their attachment to membranes (16). None of these proteins has yet been assigned an exact physiological function, but it is generally assumed that they are components of pathways that transmit and ramify signals required to govern cellular phenotype.

Structural features of the *HCK*-encoded protein product suggest that it, too, is myristylated and attached to membranes. Since expression of *HCK* may be limited to certain hemopoietic cells and increases when at least some of these cells differentiate, it is possible that the *HCK*-encoded protein interacts with a receptor for a hemopoietic growth factor. This notion conforms to the fact that a growing number of protein-tyrosine kinases appear to play specialized roles in the development or function of hemopoietic cells. (i) The product of c-*fms* is the cell surface receptor for the macrophage-specific growth factor M-CSF/CSF-I and is expressed only late in monocytic differentiation (47, 52). (ii) The expression of *SRC* is especially abundant in human platelets (11) and increases appreciably from a low level when either neoplastic or normal myeloid hemopoietic cells are induced to differentiate to monocytes (10). (iii) The *lck* gene may be expressed only in lymphoid cells and in T cells more than in B cells (32). (iv) The expression of c-*fps* may be restricted to macrophages and granulocytes (48). (v) The product of v-*abl* can free the growth of certain leukemia cells from requirements for the growth factors interleukin-3 or GM-CSF (33, 43), raising the possibility that c-*abl* represents a function that is activated during the response of normal hemopoietic cells to the same growth factors. Our findings with *HCK* add to this diversity by identifying yet another protein-tyrosine kinase that may have a specialized function in hemopoietic cells.

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