Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6312–6316, September 1986
Biochemistry

pks, a *raf*-related sequence in humans

(oncogene homology/*src* family/serine/threonine kinase/lymphoid cell)

GEORGE E. MARK*, TODD W. SEELEY*, THOMAS B. SHOWS†, AND JOHN D. MOUNTZ‡

*Laboratory of Human Carcinogenesis, National Cancer Institute, and ‡Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, MD 20892; and †Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, NY 14263

Communicated by Thomas F. Anderson, May 9, 1986

ABSTRACT **A human fetal liver cDNA library was screened at reduced hybridization stringency for v-*raf*-related sequences. In addition to the expected c-*raf-1* cDNA, a second sequence was isolated. Comparison of the second gene (*pks*) to the other *raf*-related sequences revealed nucleotide homologies of 71%. The predicted amino acid sequence of the kinase domain is sufficiently similar to that of v-*raf* to suggest that *pks* may encode a polypeptide that exhibits serine/threonine kinase activity. The expression of *pks* mRNA (2.7 kilobases long) is elevated in peripheral blood mononuclear cells isolated from two patients with angioimmunoblastic lymphadenopathy with dysproteinemia, a disease in which autoantibodies are produced following the lymphoproliferative activation of B cells. Analysis of somatic cell hybrids for segregation of the *pks* locus revealed the presence of an additional locus closely related to the *pks* sequence.**

Normal cellular growth appears to be under the control of a series of interrelated regulatory signals. There is increasing evidence that major elements in this system are the normal counterparts (protooncogenes) of already-described viral oncogenes. The list of protooncogenes expands as viral and cellular oncogenes are identified; recent additions have been v-*ski* (1), L-*myc* (2), *neu* (3), and *met* (4) sequences. Thus, as the number of possible targets of transforming lesions increases, so does the identity of loci involved in normal cellular growth and differentiation. Of the four major classes of oncogenes, the largest class encodes the members of the "*src* family." These proteins, many of which have been shown to possess either a tyrosine or serine/threonine kinase activity, are found associated with the cell membrane and are believed to be components of the cell's receptor system (5). It is the stimulation of one or more of these receptors that initiates a cascade of events leading to cellular proliferation. Whereas the amino and carboxyl termini of these gene products are unique to each member, they share a region of approximately 250 amino acids that defines an evolutionarily conserved kinase domain. We describe here the use of a probe corresponding to the sequence encoding this conserved domain to identify another oncogene-related human gene.

A previous report (6) described the nucleotide sequence of v-*raf*, the viral oncogene of murine sarcoma virus 3611. Comparison of the predicted amino acid sequence of v-*raf* with other known oncogenes revealed it to be related to members of the *src* family. It differed strikingly from most of these members within a 32 amino acid region encompassing the tyrosine acceptor site of the tyrosine kinase-encoding oncogenes. The substitution in the v-*raf* sequence of a serine for the tyrosine commonly present in the phosphate-accepting position suggested that v-*raf* specifies a serine kinase, not a tyrosine kinase. In keeping with this observation, Moelling *et al.* (7) have demonstrated that the v-*raf* gene product is a kinase that phosphorylates serine and threonine residues.

When used to screen a human fetal liver cDNA library under low-stringency conditions, a v-*raf*-specific probe identified two sequences. One was found to represent the c-*raf-1* locus (*RAF1* in standard human gene nomenclature), whereas the other represents a gene, which we call *pks*, whose sequence suggests it too may encode a serine/threonine kinase.

MATERIALS AND METHODS

Materials. A human fetal liver cDNA library in λgt10 representing transcripts obtained from a 20-week fetus was kindly provided by E. Fritch (Genetics Institute, Boston, MA). Radioisotopes and DNA-modifying enzymes were purchased from Amersham and New England Biolabs, respectively.

Screening of the λgt10 Fetal Liver cDNA Library. About 500,000 plaque-forming units of recombinant phage were screened at reduced stringency with a murine v-*raf* probe [675-base-pair (bp) *Xho* I–*Sst* II fragment] representing the kinase domain of this oncogene. Filters lifted off the phage plates (plaque "lifts") were hybridized to the ^{32}P-labeled nick-translated (8) DNA fragment (specific activity 3×10^8 cpm/μg) for 22 hr at 60°C in 5× standard saline citrate (SSC)/1× Denhardt's solution/10% (wt/vol) dextran sulfate. (1× SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7.4; Denhardt's solution is 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone.) Lifts were washed five times (15 min per wash) at 55°C in 2× SSC/0.1% $NaDodSO_4$ and autoradiographed at −70°C with intensifying screens.

DNA Sequencing. DNA sequence was determined by the method of Maxam and Gilbert (9) from uniquely labeled restriction site termini, following isolation by SeaPlaque (FMC, Rockland, ME) agarose gel electrophoresis as described (10).

Blot Hybridization Analysis of Poly(A)$^+$ RNA. RNAs were isolated from lymphoid tissues by homogenization in guanidinium thiocyanate followed by centrifugation through cesium chloride (11, 12); poly(A)$^+$ RNA was enriched by two selections on oligo(dT)-cellulose (13). RNA (10 μg), denatured by 14 mM methylmercury(II) hydroxide, was electrophoresed in a 1.5% agarose gel and transferred to *O*-diazophenylthioether paper (14). Hybridization to ^{32}P-labeled nick-translated cDNA probes in 50% (vol/vol) formamide/5× SSC/1× Denhardt's solution/yeast tRNA (250 μg/ml) at 42°C for 12 hr was followed by washes in 0.1× SSC at 65°C and autoradiography at −70°C with an intensifying screen.

Southern Blot Analysis. Genomic DNA (10 μg) was digested to completion with restriction endonucleases, electropho-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AILD, angioimmunoblastic lymphadenopathy with dysproteinemia; SLE, systemic lupus erythematosus; bp, base pair(s); kb, kilobase(s).

resed in a 0.7% agarose gel, and transferred to nitrocellulose paper by the method of Southern (15). The blots were hybridized overnight to cDNA fragment probes (specific activity 3×10^8 cpm/μg) at 65°C in 5× SSC/5× Denhardt's solution/10% (wt/vol) dextran sulfate/50 mM sodium phosphate buffer, pH 6.6/salmon sperm DNA (250 μg/ml)/0.1% (wt/vol) $NaDodSO_4$. After washing under stringent conditions (0.1× SSC/0.1% $NaDodSO_4$ at 68°C), the blots were autoradiographed as above. Low-stringency washes were performed at 50°C when DNAs from somatic cell hybrids were analyzed.

RESULTS

***raf*-Related Genes Expressed in Humans.** A human fetal liver cDNA library was screened at reduced stringency with a v-*raf* probe representing the kinase domain (a 675-bp *Xho* I–*Sst* II fragment; ref. 6). Strongly and weakly hybridizing plaques were picked, and the structures of their recombinant DNAs were analyzed by restriction mapping. Most of the isolates exhibited restriction maps similar to that of clone pHB2 (Fig. 1*a*) and were of the strongly hybridizing variety. When these clones were used as hybridization probes for restriction endonuclease-digested human DNA, bands corresponding to those expected for the c-*raf-1* locus (16) were detected. A sequence (pHD2, Fig. 1*a*) whose restriction map seemed unrelated to the c-*raf-1* gene was isolated from one of

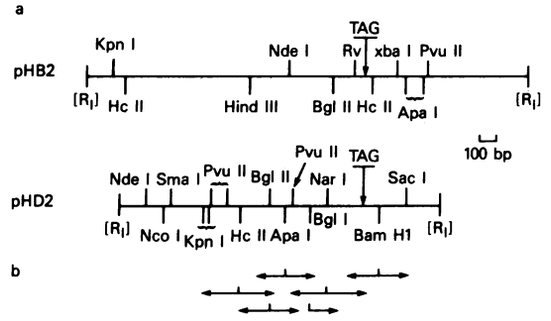

FIG. 1. (*a*) Restriction endonuclease maps of v-*raf*-related cDNA clones pHB2 and pHD2. *Eco*RI inserts from positive phage were subcloned in pBR322. Brackets indicate the site was introduced as a linker. The amber (TAG) translation termination codons (arrows) were used to align the sequences. (*b*) Strategy for sequencing pHD2. DNA sequence was determined by the method of Maxam and Gilbert (9) from labeled restriction site termini (vertical lines). Arrow lengths reflect number of nucleotides sequenced.

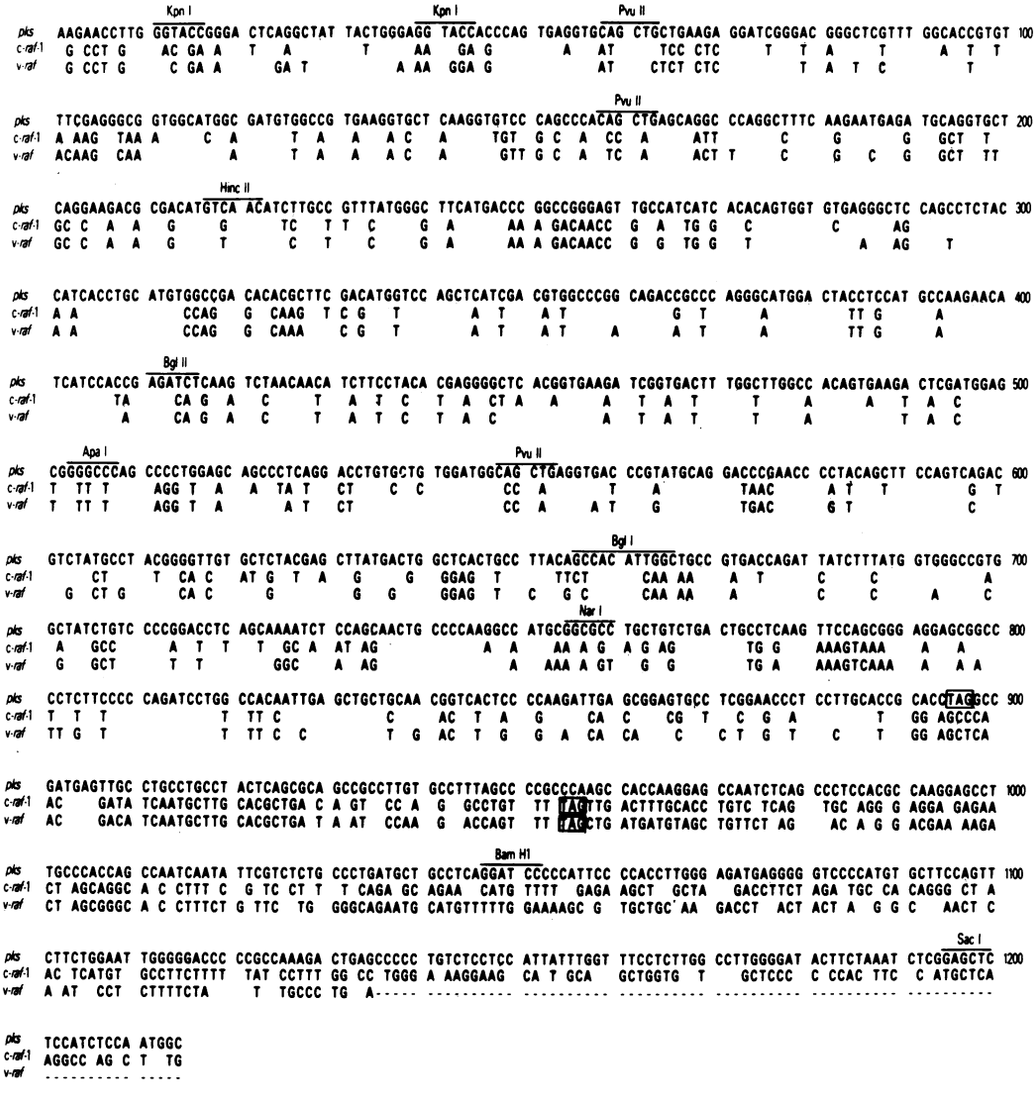

FIG. 2. Nucleotide sequence of *pks* compared to human c-*raf-1* and to v-*raf* sequences. Sequences other than *pks* are shown only where they differ from *pks*. Dashes indicate gaps; unique restriction endonuclease sites are underlined, and the amber termination codons are boxed. The c-*raf-1* sequence (16) begins at nucleotide 310 and the v-*raf* sequence (6) begins at nucleotide 126.

the weakly hybridizing plaques. To determine whether this sequence was closely related to the c-*raf-1* gene or the pseudogene c-*raf-2* (16), *Hin*dIII, *Pvu* II, *Eco*RI, and *Bam*HI digests of human DNA were blotted and hybridized at reduced stringency to a probe representing the central portion of clone pHD2 DNA. The result showed no bands that were characteristic of either the c-*raf-1* locus, located on chromosome 3, or the c-*raf-2* pseudogene sequence, located on chromosome 4 (17).

To demonstrate that sequences within clone pHD2 were related to the human c-*raf-1* gene, as well as to localize these similarities, heteroduplexes were constructed between plasmid DNAs of pHB2 (c-*raf-1*) and pHD2 and spread from a medium-stringency (50% formamide) hyperphase. These heteroduplexes showed an area of homology, ≈450 bp long, flanked by two regions of nonhomology. The location of the homologous region could be calculated, since the orientation of the pHB2 DNA sequence is known, as is the location of its translation-termination codon (16). Contour-length measurements of the substitution loops placed the beginning of the related portion of these molecules 0.80 and 0.44 kilobases (kb) from the 3′ end of the pHB2 and pHD2 clones, respectively. Thus, the similarity between these two genes appears to lie in that region of the c-*raf-1* sequence which encodes the kinase domain. To ascertain the exact relatedness of the pHD2 sequence to the known *raf* homologs, appropriate regions of this new gene, which we call *pks*, were sequenced.

Nucleotide and Predicted Amino Acid Sequence of *pks*. The DNA sequence of 1215 nucleotides representing part of the *pks* transcript was determined according to the strategy indicated in Fig. 1*b*. This was compared to the sequences of v-*raf* (6) and c-*raf-1* (ref. 17 and Fig. 2). The first 890 nucleotides of *pks* exhibit 70.5% and 72.0% homology with the DNA sequences of c-*raf-1* and v-*raf*, respectively. For comparison, c-*raf-1* and v-*raf* are 89.7% homologous over this same region. The DNA homology to the known *raf*-related genes ends at the translation-termination codon of *pks*, ≈250 nucleotides before the termination of homology between v-*raf* and c-*raf-1* (16). Comparison of the amino acid sequence specified by *pks* to those specified by human c-*raf-1* (16), murine v-*raf* (6), and avian v-*mht* (18, 19) (Fig. 3) reveals ≈75% homology beginning with amino acids initiating exon 4 of the human sequence (16) and ending with the termination codon of *pks*. The similarity is even more striking when one considers that at least 34 of the 71 amino acid differences between the two human sequences are conservative. Taking this into account, the carboxyl ends of these proteins are 88% homologous. The putative *pks* polypeptide, like all of the *src*-family oncogene products, contains a region (Arg-Ile-Gly-Thr-Gly-Ser-Phe-Gly-13 amino acids-Lys; amino acid positions 24–45) believed to be responsible for nucleotide (ATP) binding (20). The most highly conserved region within the *raf* gene subfamily correspond to the 50 amino acids (amino acid positions 114–163) that precede a domain analogous to that of the tyrosine acceptor region of v-*src* (ref. 21; amino acid positions 163–197). The homology of the *pks* sequence to the other *raf* genes is 96% in this region, and the two alterations are probably conservative amino acid changes. Each member of the *src*-family exhibits a unique carboxyl-terminal sequence, whereas the vertebrate homologs of at least one member (*raf*) terminate almost identically. The carboxyl end of the *pks* gene product appears truncated relative to the other members of the *raf*-subfamily, suggesting the unique nature of this gene. Like the other *raf*-subfamily members, *pks* has no tyrosine at the position corresponding to the tyrosine acceptor site of the tyrosine kinase-encoding oncogenes. Instead, *pks* has an alanine (amino acid position 169), whereas most of the other *raf*-subfamily members have serine residues (the protein encoded by the v-*raf* homolog in *Drosophila*, Draf1, has glutamic acid at this position; unpublished data). This suggests that if the activity of these proteins is regulated through phosphorylation of this region, then the common serine at position 167 may be the acceptor.

Expression of *pks*. The nucleotide and amino acid sequences of *pks* indicated that this gene is related to the c-*raf-1* gene. Previous work showed that the c-*raf-1* gene is expressed in mouse thymus (22) and in the activated B and T cells of patients suffering from systemic lupus erythematosus (SLE; ref. 23). To determine whether murine and human lymphoid tissues also contained mRNA homologous to the

```
pks      KNLGYRDSGY  YWEVPPSEVQ  LLKRIGTGSF  GTVFRGRWHG  DVAVKVLKVS  50
c-raf-1  RPR Q   S      IEA   M   ST    S        YK K          I   V
v-raf    RPR Q   S     KMEA   M   ST    S        YK K          I   V
v-mht    RPR Q   S      IEA   L   ST    S        YK K          I   V

pks      QPTAEQAQAF  KNEMQVLRKT  RHVNILPFMG  FMTRPGVAII  TQWCEGSSLY  100
c-raf-1  D   P   F    R   VA          L      Y   KDNL   V
v-raf    D   P   L    R   VA          L      Y   KDNL   V
v-mht    D   P   F    R   VA          L      Y   KDNL   V

pks      HHLHVADTRF  DMVQLIDVAR  QTAQGMDYLH  AKNIIHRDLK  SNNIFLHEGL  150
c-raf-1  K     QE K  Q F     I                      M
v-raf    K     QE K  Q F     I                      M
v-mht    K     QE K  Q F     I                      M

pks      TVKIGDFGLA  TVKTRWSGAQ  PLEQPSGPVL  WMAAEVTRMQ  DPNPYSFQSD  200
c-raf-1             S     S     QV    T S      P   I      N   F
v-raf               S     S     QV    T S      P   I      D   F
v-mht               S    ES     QV    T SI     P   I      S   F

pks      VYAYGVVLYE  LMTGSLPYSH  IGCRDQIIFM  VGRGYLSPDL  SKISSNCPKA  250
c-raf-1   S   I         E        NN              A        LYK
v-raf     S   I       A E   A    NN              A        RLYK
v-mht     S   I         E        NN              A        LYK

pks      MRRLLSDCLK  FQREERPLFP  QILATIELLQ  RSLPKIERSA  SEPSLHRT*  300
c-raf-1   K  QVA   V  KVK            SS        H      N T          AAH
v-raf    IK   VA   V  KVK            SS        H      N   P        AAH
v-mht     K   VA      KV             SS  A     H      N            ASH

pks
c-raf-1  TEDINACTLT  TSPRLPVF*
v-raf    TEDINACTLT  TSPRLPVF*
v-mht    TEDINSCTLT  ST-RLPVF*
```

FIG. 3. Comparison of the deduced amino acid sequences of the *raf* subfamily of kinases. Only the amino acids that differ from *pks* are shown. Overlined region represents the nucleotide binding region (20) and its reactive lysine (K). Single-letter abbreviations for amino acid residues are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

pks gene, distinguishable from the c-*raf-1* gene, RNA blots were probed with the appropriate sequences (Fig. 4). Murine thymus expresses a c-*raf-1*-specific mRNA of 3.4 kb. In contrast, the size of the *pks* mRNA is 2.7 kb. Assuming a poly(A) tail of 200 nucleotides and a 3′ untranslated region of 450 nucleotides (see Fig. 1), the maximal coding capacity of the *pks* gene is ≈680 amino acids (≈75 kDa). Normal peripheral blood mononuclear cells express very little c-*raf-1* or *pks* mRNA. Unique to the *pks* sequence is its extremely high expression in a patient with angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) (Fig. 4), a disease characterized by lymphoproliferative activation of B cells leading to the production of polyclonally derived autoantibodies. High *pks* mRNA expression also was found in the other AILD patient thus far examined. The lack of c-*raf-1* mRNA suggests that the proliferative imbalance associated with AILD is different than that exhibited by another autoimmune disease, SLE.

Two *raf*-Related Genes in the Human Genome. To determine the number of *pks* genes in the human genome, Southern blot analyses were performed on DNAs from several mouse–human somatic cell hybrids (Fig. 5). With the entire cDNA insert as a hybridization probe, four *Bgl* II restriction fragments (8.7, 3.6, 2.9, and 1.4 kb) were detected in human DNA, while mouse DNA showed only two such bands (5.3 and 2.1 kb). When several other restriction enzymes were used, human DNA consistently exhibited a more complex pattern than mouse DNA. Three patterns of segregation of *pks*-related fragments were observed in somatic cell hybrids, indicating that the 8.7-kb *Bgl* II fragment is located on a chromosome distinct from that which carries the other three *Bgl* II fragments (Fig. 5). To determine which cellular locus represented the *pks* gene, probes representing the 5′ and 3′ halves of the kinase domain, as well as the 3′ untranslated region of the *pks* cDNA clone, were hybridized to restricted human DNA under conditions of high stringency (Fig. 6). The three *Bgl* II fragments that segregated together and were unlinked to the 8.7-kb fragment all hybridized well

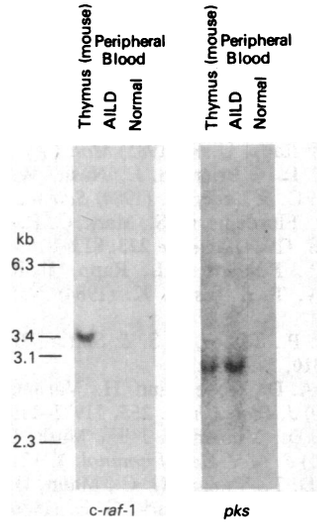

FIG. 4. Blot hybridization analysis of poly(A)$^+$ RNA (10 μg) isolated from murine and human lymphocytes. RNAs were obtained from normal mouse thymus or human peripheral blood mononuclear cells collected from an individual with the autoimmune disease AILD or from normal donors. Hybridization probe was a radiolabeled c-*raf-1* cDNA fragment obtained from pHB2 (0.85 kb, *Hin*dIII–*Xba* I) or a radiolabeled *pks* cDNA fragment obtained from pHD2 (0.64 kb, *Bgl* II–*Bam*HI). After washing under stringent conditions, blots were exposed overnight at −70°C to Kodak AR-2 film with an intensifying screen. RNAs from tobacco mosaic and brome mosaic viruses were used as size markers.

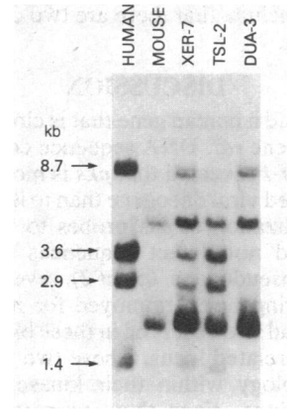

FIG. 5. Southern blot analysis of *pks* sequences in human–mouse somatic cell hybrids. High molecular weight DNA (10 μg) from human (WI-38) and mouse [L-M(TK$^-$)]fibroblasts and human–mouse hybrids (XER-7, TSL-2, and DUA-3) were digested with *Bgl* II, electrophoresed in a 0.7% agarose gel, transferred to nitrocellulose paper, and hybridized to a ^{32}P-labeled nick-translated *pks* cDNA fragment (1.85 kb, *Eco*RI). Low-stringency washes were used to facilitate hybridization to both murine and human sequences. Wild-type bacteriophage λ DNA digested with *Hin*dIII was used as size marker; sizes of the human DNA fragments are indicated at left.

to the *pks* probes. The 8.7-kb fragment hybridized poorly to the middle- and 3′-untranslated-region probes, and no weakly hybridizing bands were seen using the 5′-end probe. When the DNA was digested with *Bam*HI, a similar pattern was seen (i.e., the 8.2-kb fragment hybridizes weakly and segregates independently of the other fragments in somatic cell

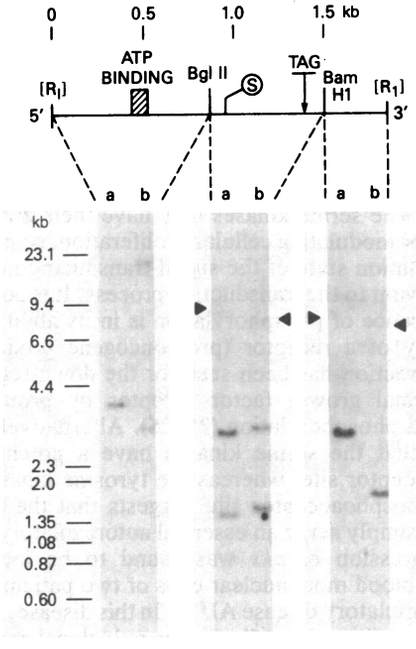

FIG. 6. Southern blot analysis of *pks* sequences detected in DNA from fresh human T cells, under high-stringency hybridization conditions. Schematic representation of the *pks* cDNA insert indicates the location of the ATP binding region, possible serine phosphoacceptor, and termination codon (TAG). Broken lines indicate the regions that give rise to the hybridization pattern shown. Arrowheads locate fragments that hybridize poorly to the probes. High molecular weight human DNA (obtained from fresh T cells) was digested with an excess of *Bgl* II (lanes a) or *Bam*HI (lanes b), blotted following electrophoresis, and hybridized to the nick-translated *pks* cDNA fragment probes. *Hin*dIII-digested wild-type λ DNA and *Hae* III-digested ΦX174 DNA were used as size markers.

hybrids). We conclude that there are two closely related *pks* genes in humans.

DISCUSSION

We have described a human gene that is closely related to the *src*-family oncogene *raf*. DNA sequence comparisons of *pks* to v-*raf* and c-*raf-1* revealed that *pks* is more homologous to the murine-derived viral oncogene than to its human homolog (c-*raf-1*). Hybridization of *pks* probes to Southern blots of human DNA did not detect sequences corresponding to c-*raf-1*, or its pseudogene (c-*raf-2*), even when the low hybridization stringencies employed for its initial isolation were used. Instead, we observed in these blots *pks* sequences as well as a *pks*-related locus. These two genes must share significant homology within their kinase domains and 3′ untranslated regions, since they were still detected after high-stringency washes of the filter. Since sequences derived from the 3′ untranslated region would be expected to diverge so rapidly within a gene family that related members should not be detected by hybridization to such a probe, it was surprising to find that both *pks* loci hybridized to the 3′ probe. Analysis of mouse–human cell hybrids and *in vitro* hybridization placed this *pks*-related sequence on human chromosome 7 very close to the centromere, whereas the *pks* gene has been localized to the X chromosome at Xp11.4 (unpublished data).

The predicted amino acid sequence of *pks* shows all the features characteristic of a *src*-family protein kinase, as well as the absence of a tyrosine acceptor site, which exemplifies both the *raf* and the *mos* oncogenes. The conservation of amino acid sequence suggests that *pks* may encode a serine/threonine kinase activity like that observed for *raf* (7). As suggested previously (6), v-*raf* may represent one of the more ancient members of the *src* family, one that evolved prior to the acquisition of tyrosine specificity. We may tentatively include *pks* and the *pks*-related gene in a subfamily of serine/threonine-specific kinases that includes *raf* and *mos* (24). Recombinants containing the kinase domain of c-*raf-1* transform NIH 3T3 cells (16). The capacity of non-tyrosine-specific kinases to transform susceptible cells leads one to speculate that all members of the *src* family may have a common cellular target, whose phosphorylation leads to neoplasia. The serine kinases may have their own separate pathway for modulating cellular proliferation, or perhaps the phosphorylation state of the signal-transducing moiety may not be relevant to the transduction process. It is possible that the significance of phosphorylation is in its ability to affect the activity of a receptor (protooncogene product); such ligand interaction has been seen for the down-regulation of the epidermal growth factor receptor by protein kinase C-mediated phosphorylation (25, 26). Alternatively, the observation that the serine kinases have a potential serine phosphoacceptor site, whereas the tyrosine kinases have a tyrosine phosphoacceptor site suggests that the kinase activity may simply serve an essential autoregulatory function.

The expression of *pks* was found to be increased in peripheral blood mononuclear cells of two patients with the immunostimulatory disease AILD. In this disease, B cells are abnormally activated, leading to the polyclonal expansion of these cells and their secretion of autoantibodies. That this may be associated with the abnormal *pks* expression in AILD is suggested by the repeated observation that no enhancement of mRNA expression was found in the patients' peripheral blood cells when c-*raf-1*, *fos*, *abl*, or *myc* probes were used (data not shown). We have, however, examined only two individuals thus far.

We speculate that proliferative responses in lymphoid cells may be associated with the *pks* and/or c-*raf-1* gene products, based on observations (unpublished) of high expression of one or the other gene in mitogen-activated normal B and T cells, in SLE-activated B and T cells (23), and in several B-cell lymphomas (both of the Burkitt and of the non-Burkitt type). In support of this hypothesis, we have observed that the greatly increased expression of c-*raf* RNA found in spleens of autoimmune mice (MRL *lpr/lpr*) is absent from congeneic mice carrying the *xid* locus (MRL *lpr/lpr* × *xid*) (22). The *xid* gene, an X-linked gene, retards the expression of murine lupus by impairing the maturation of splenic Lyb5$^-$ B cells, which are critical to autoantibody production (27). The localization of *pks* to the human X chromosome suggests that this gene may play a role in some of the X-linked immunodeficiency diseases.

This study was partly supported by Grants GM20454 and ACS-CD62 from the National Institutes of Health and the American Cancer Society, respectively, to T.B.S.

1. Stavnezer, E., Gerhard, D. S., Binari, R. & Balazs, I. (1981) *J. Virol.* **39**, 920–934.
2. Nau, M., Brooks, B., Battey, J., Sausbille, E., Gazdar, A., Kirsch, I., McBridge, O. W., Bertness, V., Hollis, G. & Minna, J. (1985) *Nature (London)* **318**, 69–73.
3. Schechter, A. L., Stern, D. F., Vaidyanathan, L., Decker, S. J., Drebin, J. A., Greene, M. I. & Weinberg, R. A. (1984) *Nature (London)* **312**, 513–516.
4. Cooper, C. S., Park, M., Blair, D. G., Tainsky, M. A., Huebner, K., Croce, C. & Vande Woude, G. F. (1984) *Nature (London)* **311**, 29–33.
5. Bishop, J. M. (1983) *Annu. Rev. Biochem.* **52**, 301–354.
6. Mark, G. E. & Rapp, U. R. (1984) *Science* **224**, 285–289.
7. Moelling, K., Heimann, B., Beimling, P., Rapp, U. R. & Sander, T. (1984) *Nature (London)* **312**, 558–561.
8. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
9. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–559.
10. Mark, G. E. & Rapp, U. R. (1984) *J. Virol.* **49**, 530–539.
11. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, R. J. (1979) *Biochemistry* **18**, 5294–5299.
12. Glisin, V. R., Crkvenjakov, R. & Byus, C. (1974) *Biochemistry* **13**, 2633–2637.
13. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
14. Seed, B. (1982) *Nucleic Acids Res.* **10**, 1799–1810.
15. Southern, E. M. (1973) *J. Mol. Biol.* **98**, 503–517.
16. Bonner, T. I., Kerby, S. B., Sutrave, P., Gunnell, M. A., Mark, G. & Rapp, U. R. (1985) *Mol. Cell. Biol.* **5**, 1400–1407.
17. Bonner, T. I., O'Brien, S. J., Nash, W. G., Rapp, U. R., Morton, C. C. & Leder, P. (1984) *Science* **223**, 71–74.
18. Kan, N. C., Flordellis, C. S., Mark, G. E., Duesberg, P. H. & Papas, T. S. (1984) *Science* **223**, 813–816.
19. Sutrave, P., Bonner, T. I., Rapp, U. R., Jansen, H. W., Patschinsky, T. & Bister, K. (1984) *Nature (London)* **309**, 85–88.
20. Kamps, M. P., Taylor, S. S. & Sefton, B. M. (1984) *Nature (London)* **310**, 589–592.
21. Levinson, A. D., Oppermann, H., Varmus, H. E. & Bishop, J. M. (1980) *J. Biol. Chem.* **255**, 11973–11980.
22. Mountz, J. D., Mushinshi, J. F., Mark, G. E. & Steinberg, A. D. (1985) *J. Mol. Cell. Immunol.* **2**, 121–131.
23. Boumpas, D. T., Tsokos, G. C., Mann, D. L., Eleftheriades, E. G., Harris, C. C. & Mark, G. E. (1986) *Arthritis Rheum.* **29**, 755-760.
24. Kloetzer, W. S., Maxwell, S. A. & Arlinghaus, R. B. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 412–416.
25. Cochet, C., Gill, G. N., Meisenhelder, J., Cooper, J. A. & Hunter, T. (1984) *J. Biol. Chem.* **259**, 2553–2558.
26. Friedman, B., Frackelton, Jr., A. R., Ross, A. H., Connors, J. M., Fujiki, H., Sugimura, T. & Rosner, M. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3034–3038.
27. Scher, I., Steinberg, A. D., Berning, A. K. & Paul, W. E. (1975) *J. Exp. Med.* **142**, 637–650.