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Additional member of the protein-tyrosine kinase family: The *src*- and *lck*-related protooncogene c-*tkl*

(cDNA cloning/nucleotide sequence/cell transformation)

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ABSTRACT **We report the isolation and nucleotide sequence of a 3.7-kilobase (kb) cDNA clone from chicken spleen corresponding to a previously undescribed member of the *src* family of protooncogenes. It encodes a protein with a C-terminal domain related to the src family of protein-tyrosine kinases (EC 2.7.1.112) and, among these, has most significant homology to the *lck* gene isolated from a murine leukemia virus-induced thymoma cell line. The gene is therefore referred to as c-*tkl* for cellular *t*yrosine *k*inase related to *l*ck. Analysis of genomic DNA reveals that c-*tkl* is a chromosomal locus distinct from c-*src* and c-*lck*. Furthermore, the size of c-*tkl* mRNA as well as its pattern of expression indicates that it is not the chicken homologue of *lck* but a different gene. A 3.8-kb transcript of the c-*tkl* gene, identical to the size determined for c-*src* mRNA, was observed in cultured chicken embryo fibroblasts and in chicken spleen and brain. In contrast, detection of a definite c-*src* mRNA signal with mRNA from spleen was not possible under the hybridization conditions employed when the 5′ end of v-*src* was used as the probe, and none of the 11 clones obtained from the cDNA library corresponded to a c-*src* transcript. Thus previous studies of c-*src* mRNA expression in spleen may have actually detected c-*tkl* transcripts.**

The protein-tyrosine kinase activity (EC 2.7.1.112) originally identified was associated with the viral transforming protein of the Schmitt–Ruppin strain of Rous sarcoma virus (1, 2). Later, similar kinase activities were found in the transforming proteins of other tumor viruses of chicken as well as of other species. To date, the products of the oncogenes v-*src* (1–3), v-*yes* (4), v-*fgr* (5), v-*fps* (6–8), v-*fes* (9), v-*abl* (10, 11), and v-*ros* (12) are known to exhibit tyrosine-specific protein kinase activity.

The provenance of these transforming proteins is cellular, since all of the viral transforming genes are closely related to cellular genes (13, 14). The cellular counterparts of viral oncogenes, termed protooncogenes, are generally expressed in very low amounts. Some of them are expressed only in very specific cells or at specific stages of development.

Apart from the protooncogene-encoded tyrosine kinase activities, whose function is unknown, cellular protein-tyrosine kinase activities are also associated with receptors for cellular growth factors (13), such as the epidermal growth factor receptor (15), the receptor for insulin (16), or the receptor for the platelet-derived growth factor (17).

Recently, an overlap between the families of protooncogenes and growth factor receptors was found: c-*erb* B (18) and c-*fms* (19) encode genetic information related to the epidermal growth factor receptor and the receptor for the mononuclear phagocyte growth factor (CSF-1), respectively. Thus tyrosine kinases of normal cells seem to play a key role in regulation of the growth and differentiation of cells.

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The level of tyrosine phosphorylation in normal cells is low (2). However, it is not known how many tyrosine-specific protein kinases exist in normal cells and how they exert their supposed regulatory functions. In an attempt to characterize the mRNA of c-*src*, the cellular homologue of the viral transforming gene v-*src*, and possibly to find more *src*-related genes that are expressed in certain cells, we analyzed expression of the gene in adult chicken tissues. We describe here the isolation and characterization of a *src*-related gene (c-*tkl*)§ from a chicken cDNA library, and we suggest that this gene likely corresponds to most of the actual mRNA detected in previous studies of c-*src* expression.

MATERIALS AND METHODS

Cells. Chicken embryo fibroblasts were prepared from 11-day-old SPF chicken embryos (SPAFAS) and maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum, in a humidified incubator at 5% CO_2.

Construction of a cDNA Library. Total RNA from adult chicken spleen (SPAFAS) was isolated by using the guanidine isothiocyanate/cesium chloride cushion technique (20). Polyadenylylated RNA was prepared by two cycles of binding to oligo(dT)-cellulose (21). The cDNA was synthesized according to a modification (22) of the Gubler and Hoffmann cDNA synthesis technique (23). In a subsequent step the cDNA was annealed to λgt10 arms (24) prepared as described and ligated with T4 DNA ligase (25).

Screening of the cDNA Library. The 0.8-kilobase (kb) *Pvu* II fragment of v-*src* was nick-translated to a specific activity of 2–8 × 10^5 cpm/ng and used at 1 × 10^6 cpm/ml to screen (26) approximately 5 × 10^5 λgt10 recombinant bacteriophage. Replica nitrocellulose filters were incubated at 42°C in a hybridization mix containing 10% dextran sulfate and 50% formamide as previously described (25). Filters were washed at 50°C in 0.2× SSC (30 mM NaCl/3 mM sodium citrate, pH 7.0). Autoradiography using intensifying screens was performed at −70°C for 1–2 days.

RNA Blot Hybridization Analysis. Samples (10 μg) of poly(A)$^+$ RNA prepared as described above were separated on 1% agarose/methylmercury gels (27) and transferred to nitrocellulose (28). As a probe, the 1.7-kb *Eco*RI fragment of *lck* (29, 30) or the probes indicated in the text, at 1 × 10^6 cpm/ml, were hybridized as described above.

Southern Blot Analysis. High molecular weight DNA was extracted from chicken spleen (20), digested with restriction endonucleases, electrophoresed in 1% agarose gels, transferred to nitrocellulose filters, and hybridized to radiolabeled probe.

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§This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03579).

Nucleotide Sequence Determination. Deletion fragments derived from the cDNA clone were generated with the exonuclease BAL-31 (31) and cloned in bacteriophages MP18 and MP19. The nucleotide sequence was determined by the dideoxy chain-termination method of Sanger *et al.* (32).

RESULTS AND DISCUSSION

Isolation of a *src*-Related Gene. Chicken spleen was used for the establishment of a cDNA library because the amount of mRNA yielding a positive signal with a *src*-specific probe had shown to be relatively high in that tissue (33). The library was screened with the 0.8-kb *Pvu* II fragment of v-*src* from the Schmidt–Ruppin strain of Rous sarcoma virus; this fragment encodes a part of the C-terminal catalytic domain of the kinase (34).

Eleven positive clones with insert sizes ranging from 1.3 to 3.7 kb (the latter was named clone C1) were obtained from 500,000 plaques screened. Restriction site analysis of these clones demonstrated that they had very similar restriction patterns. These patterns, however, differed from the pattern published for the v-*src* gene. They were also distinct from those known for the viral oncogenes v-*fgr* and v-*yes*.

To further characterize the cDNA clones, restriction enzyme digests of genomic DNA from chicken spleen were separated on agarose gels and probed by hybridization with all of the cDNA clones and with the v-*src* fragment. The latter revealed bands of 9.5, 4.9, and 2.2 kb when the DNA was cut with *Hin*dIII and *Eco*RI. When the same DNA was hybridized with the cDNA clones, bands of 18, 2.2, 1.2, 1.0, and 0.8 kb were visible, indicating that the cDNAs were derived from a locus that is not c-*src* (Table 1). A similar conclusion could be drawn from the results when *Bam*HI was used as restriction enzyme. This analysis also showed that the new clones were derived from a locus that is distinct from *lck*.

Blot hybridization analysis of the mRNA from chicken embryo fibroblasts, using the largest clone, C1, as a probe, showed a band of 3.8 kb (Fig. 1), which is identical in size to the c-*src* mRNA determined by Gonda *et al.* (33).

Structure of the mRNA of the *src*-Related Gene. The complete nucleotide sequence of C1 determined by the dideoxy chain-termination method (32) is shown in Fig. 2. The total length of the clone is 3712 nucleotides. A 1371-nucleotide open reading frame extends from its 5′ end and ends at an in-frame stop codon at position 1393. Since the ATG (Met) codon at position 22 matches with Kozak's rules for the initiation of translation (35) in that the position −3 is a purine and −2 and −1 are cytosine, this Met residue could be the start of a protein specified by the open reading frame

Table 1. Restriction analysis of genomic loci: Fragment sizes (kb) and hybridization intensities

C1 probe		v-*src* probe		*lck* probe
*Hin*dIII + *Eco*RI	*Bam*HI	*Hin*dIII + *Eco*RI	*Bam*HI	*Bam*HI
18 +++	11.2 +	9.5 +++	11 +	8.5 ++
2.2 +	4.8 ++	4.9 +	7.3 +++	4.7 +
1.2 +	2.7 +++	2.2 +	5.6 +	4.5 +++
1.0 +			5.2 +	2.8 ++
0.8 +			4.7 +	1.6 +
			3.6 +	
			2.6 ++	

Southern blotting experiments were carried out with 10 μg of chicken spleen DNA per lane and the nick-translated probes of the genes indicated. Hybridization was under medium-high stringency for C1 and v-*src*. For *lck*, low-stringency conditions (30% formamide, 37°C) were used, because the *lck* gene was derived from mouse. Hybridization intensities are indicated by + (very weak), ++ (weak), and +++ (strong).

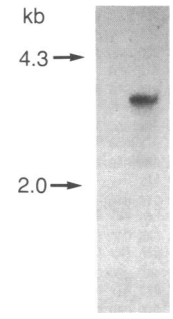

FIG. 1. Blot hybridization analysis of RNA from chicken embryo fibroblasts. A 10-μg sample of poly(A)$^+$ RNA was analyzed. The blot was hybridized with ^{32}P-labeled cDNA of clone C1 (1×10^6 cpm/ml) for 18 hr, washed with 0.2× SSC at 50°C, and exposed for 48 hr.

with a relative molecular mass of about 51,596. However, as discussed below, protein sequence alignments strongly suggest that approximately 150 bases derived from the 5′ end of the coding sequence of this gene are missing from the cDNA clone C1. The 3′ region has a length of 2319 base pairs (bp), which encompasses a signal for the poly(A) addition at position 3683 and a short poly(A) sequence at its very end. Within this region, sequence information for two short proteins is found, the first containing 169 amino acids (nucleotides 1810–2317) and the second containing 93 amino acids (nucleotides 3172–3451). However, in both cases, the consensus sequence for eukaryotic initiation sites (35) is not observed.

The Gene in C1 Is Related to the Tyrosine Kinase Family. A computer homology search of the sequence of C1 revealed that its putative translation product is highly related to the catalytic domains of tyrosine kinases (Table 2). This homology is most striking for the products of *lck* (29, 30) and *lyn* (37) and less for those of *yes*, *src*, and *syn* (36). Since the homology encompasses the entire catalytic domain of the related tyrosine kinases listed in Table 2, we supposed that C1 encodes a protein with tyrosine kinase activity and hence named it tkl (*t*yrosine *k*inase related to *l*ck).

This idea was, furthermore, strongly supported by a comparison of the catalytic domains of src (43), yes, lyn, and lck with tkl (Fig. 3*A*). The consensus sequence of the ATP-binding region (37), Gly-Xaa-Gly-Xaa-Xaa-Gly (first box in Fig. 3*A*) is perfectly conserved in tkl at amino acid positions 199–204. Furthermore, a lysine residue is present at position 220 (arrow in Fig. 3*A*) in tkl, which is supposed to be involved in the binding of ATP (38) in other tyrosine kinases. Interestingly, a tyrosine site at position 341 (second box in Fig. 3*A*) in tkl could represent an autophosphorylation site like Tyr-416 in src.

In contrast to the catalytic domain described above, much less homology with the other tyrosine kinases is found in the N-terminal 200 amino acids of tkl (Table 2). Generally, this region is believed to determine the specificity of individual tyrosine kinases with respect to their protein substrates (30). Again tkl shows the highest degree of homology with lck (77%) (Table 2). The homology between lck and tkl extends upstream and downstream of the first AUG codon in tkl (Fig. 3*B*). Furthermore, in contrast to other tyrosine kinases of the src family, the first methionine is followed by serine and proline residues, rather than by glycine and serine/cysteine, the glycine being the attachment site for myristic acid in these proteins. It therefore appears that the C1 cDNA clone is missing the 5′ end of the *tkl* transcript. Comparing the size of the C1 clone (3.71 kb) with the observed size of the tkl mRNA (3.8 kb) and the alignment shown in Fig. 3*B*, we estimate that we are missing at least 100–150 bp of sequence.

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CCC CTG GTG TCC TAC GAG GCC ATG TCT CCG CCG TGC TCC CCG CTG CAA GAC AAG CTC GTG GTG GCC CTG TAT GAC TAT GAA CCC ACT CAC     90
                          Met Ser Pro Pro Cys Ser Pro Leu Gln Asp Lys Leu Val Val Ala Leu Tyr Asp Tyr Glu Pro Thr His
GAT GGG GAC CTG GGA CTT AAG CAG GGC GAG AAG CTG CGC GTC CTG GAA GAG AGC GGA GAG TGG TGG AGG GCG CAG TCG CTC ACC ACG GGC    180
Asp Gly Asp Leu Gly Leu Lys Gln Gly Glu Lys Leu Arg Val Leu Glu Glu Ser Gly Glu Trp Trp Arg Ala Gln Ser Leu Thr Thr Gly
CAG GAG GGT TTG ATC CCC CAC AAC TTC GTG GCC ATG GTG AAC AGC CTG GAG CCG GAG CCG TGG TTC TTC AAG AAC CTC AGC CGC AAG AAC    270
Gln Glu Gly Leu Ile Pro His Asn Phe Val Ala Met Val Asn Ser Leu Glu Pro Glu Pro Trp Phe Phe Lys Asn Leu Ser Arg Lys Asn
GCG GAG GCC AGG CTG CTG GCG TCG GGC AAC ACG CAC GGC TCC TTC CTC ATC CGG GAG AGC GAG ACC TCT AAA GGC TCC TAC TCG CTG TCA    360
Ala Glu Ala Arg Leu Leu Ala Ser Gly Asn Thr His Gly Ser Phe Leu Ile Arg Glu Ser Glu Thr Ser Lys Gly Ser Tyr Ser Leu Ser
GTG AGG GAC TTC GAC CAG AAC CAG GGC GAG ACA GTG AAG CAC TAC AAG ATT CGC AAC ATG GAC AAC GGG GGG TAC TAC ATC TCC CCC CGG    450
Val Arg Asp Phe Asp Gln Asn Gln Gly Glu Thr Val Lys His Tyr Lys Ile Arg Asn Met Asp Asn Gly Gly Tyr Tyr Ile Ser Pro Arg
GTC ACC TTC AGC AGC CTG CAC GAG CTG GTG GAG TAT TAC TCA AGC AGC TCG GAT GGG CTG TGC ACC CGC CTT GGC AAG CCC TGC CGG ACG    540
Val Thr Phe Ser Ser Leu His Glu Leu Val Glu Tyr Tyr Ser Ser Ser Ser Asp Gly Leu Cys Thr Arg Leu Gly Lys Pro Cys Arg Thr
CAG AAG CCG CAG AAG CCG TGG TGG CAG GAC GAG TGG GAG GTG CCA CGA GAG TCG CTG AAG CTG GTG GAG AAG CTG GGA GCC GGG CAG TTT    630
Gln Lys Pro Gln Lys Pro Trp Trp Gln Asp Glu Trp Glu Val Pro Arg Glu Ser Leu Lys Leu Val Glu Lys Leu Gly Ala Gly Gln Phe
GGA GAA GTC TGG ATG GGC TTC TAC AAC GGC CAC ACC AAG GTA GCC ATC AAG AAC CTG AAG CAG GGC AGT ATG TCC CCC AGC GCC TTC CTG    720
Gly Glu Val Trp Met Gly Phe Tyr Asn Gly His Thr Lys Val Ala Ile Lys Asn Leu Lys Gln Gly Ser Met Ser Pro Ser Ala Phe Leu
GCC GAG GCC AAC CTG ATG AAG AAC CTG CAG CAC CCA CGG CTG GTG CGG CTC TAC GCT GTG GTG ACC AAG GAG CCC ATC TAC ATC ATC ACA    810
Ala Glu Ala Asn Leu Met Lys Asn Leu Gln His Pro Arg Leu Val Arg Leu Tyr Ala Val Val Thr Lys Glu Pro Ile Tyr Ile Ile Thr
GAG TAC ATG GAG AAG GGC AGC CTG GTG GAC TTC CTC AAG ACC TCA GAG GGC ATC AAG CTC AGC ATC AAC AAA CTT CTG GAC ATG GCC GCA    900
Glu Tyr Met Glu Lys Gly Ser Leu Val Asp Phe Leu Lys Thr Ser Glu Gly Ile Lys Leu Ser Ile Asn Lys Leu Leu Asp Met Ala Ala
CAG ATT GCT GAA GGC ATG GCC TTC ATC GAA GCC AAG AAC TAC ATC CAC CGT GAC CTG CGG GCT GCC AAC ATC CTC GTG TCG GAG GCC CTG    990
Gln Ile Ala Glu Gly Met Ala Phe Ile Glu Ala Lys Asn Tyr Ile His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val Ser Glu Ala Leu
TGC TGC AAA ATC GCT GAC TTC GGG CTG GCG CGC CTC ATC GAG GAC AAC GAA TAC ACA GCA CGA GAA GGG GCT AAA TTC CCC ATC AAG TGG   1080
Cys Cys Lys Ile Ala Asp Phe Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Glu Gly Ala Lys Phe Pro Ile Lys Trp
ACA GCA CCG GAG GCT ATC AAT TAC GGC ACG TTC ACC ATC AAG TCT GAC GTC TGG TCC TTT GGC ATC CTG CTC ACT GAG ATT GTT ACC TAC   1170
Thr Ala Pro Glu Ala Ile Asn Tyr Gly Thr Phe Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Ile Val Thr Tyr
GGC CGG ATC CCG TAT CCA GGG ATG ACC AAC CCC GAG GTG ATC CAG AAC CTG GAG CGC GGC TAC CGC ATG CCG CAG CCC GAC AAC TGC CCG   1260
Gly Arg Ile Pro Tyr Pro Gly Met Thr Asn Pro Glu Val Ile Gln Asn Leu Glu Arg Gly Tyr Arg Met Pro Gln Pro Asp Asn Cys Pro
CAG GAG CTG TAC GAA CTG ATG ATG CAG TGC TGG AAG GAG CAG CCT GAG GAG CGG CCC ACC TTC GAG TAC ATG AAG AGC GTG CTG GAG GAC   1350
Gln Glu Leu Tyr Glu Leu Met Met Gln Cys Trp Lys Glu Gln Pro Glu Glu Arg Pro Thr Phe Glu Tyr Met Lys Ser Val Leu Glu Asp
TTC TTC ACC GCC ACC GAG GGA CAA TAC CAG CAG CAG CCG TGA GGCCCCGC GCAGCCTGGG GGCACGCCGG GCCCCCCAGA CTCCACGAGC ATCCCATGAG   1450
Phe Phe Thr Ala Thr Glu Gly Gln Tyr Gln Gln Gln Pro End
CACCGCCTGG CAGTGAAGAT TTCGTGCTTC CCAAAGAGCT TTGCCCCTGA ATGGTGACAG CCTAACTGAA GTGCCCGTGG ACTGGTGTGC GCTGGGGACG TGTCGCACGC   1560
CGCCGTGCCC ATGGCTGTCA CCTCCCCGCC TTGCCCCACA GCCCCCACAC ACAGCACGGA GCTCCCAGCG TGGGGCGGCC CCTCACGGCC TCGGCCGGGC TCAGCAGAG   1670
GCCATGGGCC CTCACGGCTT GGGCAGAGCG GTGCCCCTCA GATGGAGGCC ATGGGCCGAG GCCACGGGGT TGGGGTCACT GCCAACCAGG CCCGGCTCTC CCTGCTGCCA   1780
GTCTCTCTTG CCCGCAGTGG GGGTAGGAAA TGCCTGGGAC TGCAGCGCGG GCCGCAGCCC ACAGGGACCG GGGCAAGGCC GTGGGCTCGA GGCCCTGCTG CTGCCACCCC   1890
CGGGGCCCGC GCTCCGCCAC GCCAGGCCCC GCTGACCCGT GCCGGCACTG CCCGGCCTTC CAGCCCCGCT CGCCCCAGGC CCGGGCCTCA GGCACTGGGA AGCCCCGTGC   2000
CGCGCCCCCC CGGCACGTTG GGAATGGTTC GGCCCCACCT GTTCCCCCCC TCCGCGCAGA ATGGTTCCGC CCCGCCACCT GGCCCCGCGC CCCGGTGGTG CAGCCCGCCG   2110
CTCCGCCTAG CCTGCTGGCT CCGCCGCGCC GCCGCCTCGA CTTCTCATTG GACGGCGGGC TGGAAGGGGT GATGTTGAAA CTATTACTAT GGCCAGGGGC ACCCATGAAG   2220
CCCCACAGGA TCCGGATGAC CCACAACCTA CTGCTGAACT ACGGCCTTTA CAGGAAGATG GAGATATATC GCCCTCACAA GGCGAACGGG AGGAGATGAC CAAGTACCAC   2330
AGTGATGACT ACATCAAATT CCTGAGATCC ATCCGCCCAG ACAACATGTC TGAGTACAGC AAGCAGATGC AAAGATTTAA CGTCGGGGAG GACTGCCCTG TGTTCGATGG   2440
GCTGTTTGAG TTCTGTCAGC TCTCTGCTGG AGGCTCCGTT GCCAGCGCTG TGAAGCTGAA CAAGCAACAG ACAGATATTG CTGTGAATTG GGCAGGAGGC CTTCACCACG   2550
CTAAGAAGTC GGAGGCTTCT GGCTTCTGTT ATGTCAACGA TATTGTCCTG GCTATCTTGG AGCTCTTAAA GTATCACCAG AGGGTCCTGT ATATTGACAT TGATATTCAC   2660
CATGGAGATG GTGTGGAGGA AGCCTTCTAT ACCACAGACC GTGTGATGAC CGTGTCCTTT CATAAGTATG GAGAGTACTT CCCAGGAACA GGGGACCTGC GGGACATTGG   2770
TGCAGGCAAA GGCAAATACT ATGCTGTCAA CTATCCCCTC CGGATGGGAT TGATGATGAG TCCTACGAGG CAATATTCAA GCCGGTGATA TCTAAAGTGA TGGAGACATT   2880
CCAGCCTAGT GCAGTTGTCC TGCAGTGCGG GTCGGATTCT CTGTCCGGGG ACAGGCTGGG TTGTTTTAAT CTGACCATCA AAGGTCATGC CAAGTGTGTG GAGTTTGTCA   2990
AAAGTTTTAA TTTGCCTATG CTGATGCTGG GAGGAGGTGG CTATACGATC CGCAACGTGG CCAGATGCTG GACCTATGAG ACTGCTGTGG CTTTGGACAT GAGATCCCAA   3100
ATGAGCTCCC ATATAATGAC TATTTTGAGT ACTTCGGACC AGACTTTAAG CTGCACATCA GTCCCTCAAA CATGACCAAC CAGAATACCA ATGAGTATCT CGAGAAGATC   3210
AAGCAGCGTC TCTTTGAGAA TCTGCGCATG CTGCCTCATG CCCCTGGCGT CCAGATGCAG CCAATTCCTG AGGATGCTGT TCAGGAAGAC AGTGGGGATG AAGAAGAAGA   3320
AGATCCTGAG AAGCGCATTT CAATCCGCAA TTCTGATAAG AGAATATCCT GTGATGAAGA ATTCTCTGAC TCTGAAGATG AAGGGGAAGG AGGGCGCAAA AATGTGCCAA   3430
CTTTAAGAAG GCCAAGCGTG TGAAAACAGA GGAGGAAAAG GAGGAGGAGG AAGAAGAAGG ATGAGAAAGA AGAGGAAAAA GGCAAAAGAG GAGAAAGGCC GAACCAAGGG   3540
GTGAAGGAAG AGACAAAATC CACCTAAGAT GGCTGCAGCT GGACAGTACC TGTCAGTGCA TAGTTATCGT AGGTTAGCTT CTCTGGTTGC CTCCCATTCC ACAGGATTTA   3650
TATTTTATAT ATGTTTCTGT ATATATTTCT ATATAAATAT ATAAATGACT TGAAAAAAAA AA                                                        3712
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FIG. 2. Total nucleotide and amino acid sequence of the clone C1. The longest open reading frame, which generates a 457-amino acid protein, begins at position 22. The putative translation product of this reading frame is noted above in the three-letter code. Nucleotides are numbered on the right.

Transcription of *tkl* and Related Genes in Chicken Tissues and Cell Lines. The relationship between *tkl*, *lck*, and *src* and the pattern of expression of *tkl* were studied by blot hybridization analysis of RNA derived from chicken spleen and brain and from chicken embryo fibroblasts. When spleen mRNA was hybridized with ^{32}P-labeled *tkl* under reduced stringency (Fig. 4) a strongly hybridizing 3.8-kb fragment and a very weakly hybridizing 2.2-kb fragment (clearly visible only in the original autoradiogram) were observed. In contrast, the *lck* probe detected a fragment at 2.2 kb and a very weakly hybridizing fragment at 3.8 kb. This result suggests that *lck* and *tkl* cDNAs are derived from two different mRNA species, in agreement with Southern blot data reported in Table 1. Furthermore, *lck* (isolated from murine cells) was previously found to be expressed exclusively in mouse lymphocytes (29), while *tkl* is expressed in chicken spleen (Fig. 4) and brain (data not shown) and in chicken embryo fibroblasts (Fig. 1). It should be noted, however, that we did observe a weakly hybridizing 2- to 2.2-kb band in blots from chicken brain mRNA when the blots were probed under low stringency with *lck*.

As it appears that tkl and lck are encoded by distinct chromosomal loci, tkl also does not appear to correspond to the chicken homologue of the newly transcribed gene *hck* (39): (*i*) it displays weaker amino acid sequence homology with tkl than does lck (Fig. 3*A*); (*ii*) in contrast to *tkl*, *hck* does not appear to be expressed in brain; and (*iii*) the *hck* probe detects a message of 2.1 kb in human lymphocytes, whereas the *tkl* probe detects a message of about 4 kb (data not shown).

Table 2. Relationship at the amino acid level of the protein coded for by the open reading frame of clone C1 to known tyrosine-specific protein kinases and related genes

Gene of homologous protein (origin)	Region of homology, amino acids	% amino acid identity	% identity of N termini*
fes (FeSV)	246–594	40.5	<30
gag-fps (PRC II)	263–518	42.8	<30
ros (avian)	145–364	43.4	<30
fps (Fujinami ASV)	603–858	43.2	<30
fms (FeSV)	770–942	43.7	<30
abl (Abelson MuSV)	5–372	47.2	<30
src (fruit fly)	27–303	51.3	<30
fgr (FeSV)	151–539	61.2	36
yes (avian)	73–524	62.9	48
src (chicken)	87–528	63.6	50
src (RSV)	87–511	63.5	50
syn (human)	89–497	64.4	51
lyn (human)	67–557	70.9	59
hck (human)	52–458	74.8	62
lck (mouse)	57–471	81.7	77

Note that the region of homology includes the kinase domain. MuSV, murine sarcoma virus; FeSV, feline sarcoma virus; ASV, avian sarcoma virus; PRC II, strain of ASV; RSV, Rous sarcoma virus.
*Comparison with tkl amino acid residues 1–200.

A

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c-tkl     152/  LVEYYSSSSDGLCTRLGKPCRTQKP----Q-KPWWQDEWEVPRE
c-lck     206/  ..RH.TNA.....K.SR..Q.....-----.-....E.......
c-lyn     207/  MIKHYQKQA....R..EKA.ISP..----.-...DK.A..I...
c-src     227/  ..A...KHA....H..TNV.P.S..-----.TQGLAK.A..I...
c-syn     228/  ..QH..ERAA...C..VV..HKGM.RLTDL-SVKTK.V..I...
c-slk     228/  ..QH..ERAA...C..VV..HKGM.RLTDL-SVKTK.V..I...
v-yes     227/  ..KH.REHA....HK.TTV.P.V..-----.TQGLAK.A..I...

c-tkl           SLKLVEKLGAGQFGEVWMGFYNGHTKVAIKNLKQGSMSPSAFL
c-lck           T.....R.......|.....Y........V.S........D...
c-lyn           .I...KR.......|.....Y..NS....V.T..P.T..VQ...
c-src           ..R.EV..Q.C...|.....TW..T.R....T..P.N...E...
c-syn           ..Q.IKR..N....|.....TW..N......T..P.T...ES..
c-slk           ..Q.IKR..N....|.....TW..N......T..P.T...ES..
v-yes           ..R.EV..Q.C...|.....TW..T......T..L.T.M.E...

c-tkl           AEANLMKNLQHPRLVRLYAVVT-KEPIYIITEYMEKGSLVDFLK
c-lck           .......Q.............-Q...........N........
c-lyn           E......T...DK.........RE..........A....L....
c-src           Q..QV..K.R.EK..Q.....S-E.....V....S....L....
c-syn           E..QI..K.K.DK..Q.....S-E.....V....N....L....
c-slk           E..QI..K.K.DK.LQ.....S-E.....V.H..N....L....
v-yes           Q..QI..K.R.DK..P.....S-E.....V..F.T....L....

c-tkl           TSEGIKLSINKLLDMAAQIAEGMAFIEAKNYIHRDLRAANILV
c-lck           .PS....NV.................EQ..............
c-lyn           SD..G.VLLP..I.FS........Y..R...........V..
c-src           GEM.KY.RLPQ.V.......S...YV.RM..V..........
c-syn           DG..RA.KLPN.V.....V.A...Y..RM.........S.....
c-slk           DG..RA.KLPN.V.....V.A...Y..RM.........S.....
v-yes           EG..KF.KLPQ.V.......D...Y..RM.............

c-tkl           SEALCCKIADFGLARLIEDNEYTAREGAKFPIKWTAPEAINYG
c-lck           .DT.S.....|.....|.....................
c-lyn           ..S.M.....|.....|.....|.....Q....F.
c-src           G.N.V..V..|.....|.....|Q.............AL..
c-syn           GNG.I.....|.....|.....|Q.............AL..
c-slk           GNG.I.....|.....|.....|Q.......S...DRAL..
v-yes           GDN.V.....|.....|.....|Q.............AL..

c-tkl           TFTIKSDVWSFGILLTEIVTYGRIPYPGMTNPEVIQNLERGYR
c-lck           .....................H.....................
c-lyn           C..............Y......K.....R..AD.MTA.SQ...
c-src           R..............LT.K..V.....V.R..LDQV.....
c-syn           R..............L..K..V.....N.R..LEQV.....
c-slk           R..M...........LL.K..V.....N.R..LEQV.....
v-yes           R..............L..K..V.....V.R..LEQV.....

c-tkl           MPQPDNCPQELYELMMQCWKEQPEERPTFEYMKSVLEDFFTAT
c-lck           .VR.D.......H...L....R..D....D.LR...D......
c-lyn           ..RVE...D...DI.KM....KA.....D.LQ...D..Y...
c-src           ..C.PE...S.HD..C...RRD.........LQAF...Y..S.
c-syn           ..C.QD..IS.H...IH...KD.........LQ.F...Y....
c-slk           ..C.QD..IS.H...IH...ND.........LQ.F...Y....
v-yes           ..C.QG...S.H...KL...KD.D.......IQ.F...Y...A

c-tkl           EGQYQQQP
c-lck           .....P..
c-lyn           ........
c-src           .P...PGENL
c-syn           .P...PGENL
c-slk           .P...PGENL
v-yes           .PSGY
```

B

```
c-lck   1/MGCVCSSNPEDDWMENIDVCENCHYPIVPLDSKISLPIRNGSEVRDPLVT
c-tkl   1/-------------------------------------------------...S
c-hck   1/...MK.KFLQVGGNTFSKTETSASPHCPVYVPDPTST.KP.PNSHNSNTP

c-lck     YEGSLPPASPLQDNLVIALHSYEPSHDGDLGFEKGEQLRILEQSGEWWKA
c-tkl     ..AMS..C.....K..V..YD...T......LKQ..K..V..E.....R.
c-hck     ----GIREAGSE.II.V..YD..AI.HK..S.Q..DQMVV..E.......

c-lck     QSLTTGQEGFIPFNFVAKANSLEPEPWFFKNLSRKDAERQLLAPGNTHGS
c-tkl     .........L..H....MV........PPL.....N..AR...S......
c-hck     R..A.RK..Y..S.Y..RVD...T.E....GI..............ML..
```

FIG. 3. Comparison of the various domains of known tyrosine-specific kinases with tkl. (*A*) Catalytic domains of different protein-tyrosine kinases. The amino acid sequence of a putative tkl protein is shown in one-letter code on the top line of each group. Residues that are identical to those in the tkl sequence are denoted with dots. The numbering of amino acids reflects the translation initiation codon of each protein. The first boxed sequence, beginning at residue 336, specifies a conserved region suggested to be involved in nucleotide binding. A lysine residue, which belongs to the ATP-binding region as well, is located at position 220 (indicated by an arrow). The second box shows the autophosphorylation site of pp60$^{c\text{-}src}$. (*B*) N termini of hck, lck, and tkl.

The size of the *tkl* mRNA we observe is identical to the size previously assigned to c-*src* (33). If both mRNAs were present in nearly comparable amounts in chicken spleen, some c-*src* clones should have been obtained, since the library was screened with a *src* probe. However, all of the 11 clones obtained corresponded to *tkl* cDNAs, suggesting that the c-*src* mRNA is significantly less abundant in this tissue.

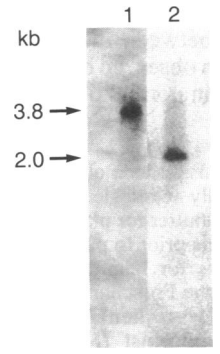

FIG. 4. Blot hybridization analysis of mRNA from chicken spleen. For lane 1, 10 μg of mRNA from chicken spleen was analyzed. The blot was hybridized with ^{32}P-labeled cDNA clone C1 (1×10^6 cpm/ml for 12 hr), washed with 0.2× SSC at 50°C, and exposed for 18 hr. For lane 2, the blot with 10 μg of mRNA from chicken spleen was hybridized under low-stringency conditions (30% formamide, 37°C) with ^{32}P-labeled cDNA clone *lck* (1×10^6 cpm/ml for 12 hr), washed with 5× SSC at 37°C, and exposed for 18 hr.

To evaluate the expression of c-*src* versus c-*tkl*, blots of chicken spleen mRNA and total RNA were first probed with the v-*src* *Pvu* II fragment and subsequently with cDNA corresponding to the N terminus of tkl. With mRNA (Fig. 5) and total RNA a significantly hybridizing 3.8-kb band was seen only with the 5′ end of c-*tkl* (0.8 kb, *Pst* I) as probe. Although probing with the *Pvu* II fragment of v-*src*, which contains the kinase domain, did result in some background radioactivity (Fig. 5, lane 1), the blot was clear when the 5′ end of *src* (*Bam*HI–*Sma* I) was used (data not shown). When the same blot was counter-probed with the 5′ end of c-*tkl*, again a 3.8-kb band was found (lane 2).

Our results demonstrate that in spleen the abundance of a 3.8-kb c-*src* mRNA is at or below the limit of detection. These data agree with the findings of Golden *et al.* (40), who were unable to precipitate the pp60$^{c\text{-}src}$ protein from spleen lysates by using a monoclonal antibody raised against v-src. The data also give one explanation why we did not obtain any c-*src*-specific cDNA clones from the spleen library. Previous experiments demonstrating a relatively high expression of c-*src* in spleen (33) may thus have been due to cross-hybridization of the v-*src* probe with c-*tkl* mRNA.

Preliminary data from our laboratory indicate that in embryonic chicken brain also the c-*src* message may be significantly less abundant than the c-*tkl* message. In future experiments it will thus be very important to use only the 5′ termini rather than the kinase domains of *src* and *tkl* as probes

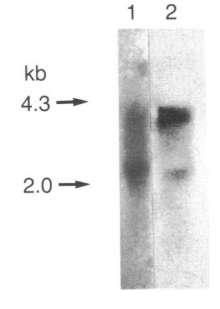

FIG. 5. Blot hybridization analysis of mRNA from chicken spleen. An 8-μg sample of mRNA was analyzed. The blot was hybridized to a ^{32}P-labeled *Pvu* II fragment of v-*src* (1×10^6 cpm/ml) for 18 hr, washed with 0.2× SSC, and exposed for 2 days (lane 1). After the probe had been removed the filter was hybridized again with ^{32}P-labeled cDNA of clone C1 under the same conditions (lane 2). For C1 exposure time was 18 hr.

and/or to use high stringency conditions for hybridization in order to distinguish between the messages of these genes. Generally, as has been observed by other authors also (41, 42, 44), c-*src* expression in normal tissues may be fairly restricted.

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