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Isolation of Chicken Cellular DNA Sequences with Homology to the Region of Viral Oncogenes That Encodes the Tyrosine Kinase Domain

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A library of chicken genomic DNA was screened for sequences that could hybridize to a cloned DNA fragment containing the transforming gene (v-*fps*) of Fujinami sarcoma virus. In addition to c-*fps*, two unique chicken cellular DNA sequences were isolated that hybridized weakly to v-*fps*. These sequences hybridized with many other viral oncogenes encoding tyrosine kinases. Sequence analysis of the region where homology was detected revealed a region that is highly conserved among the tyrosine kinases both at the nucleotide and amino acid levels. Although we were unable to detect expression of either chicken cellular DNA sequence in a variety of avian tissues, the data suggest the existence of additional members of the tyrosine kinase gene family. Screening genomic libraries for sequences that hybridize weakly to functional regions of other genes may prove useful for the isolation and characterization of additional members of other gene families.

Since the discovery that the *src* gene of Rous sarcoma virus encodes a gene product that possesses a protein kinase activity that is specific for tyrosine (5, 20), a number of other viral transforming genes have also been shown to encode tyrosine-specific protein kinases (2, 19). Sequence analysis has revealed a conserved domain in the region of these genes where the enzymatic activity has been mapped (19). These genes include *fps* of Fujinami sarcoma virus (37), *yes* of Y73 avian sarcoma virus (24), *src* of Rous sarcoma virus (36), *ros* of UR2 avian sarcoma virus (31), *erb-B* of avian erythroblastosis virus (49), *fgr* of Gardner-Rasheed feline sarcoma virus (28), *fms* of Susan McDonough feline sarcoma virus (16), and *abl* of Abelson murine leukemia virus (34). Other viral oncogenes that do not possess a tyrosine-specific protein kinase activity also share amino acid homology with the tyrosine kinase genes. Among these are *mos* of Moloney murine sarcoma virus (33, 44), *rel* of reticuloendotheliosis virus (41), and *mil* of MH2 (22, 42). In addition to the viral oncogenes that have been characterized, other proteins have been isolated that possess tyrosine kinase activity. These include the growth factor receptors for epidermal growth factor (4, 9), for platelet-derived growth factor (11, 17), for insulin-like growth factor-1 (21), and for insulin (10, 18, 23, 43). TPK75 is a tyrosine-specific protein kinase that was isolated from rat liver (48), and NCP94 is a tyrosine-specific protein kinase that was immunoprecipitated from bone marrow and other cells with an antibody raised against a peptide isolated from the tyrosine kinase domain of *fps* (13).

The ability to immunologically identify additional tyrosine specific protein kinases (13) suggests that tyrosine kinase genes could be detected by hybridization with DNA probes isolated from the conserved kinase region. Using this approach, we isolated two chicken cellular DNA sequences that likely represent additional members of the tyrosine kinase gene family.

Isolation of chicken cellular DNA sequences with homology to the tyrosine kinase domain of *fps*. We previously described the cloning of c-*fps* from a chicken genome library (14a). Foster, M. Shibuya, and H. Hanafusa, Cell, in press). In addition to c-*fps*, three other chicken cellular DNA clones were isolated by screening a chicken genomic library (7, 45) with a v-*fps* probe. The probe used was pBR-F04 (Fig. 1C) (38) which contains a portion of v-*fps* that shares homology with several viral tyrosine kinase genes (19). The level of hybridization between these clones and pBR-F04 was very weak relative to that observed between c-*fps* and pBR-F04, but still well above background at the moderately stringent conditions used (Fig. 1B). Using the Benton and Davis method for the screening of genomic libraries (1) allows hybridization to relatively high levels of cloned DNA. Thus, it is possible to isolate clones that hybridize weakly to the probe being used. The three chicken genomic clones that hybridized weakly with pBR-F04 (λTKR24, λTKR11, and λTKR16) are shown in Fig. 2. Two of them (λTKR24 and λTKR11) appear to be overlapping based on restriction mapping and hybridization experiments. None of the three genomic clones hybridized with DNA from other regions of v-*fps* (Fig. 1B).

Hybridization of cloned cellular sequences to other tyrosine kinase genes. Since pBR-F04 contains the region of v-*fps* that shares homology with other tyrosine kinases (37, 38), the possibility existed that these cellular DNA sequences (represented by λTKR24, -11, and -16) would hybridize with other tyrosine kinases or tyrosine kinase-related genes. To test this possibility, plasmid DNAs containing cloned viral oncogene sequences with tyrosine kinase regions were hybridized with nick-translated DNA fragments isolated from λTKR24 and λTKR16. All plasmids were cut with restriction enzymes such that oncogene sequences were released from vector DNA sequences. The DNA fragments were separated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with the labeled DNA fragments from λTKR24 and λTKR16. Table 1 summarizes the results of this analysis. DNA from both λTKR24 (representing λTKR11 as well) and λTKR16 hybridized weakly with several cloned DNAs coding for tyrosine kinases. Hence, these cellular

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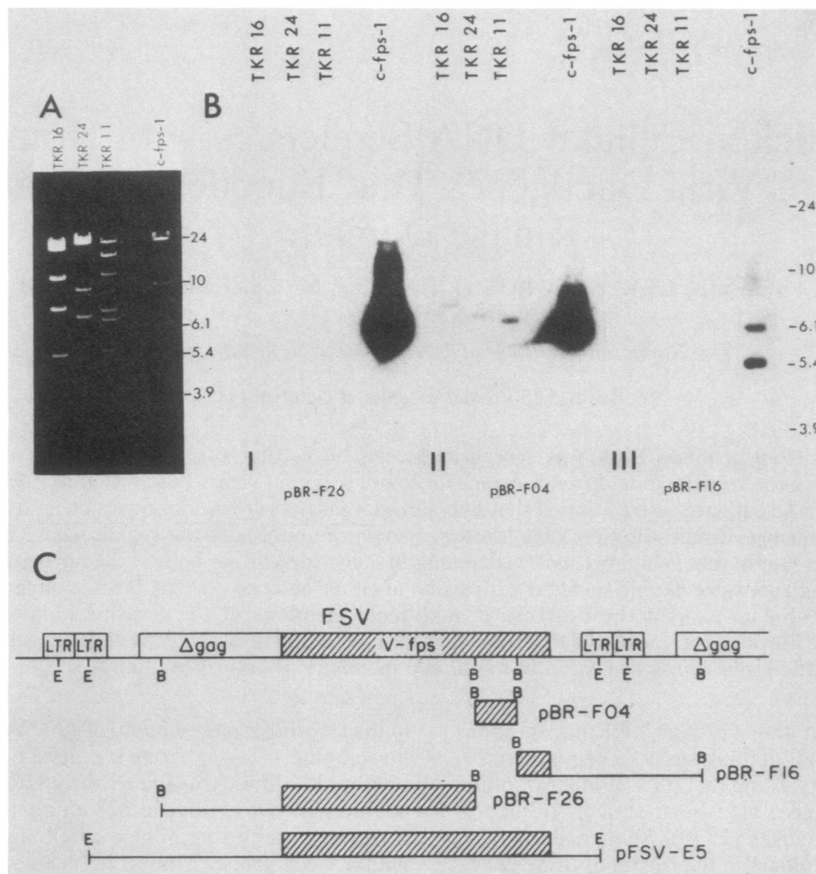
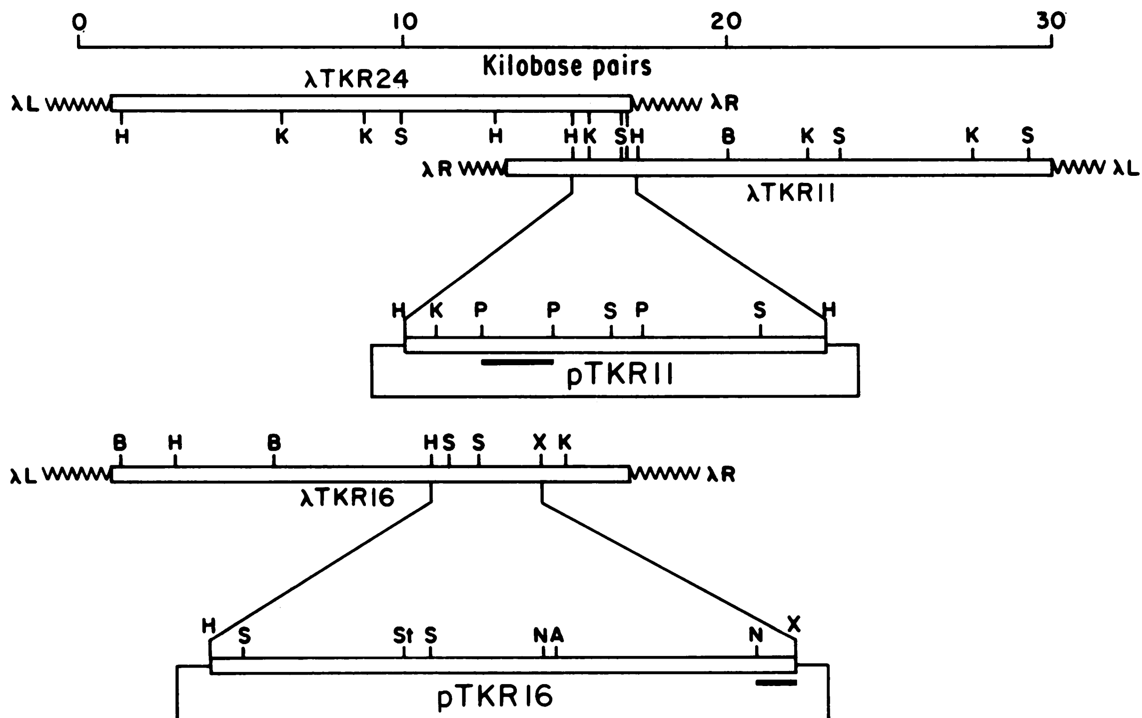


FIG. 1. Hybridization of v-*fps* DNA with chicken cellular DNA clones. The λ DNA clones were digested with restriction enzymes and electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose (40), and hybridized with v-*fps* probes under conditions of moderate stringency (3× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 1× Denhardt, 50% formamide, 50 mM Tris hydrochloride [pH 7.4], 0.02 mg of yeast tRNA per ml, 0.02 mg of sheared denatured salmon sperm DNA per ml, 1 mM EDTA; 37°C; 48 h). The filters were washed two times for 30 min each in 0.1% sodium dodecyl sulfate–0.1× SSC at 50°C. An ethidium-stained gel before transfer is shown in panel A. λTKR16 was digested with both *Bam*HI and *Xho*I; λTKR24 was digested with *Sst*I; λTKR11 was digested with *Kpn*I; and λ c-*fps*-1 (14a) was digested with *Xho*I (See Fig. 2 and Foster et al. [in press] for restriction maps of these sites). Autoradiographs are presented in panel B. In panel C the Fujinama sarcoma virus (FSV) DNA sequences that were used as probes for the analysis of λ DNA clones are shown. The cloning of pBR-F26, pBR-F04, pBR-F16, and pFSV-E5 was previously described (14a, 37). They contain 1.9, 0.4, 0.3, and 2.6 kilobases of v-*fps*-specific sequence, respectively. pBR-F04 contains the portion of v-*fps* that shares homology with other tyrosine kinases (19, 31, 38). The shaded regions indicate v-*fps*-specific sequences. Abbreviations for restriction endonuclease sites are: B, *Bam*HI; E, *Eco*RV. LTR, Long terminal repeat.



DNA sequences are designated TKR for tyrosine kinase-related sequences. λTKR24 and λTKR16 DNA sequences did not hybridize with nonconserved regions of tyrosine kinase genes (e.g., 5' or 3' *fps*, Fig. 1B; 5' v-*fes* or 3' v-*erb-B*, Table 1). In addition to the hybridization data presented in Table 1, weak hybridization was observed between v-*fps* and v-*yes*, between v-*src* and v-*yes*, and between TKR24 and TKR16 under the same hybridization conditions (data not shown). The level of hybridization was comparable to that observed between *fps* and the two TKR clones. Hybridization has also been observed between v-*fps* and v-*ros* (30).

Sequence analysis of the TKR homology region. The region of homology to the v-*fps* gene within the TKR clones was further delimited by Southern blot analysis of restriction endonuclease-digested DNA from the subclones (pTKR11 and pTKR16) shown in Fig. 2. For pTKR11, the homology was contained between two *Pvu*II sites as shown in Fig. 2, and for pTKR16, the homology was located between an *Nco*I site and the *Xho*I site (Fig. 2). Both of these restriction fragments were cloned into M13 (26) and sequenced by the procedure of Sanger et al. (35). The sequences obtained are shown in Fig. 3 and are compared with that of v-*fps* and the other avian tyrosine kinase genes. A highly conserved nucleotide sequence is seen between nucleotides 50 and 95 in Fig. 3. This region most likely accounts for the observed hybridization.

Using the DNA sequence from the strand that contained the homology to *fps* and the other tyrosine kinase genes, the amino acid sequences in three frames were examined. Open reading frames in both sequences were found that were homologous to other tyrosine kinase genes. In Fig. 4 the amino acid sequences of the two TKR sequences are compared with those of other genes known to encode a tyrosine kinase or have an amino acid sequence that is related to the tyrosine kinases. The very highly conserved regions are boxed. Both of the TKR clones contain open reading frames that are highly homologous to the boxed regions. The percentage of homology with the various genes compared in Fig. 4 is summarized in Table 2.

The amino acid sequence of TKR16 sharply diverges upstream from amino acid 14 (Fig. 4) which corresponds to nucleotide 57 (Fig. 3). This could be explained by a potential splice acceptor site (27) at nucleotide 55 in the DNA sequence (Fig. 3). Similarly, the TKR11 sequence diverges downstream from about amino acid 60 (nucleotide 182). There are two potential splice donor sites (27) at nucleotides 185 and 211 before a TGA stop codon (Fig. 3). Both of the splice donor sites are upstream from a highly conserved region that is absent in pTKR11 (Fig. 4). These data suggest that the coding sequences of both TKR sequences are interrupted with introns.

Expression of TKR sequences. RNA was isolated from a

TABLE 1. Hybridization of TKR clones to other tyrosine kinase or tyrosine kinase-related viral oncogenes[a]

Viral oncogene	Clone	Reference	Relative hybridization to:	
			TKR24/TKR11	TKR16
Tyrosine kinases				
fps	pBR-F04	38	+++	+++
fes (5')	pPst3	12	−	−
fes (3')	pPst4	12	−	++
src	pSRA2	6	++	++
ros	pros	30	+++	+++
yes	pY73[b]		+++	+++
abl	pAB3sub3	15	++	+
fgr	pGRFeSV	29	−	+
fms	pSM3	8	++	+
erb-B (3')	perb2[c]		−	−
erb-B (5')	perb5[c]		++	ND[d]
Tyrosine kinase related				
mos	pc-mos[e]		−	−
rel	pEcoRel	3	−	−
raf	pv-raf	32	+	+

[a] All clones containing the indicated viral oncogene sequences were digested wih restriction enzymes to liberate completely the oncogene sequences from pBR322 vector sequences. The digested DNA was then run on a 1% agarose gel transferred to nitrocellulose, and probed with nick-translated TKR DNA (isolated from λ DNA clones to avoid contamination with pBR322). For the TKR24/TKR11 probe, the 7-kilobase *Sst*I λ TKR24 fragment that hybridized with pBR-F04 (Fig. 1B, panel II) was isolated, and for the TKR16 probe, the 8-kilobase *Bam*HI to *Xho*I fragment that hybridized with pBR-F04 was isolated from λTKR16. Hybridizations were carried out under the same moderately stringent conditions used in Fig. 1. The relative hybridization was estimated based on the degree of hybridization of the TKR sequences with v-*fps* (Fig. 1B, panel II, for a comparison of the hybridization of TKR sequences with v-*fps* and c-*fps* sequences with the v-*fps*). The level of hybridization of v-*fps* with the TKR sequences was given a value of +++; barely detectable hybridization of the TKR sequences was given a value of +; and intermediate levels of hybridization were given a value of ++. The values are not intended to be rigorously quantitative at this low level of hybridization but rather to point out reproducible differences in the pattern of hybridization of the two TKR sequences to the various tyrosine kinase or tyrosine kinase-related DNAs.

[b] pY73 was constructed by liberating Y73 sequences from a λ DNA clone (24) with *Sst*I, ligating, cutting with *Bam*HI, and inserting into the *Bam*HI site of pBR322.

[c] perb5 contains the region of *erb-B* that is homologous to the tyrosine kinases. It contains a *Bam*HI containing this region cloned into this site in pBR322 (46, 49). perb2 is directly 3' to this region and does not contain the region of *erb-B* that is homologous to other tyrosine kinases. It contains a downstream *Bam*HI to *Eco*RI fragment (46, 49).

[d] ND, Not determined.

[e] pc-mos was obtained from G. Vande Woude. This clone has not been published. Oncogene sequences were liberated according to his instructions and restriction data. pc-mos was cut with *Ava*I and *Hin*dIII.

FIG. 2. Isolation of chicken cellular DNA sequences homologous to v-*fps*. A chicken genomic library (45) was screened for sequences homologous to v-*fps* DNA (pBR-F04 was used as a probe; Fig. 1) by the technique of Benton and Davis (1). Five clones were isolated: two that hybridized strongly as previously reported for the c-*fps* gene (14a), and three that hybridized weakly. The conditions for hybridization are given in the legend to Fig. 1. The weakly homologous λ DNA clones were grown up by standard techniques (25), and restriction enzyme cleavage sites were mapped. Hybridization with DNA representing the entire v-*fps* sequence (pFSV-E5 in Fig. 1) limited the region of homology to v-*fps* to the expanded portions of the λ clones. These regions of the λ DNA clones that hybridized with v-*fps* DNA were subcloned into pBR322. A *Hin*dIII fragment from λTKR11 containing the portion of the cloned DNA that hybridized with *fps* was cloned into the *Hin*dIII site of pBR322 to generate pTKR11. A *Hin*dIII to *Xho*I fragment from λTKR16 that hybridized with *fps* was cloned into pBR322 cut with *Hin*dIII and *Sal*I to generate pTKR16 (*Sal*I and *Xho*I have compatible sticky ends). The regions of pTKR11 and pTKR16 that hybridize with *fps* were further localized to the regions underlined with the heavy bars. Enzyme abbreviations are as follows: A, *Ava*I; B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; N, *Nco*I; P, *Pvu*II; S, *Sst*I; St, *Stu*I; X, *Xho*I. L and R refer to the left and right arms of the λ DNA clones, respectively.

```
               10         20         30         40         50         60
tkr11   CTGGTGGCAT GTTGCGTGGC ATTGCAGCAG GCATGAACTA CCTGGCTGAT ATGAACTACG
tkr16   -AT-GACAGA -C--T-ACAA CAG-----GC TTGAAGTTGT TTACTTCTC- T--C-G-G-A
fps     TGATCAAG-- -A--GAGAAT GCC--G--G- -----G-G-- -----AAAGC -A-C---G-A
ros     TCTTG-AT-- A-GCTTG-AT ---TGCAA-- -TTGTGT--- TT-A-AGA-A ---CGT-T-A
yes     -G-TG-A--- -GCTGC-CAG -----T-AT- -----GCT-- -A-T-AAAGA ---------A
src     TC-TC-AT-- -GCTGC-CAG ------T-C- -----GC--- TGT--AAAGA -----A----
erbB    T-CTCAA-TG --GTGTGCAG ------AAG- -A-------- -----AGG-A CGTCG-CT-G

               70         80         90        100        110        120
tkr11   TGCACCGGGA CCTGGCTGCC CGCAACATCC TGGTCAACAG CAACCTGGTC TGCAAGGTCT
tkr16   -C---A---- ---------T --T--T---- -CT-GTCAGA ---TAAC--G GT---AA---
fps     -C---A---- ---------- ------TG-- ----G-CAGA A--GAACAC- CTG--AA--A
ros     -A---A---- ---------T ------TG-- -T--GTCTGA G--G-AATAT G-G-GCTG--
yes     -C---A---- T--CCGG--A GC-------- -T--AGGAGA ---T--T--G --T--AA-AG
src     -------A-- ----CGG--G GC-------- ----GGGGGA G--------G --------GG
erbB    -------T-- ---T------ A-G---G-G- -T--T--G-C TCCA-AACAT GTG--AA--A

              130        140        150        160        170        180
tkr11   CCGACTTCGG CCTCTCCCGT TTCCTGGAGG ATGACACCTC TGATCCCACT TACACCAGCG
tkr16   GT--T--T-- -T-GG-T--A
fps     GC-----T-- GGATGT-GCG GCAGGA-GA- GATGGTGTCT ATGC-T-CAC GGGGGGCAT-
ros     --CGAG-G-T AAAGATTG-- GATT-T-GAC T--C--GAGA -ATCTAT-AA AATGATTA-T
yes     -A-------- T---G-AA-G --AA-A---- -CA-T-GATA CAC-G-G-GG C-AGGAGCTA
src     -T-------- G--GG-A--C C--A-C---- -CA--GAG-A CACAG-ACGG C-AGGTGC-A
erbB    -A-----T-- G--GG-AAAG C-G--T-G-- CA--TGAGAA G--GTATCAC GCAGAGG-A-

              190        200        210        220        230        240
tkr11   CACTGGTAAA GCTGCTTTTC GACAGCAGGA GTGATGGGTG GGGAGGAAAG GAGAGTGAGG
fps     A-GCA-ATCC C-GTGAAA-G ---T--CCCC -A-GCTCTGA ATT-C-GCT- -TACAGCTC-
ros     A-AGGAA--G AGGAGAAGG- CTACT-CCTG TCAGAT--AT --CTCCTG-A AGCCTCATT-
yes     A-T-TCC--T TAAATGGACT -CTCCAGAAG CA-CATT--A T--TC-GTTT ACA-TCA--T
src     AGT-CCCC-T CAA-TGGACA -C-CC-GA-G CA-CCCTC-A T--CC-GTTC ACC-TCA--T
erbB    GCAA---TCC TA-TAAA-GG ATGGCATT-G AGTCAATTTT ACACC---TT T-T-C-C-TC

              250        260        270        280        290        300
tkr11   GAGAAGTCTC CTGTTGATGT TAAGAATGCT TTTCTCAAGT GCCAACAATG GAATAATGTG

              310        320        330        340        350        360
tkr11   CCAAGTGGAA GTATCAATCA CATGAATGAA GGAAGGTCTT CTCTTGCAAG TTTGTGCCCA

              370
tkr11   GCTATTCCAG CAG
```

FIG. 3. Nucleic acid sequence of TKR11 and TKR16 and comparison with the sequence of other avian tyrosine kinases in the conserved region. The regions of pTKR11 and pTKR16 that hybridized with *fps* DNA (Fig. 2) were sequenced as described in the text. The homologous regions of the two sequences are shown here and compared with the corresponding sequences of *fps* (37), *ros* (31), *yes* (24), *src* (36), and *erb-B* (49). The sequences were aligned according to the amino acid homologies shown in Fig. 4 and those reported previously (19, 31). Where the nucleotides are identical, a dash is substituted for the nucleotide. A potential splice acceptor site for TKR16 is underlined at nucleotide 55, and two potential splice donor sites for TKR11 are underlined at nucleotides 186 and 211.

variety of chicken tissues by the methods described by Maniatis et al. (25). This poly(A)-selected RNA was subjected to agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with radioactively labeled TKR DNA as described previously (47). pTKR11 and pTKR16 (Fig. 2) were used as probes to test for the expression of the TKR sequences. We were unable to detect significant levels of expression of either TKR sequence in any of several tissues examined (leg muscle, intestine, lung, spleen, liver, kidney, bone marrow, and brain). The lack of expression in liver and bone marrow suggests that the sequences reported here do not represent the tyrosine kinases recently characterized from these tissues by immunological and biochemical techniques (13, 48).

Conservation of TKR sequences. Chromosomal DNA isolated from several sources was digested with *Hin*dIII, run on an agarose gel, transferred to nitrocellulose (40), and probed with pTKR11 and pTKR16. Hybridizing fragments of different sizes were found in all of the avian and mammalian DNAs examined (Fig. 5). In the mammalian DNAs, it appeared that both TKR sequences hybridized to the same band. Thus, both TKR sequences are apparently fairly well conserved among the avian species and possibly in mammals as well. Upon long exposure (Fig. 5A), lower-level hybridization to several other bands could be detected in all of the DNAs, suggesting that there are other TKR sequences in addition to those reported here.

It is possible that the TKR sequences represent highly

```
TKR11      1          G G M L R G I A A G M N Y L A D M N Y V H R D L A A R N I
TKR16      1                    Q Q A A L E V V Y F S L Q C I H R D L A A R N I

fps     1028    K K L I K M M E N A A A G M E Y L E S K H C I H R D L A A R N C
ros      357    T D L L D I C L D I C K G C V Y L E K M R F I H R D L A A R N C
yes      645    P Q L V D M A A Q I A D G M A Y I E R M N Y I H R D L R A A N I
src      361    P Q L V D M A A Q I A S G M A Y V E R M N Y V H R D L R A A N I
erbB     231    S QYL L N W C V Q I A K G M N Y L E E R R L V H R D L A A R N V
mil      177      Q L I D I A R Q T A Q G M D Y L H A K N I I H R D M K S N N I
fgr      498    P Q L V D M A A Q V A E G M A Y M E R M D Y I H R D L R A A N I
fms     1248    R D L L H F S S Q V A Q G M A F L A S K N C I H R D V A A R N V
abl      457    V V L L Y M A T Q I S S A M E Y L E K K N F I H R D L A A R N C
mos      204    G K C L K Y S L D V V N G L L F L H S Q S I L H L D L K P A N I
Ins R   1109    Q E M I Q M A A E I A D G M A Y L N A K K F V H R D L A A R N C
BOV-PK   139    P H A R F Y A A Q I V L T F E Y L H S L D L I Y R D L K P E N L

                                                                  *
TKR11     30    L V N S N L V C K V S D F G L S R F L EDDTSD P T Y T S A L V   K L
TKR16     25    L L S D N N V V K I C D F G L A R

fps     1060    L V T E K N T L K I S D F G M S R Q E E      D G V Y A S T G G M K QI
ros      389    L V-S C S R V V K I G D F G L A R D I Y KN D Y     Y R K R G E G L L
yes      677    L V G D N L V C K I A D F G L A R L I E      D N E Y T A R Q G A K F
src      393    L V G E N L V C K V A D F G L A R L I E      D N E Y T A R Q G A K F
erbB     264    L V K T P Q H V K I T D F G L A K L L G  A DEK E Y H A E G G   K V
mil      208    F L H G G L T V K I G D F G L A T V K S      R W S G S Q Q V E Q P TG
fgr      530    L V G E R L V C K I A D F G L A R L I E      D N E Y N P R Q G A K F
fms     1280    L L T S G R V A K I G D F G L A R D I M    N D S N Y I V K G N A R L
abl      489    L V G E N H L V K V A D F G L S R L M T    G D T   Y T A H A G A K F
mos      236    L I S E Q D V C K I S D F G C S Q K L Q      D L R G     R Q A S P P
Ins R   1141    M V A H D F T V K I G D F G M T R D I Y ET D Y     Y R K G G K G L L
BOV-PK   171    L I D Q Q G Y I Q V T D F G F A K R V K      G R T W T L C G       T

TKR11     62    L F D S R S D G W G G K E S E G E V S C O

fps     1083    P V K W T   A P E A L N Y G W Y S S E S D V W S F G I L L W E A F
ros      428    P V R W M   A P E S L I D G V F T N H S D V W A F G V L V W E T L
yes      709    P I K W T   A P E A A L Y G R F T I K S D V W S F G I L L T E L V
src      425    P I K W T   A P E A A L Y G R F T I K S D V W S F G I L L T E L T
erbB     297    P I K W M   A L E S I L H R I Y T H Q S D V W S Y G V T V W E L M
mil      241    S I L W M   A P E V IRMQDSN P F S F Q S D V Y S Y G I V L Y E L M
fgr      562    P I K W T   A P E A A L F G R F T I K S D V W S F G I L L T E L I
fms     1313    P V K W M   A P E S I F D C V Y T V Q S D V W S Y G I L L W E I F
abl      521    P I K W T   A P E S L A Y N K F S I K S D V W A F G V L L W E I A
mos      266    H I G G T - A P E I L K G E I A T P K A D I Y S F G I T L W Q M T
Ins R   1173    P V R W M   A P E S L K D G V F T T S S D N W S F G V V L W E I T
BOV-PK   200    P E Y L     A P E I I L   S K G Y N K AVD W W A L G V L I Y E M A
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FIG. 4. Amino acid sequence of TKR11 and TKR16 and comparison with the sequence of other tyrosine kinases in the conserved region. The amino acid sequences of TKR11 and TKR16 were determined from the sequence data presented in Fig. 3. The sequences have been aligned to give the maximum homology with other genes sharing homology in this region as previously described (19, 31). The sequences used for comparison are as follows: the *fps* gene of Fujinami sarcoma virus (37), the *ros* gene of UR2 avian sarcoma virus (31), the *yes* gene of Y73 avian sarcoma virus (24), the *src* gene of the Pr-C strain of Rous sarcoma virus (36), the *erb-B* gene of avian erythroblastosis virus (49), the *mil* gene of MH2 avian carcinoma virus (22, 42), the *fgr* gene of Gardner-Rasheed feline sarcoma virus (28), the *fms* gene of Susan McDonough feline sarcoma virus (16), the *abl* gene of Abelson murine leukemia virus (34), the *mos* gene of Moloney murine leukemia virus (33, 44), the insulin receptor (Ins R) (10, 43), and the bovine cyclic AMP-dependent protein kinase (Bov-PK) (39). Boxes have been drawn around the highly conserved regions. The asterisk indicates the major site of tyrosine phosphorylation for several of the viral oncogenes. The dash in the *ros* sequence represents an insert of the amino acids SEKQYG at position 391 (31). The dash in the *mos* sequence represents an insert of the sequence YTHQ at position 270 (44).

TABLE 2. Comparison of the homology of TKR sequences with other related genes at the amino acid level[a]

Gene	% Homology with:	
	TKR11	TKR16
fps	50	63
ros	44	78
yes	60	74
src	62	63
erb-B	43	63
mil	37	56
fgr	54	67
fms	45	70
abl	49	67
mos	33	44
Ins R	43	48
Bov-PK	23	37

[a] The amino acid sequences of TKR11 and TKR16 were compared with the amino acid sequences of the other related gene sequences shown in Fig. 4 through the region of homology, and the percentage of homology was determined. In the case of TKR11, the region of comparison includes amino acids 1 to 52; for TKR16 it includes amino acids 15 to 41.

diverged pseudogenes. However, the homology with many tyrosine kinase genes (Table 1), the apparent presence of introns (Figs. 3 and 4), and the extent of conservation of these sequences in other species (Fig. 5) makes this possibility unlikely. It is also unlikely that these sequences encode a previously identified tyrosine kinase since the TKR sequences do not show particularly strong homology with any of the known tyrosine kinases (Fig. 4; Tables 1 and 2). It is likely that the unique genomic sequences reported here represent additional tyrosine kinases that differ from those already reported. We cannot be sure that these sequences encode tyrosine-specific protein kinases since they also share homology with genes that do not encode a tyrosine kinase. Of particular interest in this regard are the highly conserved amino acids LAARN (beginning with amino acid 24 for TKR11 in Fig. 4). All of the tyrosine kinases contain either this sequence or the closely related sequence LRAAN. All the related proteins that phosphorylate serine or threonine rather than tyrosine (*mos*, *mil*, Bov-PK) are different in this region (Fig. 4). Whether this difference is important for the specificity of protein kinases for substrate is not known. However, both of the TKR clones have a sequence common to the tyrosine kinases.

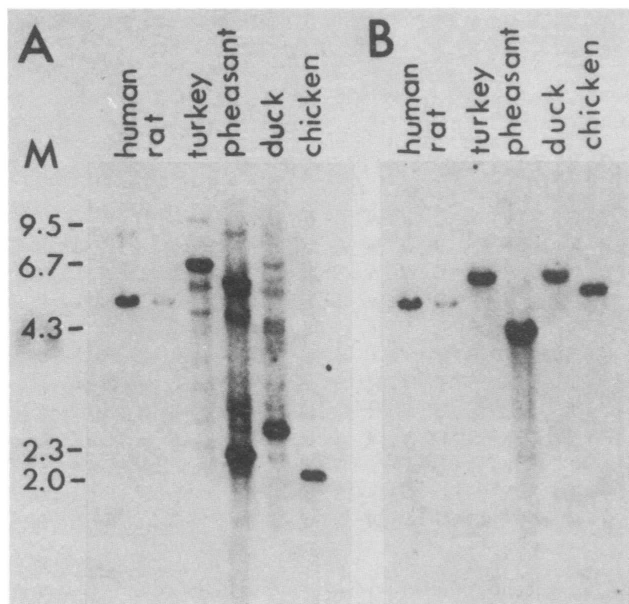

FIG. 5. Conservation of the TKR sequences among other species. Chromosomal DNA was isolated from a variety of avian and mammalian sources. The DNAs were digested with *Hin*dIII, subjected to agarose gel electrophoresis, transferred to nitrocellulose and probed with pTKR11 (A) or pTKR16 (B) as described previously (14). The DNAs were isolated from the livers of Peking ducks, ring-necked pheasants, and turkeys and from chicken embryo fibroblasts, HeLa cells (human), and Rat-1 cells. Lane M, *Hin*dIII-cut λ DNA markers.

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