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SHORT REPORT

Characterization of the human CSK locus

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The CSK-gene encodes an intracellular protein-tyrosine kinase (PTK). In contrast to members of the *src*-family, an autophosphorylation site corresponding to Tyr^{416}, as well as the equivalent of the regulatory Tyr^{527} in p60$^{c\text{-}src}$ are missing in the amino acid sequence deduced from the gene. CSK phosphorylates other members of the *src*-family of tyrosine kinases at their regulatory carboxy-terminus. By regulating the activity of these kinases, CSK may play an important role in cell growth and development. Here we describe the structure of the human CSK gene. The entire coding region spans a genomic distance of only 4.9 kb. It encompasses 12 exons ranging between 66 and 220 bp in size. The introns between coding exons vary between 76 and 920 bp in length. An exon coding for the 5′-untranslated region of CSK is separated from the first coding exon by an intron of more than 6400 bp. Based on comparisons of sequence homologies within the catalytic domains, the intracellular PTKs are divided into the *src*-family, the *fes/fer*- and the *abl/arg*-group. The genomic structure of four members of the SRC-family revealed nearly identical exon/intron boundaries within the catalytic domain of this family. They differ from those described for FES. Comparing the genomic structure of CSK with the exon/intron organisation of both, it is obvious that the exon/intron boundaries are in common either with those of the SRC-type or the FES boundaries. This intermediate exon/intron structure of CSK between FES and the SRC-family agrees with the position of CSK in a phylogenetic tree based on sequence homology within the kinase domain.

The *src*-family of proto-oncogenes encodes PTKs, which are associated with the inner surface of the plasma membrane. The regulation of these PTKs is still not fully understood, but the transforming capacity of their activated forms indicates a central role in cellular growth and differentiation (Hunter & Cooper, 1985; Hanks *et al.*, 1988; Cantley *et al.*, 1991). p56^{lck} and p56^{fyn} are expressed in cells of lymphoid origin and interact with the transmembrane surface glycoproteins CD4/CD8 and CD3, respectively (Rudd *et al.*, 1988; Veillette *et al.*, 1988; Cooke *et al.*, 1991). One principle of regulating these PTK activities is phosphorylation/dephosphorylation of a carboxyterminal Tyr-residue like Tyr^{527} in p60$^{c\text{-}src}$ or Tyr^{505} in p56^{lck} (Cooper *et al.*, 1986; Piwnica-Worms *et al.*, 1987; Amrein & Sefton, 1988; Marth *et al.*, 1988). Such tyrosine phosphorylation can be counteracted by tyrosine phosphatases. In the case of p56^{lck}, the CD45 tyrosine phosphatase suppresses its phosphorylation at Tyr^{505} (Ostergaard *et al.*, 1989).

A good candidate for an enzyme phosphorylating these regulatory tyrosines seems to be *csk* from neonatal rat brain. If *csk* is coexpressed in yeast cells it specifically phosphorylates p60$^{c\text{-}src}$ at Tyr^{527} (Nada *et al.*, 1991). *In vitro*, *csk* has also been shown to specifically phosphorylate Tyr^{527} of p60$^{c\text{-}src}$ and equivalent tyrosine residues of p56^{lck}, p59^{fyn} and p56^{lyn}, thereby down-regulating their catalytic activities (Okada *et al.*, 1991; Bergman *et al.*, 1992). In transformed rat fibroblasts, *csk* represses the transforming activity of p60$^{c\text{-}src}$, activated by v-*crk*. The transforming activity of v-*src* and *src*F527 are not suppressed (Sabe *et al.*, 1992b).

Clones encoding the human (Partanen *et al.*, 1991; Bräuninger *et al.*, 1992) and chicken (Sabe *et al.*, 1992a) equivalents of rat *csk* have been isolated from cDNA libraries. In all three species the open-reading-frame of *csk* encompasses 450 amino acids. Human CSK is 98% identical at the amino acid level to the corresponding rat sequence (Bräuninger *et al.*, 1992) and 93% identical to the chicken sequence. This high degree of interspecies conservation again suggests an important general role of *csk* in cellular physiology.

In contrast to other intracellular PTKs an autophosphorylation site corresponding to *src*-Tyr^{416}, as well as the equivalent of the regulatory *src*-Tyr^{527} of the *src*-family are missing in the deduced amino acid sequence of CSK. Similarly, a Gly residue located at position 2 in all *src*-family PTKs, which is necessary for myristylation and membrane attachment, was not detected in CSK. To gain further insight in the evolution of intracellular PTKs and to compare functional units of CSK with the structure of the gene, a genomic CSK clone was isolated and analysed.

Organisation of the human CSK gene

A CSK clone was isolated from a human genomic library in lambda EMBL4/EcoRI, using a radiolabelled probe of 400 bp representing the aminoterminal portion of CSK. Restriction fragments containing exons were identified by PCR with primers from different regions of the CSK cDNA sequence. After subcloning of various restriction fragments all exons and introns were sequenced, except the intron separating exon 1a and 1. Exon/intron junctions of CSK were subsequently identified by comparison of the cDNA (Partanen *et al.*, 1991; Bräuninger *et al.*, 1992) with the genomic sequence. Position and length of introns were verified

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by PCR across the introns using genomic DNA of another individual as template and subsequent size determination by gel electrophoresis.

The 3′ exon/intron boundary of exon 1a is not contained within the isolated clone and was determined using ligation-mediated PCR adapted from Edwards *et al.* (1991) for genomic DNA: a 3′ amino modified linker was ligated to DNaseI treated, heat denatured genomic DNA (Edwards *et al.*, 1991). In the following PCRs the genomic fragment of interest was amplified: 20 ng of linker-ligated DNA fragments were used as template in a 30 cycle PCR (1 min 97°C, 3 min 76°C) with a specific primer from exon 1a and the second complementary to the linker. 1 μl of the PCR product was subjected to a second amplification using another specific, nested primer from exon 1a with PCR conditions as above. The procedure was repeated once again with a third specific, nested primer. An amplified genomic fragment of 300 bp consisted of 50 bp 3′ region of exon 1a and 250 bp of intervening sequence. PCR across this exon/intron boundary with different primers from exon 1a and the 250 bp intron sequence yielded fragments of the expected sizes.

An EcoRI/KpnI restriction map of the isolated EMBL4-CSKI clone as well as the exon/intron structure are shown in Figure 1.

The coding region of CSK is organized in 12 exons which span a genomic distance of 4.9 kb. Compared to genomic loci of PTKs of the *src*-family, which encode for cDNAs of comparable length, the CSK locus is very small (exons 6–12 of HCK span 16 kb (Hradetzky *et al.*, 1992), exons 1–12 of LCK span 12.8 kb (Rouer *et al.*, 1989), exons 2–12 of FGR span 10 kb (Parker *et al.*, 1985; Nishizawa *et al.*, 1986; Patel *et al.*, 1990) and exons 2–12 of SRC cover a length of 15 kb (Anderson *et al.*, 1985; Tanaka *et al.*, 1987)). The exons of CSK are relatively uniform in size ranging from 66 to 220 bp (Figure 2). The only exception is the last exon which contains the entire 3′-untranslated region of 686 bp in addition to the 180 C-terminal coding nucleotides. The organization of the entire 3′-untranslated region and some coding C-terminal sequence in one large exon is a feature of CSK which is in common with all other intracellular PTKs whose genomic loci are known (SRC, FGR, LCK, HCK, FES (Roebroeck *et al.*, 1985)). Exon 1 encodes the five N-terminal amino acids of CSK and contains 65 bp of the 5′-untranslated region. The sizes of introns between coding exons vary between 76 and 920 bp. In contrast to these quite small introns, the size of the intron between exon 1a (which contains only 5′-untranslated sequence) and exon 1 (which contains 5′-untranslated region as well as coding sequence) is more than 6.400 bp.

Figure 2 also shows the nucleotide sequences at the exon/intron boundaries. The 5′ and 3′ ends of all intron sequences match the known consensus sequence for splice donor and splice acceptor sites (GT/AG rule (Mount, 1982)). All splice acceptor sites are preceeded by pyrimidine-rich stretches. An EcoRI site in the cDNA sequence which encompasses the five last nucleotides of exon 11 and the first nucleotide of exon 12 is split by an intron in the genomic sequence.

Comparing functional units of the protein (Figure 3) like the SH3, the SH2 and the kinase domain with genetic units, i.e. exons, no obvious relationship was found. The entire kinase domain of CSK is subdivided into seven exons. In the kinase domain only one functional motiv has been identified to date, the ATP-binding motiv GlyXGlyXXGly with a Lys 15–22 amino acids downstream (Hunter & Cooper, 1985). In CSK these two structural features of one functional unit are split by an intron. Regarding the SH2 domain an intron/exon junction preceeds the sequence coding for the SH2 domain by 1 bp. Another splice junction lies within a highly conserved motiv 16 amino acids before the end of the SH2 domain (Koch *et al.*, 1991). The SH3 domain is also split into two exons.

Comparison of the genomic structure of CSK with other intracellular PTKs of human origin

Based on the sequence homology in the kinase domain the intracellular PTKs are divided into the *src*-, the *fes/fer*- and the *abl/arg*-families (Hanks *et al.*, 1988). Within the *src*-family, the complete genomic structures are known for SRC, FGR and LCK. For HCK the organization of exons 6–12 has been determined (Hradetzky *et al.*, 1992). The boundaries of exons 3–12, encompassing the SH3, SH2 and kinase domains, are

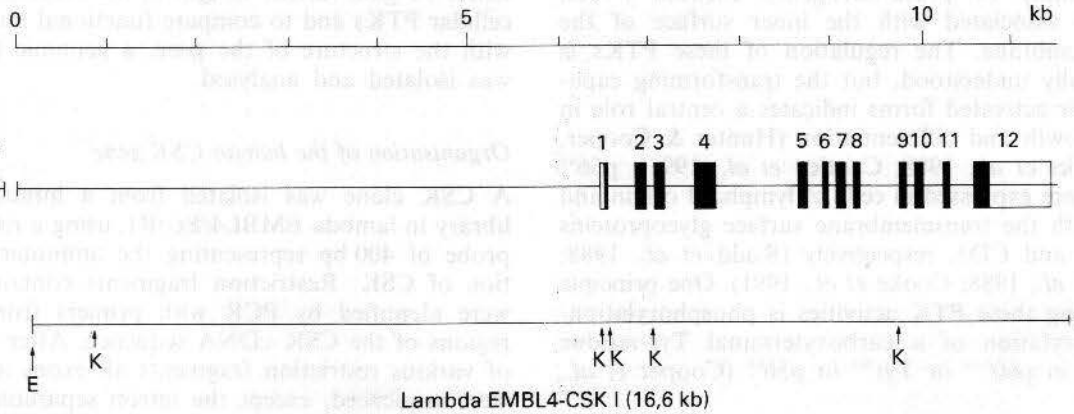

Figure 1 Genomic structure of the human CSK. Exon/intron structure of human CSK is schematically shown. Coding exons are represented by black, noncoding exons by white boxes. Exon numbering is according to SRC (Anderson *et al.*, 1985; Tanaka *et al.*, 1987). An EcoRI (E) and KpnI (K) restriction map for lambda EMBL4-CSKI, containing exons 1–12, is given below. The 3′ exon/intron boundary of exon 1a was determined using ligation-mediated PCR

Intron Splice acceptor	Exon No. (bp)			Intron Splice donor	(bp)
		1a (>68)	.. CCA AGA GAG	GTGAGT	(> 6400)
			A I Q		
TCTTCCCCACAG	CTC TAA TGG...	**1** (80)	... GCA ATA CAG	GTACCA	(302)
	A A W		V T K		
CCTGCCTTGCAG	GCC GCC TGG...	**2** (114)	.. GTC ACC AAG	GTAATC	(97)
	D P N		L M P		
CCCTCTCCCCAG	GAC CCC AAC...	**3** (113)	... CTC ATG CC	GTGAGT	(333)
	W F H		L V E		
GCTGGTCCCCAG	T TGG TTC CAC...	**4** (220)	.. CTG GTG GAG	GTGAGC	(920)
	H Y T		F Y R		
CACACCCTGCAG	CAC TAC ACC...	**5** (94)	... TTC TAC CGC A	GTGAGT	(175)
	S G W		E F G		
CCCTCCCCACAG	GC GGC TGG...	**6** (66)	... GAG TTC GGA G	GTGAGC	(76)
	D V M		V M T		
CTCTGCCCCCAG	AC GTG ATG...	**7** (100)	. GTC ATG AC	GTGAGT	(89)
	Q L R		M A K		
GTCCTGCCCCAG	G CAA CTG CGG...	**8** (91)	... ATG GCC AAG	GTGGGC	(420)
	G S L		F S L		
TCCTGCCCCCAG	GGG AGC CTT...	**9** (74)	... TTC TCG CT	GTGAGT	(99)
	D V C		E K K		
GCCTACCCCCAG	A GAT GTC TGC...	**10** (196)	.. AGA GAG AAG	GTGGGG	(106)
	K F S		P R I		
TCCACATGGCAG	AAA TTC TCC..	**11** (87)	... CCA AGA ATT	GTGAGT	(247)
	P L K				
CCCTGGCCACAG	CCC CTG AAG..	**12** (866)			

Figure 2 Exon/intron length and boundaries of human CSK. The CSK amino acid sequence at the exon boundaries is given in bold letters above the nucleotide sequence. The lengths of exons and introns including the nucleotides shown in the Figure are given in brackets beside the boldlettered exon numbers and the splice donor sites, respectively

highly conserved for SRC, FGR and LCK. Especially in the kinase domain splice sites lie within or betwen the codons for the corresponding amino acids of the proteins. This is also found for exons 6–12 of HCK. A conservation of the location of exon boundaries in the kinase domain within subfamilies of PTKs has also been described for the insulin-receptor (Seino *et al*., 1989) and ROS (Matsushime *et al*., 1986) as well as for KIT and CSFI-R (Andre *et al*., 1992; Vandenbark *et al*., 1992). In contrast to the SRC-family, where the genes of several members have been analysed, for the other intracellular PTKs only the genomic structure of FES is completely known. For the *abl/arg* family only two genomic fragments of ARG have been described so far (Kruh *et al*., 1986).

As shown schematically in Figure 3 and in detail in Figure 4, the exon organization of the SRC-family is quite different from the one of FES, except for the boundary 8/9 of SRC which is in common between the genes. The genomic structures of receptor protein-tyrosine kinases are different from both, the SRC-type and FES (Semba *et al*., 1985; Matsushime *et al*., 1986; Seino *et al*., 1989; Johnson *et al*., 1991; Andre *et al*., 1992; Vandenbark *et al*., 1992).

Interestingly, within the kinase domain the exon boundaries of CSK either match those of the SRC-type or those of FES. The exon boundary of CSK located before the kinase domain is in common with the one of FES. There is no corresponding boundary in the SRC-type in this region. The next exon boundaries for SRC,

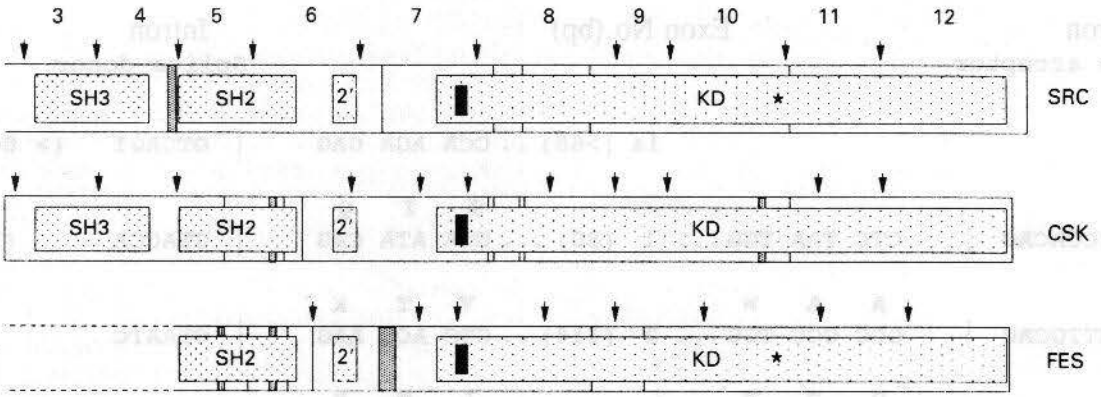

Figure 3 Schematic comparison of the exon boundaries of intracellular protein-tyrosine kinases. The amino acid sequences of CSK, SRC and FES were aligned. Gaps introduced for optimal alignment are shown as vertical lines or grey rectangles. The *src*-homology regions (SH2, SH2', SH3) as well as the kinase domains (KD) are boxed. The ATP-binding sites are represented by black bars, the autophosphorylation sites (corresponding to Tyr-416 of *src*) by asterisks. The exon boundaries are indicated by arrows

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CSK          MSAIQAAWPSGTECIAKYNFHGTAEQDLPFCKGDVLTIVAVTKDPN   WYK  AKNKVGREGI
SRC      ......PQRAGPLAGGVTTFVALYDYESRTETDLSFKKGERLQIVNNTEGDW   WLA  HSLSTGQTGY
FES      ......LCSQAKLQAQQELLQTKLEHLGPGEPPPVLLLQDDRHSTSSSEQEREGGRTPTLEILKSHISGI

CSK      IPANY VQKREGVKAGTKLSLMP  WFHGKITREQAERLLYPPET  GLFLVRESTNYPGDYTLCVS
SRC      IPSNY VAPSDSIQAEE_____WYFGKITRRESERLLLNAENPRGTFLVRESETTKGAYCLSVSDFD
FES      FRPKFSLPPPLQLIPEVQKPLHEQLWYHGAIPRAEVAELLVHS    GDFLVRESQG KQEYVLSVL

CSK       CDG KVEHYRI MYHASKLSIDEEVYFENLMQLVEHYTSDADGLCTRLIKPKVMEGTVAAQDEFYRSGW
SRC      NAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQQLVAYYSKHADGLCHRL  TTVCPTSKPQTQGLAKDAW
FES       WDGL PRHFIIQSLD  NLYRLEGEGFPSIPLLIDHLLSTQQPLTKK       SGVVLHRAVPKDKW

CSK      ALNMKELKLLQTIGKGEFGDVMLGDYRG NK VAVKCIKNDA T  AQAFLAEASVMTQLRHSNLVQLLG
SRC      EIPRESLRLEVKLGQGCFGEVWMGTWNGTTR VAIKTLKPGTMS  PEAFLQEAQVMKKLRHEKLVQLYA
FES      VLNHEDLVLGEQIGRGNFGEVFSGRLRADNTLVAVKSCRETLPPDLKAKFLQEARILKQYSHPNIVRLIG

CSK      VIVEEKGGLYIVTEYMAKGSLVDYLRSRGRSVLGGDCLLKFSLDVCEAMEYLEGNNFVHRDLAARNVLVS
SRC      VVSEE  PIYIVTEYMSKGSLLDFLKGETGKYLRLPQLVDMAAQIASGMAYVERMNYVHRDLRAANILVG
FES      VCTQKQ PIYIVMELVQGGDFLTFLRTEGAR LRVKTLLQMVGDAAAGMEYLESKCCIHRDLAARNCLVT

CSK      EDNVAKVSDFGLTK    EASSTQDTGK LPVKWTAPEALREKKFSTKSDVWSFGILLWEIYSFGRVPYP
SRC      ENLVCKVADFGLARLIEDNEYTARQGAK FPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYP
FES      EKNVLKISDFGMSREEADGVYAASGGLRQVPVKWTAPEALNYGRYSSESDVWSFGILLWETFSLGASPYP

CSK      RIPLKDVVPRVEKGYKMDAPDGCPPAVYEVMKNCWHLDAAMRPSFLQLREQLEHIKTHELHL
SRC      GMVNREVLDQVERGYRMPCPPECPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEPQYQPGENL
FES      NLSNQQTREFVEKGGRLPCPELCPDAVFRLMEQCWAYEPGQRPSFSTIYQELQSIRKRHR
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Figure 4 Exon boundaries in CSK, SRC and FES amino acid sequences. The amino acid sequences of CSK, SRC and FES were aligned using the Tree program of HUSAR (DKFZ; Heidelberg). Gaps were introduced for optimal alignment. Amino acids at exon/intron boundaries are underlined and in bold letters. If two amino acids are underlined and in bold letters the exon/intron boundaries are between the two codons. If only one amino acid is marked the exon/intron boundary is within the codon

FES and CSK are spread over a region of 11 amino acids around the consensus ATP-binding motiv (GlyX-GlyXXGly). The boundary between the SRC exons 8 and 9 is exactly at the same site in CSK as well as in FES, but in CSK and FES exon 8 of SRC is split into two exons. SRC exon boundaries 9/10 and 11/12 are in common with the corresponding junctions of CSK, whereas in FES the boundaries are shifted downstream for 16 and 11 amino acids, respectively. SRC exon boundary 10/11 is shifted 17 amino acids downstream

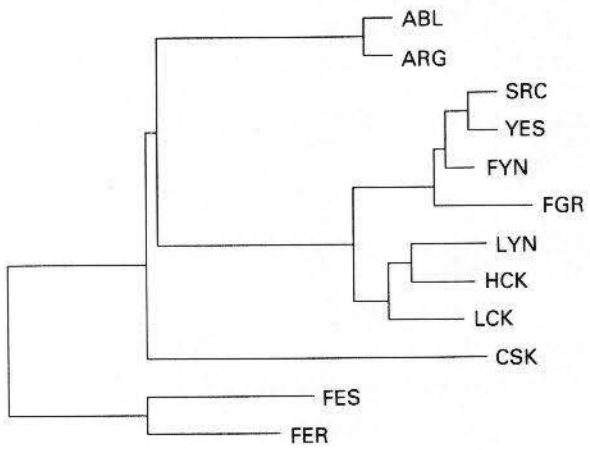

Figure 5 Phylogenetic tree of the kinase domains of human intracellular protein-tyrosine kinases. Amino acid sequences of the catalytic domains of human intracellular protein-tyrosine kinases were used to calculate the phylogenetic relationship with the Tree program (DKFZ; Heidelberg), which is based on the progressive sequence alignment method of Feng and Doolittle (1987)

in CSK and in FES.

In a phylogenetic tree based on sequence homologies within the kinase domain CSK is located in an intermediate position between the FES/FER-family and the SRC-family (Figure 5). This intermediate position of CSK seems to be reflected by its genomic structure. It seems likely, that the boundaries of CSK in common with FES have been conserved and that different sites in the SRC-type have evolved after the split of the SRC-precursor from CSK. According to this assumption, the exon boundaries common in the SRC-type and CSK but different from FES would have been created in the period after the split in a FES/FER family on the one hand and a SRC-family/CSK precursor on the other hand and before the split of CSK and the SRC-family precursor occurred.

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