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DNA Sequence Encoding the Amino-Terminal Region of the Human c-*src* Protein: Implications of Sequence Divergence among *src*-Type Kinase Oncogenes

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We sequenced the 5′-coding region of the human c-*src* gene, exons 2 through 5, corresponding to one-third of the human c-*src* protein consisting of 536 amino acids. Sequence analysis of the *src* type of protein kinases revealed that the amino-terminal region encoded by exon 2 contains sequences specific for the *src* proteins and raised the possibility that this region is involved in the recognition of a *src*-specific substrate(s) or receptor(s).

The *src* proteins encoded by both a proto-oncogene c-*src* (43) and the v-*src* gene of Rous sarcoma virus (18, 49) have molecular masses of about 60 kilodaltons and possess tyrosyl-specific protein kinase activity (3, 7, 10–12, 20, 50). The v-*src* protein induces cell transformation, whereas the c-*src* protein does not under usual conditions (21, 22, 32, 40, 47). The human c-*src* gene (c-*src*-1), localized on chromosome 20, contains 11 coding exons spanning a distance of 19.5 kilobases, more than three times that of the chicken c-*src* coding regions (6 kilobases) (1, 16, 31, 45). The DNA sequence of exons 6 to 12, corresponding to the 3′ two-thirds of the human c-*src* coding region, was determined previously (1). This region encodes the putative regulatory domain at the carboxy terminus of the c-*src* protein and the protein kinase domain (33, 47, 51). Here, we report the DNA sequence of the 5′ region (exons 2 through 5) encoding the amino-terminal one-third of the human c-*src* protein. This region appears to contain two domains, the membrane-binding domain and the putative recognition domain (13, 33, 34), and also part of the putative modulatory domain, as will be discussed later.

Previously, we studied the exon-intron structure of the human c-*src* gene by hybridization to v-*src* DNA probes and also by comparison with that of the chicken c-*src* gene (1, 16). For exon 3, however, its tentative localization was ambiguous (16). To localize exon 3 more precisely, we used the calf thymus DNA random primer method (35) to obtain a human c-*src* cDNA probe prepared from 70S virion RNA of a retrovirus (WO CVS virus) whose genome contains a chimeric, intronless form of human c-*src* sequence (47). This approach, together with DNA sequencing and previous mapping data, allowed us to complete our analyses of exon-intron structure of the human genomic c-*src*-1 coding region spanning 19.5 kilobases.

The DNA sequence, obtained as depicted in Fig. 1, and the deduced amino acid sequence of the 5′ human c-*src* gene were compared with the corresponding chicken c-*src* region (45) (Fig. 2; Table 1). The average amino acid sequence homology of the region encoded by exons 3 to 5 is 98%, which is comparable to that of the region encoded by exons 6 to 12 (98%) (1). Thus, the human and chicken c-*src* protein sequences are highly conserved not only in the carboxy-terminal two-thirds region (1), but also in the region corresponding to exons 3 to 5, which is outside the kinase domain (13, 15, 24, 33). However, the region encoded by human exon 2 exhibits unusual features when compared with the chicken c-*src*. The amino acid sequence homology is 71%, which is significantly low when compared with the average homology of exons 3 through 12 (98%) (Table 1), and human exon 2 encodes three additional amino acids which are not present in the chicken c-*src* protein (Fig. 2). Thus, the human c-*src* gene encodes 536 amino acids (1), whereas the chicken c-*src* gene encodes 533 amino acids (45). However, it should be pointed out that the profile of hydrophilicity plots of the region encoded by human c-*src* exon 2 is very similar to that of the chicken c-*src* exon 2 (data not shown).

The following observations about exon-intron structures were made by comparison of the human and chicken c-*src* regions from the 3′ end of exon 2 to exon 12 (1) (Tables 2 and 3): the total nucleotide number as well as the total number of amino acid residues encoded in corresponding exons are the same; the boundaries between exons and introns are identical; the noncoding regions are not conserved; intron size is

TABLE 1. Nucleotide and amino acid sequence homologies in corresponding exons of human and chicken c-*src* genes[a]

Exon	Nucleotide homology (%)	Amino acid homology (%)
2	190/250 (76.0)	59/83 (71.0)
3	82/100 (82.0)	34/34 (100.0)
4	85/99 (85.8)	32/33 (97.0)
5	80/104 (76.9)	34/35 (97.1)
6–12[b]	937/1,055 (88.8)	346/351 (98.5)
3–12	1,184/1,358 (87.1)	446/453 (98.3)
2–12	1,374/1,608 (85.4)	505/536 (94.2)

[a] Homology was determined by the number of identical chicken c-*src* nucleotides or amino acid residues (45) within each exon of the human c-*src* gene.

[b] The average homologies from exons 6 through 12 were obtained from results previously published (1). The nucleotides in noncoding regions of exon 2 and exon 12 are not included in the calculations; only the nucleotides within the coding region are included.

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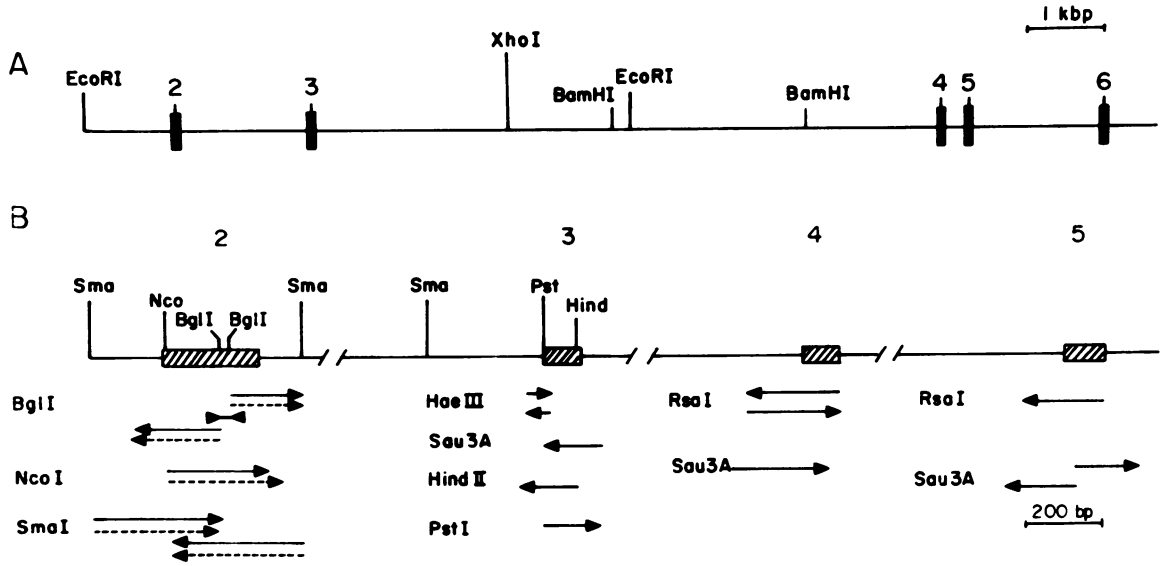

FIG. 1. Location of exons and DNA-sequencing strategy. (A) Location of exons 2 through 5. The number shows the exon number. The size of each exon is not drawn to scale. (B) DNA-sequencing strategy. All exons were sequenced by the dideoxy chain termination method (1, 29) (shown by solid lines). Exon 2 was also sequenced by the Maxam-Gilbert method (28) (shown by broken lines). DNA fragments containing exon 2 were derived from λ clones S11H (16) and RA-1 which contains an insert different from that of λ S11H (R. R. Arthur and D. J. Fujita unpublished data). Exons 3 through 5 were from λ S3H (16). All λ clones originated from the same library (16). Arrows indicate the orientations of DNA sequencing. bp, Base pairs.

not conserved. However, the intron-exon boundary at the 5′ end of human exon 2 and the total number of nucleotides or amino acid residues in exon 2 are different from those of chicken exon 2 (Table 2). The noncoding region of human exon 2 is 4 base pairs long, whereas that of chicken exon 2 is 9 base pairs long (45) (Table 2). The presence of the consensus sequence for the initiator (purine)-C-C-ATG-G (25) surrounding the ATG at residue 1 (Table 2; Fig. 2) strengthens the belief that this ATG codon is the authentic initiation site for c-*src* protein synthesis.

The amino-terminal region presented in this paper contains the 18-kilodalton peptide generated by proteolytic digestion of the *src* protein with *Staphylococcus aureus* V-8 protease (10, 44) (Fig. 2). At least four possible serine phosphorylation sites are present within the 18-kilodalton peptide of the chicken c-*src* or the v-*src* protein (6, 17, 44), and there is another phosphorylation on a tyrosine residue(s) (4, 5, 10, 52) which appears to be present between v-*src* residues 81 and 149 (13). All the corresponding serine phosphorylation sites are present in the 18-kilodalton region of the human c-*src* protein (Fig. 2). As for the tyrosine phosphorylation, all the tyrosine residues are conserved in the corresponding human c-*src* region (residues 84 to 152) (Fig. 3).

The *src* protein is myristylated at residue 2 (Gly) after residue 1 (Met) is removed (15, 21, 34, 36, 39). We compared amino-terminal regions of *src*-related proteins (2, 9, 41, 42, 45) and other myristylated proteins (19, 30) (Fig. 3). There was no single consensus sequence observed among them, although the human, chicken, and *Xenopus laevis* c-*src* proteins have the consensus of Gly-X-X-Lys-Ser-Lys-Pro-Lys(Arg)-Asp(Glu) since Arg and Glu are conservative alterations for Lys and Asp, respectively (37). There are two possible explanations for this. One is that conformation of the amino-terminal region, rather than a specific amino acid sequence, may play a key role in the myristylation pro-

TABLE 2. Splice donor and splice acceptor sequences of the human c-*src* gene[a]

Exon no.		Intron (splice acceptor)	Exon size (bp)[b]	Intron (splice donor)
2				
	H	CTGCCAG	———GACCATG..254..CCCGCTGGCG	GTCAGTGCGC
	C	C	CCCACC ..250..GG A T	T G
3				
	H	CTCTCTGCAG	GTGGAGTGAC.....100..TCAACAACAC	GTGAGTGC
	C	GTGT	C C C	T
4				
	H	CCTGCTCAG	AGAGAGGGAG.....99..AGGCTGAGGA	GTTAG
	C	TCTTG	G AG T C A	A T
5				
	H	CCCCCAG	GTGGTATTTT.....104..ACCACGAAAG	GTAC
	C	A	C G A	GA

[a] DNA sequences at intron-exon boundaries of the human c-*src* gene (H) are compared with the corresponding regions of the chicken c-*src* gene (C) (45).
[b] Exon size is shown by the number of base pairs in each exon. Only nuleotide differences are shown for the corresponding chicken c-*src* sequence. In exon 2, a dash (—) indicates no corresponding DNA sequence in human c-*src*. ATG, Initiation site for *src* protein synthesis.

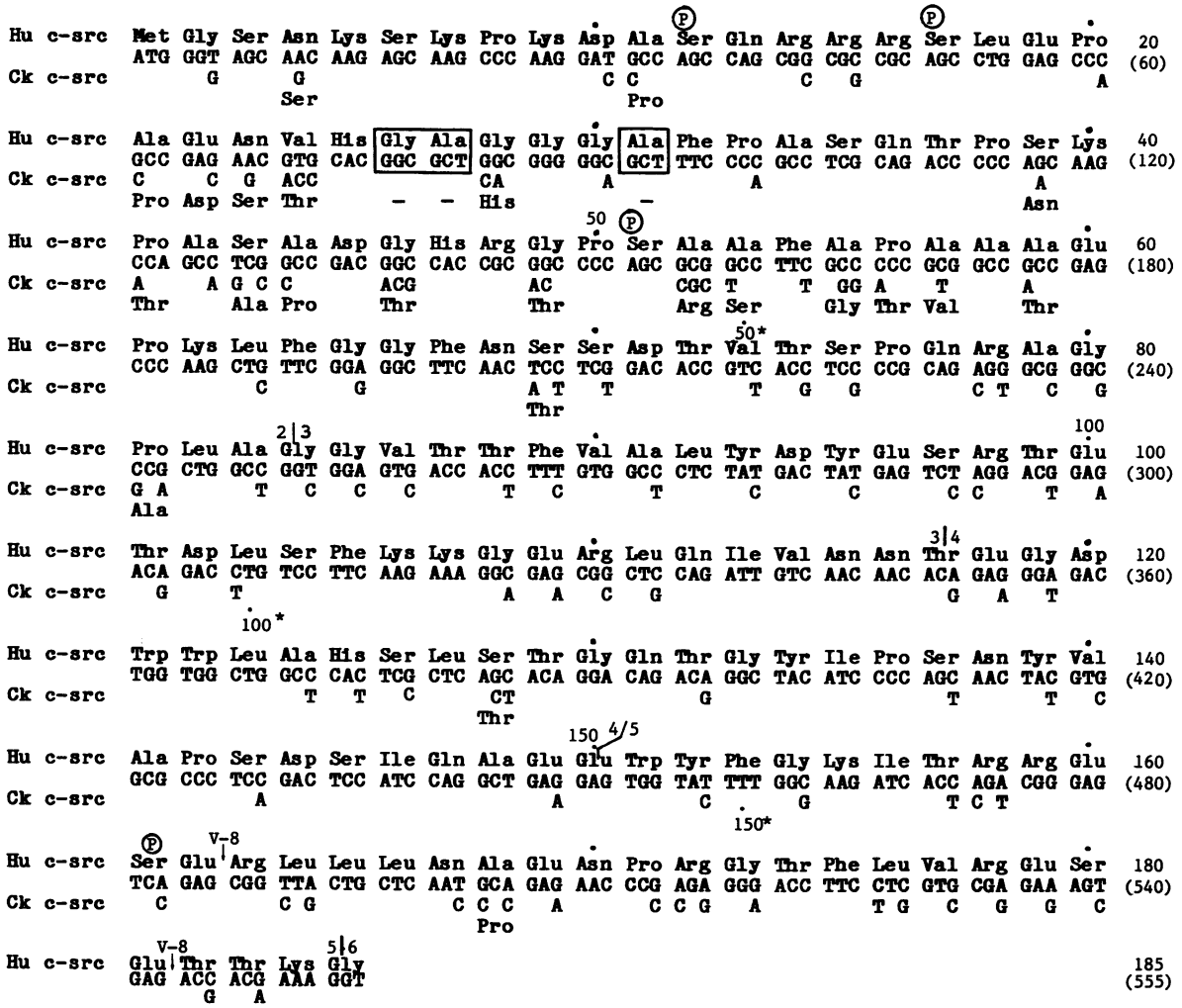

FIG. 2. DNA sequence of exons 2 to 5 and the deduced amino acid sequence. The DNA sequence of the human (Hu) c-*src* gene from exons 2 to 5 and the deduced amino acid sequence are compared with the corresponding chicken (Ck) c-*src* sequences. For the chicken c-*src* sequence, only nucleotides or amino acid residues which differ from those of human c-*src* are shown. Boxed regions show the sequence observed only in the human c-*src* gene. A dash (–) indicates the absence of the corresponding amino acid in the chicken c-*src* sequence. A vertical bar with two numbers shows a boundary between the numbered exons. Ⓟ, Possible site for phosphorylation (17, 44); V-8, Possible cutting site by proteolytic digestion with *S. aureus* V-8 protease (44); *, amino acid residues of the chicken c-*src* protein (45).

cesses, i.e., the steps to remove residue 1 (Met) and to myristylate residue 2 (Gly). Another explanation is that there are several types of the myristylation processes, one of which is specific for the *src* consensus sequence.

When the amino acid sequences of exon 2-encoded regions of the human and chicken c-*src* proteins were compared, their divergence was mainly localized to two smaller subregions (α and β in Fig. 4B; also see Fig. 2). In contrast, relatively high homology was observed in the rest of the exon 2-encoded region (subregions I, II, III, and IV in Fig. 4B). Subregion I has been shown to be essential for myristylation or membrane binding (34). Thus, it is possible that some or all of the other conserved subregions play important roles common to c-*src* proteins since some of the conserved regions (II, III, and IV in Fig. 4B) appear to be specific for the *src* protein, as will be discussed below.

Figure 4B also illustrates the extremely low degree of homology observed in the region corresponding to exon 2 between the human c-*src* protein and the v-*yes* protein, which shares 80% amino acid homology to the rest of the *src* protein (23). We presume that the region of the v-*yes* gene corresponding to c-*src* exon 2 (Fig. 4B) had been derived from the c-*yes* gene and that the c-*yes* gene belongs to the *src* type of tyrosyl protein kinase oncogenes whose products do

TABLE 3. Size comparison of corresponding human and chicken c-*src* introns[a]

Intron no.	Intron size (bp)	
	Human	Chicken
2	2,000	50
3	8,000	2,040
4	350	390
5	1,750	1,010

[a] Each intron is numbered with the number of the exon located at the immediate 5′ end of the intron. Sizes of the human c-*src* introns were determined from restriction mapping nd DNA sequencing information. Sizes of the chicken c-*src* introns were obtained from reference 45.

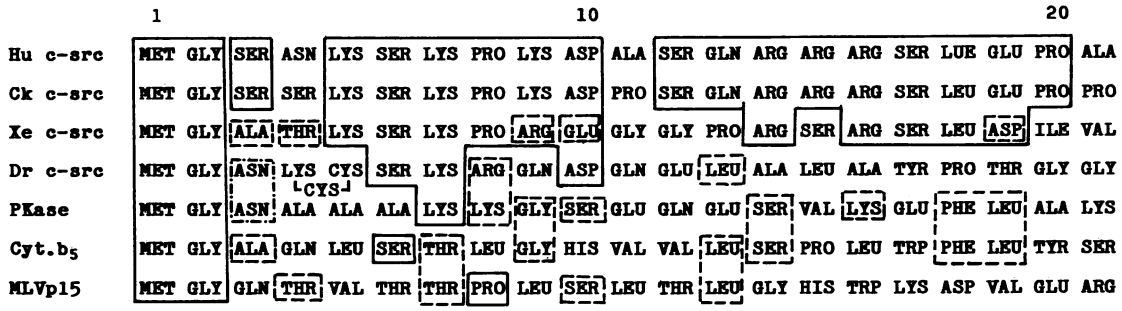

FIG. 3. Comparison of amino-terminal regions of myristylated proteins. The amino-terminal 21 amino acid residues are compared among the human (Hu), chicken (Ck) (45), *Xenopus* (Xe) (42), and *Drosophila* (Dr) (41) c-*src* proteins and the bovine cyclic AMP-dependent protein kinase (PKase) (9). Two other proteins, NADH cytochrome b_5 (Cyt.b_5) (30) and Moloney murine leukemia virus *gag* protein (MLVp15) (19) are not related to the *src* protein but are myristylated. The boxes enclose common amino acid residues; Arg and lys are treated as conserved residues, as are Glu and Asp.

not have transmembrane and extracellular domains (3, 50), because there are limited areas of significant nucleotide and amino acid sequence homologies observed between the corresponding v-*yes* and c-*src* regions and because there is a sequence related to myristylation or membrane association in the *yes* protein (Fig. 4A and B). Similarly, when the human c-*src* protein sequence is compared with other *src*-type protein kinases, such as the murine *lsk*^{T}/*tck* protein (27, 48) and the *Drosophila* c-*src* protein (41), the greatest divergence is observed within the amino-terminal region. These results, taken together, suggest that the region encoded by c-*src* exon 2 contains sequences that are important for c-*src*-specific functions, such as recognition of a c-*src*-specific substrate(s) or receptor(s). Similarly, it is possible that the corresponding regions of other *src*-type kinase proteins, such as *yes*, *lsk*^{T}/*tck*, and D-*src*, play similar specific roles.

If the recognition domain is present within the region encoded by exon 2, then another functional domain appears to be present in a region between the putative recognition domain and the kinase domain (human c-*src* residues 84 to 240). The v-*src* protein forms complexes with p50 and p90 proteins (8, 26), whose possible binding sites on the v-*src* protein have been placed at both its carboxy-terminal end (38) within or near the putative regulatory region (33, 47) and the region near or at the v-*src* amino acid residues 155 to 160 (15, 46). Mutations of the v-*src* gene within the region corresponding to human c-*src* residues 84 to 240. do not abolish either transforming ability or protein kinase activity, but result in generation of various partial transforming mutants, such as fusiform mutants (13, 14, 24; S. K. Anderson and D. J. Fujita, J. Virol., in press) and temperature-sensitive mutants for cell transformation (33). Phosphorylation of a certain tyrosine residue(s) within this region appears to activate v-*src* protein kinase activity (10, 15). It is thus possible that this region participates in functions modulating expression of the protein kinase activity.

As discussed above, it is very possible that the recognition domain is encoded by exon 2. However, at present we cannot rule out the possibility that diverged regions observed among the *src* type of protein kinases are not involved in specific functional roles since our discussion is based solely on amino acid sequence homologies. Further experimental data are required to resolve fully this issue.

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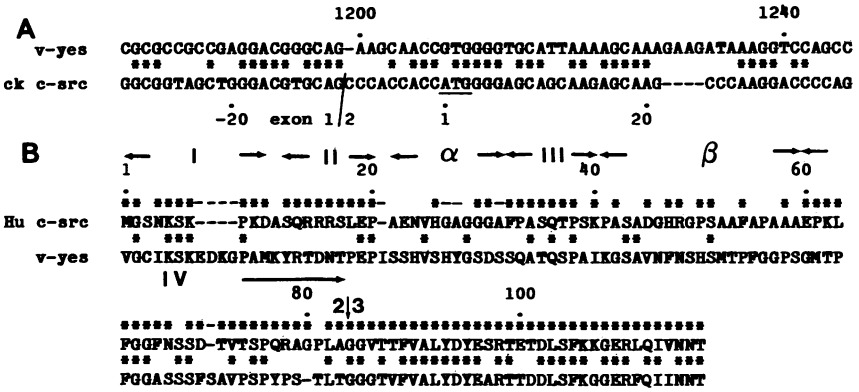

FIG. 4. Comparison of DNA and amino acid sequences between the c-*src* exon 2 region and the corresponding chicken c-*src* or Y73 v-*yes* region. (A) Nucleotide sequence homology at the boundary of exon 1 and 2 of the chicken (ck) c-*src* gene (45) compared with the corresponding Y73 v-*yes* sequence (23). ATG, Initiation codon for the c-*src* protein; −, no corresponding nucleotide. (B) Comparison of amino acid sequence homology between the human c-*src* exon 2 and 3 region and the corresponding chicken c-*src* (45) or Y73 v-*yes* (23) region. The amino acid sequence homology of the human (Hu) c-*src* protein and the chicken c-*src* protein is shown by asterisks on the top row (also see Fig. 2). −, No corresponding amino acid residue.

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