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Chromosomal Mapping of Murine c-*fes* and c-*src* Genes

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The murine homologs of two viral oncogenes associated with tyrosine-specific kinase activity have been assigned to different loci in the mouse genome. The segregation of restriction site polymorphisms, as detected by probes that are specific for endogenous c-*fes* and c-*src* sequences, was followed in the DNA of recombinant inbred strains. The c-*fes* gene was mapped to the proximal portion of chromosome 7, very close to the *Gpi-1* locus, whereas c-*src* was linked to the *Psp* locus on the distal half of chromosome 2.

Oncogenic retroviruses contain transforming genes (v-*onc* genes) as an integral part of their genomes. It is believed that these viruses are a result of genetic recombination between retroviral sequences and distinct cellular sequences (c-*onc* genes), which are responsible for their acute transforming ability. The products encoded by some identified v-*onc* genes are associated with tyrosine-specific kinase activity (*src*, *yes*, *abl*, *ros*, and *fes*; reviewed in reference 2). Nucleotide sequence analysis (9, 26) and measurements of immunological cross-reactivity (1) support the idea that some of these oncogenes (the *src* family) are evolutionarily related. The recent discovery of a gene that is homologous to v-*src* and v-*abl* in *Drosophila melanogaster* DNA further suggests that these oncogenes may have evolved from a common ancestral gene (14). It is of interest, therefore, to determine the relative locations of the cellular oncogenes in the mammalian genome and to find out whether members of the *src* family of genes are clustered on the same chromosome. Furthermore, the importance of the chromosomal locations of cellular transforming genes has been underscored by the demonstration that specific chromosomal translocations involving sites adjacent to specific c-*onc* genes are associated with murine and human lymphomas (4, 16, 23, 29). While this manuscript was still being prepared, c-*src* and c-*fes* were assigned to chromosomes 2 and 7, respectively, by the use of mouse-hamster somatic cell hybrids (A. Sakuguchi, personal communication) (18). We have mapped the mouse cellular homologs of v-*fes* and v-*src* to determine their relative chromosomal locations and to examine the possibility of their involvement in DNA rearrangements in the neoplastic transformation.

The regional chromosomal locations of c-*fes* and c-*src* were studied by following the segregation of restriction fragment length polymorphisms involving these genes within the DNA of two sets of recombinant inbred (RI) strains. These strains were derived from crosses between inbred strains A/J (A) and C57BL/6J (B) or from crosses between strains B and DBA/2J (D), which resulted in A×B and B×A RI strains (22) and B×D RI strains (Jackson Laboratory, Bar Harbor, Me.; 30, 31), respectively.

Gene mapping of RI strains is accomplished in three steps. (i) Parental strains are screened for phenotypic or genotypic variation, in this case by using a nucleic acid probe (24) that detects a specific restriction site polymorphism in the DNA. (ii) The hybridization pattern of each of the RI strains is determined, and the resemblance to one parental strain or the other is ascertained, resulting in a strain distribution pattern (SDP) for a given genetic locus (see Table 1). (iii) The newly determined SDP is compared with SDPs of previously mapped genes to ascertain the degree of linkage and, thus, the chromosomal position of the particular gene (8, 30).

Polymorphism within the murine c-*fes* locus was detected by Southern blotting of restriction endonuclease-digested DNA of the two progenitor strains A and B, followed by hybridization with a c-*fes* probe (7). The pattern obtained by using DNA digested with four different restriction enzymes suggests the existence of a single locus containing the murine c-*fes* gene (Fig. 1). Except for *Eco*RI, no other restriction enzymes used (*Hin*dIII, *Pst*I, and *Bgl*II in Fig. 1 and *Msp*I, *Pvu*II, and *Xba*I, not shown) generated a polymorphic restriction pattern among A, B, and D mice. The *Eco*RI fragments that hybridize to c-*fes* differ in size: a 13-kilobase fragment is present in the A strain, and a 12-kilobase fragment is present in the B strain. The polymorphism in the size of the *Eco*RI fragments was used to follow the segregation of the variants in the A×B and B×A RI strains. A total of 30 RI strains were tested; two representative Southern blots are shown in Fig. 2. That RI strains show both parental phenotypes is due to heterozygosis; i.e., the c-*fes* alleles were still segregating, and these RIs could not be used in the analysis. The results showed that 23 of the 30 RI strains were homozygous with respect to the size of the *Eco*RI fragment containing the c-*fes* gene. Comparison of the SDP for c-*fes* with SDPs of other genes indicated tight linkage between c-*fes* and genes located on chromosome 7 (Table 1). Thus, c-*fes* is located between *Gpi-1* (glucose phosphate isomerase-1) (15) and *Tam-1* (tosyl arginine methylesterase-1) (27), with a distance of 2.6 ± 2 centimorgans from *Gpi-1* and 6 ± 4 centimorgans from *Tam-1*.

A polymorphic pattern involving the murine c-*src* locus was obtained by using DNA of parental strains B and D, digested with *Hin*dIII and hybridized with a v-*src* probe (6). Other restriction enzymes, such as *Eco*RI, *Pst*I, and *Bgl*II, generated identical patterns among the mouse strains tested. The v-*src* probe hybridizes to a single 13-kilobase DNA fragment in D mice and hybridizes with two DNA fragments of 16.5 and 14 kilobases in the DNA of B mice. The segregation of these alleles was followed in 26 B×D RI mice, and the SDP was determined (Table 2). Analysis of SDPs indicated that the murine c-*src* gene is located on chromo-

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TABLE 1. SDP of c-*fes* and neighboring genes on chromosome 7

Locus	SDP of: A×B RI strain														B×A RI strain								
	1	2	3	4	5	6	9	10	12	14	15	20	21	25	1	3	4	6	7	13	14	19	23
c-*fes*	B	A	A	B	A	A	A	B	A	A	A	B	A	B	B	B	A	A	A	A	A	A	A
Gpi-1[a]	B	A	A	B	A	A	A	B	A	A	A	B	A	B	B	B	B	A	A	A	—[b]	B	A
Tam-1[c]	B	B	A	B	A	A	A	B	A	B	A	B	A	—[b]	B	B	A	A	B	A	A	B	A
c[d]	B	A	A	B	B	A	A	B	A	A	B	B	B	B	B	B	A	B	A	A	A	A	B
Hbb[e]	B	A	A	B	A	A	A	B	A	A	B	B	B	B	B	B	A	B	A	A	A	A	A

[a] Glucose phosphate isomerase-1 (15).
[b] —, Not tested.
[c] Tosyl arginine methylesterase-1 (27).
[d] Coat color (albino).
[e] Hemoglobin chain (32).

some 2 by linkage (2.2 ± 1.6 centimorgans) to *Psp* (parotid secretory protein) (13), which resides on the distal half of the chromosome. However, the orientation of *Psp* and c-*src* with respect to the centromere was not posssible because of a lack of SDPs for other genetic markers on this portion of chromosome 2. This result places c-*src* more than 20 centimorgans away from the c-*abl* gene, another member of the tyrosine kinase genes that maps very near to the β_2-microglobulin gene (21). It has been suggested that the genesis of myeloid leukemia is greatly influenced by genetic information of chromosome 2 since partial deletions of chromosome 2 have been associated with 49 of 52 cases of the disease (11). Further studies are necessary to examine the involvement of c-*src* and c-*abl* on chromosome 2 with leukemia.

The mouse c-*fes* gene maps to the proximal portion of chromosome 7 between the *Gpi-1* and *Tam-1* genes. It is interesting that although chromosome 7 has not been shown thus far to be involved in specific translocations in malignancies, a number of genes potentially involved in oncogenesis have been mapped to chromosome 7. These include the ecotropic murine leukemia virus loci *Akv-1* of AKR mice and *Fgv-1* of C3H/Fg mice (17), two endogenous mink cell focus-inducing viral sequences (2a), *Mtv-1* (a mammary tumor viral sequence [31]), and the c-*Ha-ras*-1 gene (18).

Studies with human-mouse somatic cell hybrids assigned the human c-*fes* gene to chromosome 15 (5, 12), and more recently it has been sublocalized to 15q25-26 by in situ hybridization (10). Comparative mapping suggests that a portion of mouse chromosome 7 is homologous to a region of human chromosome 15, since the mitochondrial isocitrate

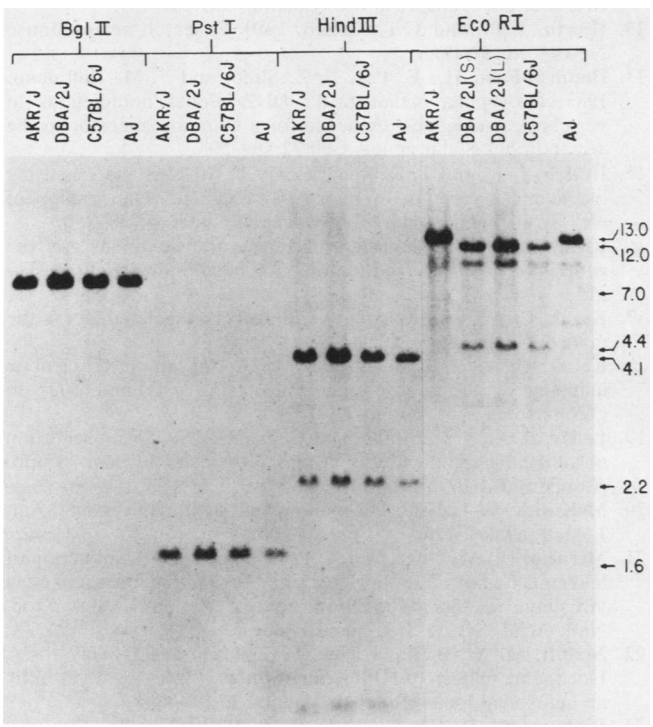

FIG. 1. Detection of c-*fes* sequences in the DNA of four inbred mouse strains. High-molecular-weight liver DNA was digested with endonuclease restriction enzymes (4 U/μg), followed by electrophoresis and Southern transfer (28). The blot was hybridized with a ^{32}P-labeled c-*fes* probe, recombinant plasmid pN26 (7). Spleen (S) and liver (L) DNAs of D mice digested with *Eco*RI exhibit an identical hybridization pattern. The variation between DNA of A and B mice was used to determine the segregation of the c-*fes* gene in the A×B RI strains. Numbers on the right indicate fragment size in kilobases.

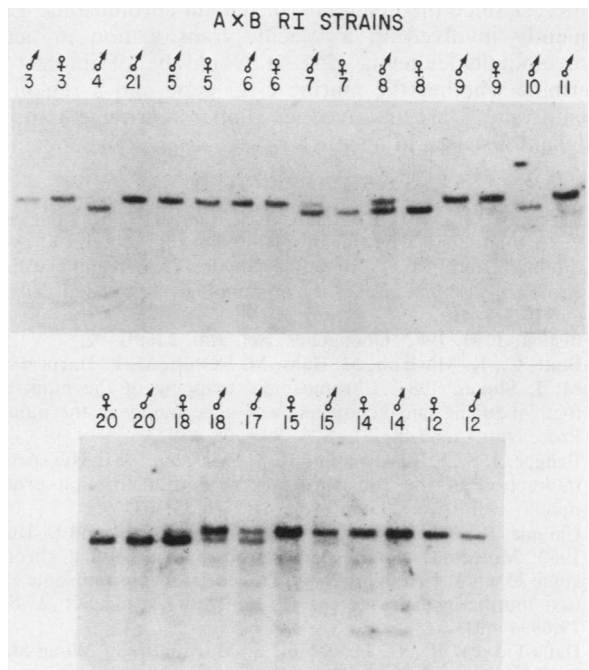

FIG. 2. Segregation of the allelic *Eco*RI restriction fragments in A×B RI strains as shown by hybridization with the c-*fes* probe. Strains heterozygous at the c-*fes* locus (A×B 7, A×B 8, A×B 17, and A×B 18) were not included in the SDP.

TABLE 2. SDP of c-*src* and *Psp*

Locus	SDP of B×D RI strains																								
	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
c-*src*	D	D	B	D	D	D	D	D	D	B	B	D	D	B	D	D	B	D	D	D	D	B	D	D	D	D
Psp[a]	D	D	B	D	D	D	D	D	B	B	B	D	D	D	D	D	B	D	D	D	D	B	D	D	D	D

[a] Parotid secretory protein (13).

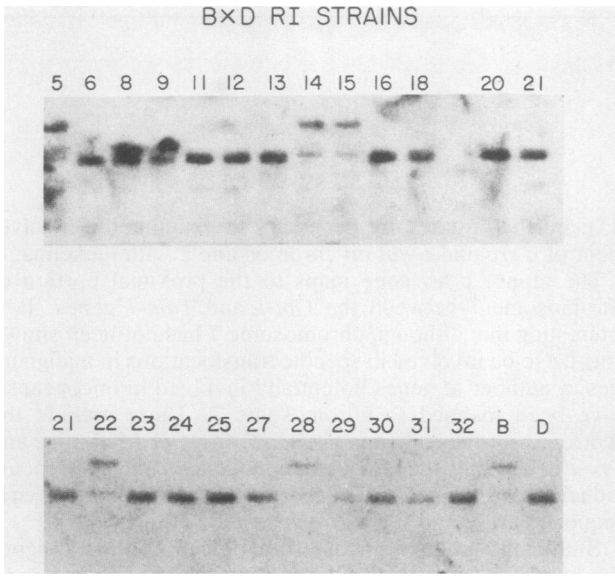

FIG. 3. Segregation of the allelic *Hin*dIII restriction fragments in B×D RI strains as shown by hybridization with the v-*src* probe (6).

dehydrogenase locus (*Idh-2*) is located on mouse chromosome 7 (19) and on 15q21-qter in humans (20). This finding is of interest since the distal half of human chromosome 15 is frequently involved in a specific translocation in acute promyelocytic leukemia (25). It would be of interest to determine whether the murine c-*fes* gene and a region of chromosome 7 are involved in similar rearrangements in malignant diseases in mice.

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