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Chromosome assignments of four mouse cellular homologs of sarcoma and leukemia virus oncogenes

(proto-oncogenes/somatic cell hybrids/conserved linkage groups/gene mapping)

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ABSTRACT **Molecular probes for the oncogenes of Rous sarcoma virus (v-*src*), avian myeloblastosis virus (v-*myb*), Kirsten murine sarcoma virus (v-Ki-*ras*), and Harvey murine sarcoma virus (v-Ha-*ras*) were hybridized to the DNA from mouse–Chinese hamster somatic cell hybrids. The v-*src*, v-*myb*, v-Ki-*ras*, and v-Ha-*ras* genes each detected one or a few homologous mouse DNA fragments whose segregation was analyzed in cell hybrids. Mouse cellular homologs c-*src*, c-Ki-*ras*, c-Ha-*ras*, and c-*myb* segregated concordantly with chromosomes 2, 6, 7, and 10, respectively. Comparison with the known locations of human c-*src* (chromosome 20) and human c-Ha-*ras1* (chromosome 11 short arm) suggests that the human and mouse homologs of these two viral oncogenes reside in conserved linkage groups. The c-Ki-*ras* gene on mouse chromosome 6 might reside also in a conserved linkage group, along with glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase. However, direct confirmation of this suggestion must await a demonstration that c-Ki-*ras* on mouse chromosome 6 is homologous to c-Ki-*ras2* on the short arm of human chromosome 12.**

Acutely transforming mammalian and avian retroviruses are recombinants between C-type RNA viruses and host cellular genes [cellular oncogenes (c-*onc*s) (1) or proto-oncogenes (2)]. The pirated cellular sequences—at least 18 have been identified (2)—constitute the viral oncogenes (v-*onc*s), which encode proteins believed responsible for malignant transformation of cells (3). The exact functions of proto-oncogene products are not understood, although essential roles in cellular biochemistry and perhaps differentiation have been suggested (4, 5).

Recently, altered forms of several proto-oncogenes have been identified in malignant cells. In several instances the proto-oncogene has undergone a rearrangement as a result of a characteristic chromosome translocation (6) or DNA insertion (7) or has undergone an apparent point mutation, changing the primary amino acid sequence of the encoded product (8, 9). These alterations are believed responsible for the attendant changes in levels of expression or properties, or both, of the c-*onc* gene products.

Consequently, proto-oncogene assignment in man and other species is of interest for several reasons. Consistent numerical and structural chromosome abnormalities have been associated with a number of different cancers, and the possibility that proto-oncogenes might reside at chromosome breakpoints (10, 11) has been realized in the case of human and mouse c-*myc* proto-oncogenes in lymphoid B-cell cancers (6). Transcriptional activation of c-*onc*s by translocation or rearrangement might involve other proto-oncogenes as well. Moreover, there are inherited susceptibilities to cancer, some of which have been associated with chromosome aberrations in man (12–15) or have been mapped to specific chromosomes in mouse (16). From an evolutionary standpoint, assignment of proto-oncogenes in different species might reveal chromosome regions representing very ancient linkage groups (17). For example, several proto-oncogenes are homologus to v-*onc*s encoding tyrosine kinases (3) and may be descendants of a common progenitor gene that evolved at least 1×10^8 years ago (17).

We report here assignment of mouse proto-oncogenes homologous to four different sarcoma and leukemia virus oncogenes to chromosome 2 (c-*src*, homolog of Rous sarcoma virus v-*src*), chromosome 6 (c-Ki-*ras*, homolog of Kirsten murine sarcoma virus v-Ki-*ras*), chromosome 7 (c-Ha-*ras*, homolog of Harvey murine sarcoma virus v-Ha-*ras*), and chromosome 10 (c-*myb*, homolog of avian myeloblastosis virus v-*myb*). Three of the proto-oncogenes (c-*src*, c-Ki-*ras*, and c-Ha-*ras*) map to chromosomes encoding other genes whose human homologs are also syntenic. Thus, they might comprise conserved linkage groups in man and mouse.

MATERIALS AND METHODS

Filter Hybridization. Isolation of high molecular weight cellular DNAs, cleavage with restriction enzymes, electrophoresis of DNA fragments, and transfer to nitrocellulose filters (Schleicher & Schuell) have been described (18). Filters were hybridized to ^{32}P-labeled oncogene probes, rinsed, and exposed to x-ray film exactly as described (18).

Oncogene Probes. Four cloned retroviral oncogene probes were used to detect homologous cellular sequences: (*i*) v-*src*—from plasmid clone pSRA-2, which contains the genome of the Schmidt–Ruppin A strain of Rous sarcoma virus (19); (*ii*) v-Ha-*ras*—from plasmid clone BS9, which contains a 460-base-pair (bp) fragment of the oncogene of Harvey murine sarcoma virus cloned with *Eco*RI linkers (20); (*iii*) v-Ki-*ras*—from plasmid clone HiHi-3, which contains a 1-kbp *Hin*cII fragment of the oncogene of Kirsten murine sarcoma virus cloned with *Eco*RI linkers (21); and (*iv*) v-*myb*—from plasmid clone pVM2, which contains part of a proviral genome of avian myeloblastosis virus (22).

Specific probes were prepared by cleaving the plasmid clones with appropriate restriction enzymes (pSRA-2 with *Eco*RI; BS9 with *Eco*RI; HiHi-3 with *Sst* II and *Eco*RI; and pVM2 with *Kpn* I and *Sst* I) and purifying the oncogene-specific fragments by agarose electrophoresis and electroelution into a dialysis bag (23). Oncogene-specific fragments were labeled with [^{32}P]dCTP and [^{32}P]dTTP by nick-translation (24) and used for filter hybridization.

Chinese Hamster–Mouse Cell Hybrids. EBS hybrid cells were originally derived from the fusion of Chinese hamster V79 lung fibroblasts (clone E36) with BALB/c mouse spleen cells. The construction and characterization of EBS hybrids has been described (25–27). The mouse chromosome content

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Abbreviations: bp, base pair(s); v-*onc*, viral oncogene; c-*onc*, cellular oncogene.

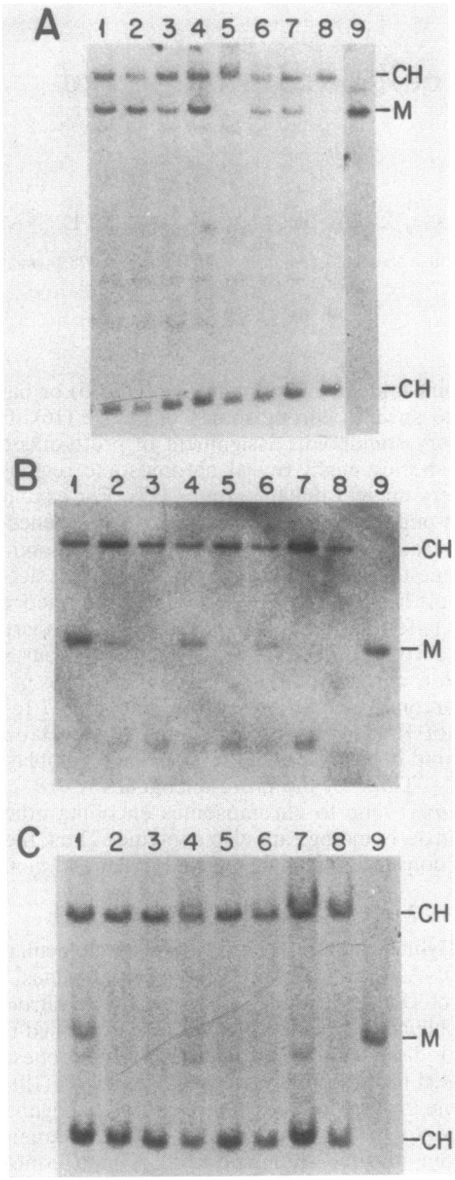

FIG. 1. Hybridization of viral oncogene probes to cell hybrid DNAs. (*A–C*) DNAs from EBS cell hybrids 15–74 (lanes 1–7, respectively), Chinese hamster cells (CHW1102, lane 8), and mouse cells (RAG, lane 9). DNAs were cleaved with *Eco*RI (*A* and *C*) and *Hin*dIII (*B*), and fragments were separated by electrophoresis through agarose and transferred to nitrocellulose. (*A*) DNAs hybridized with the 3.1-kbp *Eco*RI v-*src* probe from plasmid clone pSRA-2 (19). Sizes of major hybridizing fragments are 14 and 15.5 kbp (mouse; both contained within band M and not clearly resolved in this photograph) and 2.1 and ≈22 kbp (Chinese hamster; band CH). Cell hybrid DNAs in lanes 1–4 and 6–7 are positive for mouse c-*src*. The weakly hybridizing band in RAG DNA (lane 9), which migrates more slowly than do the 14- and 15.5-kbp bands (band M), is not present in mouse LM/TK$^-$ DNA and might represent a minor structural difference in the *src* genes of these two cell lines derived from different strains of mice. The weak signal precluded following the segregation of this band in cell hybrids. (*B*) DNAs hybridizing with the 460-kbp *Eco*RI v-Ha-*ras* probe from plasmid clone BS9 (20). A 4.2-kbp fragment (band M) and an 11-kbp fragment (band CH) are detected. Cell hybrid DNAs in lanes 1, 2, 4, 5 (weak), and 6 are positive for c-Ha-*ras*. (*C*) DNAs hybridized with a 1.7-kbp *Kpn* I–*Sst* I v-*myb* probe from plasmid clone pVM2 (22). A 5.1-kbp fragment (band M) and 3.4- and 9.4-kbp fragments (band CH) are detected. Cell hybrid DNA in lane 1 is positive for mouse c-*myb*.

of hybrid cells was determined by histochemical assay of cell homogenates and by karyotyping (25–27) of cells at the same passage as those used for DNA isolation.

RESULTS

Detection of Proto-Oncogenes. Mouse and Chinese hamster cellular homologs of the v-*src*, v-Ha-*ras*, v-Ki-*ras*, and v-*myb* oncogenes were identified by their characteristic pattern of hybridization when filters containing restriction enzyme-cleaved cellular DNAs were hybridized with a given v-*onc* probe (Figs. 1 and 2). The hybridization patterns are consistent with estimates of one to a few cellular homologs of the four v-*onc*s per haploid mouse or hamster genome (4, 21, 28, 29). However, the amplification of c-Ha-*ras* in at least one strain of mouse (*Mus pahari*) and amplification of c-Ki-*ras* in Chinese hamsters are exceptions (28). Amplification of c-Ki-*ras* in Chinese hamster cells complicated chromosome assignment studies, as discussed below.

It was apparent that c-*src*, c-Ha-*ras*, and c-*myb* were asyntenic because each was detected in a different subset of the 15-cell hybrids. (DNAs from 7-cell hybrids that were annealed with the 3 v-*onc*s are shown in Fig. 1 *A–C*.) Detailed analysis of the segregation pattern for each proto-oncogene is described below.

Assignment of Mouse *src*. The 3.1-kbp *Eco*RI fragment of pSRA-2 encompassing the v-*src* gene (30, 31) recognizes two mouse *Eco*RI fragments roughly 14 and 15.5 kbp in size (32) (both fragments were contained in the band marked "M" in Fig. 1*A*, lane 9). The individual bands were seen on shorter exposures of filters to x-ray film (not shown). In contrast,

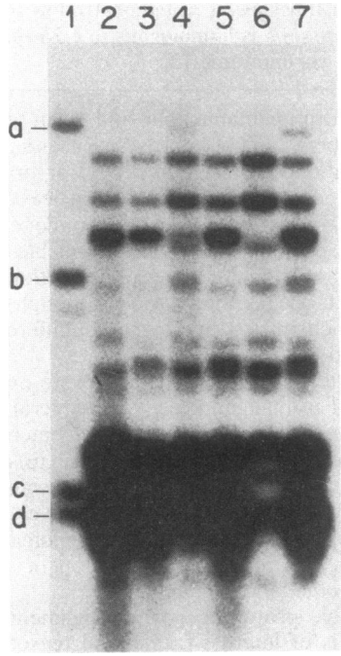

FIG. 2. Genomic DNAs from mouse (lane 1), Chinese hamster (lane 2), and mouse–Chinese hamster cell hybrids EBS 18–74 (lanes 3–7, respectively) were cleaved with the restriction enzymes *Pvu* II and *Hin*dIII and hybridized with the 600-bp *Sst* II–*Eco*RI v-Ki-*ras* probe (21). The probe recognizes four principal mouse DNA fragments (bands a–d; *ca.*, 7.0, 3.5, 1.1, and 0.9 kbp, respectively) and a multitude of Chinese hamster DNA fragments (ranging in size from about 6 to 0.7 kbp). Cell hybrid DNAs in lanes 4 (weakly positive) and 7 contain the 7.0-kbp *Pvu* II–*Hin*dIII mouse c-Ki-*ras* fragment (band a). The 3.5-kbp mouse c-Ki-*ras* fragment (band b) also seems to be present in lanes 4 and 7 and might cosegregate with the 7.0-kbp fragment. Mouse c-Ki-*ras* fragments labeled c and d are obscured by the intense hybridization of the probe to Chinese hamster DNA (compare with lanes 1 and 2).

Table 1. Segregation of mouse proto-oncogenes and mouse chromosomes in mouse–Chinese hamster hybrids

Cell hybrid	c-*src*	c-Ki-*ras*	c-Ha-*ras*	c-*myb*	Mouse chromosomes* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
EBS-1	+	+	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
EBS-2	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+
EBS-3	+	+	+	−	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	+	+	−	+	+
EBS-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+
EBS-5	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	−	+	+
EBS-9	+	+	+	−	−	+	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+
EBS-10	+	−	+	+	−	+	+	−	−	−	+	−	−	+	−	+	+	+	+	+	−	+	−	+
EBS-11	−	−	+	+	+	−	−	−	−	−	+	−	−	+	−	+	−	−	+	+	−	−	−	+
EBS-15	+	+	+	+	−	+	+	+	−	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+
EBS-17	+	−	+	−	+	+	−	−	+	−	+	−	+	−	−	−	+	+	+	−	+	−	−	+
EBS-18	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+†	−	−	−	−	+
EBS-51	+	+	+	−	−	+	−	−	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+
EBS-63	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+
EBS-71	+	−	+	−	+	+	+	−	+	−	+	−	+	−	−	+	−	+	+	+	+	+	−	+
EBS-74	+	+	−	−	−	+	+	−	−	+	−	−	−	−	−	+	−	+	+	+	−	−	−	+

The presence (+) or absence (−) of a given proto-oncogene and mouse chromosomes in the 15 EBS hybrids is listed. The segregation of each proto-oncogene and any given mouse chromosome can be analyzed by comparing their respective vertical columns. There was complete concordant segregation of c-*src* with mouse chromosome 2, c-Ki-*ras* with chromosome 6, c-Ha-*ras* with chromosome 7, and c-*myb* with chromosome 10.

*Enzyme markers also scored were adenylate kinase-1, acid phosphatase-2, and sorbitol dehydrogenase for mouse chromosome 2; triosephosphate isomerase for chromosome 6; lactate dehydrogenase A, peptidase-4, and glucose phosphate isomerase for chromosome 7; and peptidase-2, pyrophosphatase, and hexokinase-1 for chromosome 10.

†EBS-18 contained a mouse chromosome 15 in which band F3 appeared to be deleted.

Chinese hamster DNA yields two major hybridizing *Eco*RI fragments approximately 4 and 22 kbp in size. Both mouse c-*src*-specific *Eco*RI fragments cosegregated with mouse chromosome 2 markers adenylate kinase-1, acid phosphatase-2, and sorbitol dehydrogenase (Table 1). It is of interest to note the v-*yes* oncogene of the Yamaguchi 73 avian sarcoma virus encodes a tyrosine kinase activity and bears homology with v-*src* (33). Both v-*src* and v-*yes* and also v-*fps*, a third avian sarcoma virus oncogene, are believed to have arisen from a common progenitor, perhaps by a mechanism of partial gene duplication (33, 34). Mouse c-*yes*, however, appears to be asyntenic with c-*src* (unpublished data).

Assignment of Mouse c-Ha-*ras* and c-Ki-*ras*. The v-Ha-*ras* and v-Ki-*ras* oncogenes are derived from divergent members of a family of normal rat cellular genes (21). Homologous genes have been detected in the mouse, Chinese hamsters, and humans (21, 28, 35). The human c-Ha-*ras1* and c-Ki-*ras2* genes have been assigned to chromosomes 11p (36) and 12p, respectively (37, 38). Therefore, we examined the possibility that these related genes might recognize conserved chromosomal regions in the mouse.

The 460-bp v-Ha-*ras* probe detected a single major hybridizing fragment of 4.2 kbp in mouse DNA cleaved with *Hin*dIII (Fig. 1*B*, lane 9). This *Hin*dIII fragment corresponds to a c-Ha-*ras1* homolog, the most highly conserved of the cellular *ras* genes previously described in rats and humans (21, 35, 39). The v-Ha-*ras* gene detects two homologs in rats and humans, designated c-Ha-*ras1* and c-Ha-*ras2* (35, 39). The c-Ha-*ras1* genes of both species are similar in structure and contain three intervening sequences. The c-Ha-*ras2* genes may represent pseudogene members of the *ras* family (35).

Mouse c-Ha-*ras* was assigned to chromosome 7 by concordant segregation with mouse chromosome 7 markers lactate dehydrogenase A and glucose phosphate isomerase (Table 1).

BALB/c mouse spleen cells were used in the construction of the original EBS series of hybrids (25, 26). Although BALB/c DNA contains one or two c-Ki-*ras* genes, in Chinese hamsters the c-Ki-*ras* genes are amplified (28). When cell hybrid DNA is hybridized with the v-Ki-*ras* probe, mouse c-Ki-*ras* fragments are superimposed upon a panoply of Chinese hamster c-Ki-*ras* fragments. Consequently, the segregation of mouse c-Ki-*ras* could not be determined with certainty with cell hybrid DNAs cleaved with a single restriction enzyme. We found, however, that cell hybrid DNAs cleaved with *Pvu* II and *Hin*dIII yielded an interpretable hybridization pattern. Mouse DNA cleaved with the two enzymes yielded four major hybridizing c-Ki-*ras* fragments (*ca.* 7.0, 3.5, 1.1, and 0.9 kbp; Fig. 2, lane 1). The 7.0-kbp fragment was easily scored in cell hybrid DNAs (e.g., Fig. 2, lanes 4 and 7). The 3.5-kbp mouse c-Ki-*ras* fragment migrated near a weakly hybridizing Chinese hamster c-Ki-*ras* fragment. As a result, cell hybrids positive for the 7.0-kbp mouse c-Ki-*ras* fragment also displayed a broad band near 3.5 kbp, suggesting that the two fragments cosegregate. The 7.0-kbp mouse c-Ki-*ras* fragment segregated concordantly with mouse chromosome 6 marker triosephosphate isomerase (Table 1).

The 1.1- and 0.9-kbp mouse c-Ki-*ras* fragments migrated near the region of intense hybridization in Chinese hamster DNA and could not be followed in cell hybrids. Consequently we could not determine if these two fragments originate from c-Ki-*ras* on chromosome 6 or represent unlinked homologous sequences.

Assignment of Mouse c-*myb*. A 1.7-kbp *Kpn* I–*Sst* I fragment containing v-*myb* sequences, prepared from the plasmid pVM2 (22), detected a single major hybridizing fragment in mouse DNA (*ca.* 5.1 kbp) and two fragments of Chinese hamster DNA (*ca.* 3.4 and 9.4 kbp) (Fig. 1*C*, lanes 9 and 10). The c-*myb* gene of chickens, the natural host for avian myeloblastosis virus, is approximately 16 kbp in size and possesses at least seven intervening sequences (22, 40). Therefore, it is likely that we were detecting only a highly conserved portion of the mouse c-*myb* gene with the v-*myb* probe. The 5.1-kbp mouse c-*myb* band segregated concordantly with chromosome 10 markers peptidase-2, pyrophosphatase, and hexokinase-1 (Table 1).

Segregation Analysis of Proto-Oncogenes in Cell Hybrids. Table 1 compares the segregation of each c-*onc* with enzyme markers and mouse chromosomes in the 15-cell hybrid clones. There were no discordant hybrid clones for any of the four c-*onc*s assigned, and each c-*onc* was present in a distinct subset of the cell hybrids. Although mouse chromosome 11 was not retained in any of the cell hybrid clones, a

feature common to EBS hybrids (26), we can likely rule out the possibility that mouse chromosome 11 contains any of the four c-*onc*s assigned. Aside from two small Ki-*ras*-related fragments, virtually all of the hybridizing fragments observed in mouse parental DNA could be accounted for in our cell hybrids.

DISCUSSION

Three of the four mouse c-*onc*s assigned here (c-*src*, c-Ki-*ras*, and c-*myb*) warrant discussion with regard to chromosomes structurally altered in cancers and c-*onc* gene rearrangements. Recently, Muchinski *et al.* (41) have reported mouse c-*myb* rearrangements in plasmacytoid lymphosarcomas induced with pristane and Abelson murine leukemia virus, often associated with the expression of altered forms of c-*myb* RNA. They speculate that rearrangement of c-*myb* might have occurred during excision of an integrated Abelson murine leukemia provirus. Alternatively, c-*myb* alterations could be due to chromosome 10 rearrangements, although karyotypes of these tumors are not available to answer this question. Mouse chromosome 2, to which we have assigned c-*src*, is frequently structurally altered in myeloid leukemias. Hayata *et al.* (42) observed deletions in mouse chromosome 2 in 49 of 52 (94%) myeloid leukemias examined. Although the extent of deletion varied, material between bands C and D were commonly absent (see ref. 43 for mouse chromosome banding nomenclature), and the authors suggested that this region might contain genes affecting myeloid cell proliferation. Whether the c-*src* gene [or mouse c-*abl* (homolog of the Abelson murine leukemia virus oncogene v-*abl*), which also resides on chromosome 2, see below] might be rearranged or its expression elevated in the mouse myeloid leukemias is not known. Structural and numerical abnormalities of mouse chromosome 6 preferentially in granulocytic leukemias were reported in the same study (42). An amplified segment of DNA containing an expressed mouse c-Ki-*ras* gene in Y1 adrenal tumor cells has been reported (44). The amplified c-Ki-*ras* sequences were present in double-minute chromosomes and in a heterogeneously staining region in an unidentified marker chromosome. The normal location of this gene has been independently assigned to mouse chromosome 6 (38).

Although mouse c-*src* and c-*abl* code for tyrosine kinases (3) and might have a common evolutionary origin (17), their synteny (45) may not reflect any functional significance. Although the subchromosomal locations of c-*src* and c-*abl* in the mouse are not presently known, the two genes might not be tightly linked. This supposition is based primarily on the fact that human c-*abl* and c-*src* reside on chromosomes 9 (46) and 20 (32), respectively. Human c-*src* and c-*abl* appear to be situated with different groups of genes on separate chromosomes whose homologs reside on mouse chromosome 2. For example, genes for adenosine deaminase and inosine triphosphatase, and adenylate kinase-1 on mouse chromosome 2 occur on human chromosomes 20 and 9, respectively (47, 48). Human c-*abl* is known to map distal to the adenylate kinase-1 gene on the chromosome 9 long arm (49).

Mouse c-Ki-*ras* and c-Ha-*ras* genes assigned here to chromosomes 6 and 7, respectively, might also identify conserved linkage groups in man and mouse. For example, a linkage group of insulin, β-globin, lactate dehydrogenase A (see refs. 47 and 48 for review), and c-Ha-*ras1* occurs on the short arm of human chromosome 11 (36). Mouse homologs of insulin (50), β-globin (47, 48), lactate dehydrogenase A (47, 48), and c-Ha-*ras* (this paper) occur on mouse chromosome 7. Human c-Ki-*ras2*, glyceraldehyde-3-phosphate dehydrogenase, and triosephosphate isomerase are on the human chromosome 12 short arm (37, 38, 47, 48). Homologs of glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase are on mouse chromosome 6 along with a c-Ki-*ras* gene (refs. 47 and 48; this paper). However, our chromosome assignment does not reveal whether the c-Ki-*ras* gene on mouse chromosome 6 is actually a human c-Ki-*ras1* or c-Ki-*ras2* homolog (51). Thus, proof that mouse c-Ki-*ras* identifies a conserved linkage group must await an independent demonstration that this gene is homologous to human c-Ki-*ras2* rather than c-Ki-*ras1* located on human chromosome 6 (38, 51).

Homologs of certain retrovirus oncogenes can be detected in lower organisms separated evolutionarily from mammals by 100×10^6 years (17, 52). Hence, cellular proto-oncogenes appear to be useful tools to investigate genetic and molecular evolution of a phylogenetically diverse group of organisms (52, 53). Chromosome assignment of proto-oncogenes in mouse is important for determining their possible association with chromosome aberrations in cancer and their relationships to integrated and endogenous proviruses and provides additional markers for delineating the extent of chromosome rearrangements that have occurred during speciation (cf., refs. 47, 48, and 54). The present proto-oncogene assignments add to the growing list of mouse homologs of retroviral oncogenes reported by other groups as well as our own (18, 38, 45, 55–59).

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