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NOTES

Genetic Mapping of the Mouse Oncogenes c-Ha-*ras*-1 and c-*fes* to Chromosome 7

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The mouse homologs of the cellular oncogenes c-Ha-*ras*-1 of Harvey sarcoma virus and c-*fes* of feline sarcoma virus were both mapped to chromosome 7 by Southern blot analysis of hamster-mouse somatic cell hybrid DNAs.

Acute transforming viruses are recombinants between retroviruses and cellular sequences (c-*onc* genes) responsible for oncogenicity. Transcription of these virally transduced sequences (v-*onc* genes) is required for the induction or maintenance of transformation by these viruses. Recent reports now suggest that the expression of the cellular homologs of these sequences (proto-oncogenes) may also be associated with neoplastic transformation. Activation of at least one such cellular oncogene (c-*myc*) can be effected by the local insertion of nonacute avian leukosis virus (12). Similarly, both rat and human c-Ha-*ras*-1 and mouse c-*mos* can cause cellular transformation when ligated to the transcriptional control sequences in retroviral long terminal repeats (1, 2, 6).

More recent observations suggest that c-*onc* sequences may also elicit transformation by nonviral mechanisms. Cellular transforming genes have been detected in tumor cell DNAs by their ability to transform cultured cells (4). In addition, a number of chromosomes known to carry c-*onc* genes are involved in the specific karyotypic abnormalities found in certain forms of cancer. In several cases, these c-*onc* sequences have been localized to the specific breakpoints involved in these translocations and deletions (reviewed in reference 21). Therefore, the determination of the chromosomal locations of these c-*onc* sequences is of some importance in our understanding of the mechanisms of both viral and nonviral carcinogenesis.

The c-*onc* genes have been highly conserved during evolution. In this report, we used interspecific hamster-mouse somatic cell hybrids to identify the chromosomal locations of the mouse homologs of one proto-oncogene first described in cats (c-*fes*) and one first described in rats (c-Ha-*ras*-1). The c-*fes* oncogene is present in the genomes of two independent isolates of feline sarcoma virus (Snyder-Theilen and Gardner-Arnstein [8]) and is also closely related to the c-*fps* oncogene found in several avian transforming viruses (22). c-*ras* is a family of oncogenes contained in the Harvey and Kirsten sarcoma viruses, and the c-Ha-*ras*-1 oncogene is homologous to the transforming DNA sequences isolated from a human bladder carcinoma (20). c-Ha-*ras*-1 and c-*fes* show no detectable homology, and their human homologs have been mapped to chromosomes 11 and 15, respectively (13, 19). In this report, we show that in mice, both oncogenes are carried by the same chromosome.

Somatic cell hybrids were generated by the fusion of Chinese hamster cells (E36) with peritoneal cells or spleen cells of BALB/c, A/J, and NFS.*Akv-2* congenic mice. The characterization of these hybrids and their use in the chromosomal mapping of other cellular and retroviral genes have been described previously (16–18).

A characteristic 4.4-kilobase (kb) fragment was identified when mouse genomic DNA was digested with *Hin*dIII, electrophoresed on 0.4% agarose gels, transferred to nitrocellulose filters as previously described (14), and probed with any of the following c-*fes*-related DNA probes: v-*fes Pst*I-3 (9), v-*fes Pst*I-4 (9), or v-*fps Bam*HI-4 (23). This 4.4-kb fragment could easily be distinguished from the 4.0-kb c-*fes*-reactive *Hin*dIII fragment in hamster cell DNA (Fig. 1A).

DNAs extracted from 22 hybrids were analyzed by Southern blot hybridization for the presence of c-*fes*-reactive *Hin*dIII fragments (Fig. 1A). As expected, all somatic cell hybrids carried the hamster c-*fes*-reactive 4.0-kb fragment; 12 hybrid cell DNAs also contained the

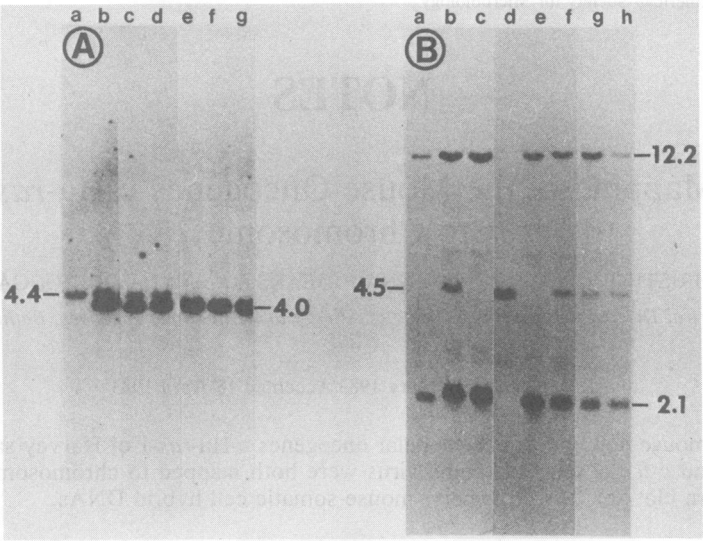

FIG. 1. Hybridization of cloned oncogene probes to DNAs from hamster-mouse somatic cell hybrids. High-molecular-weight DNA was prepared as previously described (14). DNAs were digested with *Hin*dIII, electrophoresed through 0.4% horizontal agarose slab gels, transferred to nitrocellulose membranes, and hybridized to ^{32}P-labeled pV-*fes* *Pst*I-3 (9)(A) or human pC-*ras*-1 (3)(B) DNA probes under stringent conditions (50% formamide–10% dextran sulfate–5× SSC [SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–10 ng of salmon sperm DNA per ml at 42°C for 18 to 24 h). Membranes were washed two times with 2× SSC and 0.1% sodium dodecyl sulfate at room temperature followed by two to four 15-min washes at 50°C. (A) DNAs from BALB/c mouse liver (lane a), somatic cell hybrid BM34 (lane b), somatic cell hybrid BE3-11 (lane c), somatic cell hybrid BE3-12 (lane d), somatic cell hybrid 7-2.9-2 (lane e), somatic cell hybrid 7-2.9-4 (lane f), and Chinese hamster cell line E36 (lane g). (B) DNAs from Chinese hamster cell line E36 (lane a), somatic cell hybrid VE6-4 (lane b), somatic cell hybrid VE8-1 (lane c), A/J mouse liver DNA (lane d), somatic cell hybrid MA3B (lane e), somatic cell hybrid MA6B (lane f), somatic cell hybrid MA1A (lane g), and somatic cell hybrid MA13-2 (lane h). The sizes of reacting fragments are shown in kb pairs.

4.4-kb mouse homolog of c-*fes*. A comparison of the presence of this fragment with the mouse chromosomes in the hybrid cells showed that all 12 of these positive clones carried chromosome 7 and, conversely, that none of the hybrids lacking this fragment retained chromosome 7 (Table 1). All other chromosomes showed discordant segregation with c-*fes*. Particularly striking was the fact that one c-*fes*-positive clone carried chromosome 7 as its only mouse genetic material.

Similarly, both hamster and mouse genomic DNAs had sequences homologous to rat c-Ha-*ras*-1, and these homologs could be identified when Southern blot-transferred gels were probed with either human c-Ha-*ras*-1 (3) or a *Hin*dIII fragment of v-Ha-*ras* (11) subcloned in our laboratory (Fig. 1B). Analysis of 30 somatic cell hybrids with different mouse complements showed that all hybrids had the 2.1-kb *Hin*dIII hamster fragment, all but 2 hybrids had the 12.2-kb hamster fragment, and 18 hybrids had the 4.5-kb mouse fragment. A comparison with mouse chromosomes retained by these hybrids showed a positive correlation with chromosome 7 (Table 2). The mouse homologs of c-Ha-*ras*-1 and c-*fes* were either both present or both absent in the 22 hybrids tested for both oncogenes. These data show that the mouse c-Ha-*ras*-1 and c-*fes* oncogenes are both located on mouse chromosome 7.

There are now more than 15 known oncogenes, and the determination of their location on mammalian chromosomes is of obvious interest in the study of tumorigenesis. Although this report demonstrates that c-Ha-*ras*-1 and c-*fes* are both on chromosome 7, it is clear that the cellular oncogenes are not present as a single major cluster of transformation-related genes in mice. In this species, c-*mos* has been mapped to mouse chromosome 4 (24), c-*abl* has been mapped to chromosome 2 (10), and c-*myc* has been mapped to chromosome 15 (5). However, additional studies should determine whether any other *onc* genes are present on these same four chromosomes.

A comparison of the human and mouse gene maps shows that the assignment of two oncogenes on separate human chromosomes to the same mouse chromosome is not unexpected. Both human chromosomes 11 and 15 show some

homology to mouse chromosome 7. Recent studies have localized the human c-*ras* oncogene, c-Ha-*ras*-1, to a specific region on human chromosome 11 (11p11-15) (7). This region carries the gene for lactate dehydrogenase A and the non-α-globin gene complex; the mouse homologs of both genes have been mapped to chromosome 7 (*Ldh-1* and *Hbb*). Similarly, c-*fes* is thought to be on band q22 of human chromosome 15 and is thus closely linked to the IDH2 locus (15q21-qter) (F. Wong-Staal, personal communication). Murine *Idh-2* has been mapped to mouse chromosome 7.

No tumor-specific translocations involving mouse chromosome 7 have been identified. However, it may be significant that two transforming sequences are located on a chromosome carrying several endogenous retroviral loci associated with high tumor incidence. Early studies on the genetic basis of virus-associated leukemogenesis and mammary carcinogenesis showed that this chromosome carries the ecotropic murine leukemia virus loci *Akv-1* and *Fgv-1* of AKR and C3H/Fg mice, respectively, and a locus associated with late mammary tumors in DBA and C3H mice, *Mtv-1* (reviewed in reference 15). Analysis of somatic cell hybrids of BALB/c mice shows that this chromosome also carries at least two distinct DNA fragments with sequences homologous to the xenotropic murine leukemia virus envelope (M. D. Hoggan and C. A. Kozak, unpublished data). Although *Akv-1*, *Fgv-1*, and *Mtv-1* are separated by substantial distances on this chromosome, it will be of interest to determine their physical relationship, if any, with the oncogene sequences.

TABLE 1. Correlation between specific mouse chromosomes and the c-*fes* mouse homolog in 22 somatic cell hybrids[a]

Mouse chromosome	No. of hybrid clones with mouse c-*fes*/chromosome retention				% discordant
	+/+	−/−	+/−	−/+	
1	8	4	4	6	45
2	7	9	5	1	27
3	4	7	8	3	50
4	6	8	6	2	36
5	5	10	7	0	32
6	8	9	4	1	23
7	12	10	0	0	0
8	3	8	9	2	50
9	4	10	8	0	36
10	5	10	7	0	32
11	0	10	12	0	55
12	8	6	4	4	36
13	7	9	5	1	27
14	3	9	9	1	45
15	10	5	2	5	32
16	7	9	5	1	27
17	8	4	4	6	45
18	6	8	6	2	36
19	6	6	6	4	45
X	6	7	6	3	41

[a] The mouse chromosome content was determined by direct karyology with Giemsa-trypsin banding followed by staining with Hoechst 33258 (Farberwerke Hoechst AG).

TABLE 2. Correlation between specific mouse chromosomes and the c-Ha-*ras*-1 mouse homolog in 30 somatic cell hybrids[a]

Mouse chromosome	No. of hybrid clones with mouse c-Ha-*ras*-1/chromosome retention				% discordant
	+/+	−/−	+/−	−/+	
1	11	7	7	5	40
2	13	11	5	1	20
3	8	9	10	3	43
4	7	10	11	2	43
5	4	11	14	1	50
6	11	11	7	1	27
7	18	12	0	0	0
8	6	11	12	1	43
9	8	11	10	1	37
10	6	12	12	0	40
11	0	12	18	0	60
12	15	8	3	4	23
13	8	12	10	0	33
14	4	11	14	1	50
15	17	6	1	6	23
16	8	9	10	3	43
17	10	7	8	5	43
18	6	10	12	2	47
19	9	9	9	3	40
X	5	9	13	3	53

[a] The mouse chromosomes were identified by Giemsa-trypsin banding followed by staining with Hoechst 33258.

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