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A New Oncogene, c-*raf*, Is Located on Mouse Chromosome 6

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The recently described acute transforming virus 3611-MSV contains cellular sequences designated v-*raf*. Mouse cellular DNA contains a single-copy sequence homologous to this oncogene (c-*raf*), and Southern blot analysis of hamster-mouse somatic cell hybrid DNAs showed that the mouse c-*raf* sequence is present on chromosome 6.

Mammalian chromosomes carry cellular genes (c-*onc* genes) which are homologous to the transforming sequences contained in the genomes of the acute transforming viruses (v-*onc* genes) (1). These sequences are conserved evolutionarily and are present in limited copies in chromosomal DNA. The association of these chromosomal sequences and neoplastic transformation is under active investigation, and a number of lines of evidence suggest that transformation may result from the altered structure or expression of these c-*onc* genes (6, 8, 17, 24). Perhaps the most intriguing was the finding that c-*onc* genes may be activated by their translocation to transcriptionally active chromosomal regions (4, 5, 25, 26). Many human and mouse tumors contain characteristic chromosomal abnormalities, and recent studies have localized various c-*onc* genes to the chromosomal breakpoints involved in the generation of these aberrant chromosomes (23).

These observations suggest a role for cellular oncogenes in malignancy and point to a need for a more complete description of the number, variety, and chromosomal distribution of these sequences in mammalian DNA. Recently, an experimental system has been described to specifically generate novel acute transforming viruses by growing type C viruses in chemically transformed mouse cells (18, 19). By using this approach, several transforming viruses have been isolated. One of these, designated 3611-MSV, transforms fibroblasts and epithelial cells in culture and induces fibrosarcomas in mice (21, 22). This virus carries a novel segment of DNA designated v-*raf* which is distinct from previously described *onc* sequences and which is associated with the production of two polyproteins devoid of protein kinase activity (21).

In this study, we analyzed a set of hamster-mouse somatic cell hybrids by blot hybridization to find the chromosomal location of the mouse cellular homolog of c-*raf*. Hybrids were generated by fusing cells from three inbred mouse strains (BALB, A, and NFS.*Akv-2*) with cells of the Chinese hamster line E36. These hybrids contain all of the hamster chromosomes and different subsets of mouse chromosomes. Mouse chromosomes were identified in metaphase spreads by Giemsa-trypsin banding followed by staining with Hoechst 33258 (10). The isolation and characterization of these hybrids and their use in genetic mapping have been described previously (11–13).

High-molecular-weight DNA was prepared from hamster, mouse, and hybrid cells as previously described (20). DNAs were digested with *Bgl*I or *Pst*I, electrophoresed, and examined in Southern blots with the v-*raf* probe (Fig. 1). Figure 1 shows DNAs from two negative (lanes 5 and 6) and two positive mouse-hamster hybrids (lanes 3 and 4). Lanes 2 and 1 show NFS/N mouse liver DNA and E36 DNA, respectively. In lane M are ^{32}P-labeled lambda *Hin*dIII and φX174 *Taq*I markers. Figure 1A and B display *Bgl*I- and *Pst*I-restricted DNAs, respectively.

In Fig. 1A, the 3611-MSV *Xho*I-*Bst*EII probe hybridizes to a ~12-kilobase (kb) fragment present in the mouse control and in the positive hybrids. E36 DNA and all the hybrids exhibit hybridization to a 4.45-kb fragment. In Fig. 1B, v-*raf*-specific hybridization is seen in the mouse control 5.5-kb *Pst*I fragment; this band is also present in the positive hybrids but is absent from the E36 and negative hybrid DNAs.

All 22 somatic cell hybrid lines tested after digestion with *Bgl*I contained the 4.45-kb hamster fragment, and seven of these hybrids also contained the 12-kb mouse band. Correlations with the mouse chromosome content of these 22 lines showed that the c-*raf* sequence is present in mouse chromosome 6 (Table 1). All seven hybrids with mouse c-*raf* sequences contained chromosome 6, and all of the remaining hybrids lacked both chromosome 6 and c-*raf*. All other mouse chromosomes showed discordant segregation with this sequence. This correlation was confirmed in experiments in which *Pst*I instead of *Bgl*I was used for DNA restriction.

More than 15 oncogenes have now been identified which are inherited as chromosomal genes in mammalian species (1). The assignment of the oncogene c-*raf* to mouse chromosome 6 emphasizes the fact that sequences associated with transformation are present on different chromosomes. In mice, as in humans, oncogenes have now been mapped to multiple chromosomes, including chromosomes 4 (*mos*) (25), 2 (*abl*) (7), 7 (*fes*, Ha-*ras*) (14), 15 (*myc*, *sis*) (3, 15), and now 6.

Examination of the mouse chromosome map shows that chromosome 6 carries one other genetic locus which directly affects tumor incidence. This locus, *Mov-1*, represents an integration site for Moloney leukemia virus (2). However, localization of c-*raf* and *Mov-1* to the same chromosome is most likely coincidental.

The assignment of c-*raf* to chromosome 6 is interesting in light of the fact that 6;15 as well as 12;15 chromosomal translocations are commonly found in mouse plasmacytomas (16). These typically result in the translocation of the *myc* oncogene from chromosome 15 to the immunoglobulin loci on chromosome 6 (*Igl*) or 12 (*Igh*) (9). Although we could not determine whether the c-*raf* and *Igl* sequences on this chromosome are closely linked, experiments are cur-

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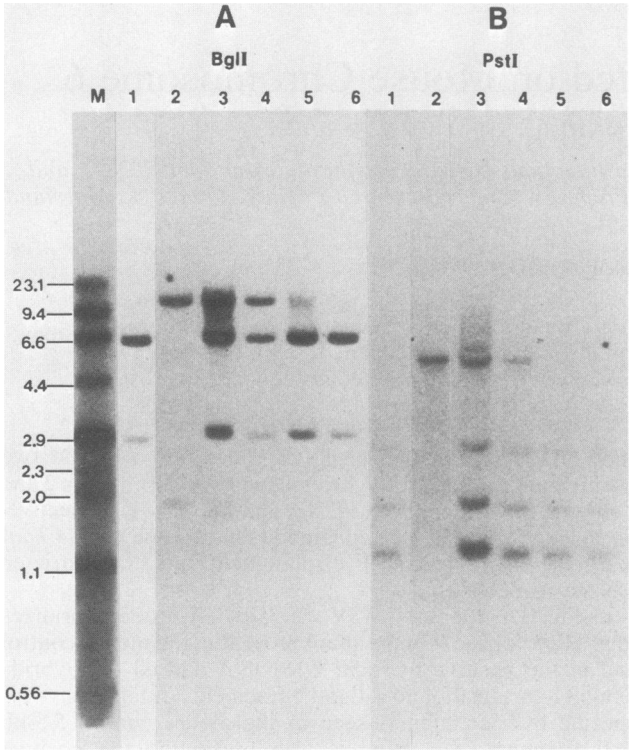

FIG. 1. DNAs from hybrid and control cells were digested with *Pst*I and *Bgl*I and electrophoresed in 1% agarose (SeaKem; FMC Corp., Marine Colloids Div., Rockland, Maine) with a Tris-acetate buffer (pH 7.8). Restriction fragments of ^{32}P-labeled lambda *Hin*dIII and ϕX174 *Taq*I were used as size markers. After electrophoresis, DNA was transferred from gels onto Genescreen (New England Nuclear Corp., Boston, Mass.) by a modification of the Southern technique as recommended by New England Nuclear Corp. A cloned *Xho*I-*Bst*EII fragment of 3611-MSV from v-*raf* was ^{32}P labeled by nick translation and hybridized to the membrane according to the protocol outlined by New England Nuclear Corp. Lanes: M, markers; 1, E36 hamster; 2, NFS/N mouse lines; 3 and 4, c-*raf* positive hybrids; and 5 and 6, c-*raf* negative hybrids. (A) *Bgl*I digest; (B) *Pst*I digest.

rently in progress to position c-*raf* by using somatic cell hybrids which carry translocation chromosomes. Finally, the examination of plasmacytomas carrying 6;15 translocations should determine whether c-*raf* sequences are rearranged or show altered expression to determine whether this *onc* sequence has any role in this neoplastic disease.

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TABLE 1. Correlation between specific mouse chromosomes with the c-*raf* homolog in 22 somatic cell hybrids

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

[a] Five hybrids contain c-*raf* and chromosome 1 (+/+), ten hybrids lack c-*raf* and chromosome 1 (−/−), seven hybrids contain only c-*raf* (+/−) or only chromosome 1 (−/+).

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