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Regional Localization of Two Human Cellular Kirsten *ras* Genes on Chromosomes 6 and 12

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Human cellular Kirsten *ras*1 and *ras*2 genes were localized to chromosomes 6p23→q12 and 12p12.05→pter, respectively, using human-rodent cell hybrids. Thus, the short arms of human chromosomes 11 (encoding lactate dehydrogenase-A and the proto-oncogene c-Ha-*ras*1) and 12 (encoding lactate dehydrogenase B and c-Ki-*ras*2) share at least two pairs of genes that probably evolved from common ancestral genes.

Kirsten murine sarcoma virus is a replication-defective mouse retrovirus isolated from a mouse tumor induced by murine leukemia virus that had been passaged in rats (16). Kirsten murine sarcoma virus causes solid tumors and erythroid leukemias in infected animals (33). The viral sequences responsible for oncogenic transformation by Kirsten murine sarcoma virus are designated v-Ki-*ras* and are derived from a low copy sequence present in the normal rat genome (9). Cellular sequences related to v-Ki-*ras* have been identified in the human genome (9). One of these genes, designated c-Ki-*ras*2, resides on chromosome 12 (32) and is the progenitor of a transforming gene identified in some human lung and colon cancer cells by DNA-mediated transfection assays with mouse NIH 3T3 fibroblasts (7, 21). The second v-Ki-*ras*-related gene, c-Ki-*ras*1, has been assigned to chromosome 6 (24).

Chromosome rearrangements are frequently observed in cancer cells (1, 28, 34) and have been proposed as a mechanism that could alter the expression of proto-oncogenes (17). To ascertain whether either of the two human c-Ki-*ras* genes resided near a known chromosomal aberration, we examined, by filter hybridization, human-rodent somatic cell hybrids containing various well-characterized portions of human chromosome 6 or 12 for the presence of c-Ki-*ras* genes. We report here the regional localization of c-Ki-*ras*1 to 6p23→q12 and c-Ki-*ras*2 to 12p12.05→pter.

Human-mouse somatic cell hybrids were characterized for human chromosome content by isozyme analysis and karyotyping as already described in detail (36). Human-mouse cell hybrids containing different regions of human chromosome 6, previously described by Grzeschik et al. (12), were constructed by fusion of the following cells: Call fibroblasts [46,XX,t(2;6)(q23;p23)] with mouse A9 fibroblasts (CallA9); IT fibroblasts [46,XX,t(6;7)(q12;p14)] with mouse A9 fibroblasts (ITA9); and GM610 fibroblasts [46,XX,t(6;18)(q21;p11)] to mouse RAG cells (GM610RAG). The RAGSU-3-1-2-3 human-mouse hybrid containing a spontaneous deletion of 6q [del(6q14→6qter)] was also examined. Human-Chinese hamster cell hybrids segregating chromosome 12 breaks have been described previously by Law and Kao (18, 19). Cellular Ki-*ras* genes were detected by using a 600-base-pair *Sst*II-*Eco*RI fragment of the v-Ki-*ras* oncogene cloned in the recombinant plasmid HiHi-3 (9). HiHi-3 contains a 1-kilobase-pair (kbp) *Hin*cII fragment of the v-Ki-*ras* oncogene cloned with *Eco*RI linkers (9). In one experiment the presence of c-Ki-*ras*2 was verified with the clone p640 containing a 640-base-pair *Eco*RI-*Hin*dIII fragment specific for human c-Ki-*ras*2 (21; given to us by R. A. Weinberg). High-molecular-weight DNAs were isolated by proteinase K-sodium dodecyl sulfate methods (26) from cells at the same passage in culture as those used for isozyme analysis and karyotyping. Cellular DNAs (10 μg) were cleaved with 30 U of restriction endonuclease in the buffer formula provided by the manufacturer for 3 h at 37°C. The DNA fragments were loaded onto a 3-mm-thick 0.8% agarose gel made up in Tris-acetate buffer, pH 8.2. A 35-volt charge was applied to the gel for 16 h, and the separated fragments were transferred onto nitrocellulose (Schleicher & Schuell Co.) by the method of Southern (37). Filters were baked in vacuo for 2 h at 80°C before use. Filter hybridization of cell hybrid DNAs with Ki-*ras* probes was performed at 42°C in a buffer containing 10% sodium dextran sulfate and 40% formamide as previously described (31). Probes were labeled with [^{32}P]dCTP and [^{32}P]dTTP by nick translation (27) to specific activities of 5×10^7 to 1×10^8 cpm per μg of DNA. Filters were rinsed briefly in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at room temperature, then in 0.1× SSC–0.1% sodium dodecyl sulfate at 50°C, and exposed to X-ray film as described previously (31). The p640 probe was used in an identical manner, except that buffers used for hybridization contained 50% formamide.

Human DNA cut with *Bgl*II yielded two major fragments hybridizing with v-Ki-*ras* (ca. >23 and 4.2 kbp, labeled a and e, respectively, in Fig. 1A, lane 5). The 4.2-kbp *Bgl*II fragment originates from c-Ki-*ras*1, whereas the >23-kbp fragment originates from c-Ki-*ras*2 (21, 22). We initially examined DNAs from several human-mouse cell hybrids that were segregating human chromosomes 3 and 6, reported to contain Ki-*ras*-related sequences (15, 24). These DNAs were cleaved with *Bgl*II and hybridized with the v-Ki-*ras* probe. The 4.2-kbp *Bgl*II c-Ki-*ras*1 fragment (labeled e in Fig. 1A) was present in cell hybrid DNAs in lanes 1, 2, and 3 (weak signal). Cell hybrid DNA in Fig. 1A, lane 4 was negative for this fragment, which was clearly distinguishable from mouse-reactive fragments in lanes 6 and 7, labeled b, c,

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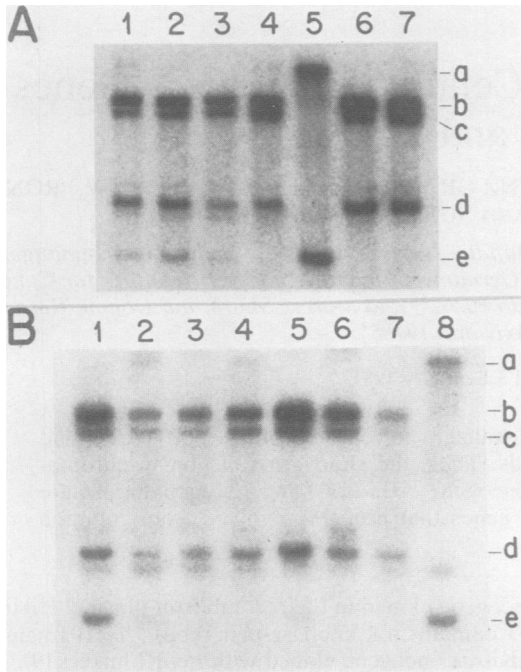

FIG. 1. Hybridization of v-Ki-*ras* to cell hybrid DNAs. (A) DNAs from human-mouse cell hybrids (lanes 1 through 4), human fibroblasts (lane 5), and mouse fibroblasts (RAG, lane 6; LM/TK$^-$, lane 7) cut with *Bgl*II and hybridized with the *Sst*II-*Eco*RI v-Ki-*ras* probe. Human DNA yielded two major hybridizing *Bgl*II fragments (ca. >23 and 4.2 kbp, labeled a and e). Mouse DNAs yielded three major hybridizing fragments (ca. 14, 11.5, and 5.5 kbp, labeled b, c, and d). The 4.2-kbp human *Bgl*II c-Ki-*ras*1 fragment is present in cell hybrid DNAs in lanes 1 through 3. (B) DNAs from cell hybrids containing chromosome 6 regions (12) are shown in lanes 1 through 6, mouse RAG cells are shown in lane 7, and human fibroblasts are shown in lane 8. DNAs were cleaved with *Bgl*II and hybridized with the v-Ki-*ras* probe. The regions of human chromosome 6 present in these cell hybrids are as follows (lanes): 1, RAGSU-3-1-2-3, 6pter→q14; 2, GM610RAG-4-5-1, 6pter→q21; 3, GM610RAG-5-2-3, 6q21→qter; 4, ITA9-1-2, 6q12→qter; 5, CallA9-1-13, 6p23→qter; 6, CallA9-1-9, 6pter→p23. These regions are depicted in Fig. 2. The 4.2-kbp *Bgl*II human c-Ki-*ras*1 fragment is present in cell hybrid DNAs in lanes 1, 2, and 5 (weak). Hybridization of the 4.2-kb *Bgl*II c-Ki-*ras*1 fragment is weak in some positive hybrids since human chromosome 6 and chromosome 6 translocations in cell hybrids in panels A and B are not selectively retained. Rather, these chromosomes are lost randomly during cell culture before harvest for DNA.

and d (ca. 14, 11.5, and 5.5 kbp, respectively). The chromosome composition of these cell hybrid lines with respect to human chromosomes 3 and 6 is shown in Fig. 1A as follows: lane 1, 3+ and 6+; lane 2, 3−, and 6+; lane 3, 3− and 6+; lane 4, 3+ and 6−. The 4.2-kbp *Bgl*II fragment equated with c-Ki-*ras*1 (21, 22, 24) thus segregated concordantly with chromosome 6 but not with chromosome 3. The other major hybridizing human *Bgl*II fragment equated with c-Ki-*ras*2

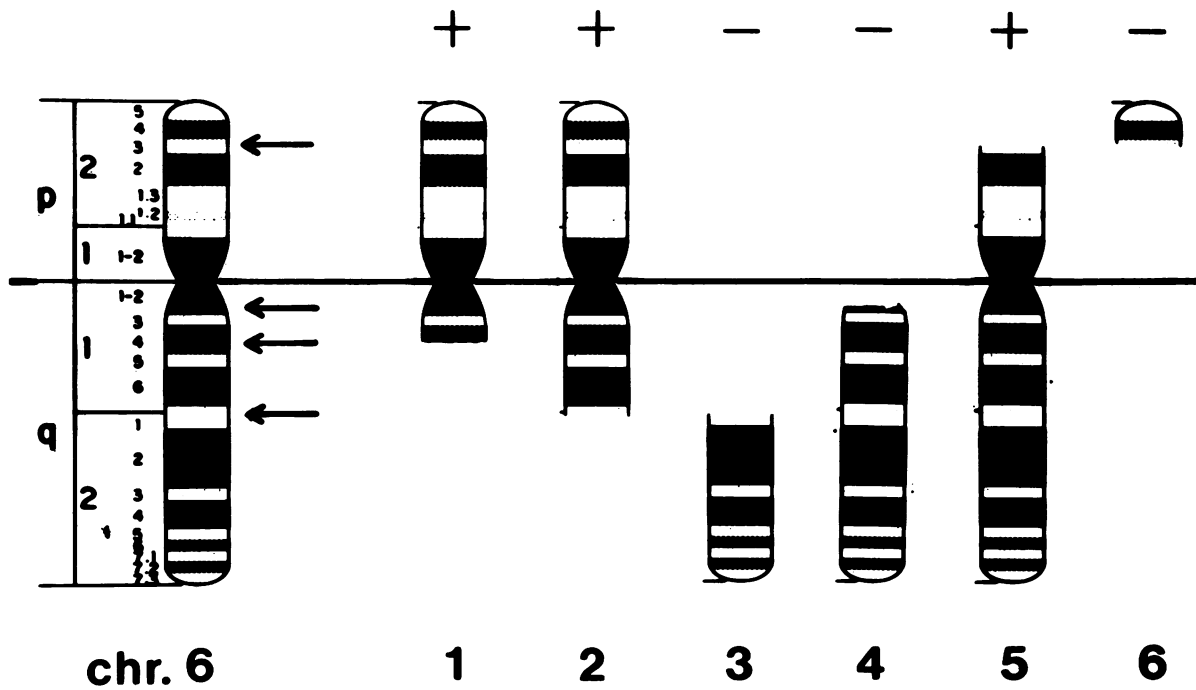

FIG. 2. The regions of human chromosome 6 segregating in CallA9, RAGSU, GM610RAG, and ITA9 cell hybrids are shown. The numbers 1 through 6 correspond to cell hybrid DNA samples with the same lane numbers in Fig. 1B. The presence (+) or absence (−) of human c-Ki-*ras*1 as determined by filter hybridization is indicated over each chromosome. The location of breaks involved in these chromosome 6 regions are indicated by arrows that point to a normal chromosome 6. These breaks are, from top to bottom: p23, q12, q14, and q21. The smallest common region of overlap of chromosome 6 segments present in cell hybrids containing human c-Ki-*ras*1 is p23→q12 (for example, compare chromosome 4 with chromosome 5).

(labeled a in lane 5, Fig. 1A) is not syntenic with either chromosome 3 or 6, and in fact, it segregated concordantly in cell hybrids shown in Fig. 1A and B with human chromosome 12, the known location of this gene (32).

The c-Ki-*ras*1 gene was regionally localized on chromosome 6 by examining DNAs from six cell hybrids that were constructed from human parental cells carrying translocations or spontaneous deletions of chromosome 6 (12). DNAs were cleaved with *Bgl*II and hybridized with the v-Ki-*ras* probe (Fig. 1B). The human 4.2-kbp *Bgl*II fragment was present in cell hybrid DNAs in lanes 1, 2, and 5 of Fig. 1B. Again several discordancies between chromosome 3 and c-Ki-*ras*1 were also noted among these cell hybrids. For example, DNAs in lanes 1 and 5 (Fib. 1B) that were positive for c-Ki-*ras*1 were isolated from hybrids without human chromosome 3, whereas DNA in lane 3 (negative for c-Ki-*ras*1) originated from a cell hybrid containing human chromosome 3. The 4.2-kbp *Bgl*II fragment segregated discordantly with all other human chromosomes except for chromosome 6 regions. The portions of chromosome 6 present in DNAs in lanes 1 through 6, Fig. 1B, are shown in Fig. 2. The smallest common region of overlap of chromosome 6 segments in hybrids positive for c-Ki-*ras*1 was 6p23→q12, thus localizing the gene to this region.

Human-Chinese hamster cell hybrids in which human chromosome 12 was selectively retained were examined to localize c-Ki-*ras*2 on chromosome 12. Cleaving DNAs with *Pvu*II and *Hin*dIII allowed human fragments hybridizing with v-Ki-*ras* (ca. 13, 8.1 and 3.6 kbp and indicated by arrows in Fig. 3, lane 6) to be distinguished from Chinese hamster hybridizing fragments (ranging in size from ca. 5.5 to 0.8 kbp). The complex pattern seen in Chinese hamster ovary DNA in Fig. 3, lane 5, results from amplification of c-Ki-*ras* genes in this species of hamster as previously reported by Chattopadhyay et al. (4). The variability in intensity of some Chinese hamster hybridizing fragments in cell hybrids could be due to aneuploidy and a variable number of the Chinese hamster chromosomes encoding c-Ki-*ras* in individual hybrid cell lines or to differential amplification of some c-Ki-*ras* sequences on chromosome homologs.

Human-Chinese hamster hybrid cell lines examined included 12A containing only human chromosome 12 (Fig. 3, lane 2); A9, a derivative of 12A, containing the portion 12p12.05→qter (lane 1); CHEN 3 containing a chromosome 12 from an unrelated individual (lane 3); and 60A2C18, a subclone of 12A that has lost chromosome 12 (lane 4). The 13- and 8.1-kbp *Pvu*II-*Hin*dIII human fragments hybridizing with v-Ki-*ras* were present in 12A and CHEN 3 but absent in A9, and as expected, they were absent in 60A2C18. DNAs cleaved with *Pvu*II and hybridized with v-Ki-*ras* yielded an 11-kbp fragment present in 12A but absent in A9 (data not shown). A fragment of the same size also hybridized with the probe p640, which specifically detects c-Ki-*ras*2 (21). Taken together, the data place human c-Ki-*ras*2 in the p12.05→pter region of chromosome 12.

The 3.6-kbp human DNA fragment was absent in 12A and CHEN 3 and was therefore not syntenic with chromosome 12. We could not determine whether the 3.6-kbp human fragment originates from c-Ki-*ras*1 on human chromosome 6 because the *Pvu*II-*Hin*dIII digest of mouse-human hybrid DNA containing chromosome 6 but not 12 (e.g., cell hybrid in lane 1, Fig. 1B) yielded a comigrating mouse fragment. This seems likely, however, since the 3.6-kbp fragment represented the only other human fragment displaying strong hybridization with the v-Ki-*ras* probe (Fig. 1A).

The c-Ha-*ras*1 proto-oncogene, which shares homology with c-Ki-*ras*2, has previously been assigned to the short arm of chromosome 11 (6, 20), also the site for the gene encoding lactate dehydrogenase-A (14). The chromosome 12 short arm encodes the gene for lactate dehydrogenase-B (14), which shares a high degree of homology with lactate dehydrogenase-A (8). Ohno has proposed that chromosomes 11 and 12 have arisen from a tetraploidation of the genome 300,000,000 years ago in an ancestral species (25). Whether c-Ha-*ras*1 and c-Ki-*ras*2 and lactate dehydrogenase-A and lactate dehydrogenase-B are the result of partial or complete chromosome duplication and subsequent divergence is not known. However, c-Ha-*ras*1 and c-Ki-*ras*2 genes have a similar exon structure and probably have evolved from a common ancestral gene (2, 10, 22, 35). It is also of interest to note that mouse chromosomes 6 and 7 that encode c-Ki-*ras* and c-Ha-*ras* genes (31a), also encode homologs of human lactate dehydrogenase-B and lactate dehydrogenase-A, respectively (14a). This raises the possibility that cellular *ras* genes and genes for lactate dehydrogenase isozymes might also be linked in other mammalian species and perhaps even in species phylogenetically distant from mammals.

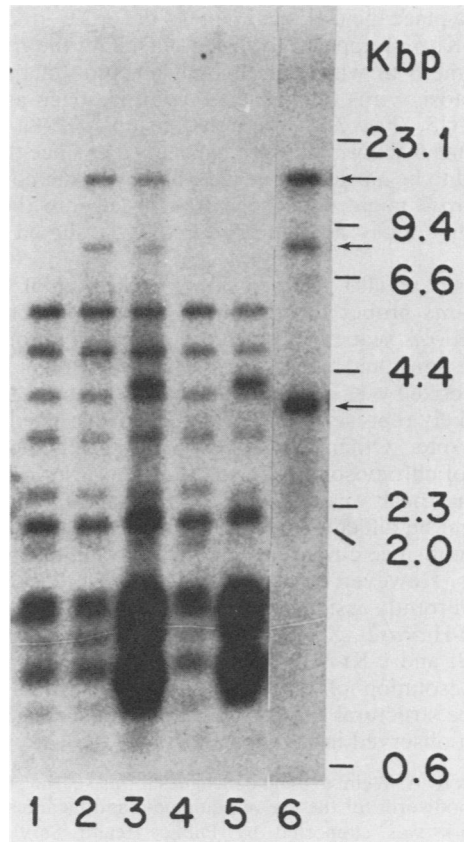

FIG. 3. Hybridization of the v-Ki-*ras* probe to cellular DNAs cleaved with *Pvu*II and *Hin*dIII. The DNAs shown are from the following cells (lanes): 1, cell hybrid A9 containing the human chromosome 12 region p12.05→qter; 2, cell hybrid 12A containing human chromosome 12; 3, CHEN 3 containing chromosome 12 from an individual unrelated to that in 12A; 4, cell hybrid 60A2C18, devoid of human chromosomes; 5, Chinese hamster ovary cells; and 6, human diploid fibroblasts. Three major hybridizing fragments occur in human DNA in lane 6 (ca. 13, 8.1, and 3.6 kbp and indicated by arrows). Chinese hamster ovary DNA yields hybridizing fragments ranging in size from ca. 5.5 to 0.8 kbp (lane 5). The positions of bacteriophage λ *Hin*dIII fragments are shown on the right in kbp.

Our data place the c-Ki-*ras*1 gene in the 6p23→q12 region. Human c-Ki-*ras*1 appears to reside outside of the region on chromosome 6 to which the human homolog of the avian myeloblastosis virus oncogene (v-*myb*) has been assigned, q15→q24 (13, 20a, 38). The relationship of c-Ki-*ras*1 to chromosome 6 abnormalities remains unclear since this gene is reported to be a pseudogene (22); however, the possibility that c-Ki-*ras*1 might have been transposed into the histocompatibility locus antigen region (p21.1) should be explored.

Jahnwar et al. (15), using in situ hybridization of v-Ki-*ras* and v-Ha-*ras* probes to human meiotic chromosomes, reported c-Ki-*ras* sequences on the short arm of chromosome 3 and the long and short arms of chromosome 12. The uncharacterized v-Ki-*ras*-related sequences they detected at 12p12.1 likely represent the c-Ki-*ras*2 gene assigned here to 12p12.05→pter. Other cellular *ras* genes segregating on the long arm of chromosome 12 or on the short arm of chromosome 3 that bear weak homology with v-Ki-*ras*, and as a result might be difficult to detect by filter hybridization, are not ruled out since c-*ras* genes constitute a multigene family in humans. However, such genes would have to be distinct from the currently assigned human *ras* genes: c-Ha-*ras*1, 11p (6, 20); c-Ha-*ras*2, X (29); N-*ras*, 1p (5, 30); c-Ki-*ras*1, 6p23→q12; and c-Ki-*ras*2, 12p12.05→pter (24, 30, 32; this paper). Resolution of these possibilities warrants further study since structural aberrations of chromosomes 3 and 12 have been observed in human cancers (11, 23).

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