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23. Abbreviations for amino acid residues: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Trp, tryptophan; Val, valine.
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Nucleotide Sequence of the Oncogene Encoding the p21 Transforming Protein of Kirsten Murine Sarcoma Virus

Abstract. *The transforming protein of Kirsten murine sarcoma virus (Ki-MuSV) is a virally encoded 21-kilodalton protein called p21* kis. *The sequences encoding p21* kis *were genetically localized to a 1.3-kilobase segment near the 5′ end of the viral genome by assaying the capacity of a series of defined deletion mutants of molecularly cloned Ki-MuSV DNA to induce focal transformation of mouse cells. Nucleotide sequencing of a portion of this region has led to the identification of an open reading frame of 567 nucleotides coding for p21* kis *protein.*

There is accumulating evidence that homologs of the viral *onc* (v-*onc*) genes of the transforming sequences of retroviruses present in the cellular genome (*1*) are incorporated into the viral genome of replication-competent leukemia viruses (*2, 3*). This process often involves substitution of a portion of the leukemia virus genome with the cellular *onc* (c-*onc*) gene (*4*). Thus, the mammalian transforming viruses are defective in their replication. Kirsten murine sarcoma virus (Ki-MuSV) (*5*) and Harvey murine sarcoma virus (Ha-MuSV) (*6*) are two such replication-defective transforming viruses. Each of the transforming sequences, derived from rat cellular DNA, was incorporated into its respective replication-competent murine leukemia virus (MuLV) genome while the MuLV's had been passaged in rats (*2*). The transforming proteins of Ki-MuSV and Ha-MuSV are virally encoded 21-kilodalton proteins called p21 *kis* and p21 *has*, respectively. The p21's of both viruses share certain antigenic determinants and the capacity to bind guanine nucleotides. However, molecular hybridization studies suggest that the p21 coding sequences of Ki-MuSV and Ha-MuSV were derived from different c-*onc* genes. We now report the genetic mapping of the transforming region of the Ki-MuSV genome and the complete nucleotide sequence of the open reading frame that encodes p21 *kis* viral protein.

The viral RNA genomes of the rat-derived Ki-MuSV [6.5 kilobases (kb) in length] and of Ha-MuSV (5.5 kb) (*7, 8*) are both composed of three distinct types of nucleotide sequences: (i) sequences homologous to MuLV, which are located in the first 0.2 kb at the 5′ end

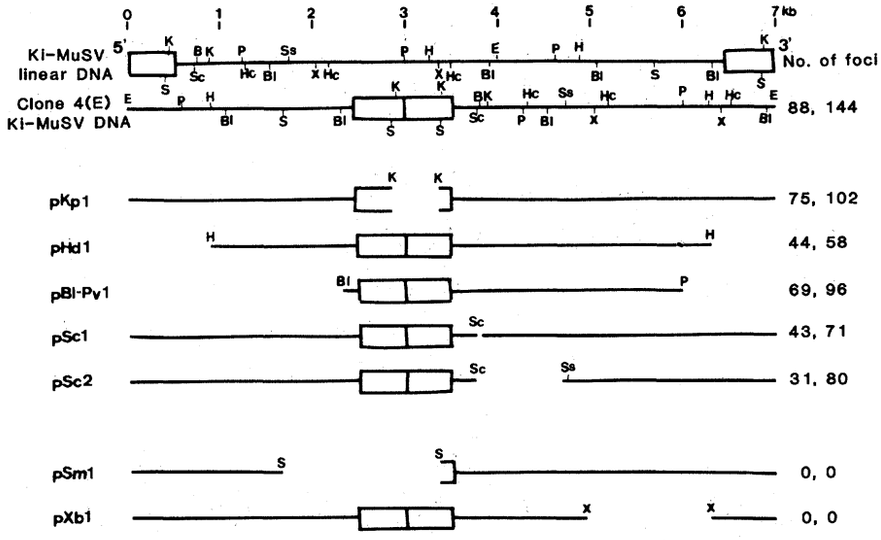

Fig. 1. Focus-forming activities of Ki-MuSV DNA clone 4(E) and deletion mutants derived from it. Recombinant DNA (1.5 μg) was used to transfect BALB/3T3 cells on duplicate plates, and foci were counted as described (*7*). The restriction enzyme sites on Ki-MuSV DNA are shown for Eco RI (E), Kpn I (K), Sma I (S), Sac I (Sc), Bam HI (B), Hinc II (HC), Pvu II (P), Bal I (Bl), Sst II (Ss), Xba I (X), and Hind III (H). The box represents the LTR sequence of Ki-MuSV linear DNA. The left end and the right end of the Ki-MuSV DNA correspond to 5′ and 3′ sides of the viral RNA, respectively. Preparation of recombinant DNA, digestion with restriction enzymes, purification of DNA fragments, ligation, transformation, and selection of *Escherichia coli* carrying a plasmid DNA were as described (*7*). For pKp1, clone 4(E) DNA, which is a circularly permuted form of the viral DNA genome cloned at the Eco RI site in pBR322, was digested with Kpn I, heated at 70°C for 5 minutes, and recircularized. The ligated DNA's were then used to transform *E. coli* χ1776. For pHd1, clone 4(E) DNA was digested with Hind III, and the resulting 5.4-kb fragment was ligated to pBR322 DNA, which had been digested with Hind III and treated with bacterial alkaline phosphatase (BAP) (*16*). The ligated DNA was used to transform *E. coli*, and a bacterial clone was selected by colony hybridization (*17*) with a ^{32}P-labeled Ki-MuSV RNA probe, prepared as described (*7*). For pBl–Pv1, clone 4(E) DNA was digested with Bal I and the 2.25-kb Bal I fragment containing two LTR's was purified and ligated to pBR322 DNA, which had been digested with Bal I and BAP. When this step was completed, DNA's were prepared from each clone and digested with Pvu II. A recombinant clone that produced 0.9- and 5.8-kb fragments after digestion was selected. The selected clone was digested with Pvu II, and the large fragment was isolated, treated with BAP, and ligated to the 1.7-kb Pvu II fragment that had been digested from clone 4(E) DNA. After transformation, a recombinant DNA clone was selected which produced 1.45-, 2.25-, and 3.75-kb fragments after Bal I restriction. For pSc1 and pSc2, clone 4(E) DNA was digested with Sac I, heated at 60°C for 5 minutes, and treated with 250 units of S1 nuclease (BRL) at 37°C for 10 minutes in 30 m*M* sodium acetate buffer (*p*H 4.6) containing 50 m*M* NaCl, 1 m*M* $ZnSO_4$ and 5 percent glycerol. DNA was extracted with a mixture of phenol, chloroform, and isoamyl alcohol, precipitated with ethanol, self-ligated, and used to transform *E. coli*. Bacterial clones that carried plasmid DNA were selected, and the plasmid DNA's were prepared. The pSc1 and pSc2 recombinant DNA's could not be restricted by Sac I, but produced Ki-MuSV DNA fragments of 7.0 and 6.15 kb, respectively, after the digestion with Eco RI (*18*). For pSm1, clone 4(E) DNA was digested with Xma I (which cleaves CCC GGG, as does Sma I) heated at 70°C for 5 minutes, circularized, and used to transform *E. coli*. For pXb1, clone 4(E) DNA was digested with Xba I, heated at 70°C for 5 minutes, recircularized, and used to transform *E. coli*.

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and the final 1.0 kb at the 3′ end of the viral RNA's (*8*); (ii) a sequence homologous to rat retrovirus-like "30*S* RNA" sequences (*9*), which are located between the two MuLV segments (*8*); and (iii) rat-derived sequences bracketed by the rat 30*S* RNA sequence but not homologous to MuLV or to the rat 30*S* RNA sequences (*10*). Except for the third sequence, Ki-MuSV and Ha-MuSV share extensive sequence homology with each other. In the third type sequence of both viruses (1.75 kb long for Ki-MuSV and 1.05 kb long for Ha-MuSV), it has been suggested that a 0.35-kb segment that was found to be partially homologous codes for part of each viral p21 protein (*10*).

The p21 transforming gene of the Ha-MuSV has been localized by genetic mapping (*11*). To confirm the location of the transforming gene of Ki-MuSV and elucidate sequences involved in its expression, we have introduced deletions of various lengths into the Ki-MuSV genomic DNA that had been molecularly cloned into the bacterial plasmid pBR322 (*7*). Seven recombinant DNA clones, with deletions in the locations indicated in Fig. 1, were isolated and used to transfect mouse cells. From the results of the transfection experiments (Fig. 1), we conclude that transformation requires at least one intact long terminal repeat (LTR), which is the viral control element, and an intact p21 structural coding sequence located between the Sst II and Pvu II restriction sites located at 1.7 and 3.0 kb, respectively (*12*).

We then determined the nucleotide sequence in the left half of the coding region described above (the Sst II–Hinc II fragment at 1.7 to 2.3 kb) (*13*). This fragment (Fig. 2) contains an open reading frame, starting with the initiation codon ATG (A, adenine; T, thymine; G, guanine) and ending with the termination codon TAA, which has a coding capacity for a protein of 189 amino acids. The estimated molecular size of such a protein is about 21,600 daltons, a value consistent with that of the p21 synthesized in vivo (*14*). The number of tryptic peptides predicted from the amino acid sequence was 23, which is also consistent with that reported (*10*).

The nucleotide sequence of the p21 transforming gene of Ki-MuSV was compared with that of Ha-MuSV (*15*). When the deduced amino acid sequences coded by each of the two genes were compared, we found an extensive sequence homology, except for the 22 amino acids at the carboxyl end of the Ki-MuSV p21 protein. In the first 165 amino acids, although 25.1 percent of the bases were different, only 17 amino acids were different, and most of these differences were neutral changes. Most of the substitutions of nucleotide sequences occurred at the third base of each codon. Since both p21 *kis* and p21 *has* proteins share immunological cross-reactivity and guanine nucleotide binding activity (*14*) and since the sequences partially homologous with that of the Ha-MuSV p21 coding region are located around the Sst II site of Ki-MuSV DNA (*10*), our results confirm the location of the p21 transforming gene in the Ki-MuSV genome and reveal the amino acid sequence of the p21 Ki-MuSV transforming protein. Thus, the p21 transforming gene is not present in either the MuLV sequences or the rat 30*S* RNA sequences; but rather this gene is located completely in rat-derived sequences that are conserved in evolution (*10*).

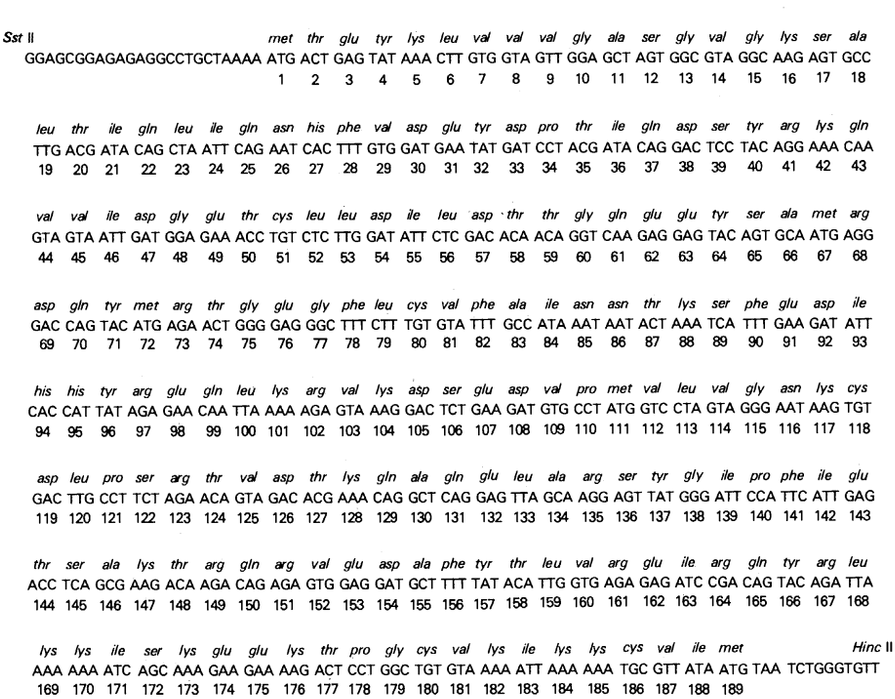

Fig. 2. Nucleotide sequence of the Sst II–Hinc II fragment, including p21 *kis*. Nucleotide sequence is shown for viral DNA (same sequences as viral RNA). The derived amino acid sequence is shown above the nucleotide sequence. Abbreviations for the amino acid residues are: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine, Cys, cysteine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Trp, tryptophan; Val, valine.

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I and S1 nuclease digestions and the subsequent ligation, had a 0.85-kb deletion and had lost all five restriction sites in the region from the Sac I site at 0.75 kb to the Bal I site at 1.55 kb of the linear DNA, the deletion of pSc2 was suggested to include the region from 0.75 kb to 1.6 kb of the linear DNA.

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Familial Renal Cell Carcinoma with a 3;11 Chromosome Translocation Limited to Tumor Cells

Abstract. *Cytogenetic studies were performed on the direct chromosome preparations of the renal cell carcinoma cells and the cultured peripheral blood lymphocytes of a patient with familial renal cell carcinoma. The results revealed a specific, acquired translocation (3p;11p) present in the majority of metaphases of the tumor, indicating that the development of renal cell carcinoma is associated with a deletion in the proximal end of 3p. Renal cell carcinoma is thus the third example—the first two being retinoblastoma and Wilms' tumor—of a chromosomal deletion occurring germinally or somatically in association with a specific tumor. This finding adds further support to the existence of specific human cancer genes.*

Nonrandom, tumor-specific chromosomal abnormalities have been observed in cells of many types of human tumors. At least for retinoblastoma and Wilms' tumor, chromosomal abnormalities have been observed to be both heritable and predisposing to the tumors and have been identified as tumor-specific, acquired abnormalities in tumors from persons with normal constitutional karyotypes (*1–4*). Recently, a heritable, balanced 3;8 chromosomal translocation was reported in association with familial renal cell carcinoma (RCC) (*5*). We now report a balanced 3;11 translocation in tumor cells from a patient with a normal constitutional karyotype and familial RCC.

The patient, a 32-year-old white man, was referred to the University of Texas M. D. Anderson Hospital and Tumor Institute at Houston after the diagnosis of a left papillary renal cell adenocarcinoma with extensive regional lymph node metastases. Peripheral blood was collected from the patient before chemotherapy for karyotyping and biochemical genetic markers; peripheral blood was also collected from family members. Ascitic fluid with cytologically confirmed malignant cells was obtained from the patient and processed immediately for cytological preparations (*6*). The familial occurrence of RCC over three generations was confirmed through death certificates and medical records (Fig. 1).

Of 50 metaphases, 30 showed a stemline number of 54 chromosomes. A minor peak (eight cells) occurred at 52 chromosomes. A total of 30 G-banded metaphases were analyzed for the identification of altered chromosomes. A typical G-banded karyotype of the major stem-line metaphase is shown in Fig. 2A. Among the G-banded cells, two cell lines were identified: in 22 cells (73.3 percent), a translocation involving 3p and the entire No. 11 chromosome was present, but in the other eight cells, chromosomes 3 and 11 were both normal. Four marker chromosomes were identified in the majority of metaphases of the major stemline. Putative identification of each of the marker chromosomes follows. M_1, an acrocentric, was similar in length to a D-group chromosome; the G-banding pattern identified this as the long arm of a chromosome 3. M_2 was a large, submetacentric chromosome the size of a normal chromosome 2; the G-banding pattern indicated that this marker was made of an entire chromosome 11, the distal end of whose p arm was translocated to 3p. This marker was also present in all 22 cells with a 3;11 translocation. Although M_2 sometimes exhibits the morphology of a dicentric chromosome, its C-banding pattern reveals only one centromeric region. M_2 was invariably accompanied by M_1. In cells with a 3;11 translocation only one normal chromosome 3 and one normal chromosome 11 were present. M_3 was the largest acrocentric chromosome in the complement. The proximal end, including the centromeric region, was similar to that of a chromosome 14, whereas the distal part was similar to that of a chromosome 2q in the G-banding pattern. This marker was also present in the majority of cells analyzed. M_4 was a small, metacentric chromosome present in the majority of the cells. It appeared to be an isochromosome of 5p. It was the only marker chromosome that was also present in the other eight G-banded metaphases in which other markers (M_1, M_2, and M_3) were absent.

A number of other altered chromosomes with minor rearrangements are shown paired with their normal homologs in Fig. 2A. For example, one chromosome 1 had a deletion in the distal segment of its q arm. Also, one chromosome 7 had added chromatin in its short arm. The minor hyperdiploid stem line had only the M_4 marker and normal chromosomes 3 and 11. Apparently M_4 was present before the translocation between 3p and 11p took place. Presence of M_4 might have been advantageous for the selection in vivo of the stem line with a 3p;11p translocation.

A diagrammatic sketch of the forma-

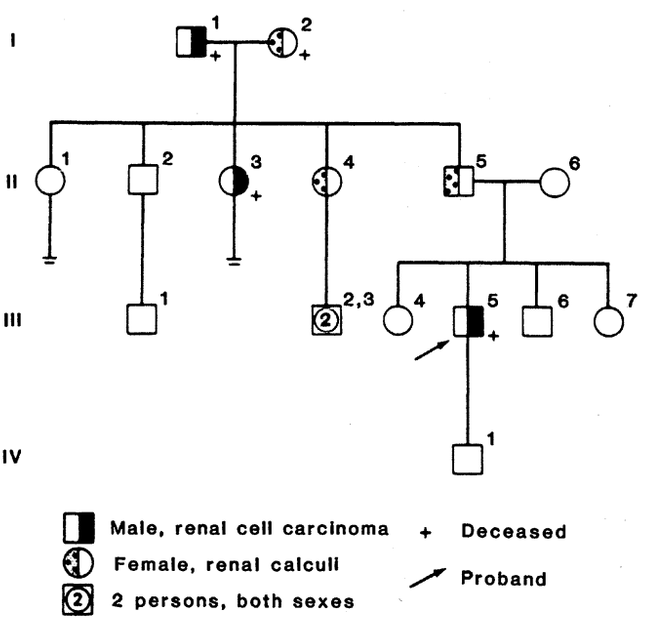

Fig. 1. Pedigree of the family members by clinical phenotype and karyotype.

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