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NUCLEOTIDE SEQUENCE OF EXON I OF THE RAT C-K-*RAS* GENE

BY

Akio IRITANI*1, Nachiko KATAYAMA*1, Tomoko TAHIRA*2,
Kenshi HAYASHI*3 and Nobuo TSUCHIDA*1

ABSTRACT

We have isolated a DNA fragment containing exon I of rat cellular proto-oncogene of K-*ras* and sequenced the exon I. The obtained sequence was compared with that of the corresponding region in viral oncogene of Kirsten strain of murine sarcoma virus (Ki-MSV). The results showed that the exon I sequences of cellular K-*ras* genes in rat and human cells could encode the same amino acid sequence, while the viral K-*ras* gene could code for two different amino acids corresponding to the 12th and 37th positions from the N-terminus of K-*ras* gene product. The amino acid substitutions found between viral and cellular K-*ras* genes are discussed in relation to the transforming ability of Ki-MSV.

Key words: Kirsten murine sarcoma virus, Sarcoma, *ras* gene, DNA sequence, Molecular cloning, Oncogene

INTRODUCTION

Acutely transforming retroviruses were known to be formed by transduction of cellular proto-oncogenes into replication-competent leukemia viruses (Swanstrom *et al.* [1]; Varmus [2]). Kirsten strain of murine sarcoma virus is one of the transforming retroviruses which has v-K-*ras* oncogene as a transforming gene. The virus was isolated initially from sarcomas induced when the Kirsten murine leukemia virus (Ki-MLV) was injected into rat (Kirsten and Mayer [3]). It has been suggested that during replication of Ki-MLV in rats, portions of the MLV genome substitute for the cellular proto-oncogene (c-K-*ras*), a cellular counterpart of v-K-*ras* gene, by transduction (Tsuchida *et al.* [4]; Ellis *et al.* [5]). The v-K-*ras* gene and human c-K-*ras*2 gene have been extensively studied (Varmus [2]). These genes are highly conserved among mammalian species and encode a protein of 21,000 daltons (p21). Although the human c-K-*ras*2 gene is comprised of four major coding exons, v-K-*ras* gene does not have an exon-intron structure (Tsuchida *et al.* [6]; Shimizu *et al.* [7]; McGrath *et al.* [8]). Results of analyses on human tumor DNAs showed that the substitution of an amino acid corresponding to either the 12th or 61st codon of c-K-*ras*2 gene could be activated to induce foci on NIH3T3 cells when tumor DNAs were transfected (Varmus [2]). Moreover the activation of the c-K-*ras* gene was suggested to result

*1 入谷昭男，片山奈知子，土田信夫: Department of Oral Microbiology (Chief: Prof. N. TSUCHIDA), Faculty of Dentistry, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
*2 田平知子: Carcinogenesis Division (Chief: Dr. M. NAGAO), National Cancer Center Research Institute.
*3 林 健志: Biochemistry Division (Chief: Dr. S. SATO), National Cancer Center Research Institute.
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in the reduction of GTPase activity of the p21 protein, although the enzymatic activities were only tested for p21 proteins of H-*ras* gene which is closely related to K-*ras* gene (Bishop [9]).

From the comparison of nucleotide sequences of v-K-*ras* with those of c-K-*ras*2 genes in normal and tumor DNAs of human cells, it has been suggested that the 12th codon of v-K-*ras* had an amino acid substitution, which conferred the oncogenicity on the viral *ras* gene (Tsuchida [6]; Shimizu *et al.* [7]; Capon *et al.* [10]). In the present study we cloned a DNA fragment containing exon I of c-K-*ras* gene from normal rat DNA and determined the nucleotide sequence of the exon I. The amino acid sequence deduced from the nucleotide sequence showed that there were two amino acid differences between v-K-*ras* and c-K-*ras* genes; one at the 12th codon and the other at the 37th codon.

Materials and Methods

Bacterial strains, plasmid and phages

LE392, JM105, and χ1776 were used as hosts for lambda phage, M13 phage, and the plasmid vector pBR322, respectively.

Enzymes

Restriction endonucleases, ligase, Klenow fragment of *E. coli* DNA polymerase I, and bacterial alkaline phosphatase were purchased from Takara Shuzo Co., and New England Biolab. Co., and used as suggested by the suppliers.

Rat genomic library

A genomic library from Sprague-Dawley rat was a kind gift from Drs. L. L. Jagodzinski and J. Bonner at the California Institute of Technology, California, U.S.A. The library had been constructed by partial digestion of rat DNA with *Hae* III, ligation to *Eco*R I linker, and insertion into Charon 4A phage DNA.

Molecular cloning and filter hybridization

All procedures were done according to the methods described by Maniatis *et al.* [11]. The rat genomic library was screened for the K-*ras* sequence, using the 380 nucleotide *Eco*R I-*Xba* I fragment of v-K-*ras* as a probe (Tsuchida *et al.* [6]; Ellis *et al.* [5]). Positive clones were isolated from single plaques, and grown in large quantity from which phage DNAs were prepared. The *Eco*R I fragment of phage DNA containing exon I sequence was subcloned at the *Eco*R I site of pBR322. The 1.45 kb *Stu* I-*Xba* I fragment (the human exon I probe) of pLC8 (Shimizu *et al.* [7]) was provided by Dr. K. Shimizu, Kyushu Univ. This fragment which contains the exon I sequence of human c-K-*ras*2 was used as a probe to identify restriction fragments of recombinant phage and plasmid DNAs containing exon I sequence in filter hybridization experiments.

Nucleotide sequencing

Nucleotide sequence was determined by dideoxy chain termination method developed by Sanger (Sanger *et al.* [12]), using M13mp18 and M13mp19 phages. Primer DNA and four dXTPs were purchased from Takara Shuzo Co., and ^{32}P-dCTP from International Chemicals and Nucleus Co.

Results and Discussion

Isolation of lambda phage containing c-K-ras

In order to isolate a DNA fragment containing exon I of c-K-*ras* gene, we first screened the rat genomic library using the 380 nucleotide fragment of v-K-*ras* gene as a probe. This fragment contains sequences corresponding to exon I, exon II and a 5′ portion of exon III, which was suggested from the comparison of nucleotide sequences of v-K-*ras* and human c-K-*ras* 2. Among 10^5 phages screened, two phage clones were

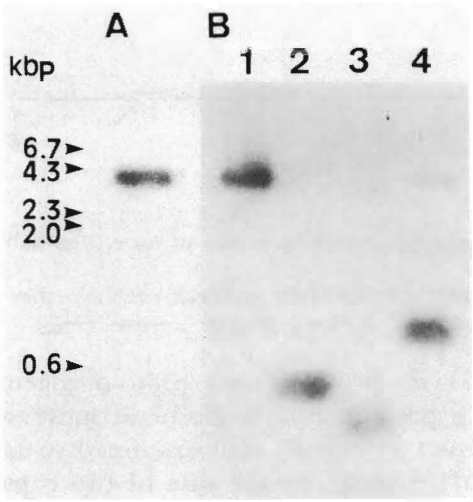

Fig. 1. Filter hybridization of DNA fragments containing exon I of rat c-K-*ras*.
(A) CKR-4 DNA was digested with *Eco*R I. (B) p3.9E DNA digested with *Eco*R I (lane 1) was further digested with *Hin*d III (lane 2), *Dra* I (lane 3), or *Bgl* II (lane 4). The digests were electrophoresed on a 1% agarose gel using *Hin*d III-digested lambda phage DNA as a marker, DNA fragments transferred to a membrane filter, and hybridized to ^{32}p-labeled human exon I probe. After the hybridization the filter was exposed to an X-ray film.

isolated and designated as CKR-4 and CKR-8, respectively. When the phage DNAs were digested with *Eco*R I, electrophoresed on a 1% agarose gel, transferred to a membrane filter, and probed with the 380 nucleotide fragment, the 3.9 kb fragment was detected in DNAs of both phages which were subsequently found to be overlapping clones (data not shown). To know whether or not the 3.9 kb fragment contains the exon I sequence, the *Eco*R I-digested CKR-4 DNA was filter-hybridized with the human exon I probe (Fig. 1, A). The figure indicates that the 3.9 kb fragment contained the exon I sequence.

Restriction mapping of the 3.9 kb fragment and localiationof the exon I sequence

The 3.9 kb fragment containing the exon I sequence was subcloned into the *Eco*R I site of pBR322, and the clone obtained was designated as p3.9E. The restriction map of the fragment was constructed for several restriction enzymes. The exon I sequence was localized, referring to the map of the same region and the location of exon I of c-K-*ras* gene in rat tumor induced by 1, 8-dinitropyrene (Tahira, Hayashi, Ochiai, Tsuchida, Nagao and Sugimura, unpublished data) (Fig. 2). To localize the exon I sequence within the 3.9 kb *Eco*R I fragment, the *Eco*R I-digested p3.9E DNA was further digested with *Hin*d III, *Dra* I, or *Bgl* II, and then filter-hybridized with human exon I probe. As shown in Fig. 1 B, the probe hybridized to the 0.5 kb *Eco*R I-*Hin*d III fragment (lane 2), the 0.3 kb *Eco*R I-*Dra* I fragment (lane 3), and the 0.8 kb *Eco*R I-*Bgl* II fragment (lane 4), suggesting that the exon I sequence was located in the region covered by the 0.3 kb *Eco*R I-*Dra* I fragment.

Nucleotide sequence of the exon I in normal rat DNA

Strategy for sequencing the exon I and its vicinity is shown in Fig. 2. The 0.5 kb *Eco*R I-*Hin*d III fragment and the 0.3 kb *Eco*R I-*Dra* I fragment were inserted between *Eco*R I and *Hin*d III sites of M13mp19, and *Eco*R I and *Hin*c II sites of M13mp18, respectively. The nucleotide sequence was determined by Sanger's method. The results are shown in Figs. 3 and 4.

Comparison of the nucleotide sequence with those of v-K-ras and human c-K-ras2

When the nucleotide sequence of the exon I and its vicinity of rat c-K-*ras* was compared with that of human c-K-*ras*2 (Shimizu *et al* [7]; McGrath *et al* [8]), both sequences showed extensive homology, including the donor and acceptor sequences for splicing, and the coding sequence. We found four nucleotide differences only in the coding frame, all of

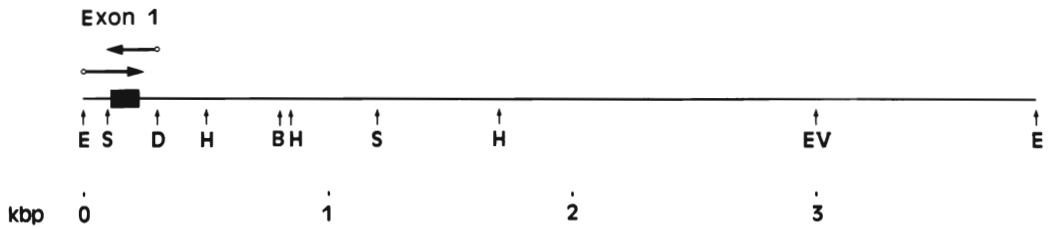

Fig. 2. Restriction endonuclease map of the 3.9 kb fragment containing exon I of rat c-K-*ras* and a strategy for DNA sequencing.
The cleavage sites for *Eco*R I (E), *Stu* I (S), *Dra* I (D), *Hin*d III (H), *Bgl* II (B), and *Eco*R V (EV) are shown. Long arrows indicate the region sequenced and the directions of the sequencing.

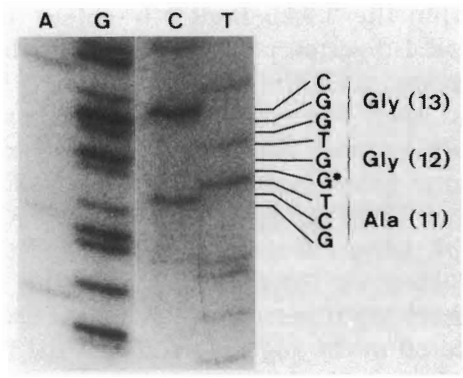

Fig. 3. Autoradiogram of a portion of the sequencing gel corresponding to the 12th codon of rat c-K-*ras* gene.
The 0.5 kb *Eco*R I-*Hin*d III fragment containing the exon I of rat c-K-*ras* gene was cloned into M13mp19, and the sequence was determined by the dideoxy chain termination method. The asterisk indicates the guanine which was changed to adenine in v-K-*ras* gene. The bracketed number are the predicted order of amino acid residues from the N-terminus of p21 K-*ras* protein.

which were located at the third letter of codons. Thus, both exons encode the same amino acid sequence. In the human genome, there are two c-K-*ras* genes, c-K-*ras*1 and c-K-*ras*2; the former is known as a pseudogene which lacks exon-intron structure and does not code for functional protein (McGrath *et al.* [8]). Although we do not know how many copies of c-K-*ras* genes rat genome has, the exon I that we cloned and sequenced most probably corresponds to that of human c-K-*ras*2 which encode p21 *ras* protein, since proto-oncogenes are present as a single or at most two copies in human cells examined so far. Furthermore, in the case of two copies one of them seems to be a processed nonfunctional pseudogene (McGrath *et al.* [8]; Bonner *et al* [14]; Chang *et al.* [15]). Therefore, it is likely that the exon I sequenced in the present study was transduced into the Ki-MSV genome. However, the possibility that other c-K-*ras* gene(s) in rat cells was/were used for the transduction is not excluded, since one of the two c-H-*ras* genes in rat cell which lacks introns is biologically active when ligated to viral LTR (DeFeo *et al.* [16]).

Comparing the exon I of normal c-K-*ras* sequence with the corresponding region of v-K-*ras* sequence, the 12th codon of glycine and the 37th codon of glutamine in the normal cell changed to serine and glutamic acid, respectively. Since both codons are the same for normal rat and human c-K-*ras* genes, these amino acid substitutions are specific for v-K-*ras* gene. Since an amino acid substitution at the 12th codon is known to activate c-K-*ras* genes, the change from glycine in normal rat c-K-*ras* gene to serine in v-K-*ras* gene is suggested to be related to the acquisition of transforming ability of v-K-*ras* gene. As for the amino acid change at the 37th codon from glutamine to glutamic acid, we do not known

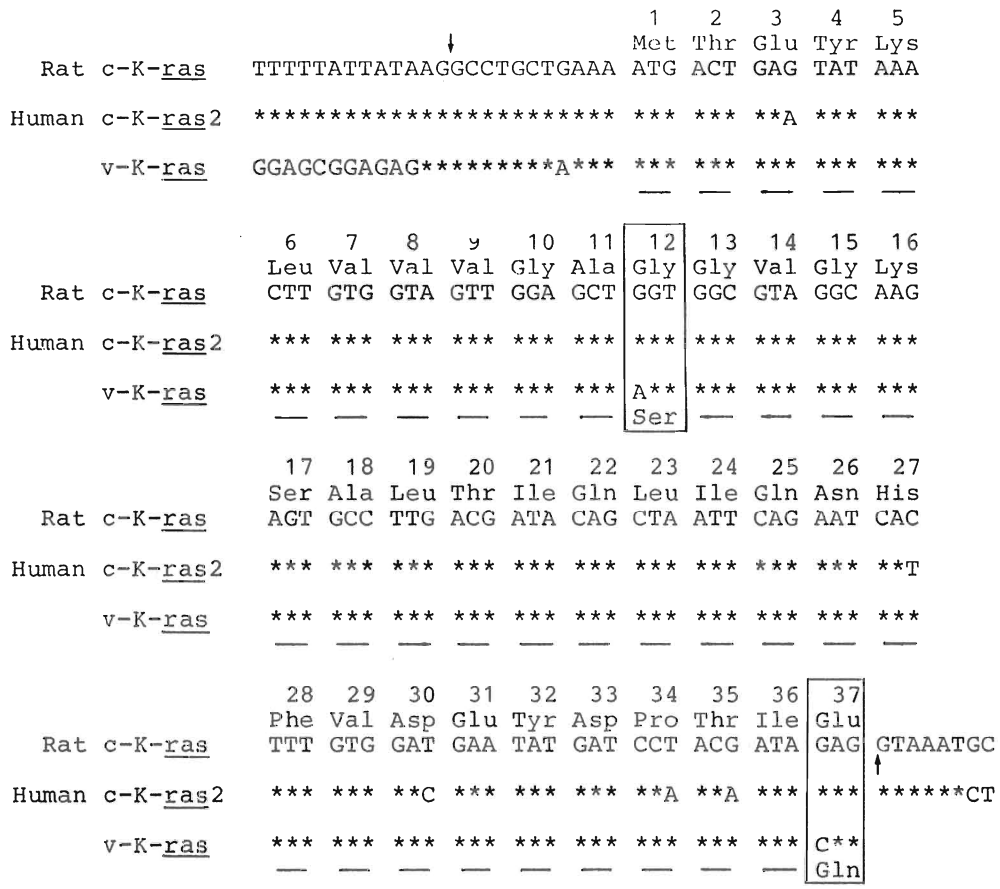

Fig. 4. Nucleotide sequence of the exon I.
The predicted amino acid sequence is shown above the nucleotide sequence. For comparison, sequences of the exon I of human c-K-*ras* and v-K-*ras* are shown. The asterisks and short lines indicate the same nucleotide and the same amino acid, respectively, as are those for the exon I of the rat c-K-*ras* gene. The positions where the amino acid sequence differs from that of v-K-*ras* are boxed. Small vertical upward and downward arrows (↑ and ↓) indicate acceptor and donor sites, respectively, for splicing.

its significance in reference to the oncogenicity of Ki-MSV. Although, Fasano *et al.* [17] introduced mutations in vitro at many positions in c-H-*ras* gene and found that oncogenic mutations occurred only at a restricted number of sites, including codons 12, 13, 59, 61, and 63.

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