An altered DNA sequence encompassing the ras gene of Harvey murine sarcoma virus

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ABSTRACT

The DNA fragment encompassing the ras gene of Harvey murine sarcoma virus was sequenced and assigned the coding region of a transforming protein, p21, to the sequence. Examination of nucleotide sequence, taken together with the result of analysis of the ras mRNAs (1), has revealed that p21 is encoded from a continuous coding region starting with the 5' proximal initiation codon but not a processed protein. However, there were found several differences between the sequence published by Dhar et al. (2) and ours, including 9 deletions, 7 substitutions and 2 insertions of nucleotides in the published sequence of 997 nucleotides in length. Among these, one of the substitutions occurring in the coding region resulted in amino acid replacement of glycine by alanine at position 122 of p21. The evidences are presented with some of actual gel autoradiographs.

INTRODUCTION

Harvey murine sarcoma virus (Ha-MuSV) was originally isolated from tumours induced by inoculation of Moloney murine leukemia virus (Mo-MuLV) into rats (3). This virus was able to transform fibroblast cells in culture and induce sarcomas in susceptible mice (4,5). In the virus-infected cells, a transformation specific protein with a molecular weight of 21 kilodaltons, p21, was produced (6,7). The closed circular DNA intermediates have been isolated from the infected cells and cloned molecularly (8) and physically characterized (13). Studies on transforming ability of the sub-genomic DNA allowed to localize it within the SmaI-PstI fragment in the proximal half of the viral genome (9,10).

We have sequenced a fragment spanning the ras gene of Ha-MuSV (H-ras) by the methods of Maxam and Gilbert (11) and of "dideoxy" sequencing (12) and assigned H-ras coding region to the sequence. Comparison of the nucleotide sequence with that reported earlier (2) has showed several discrepancies including insertion, deletion and substitution of nucleotides. One of these changes result in amino acid replacement of glycine by alanine at position 122 of p21 and in frame shift upstream from H-ras gene. The
latter suggests that p21 is encoded continuously from the 5' proximal ATG triplet in the single open reading frame. Technical problems will be discussed about DNA sequencing technology.

MATERIALS AND METHODS

[γ - 32 P]ATP and [α - 32 P] dCTP were purchased from RCC Amersham. M13 cloning and sequencing kits were obtained from either RCC Amersham or Takara Shuzo Co., Japan. Restriction endonucleases were gifts from Takara Shuzo Co. or purchased from New England Lab and Bio-Rad. T4 polynucleotide kinase was from Boehringer. Each enzyme and kit was used according to the suppliers specifications. dITP was from Boehringer.

The full length Ha-MuSV DNA cloned in λ gt WES - λ B (8) was generously provided by Dr. M. M. Martin and subsequently subcloned in pBR322 under the P2-Eκ2 conditions. The viral DNA was cleaved with appropriate restriction endonucleases to generate suitable sizes and separated by electrophoresis on polyacrylamide gels. After elution from the gels, the fragments were purified by the column chromatography on DEAE-sephacel (Pharmacia Co.).

The fragments were sequenced by the methods of Maxam and Gilbert (11) and of Sanger et al. (12).

RESULTS AND DISCUSSION

Coding region of p21

Ha-MuSV was originally isolated by passage of Mo-MuLV in rats (3). The circular duplex DNA intermediates were extracted from the Ha-MuSV infected NIH 3T3 cells and cloned molecularly (8) and the DNA was physically characterized (13). Thus, the structure of Ha-MuSV genome in an integrated form is tripartite (14): i) a 1.2 kilo base (kb) sequence derived from both termini of Mo-MuLV, ii) a 3.2 kb sequence homologous to rat 3OS RNA and iii) a 1.2 kb non-homologous sequence derived from the endogeneous transforming gene of rat, designated as c-ras. It is known that the ras gene family is widely distributed among eucaryotic species and the DNA sequences have been highly conserved since divergence of the species. It has been reported that the variants of human c-ras isolated from the bladder carcinoma cells (15,16) and neuroblastoma cells (17) carried a point mutation which resulted in the single amino acid substitution of p21, and were able to induce focal transformation of NIH 3T3 mouse cells, respectively.

Transfection experiments with the subgenomic DNA (9,10) has circumscribed the coding region of H-ras in the Smal-PstI fragment 580 nucleo-
tides downstream from the 5' long terminal repeat sequence (LTR) derived from Mo-MuLV (6). The fragment was isolated by electrophoresis of a polyacrylamide gel and then submitted to sequencing mainly according to the method of Maxam and Gilbert (11).

The AccI-PstI fragment which encompasses the SmaI-PstI fragment was composed of 1042 nucleotide in length (Fig. 1). Nucleotide sequence examination has indicated that a long reading frame was left open from position 178 to 921 of this sequence with the first ATG triplet at position 355. The frame from the first ATG has amino acid sequence information to encode a 21.3 kilo dalton protein, consistent with a molecular weight of p21. In addition, the amino acid sequence predicted from this frame was highly homologous to those from the ras genes of other eucaryotic species published so far. Therefore, the open frame from the ATG was able to be assigned as a coding frame of p21, in agreement with that reported earlier (2) except for two nucleotide substitutions; T for C at position 717 and G for C at position 719 of the present sequence (Fig. 1). The latter change results in amino acid substitution of glycine by alanine at position 122 of p21. In the case of c-H-ras, alanine is encoded from the corresponding position (15).

Discrepancies in nucleotide sequences flanked by H-ras

Several differences in the sequences were also found in non-coding regions, particularly upstream from the p21 coding region. They included 9 deletions and 5 substitutions and 2 insertions of nucleotides in the published sequences (Fig. 1). Because the viral DNA was originated from the same clone but sequenced in two separate laboratories, these discrepancies in the sequences may be partially caused by technical problems occurring inevitably in sequencing. Thus, most of the regions where many differences were found in the sequences can be characterized by GC rich regions which cause troubles against sequencing due to formation of secondary structures while the gel is running. We tried to sequence them from the both strands by the method of Maxam and Gilbert (11) as well as the "dideoxy" method (12) but failed to do them mainly due to "band compression" emerging in the ladder sequencing patterns. When dITP was added instead of dGTP to "dideoxy" reaction mixtures (18), the band compression disappeared and additional G bands appeared in the ladder patterns with the expected spaces between bands (Fig. 2a).

The active elements for gene expression resided in the 5' LTR approximately 1 kb upstream from H-ras gene. It has been presumed that transcrip-
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Fig. 1. The nucleotide sequence of the fragment encompassing H-ras gene of Ha-MuSV. The nucleotide sequence of the AccI-PstI fragment was determined by the methods of Maxam and Gilbert (11) and of Sanger et al. (12), and compared with the sequence reported earlier (2). The differences in nucleotide sequences from the published ones are indicated above the sequence line. Arrows (▼) indicate the positions where nucleotides were added to the original sequence. The deletion of dinucleotide CC occurred between position 134 and 135 shown in the box. The predicted amino acid sequence of p21 is also shown below the sequence line with one replacement of glycine by alanine at position 122.
Fig. 2. Autoradiographs of sequencing gels. Nucleotide sequences were determined by the "dideoxy" method of Sanger et al. (12). dITP was added to "dideoxy" mixture instead of dGTP in order to circumvent band compression occurring successive GC stretches (a). The nucleotide sequences different from the published by Dhar et al. (2) are indicated with the underlined capitals.

...tation of H-ras mRNA proceeds exclusively from the upstream LTR, generating the full-genome length of Ha-MuLV. The results of SI mapping (1) have indicated that the mature genomic and/or subgenomic RNAs were unspliced in this region. In accordance with this, the consensus sequences conserved around the donor and acceptor sites for splicing events were not found in the present sequence. In the sequence upstream from H-ras coding gene, all reading frames were interrupted by the presence of several stop codons and the amino acid sequences predicted from such short open frames were not homologous to those predicted from the corresponding regions of the re...
lated ras genes. These evidences suggest that H-ras mRNA carries a long non-coding region beyond the \textit{AccI} site and p21 is not the processed protein derived from a precursor protein. Probably, translation of p21 initiates from the ATG triplet at position 355. If so, the problems remain to be elucidated how ribosomes can bind the initiation codon of H-ras mRNA, scanning across such a long non-coding region, possibly from the cap site in LTR.

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\textbf{REFERENCES}