A Viable Simian Virus 40 Variant with a Deletion in the Overlapping Genes for Virion Proteins VP1, VP2 and VP3

(Accepted 8 July 1982)

SUMMARY

Nucleotide sequence analysis was used to determine the exact location of a deletion in the late region of the SP2 mutant of simian virus 40 (SV40), a viable small-plaque variant isolated from a persistent infection of rhesus monkey kidney cells. The results indicate that six base pairs are deleted from that part of the SV40 genome in which the coding regions for the three virion proteins, VP1, VP2 and VP3, overlap. This implies that all three virion proteins are affected by the deletion. This finding is discussed with respect to the viability of SP2.

Simian virus 40 (SV40) contains three structural proteins, VP1, VP2 and VP3, with respective molecular weights of 39700, 38500 and 27000 (Fiers et al., 1978; Reddy et al., 1978). The major virion protein, VP1, is encoded by nucleotide residues 1423 to 2508 (by the numbering convention of Reddy et al., 1978). The minor virion proteins, VP2 and VP3, are encoded by residues 480 to 1535 and 834 to 1535 respectively (Reddy et al., 1978). Thus, the same segment of DNA codes for the C-terminal ends of both VP2 and VP3 (Fig. 1). Furthermore, the coding sequences of VP2 and VP3, which are read in the same frame, overlap the first 113 nucleotides of the VP1 coding sequence, which is read in an alternate frame.

SP2, a viable small-plaque variant of SV40, was isolated from a persistent infection of rhesus monkey kidney cells (Norkin, 1979). Restriction endonuclease analysis indicated that SP2 contains a deletion of about six base pairs in the HindII + III fragment K (Norkin, 1979), which is bounded by nucleotide residues 1415 and 1628 (Reddy et al., 1978). Because the coding sequence of VP1 overlaps that of VP2 and VP3 for 113 of the 214 residues of fragment K (Fig. 1), it appeared possible that all three structural proteins of SP2 might be affected by the deletion, and nucleotide sequence analysis, as reported here, established that this is indeed the case. Because one might expect that a DNA sequence which contains three overlapping genes and which is transcribed in two reading frames would be highly conserved, the deletion in SP2 is discussed with respect to the viability of this variant.

The sequence gel pattern (not shown) established that residues 1446 to 1451 (or 1445 to 1450)
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Simian virus 40 (SV40) contains three structural proteins, VP1, VP2 and VP3, with respective molecular weights of 39,700, 38,500 and 27,000 (Fiers et al., 1978; Reddy et al., 1978). The major virion protein, VP1, is encoded by nucleotide residues 1423 to 2508 (by the numbering convention of Reddy et al., 1978). The minor virion proteins, VP2 and VP3, are encoded by residues 480 to 1535 and 834 to 1535 respectively (Reddy et al., 1978). Thus, the same segment of DNA codes for the C-terminal ends of both VP2 and VP3 (Fig. 1). Furthermore, the coding sequences of VP2 and VP3, which are read in the same frame, overlap the first 113 nucleotides of the VP1 coding sequence, which is read in an alternate frame.

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It might have been predicted that a segment of the virus genome which encodes three proteins and which is transcribed in two reading frames could not be altered without a concomitant change in some function. Thus, note that the rates of replication and release of SP2 are similar to that of wt virus, and that SP2 effectively competes with wt virus in mixed infections (Norkin, 1979). Consequently, the affected regions of the virion proteins of SP2 are either non-essential for virus function or serve in a role not yet identified.

The roles and mechanisms of action of the virion proteins in virus replication and maturation are not yet well understood. Temperature-sensitive (ts) mutations which affect VP1 are normal for virus DNA replication, but fail to form infectious virus at non-permissive temperature (Tegtmeyer & Ozer, 1971). The ts mutants of VP2 and/or VP3 fail to synthesize early viral mRNA, although they replicate as well as wt virus in DNA infections (Robb & Martin, 1972). This suggests that such mutants might contain a tightly binding repressor-like molecule which must be removed before any transcription can begin. It was suggested that late in infection VP2 and/or VP3 might bind to virus transcriptional complexes, thereby repressing (attenuating) transcription and promoting packaging (Llopsis & Stark, 1981).

SP2 is somewhat cold-sensitive for replication at 33°C (Norkin, 1979), suggesting that it might be impaired in assembly at that temperature. Assuming that this aspect of the SP2 phenotype results from the deletion described here, then the deletion might affect one or more of the structural proteins at a site which plays a role in the not yet well-understood maturation and assembly processes.

VP2, and possibly VP3, may indeed be dispensable for virus assembly. Viable deletion
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Because one might expect that a DNA sequence which contains three overlapping genes and which is transcribed in two reading frames would be highly conserved, the deletion in SP2 is discussed with respect to the viability of this variant.

The sequence gel pattern (not shown) established that residues 1446 to 1451 (or 1445 to 1450) are deleted in SP2. This deletion affects the C terminus of VP1 by removing 11 nucleotides which code for the 36 C-terminal amino acids (Reddy et al., 1978). The deletion also affects the C terminus of VP2 and VP3 by removing 11 nucleotides which code for the 36 C-terminal amino acids.

This investigation was supported by Public Health Service Research grant 1 R01 AI14049 from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support grant RR07048, and a grant from the American Cancer Society. Michael Piatak was supported by Public Health Service Traineeship CA09159 and was a fellow of the Leopold Scheppe Foundation.

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(Received 27 April 1982)