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Suppression of a VP1 Mutant of Simian Virus 40 by Missense Mutations in Serine Codons of the Viral Agnogene

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We isolated second-site revertants of a partially defective VP1 mutant of simian virus 40. The suppressing mutation in each of these pseudorevertants was mapped to the viral agnogene. Of six independently isolated pseudorevertants, all had a missense mutation in a serine codon, near the beginning of the agnogene, that would cause replacement of serine at position 7, 11, or 17 in the agnoprotein by a hydrophobic amino acid. Our results suggest that the agnoprotein interacts in a specific way with VP1 during the late stages of viral development.

The late region of the simian virus 40 (SV40) genome encodes three virion proteins (VP1, VP2, and VP3) and a recently identified basic protein (agnoprotein), not detected in virions, whose function is unknown (9, 10). The agnoprotein is encoded within the leader sequence of late mRNA (4–6) and has a molecular weight of about 7,900. In keeping with its high content of basic amino acids, the agnoprotein binds to nucleic acids (10). It has recently been suggested that the agnoprotein may play a role in regulating the expression of virion protein genes (1, 8). Another possible function of the agnoprotein is in the assembly of virus particles, interacting transiently with viral DNA, virion protein(s), or both. In this report we present genetic evidence for a specific interaction between the agnoprotein and VP1, the major capsid protein of the virus.

MATERIALS AND METHODS

Cell lines and viruses. SV40 and its mutants were propagated in monolayer cultures of BSC-40 African green monkey kidney cells (3). The original plaque morphology mutants used were described previously (19). Isolation of second-site revertants was carried out as described previously (20). Viral growth was measured at 37°C after infection of BSC-40 monolayers at a multiplicity of 1 PFU per cell, and duplicate cell lysates were assayed at different times after infection.

In vitro recombinants and marker rescue mapping. Fragments of viral DNA were separated by electrophoresis in 1.4% agarose gels (17). The appropriate recovered fragments were joined by incubating, for 18 h at 15°C, ca. 50 ng of each fragment in a 10-μl reaction mixture containing 0.2 U of T4 DNA ligase. After dilution with 15 mM NaCl–1.5 mM sodium citrate, the DNA was used to transfect BSC-40 cells by the DEAE-dextran method (13). Each 6-cm dish received from 1 to 20 ng of DNA, which yielded approximately 10 plaques per nanogram. All enzymes were obtained from commercial sources. Mapping by marker rescue was carried out as previously described (20).

DNA sequence analysis. DNA sequencing was done by the chemical degradation method of Maxam and Gilbert (12). For agnogene sequences, the *Hpa*II-*Hae*II fragment (0.73 to 0.83 map units) was 3′ end labeled at 0.73 with *Micrococcus luteus* polymerase and [α-^{32}P]dATP. Labeling reactions contained 1 to 3 μg of restricted mutant DNA, a twofold molar excess of [α-^{32}P]deoxynucleotide, and 2 U of *M. luteus* polymerase (Miles Laboratories, Inc.).

RESULTS

Identification of a partially defective VP1 mutant. In the course of a mutational analysis of the origin of replication (*ori*) of SV40 DNA, Shortle and Nathans (19) isolated two independent mutants (*shp*1027 and *shp*1028), after local bisulfite mutagenesis at the *Bgl*I site of SV40 DNA, that produced small, clear, sharp-edged plaques (*shp*) and contained the same cytosine-guanine to adenine-thymine change at base pair 5,237 within the origin of replication. Although the plaque phenotype of these mutants was attributed to the base substitution at the origin of replication, in neither case was it demonstrated that the phenotype was due to the altered *ori* sequence. During our subsequent investigation of second-site revertants of these isolates, we found that the *ori* region of both mutants could be replaced by the wild-type SV40 sequence without changing the plaque morphology. We then proceeded to localize the mutation responsible for the sharp-edged plaque phenotype of *shp*1027 and *shp*1028 to a DNA segment between the *Eco*RI site at nucleotide 1,782 and the adjacent *Hin*dIII site at nucleotide 1,708 by in vitro recombination and marker rescue experiments (Fig. 1). This segment encodes part of the

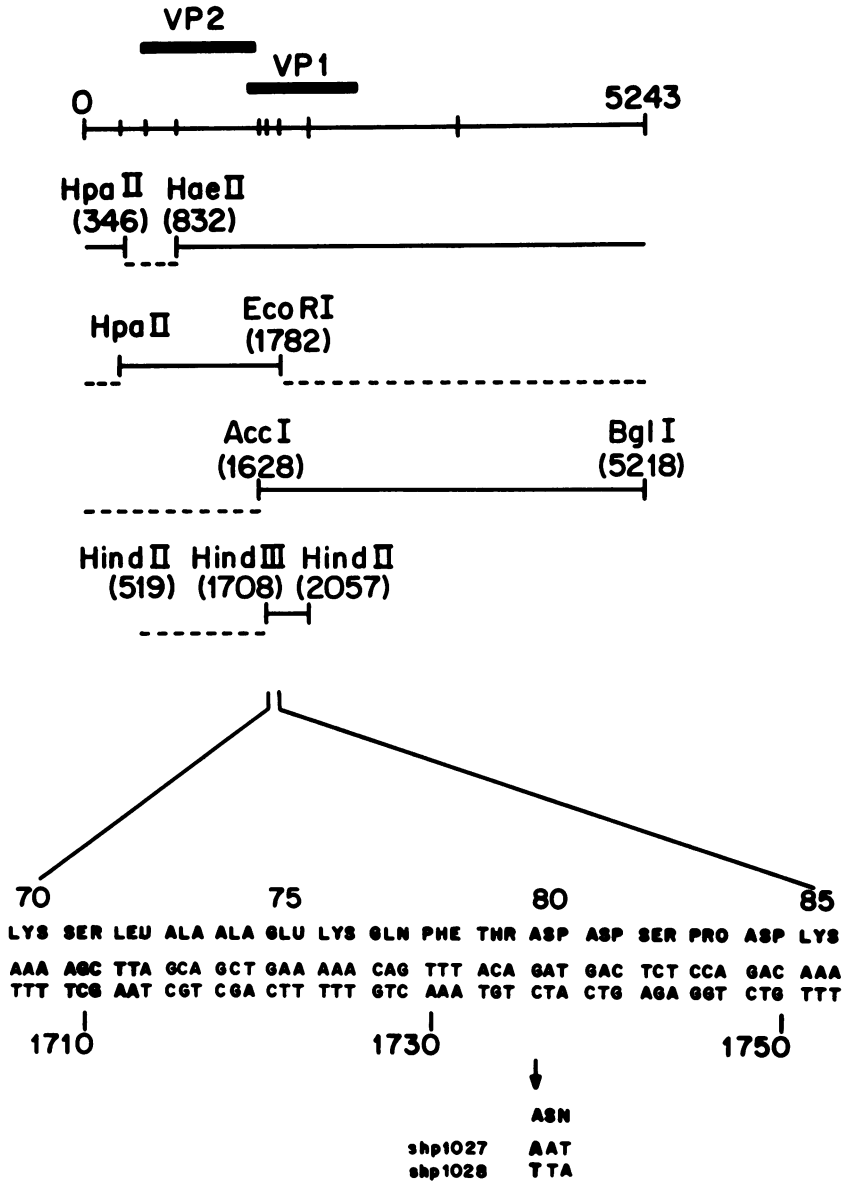

FIG. 1. Localization of the mutation causing the sharp-edged plaque phenotype to the VP1 gene of *shp*1027 and *shp*1028. At the top of the figure is shown the location of the genes for VP1 and VP2 on the 5,243-nucleotide-long SV40 genome, depicted as a linear molecule opened near the *BglI* site. Nucleotide numbers in accordance with those in reference 22 are shown below the specific restriction sites. Fragments shown as solid lines contain the *shp* mutation as determined by in vitro recombination or marker rescue experiments. Fragments shown as dashed lines do not contain the *shp* mutation. At the bottom of the figure is shown the nucleotide sequence of the shp^+ region of VP1.

major virion protein, VP1 (Fig. 1). By nucleotide sequence analysis the same single base-pair change (guanine-cytosine to adenine-thymine) was found in this region of each mutant at nucleotide 1,736, resulting in the replacement of an aspartic acid codon by an asparagine codon corresponding to amino acid 80 of the VP1 gene. (Note that the base-pair change lies beyond the coding sequence for VP2 and VP3.) Therefore, we conclude that the unusual plaque morphology of *shp*1027 and *shp*1028 is due to this single amino acid substitution in VP1. That the plaque morphology reflects a partial defect in viral development is indicated by the fact that an in

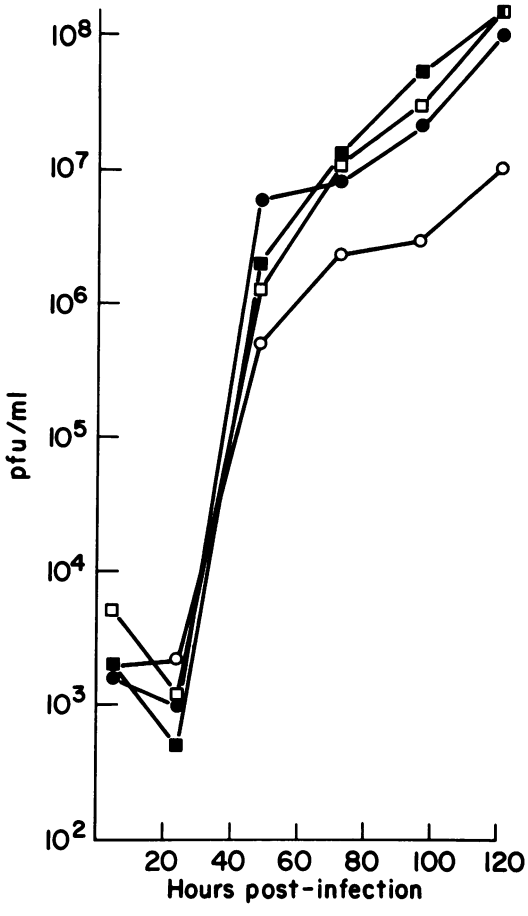

FIG. 2. Growth of mutant viruses compared with that of wild-type SV40. Symbols: ●, wild-type ○, *shp*1035; ■, *shp*1035-sr1; □, *lp*1036.

vitro recombinant (*shp*1035) in which the mutated VP1 segment of *shp*1027 between the *Hae*II and *Eco*RI sites has been joined to the complementary wild-type SV40 DNA fragment yields about one-tenth as much progeny virus as does wild-type SV40 (Fig. 2).

Second-site revertants of *shp* mutants. Before learning that the plaque phenotype of *shp* mutants was due to a mutation in VP1, Shortle et al. (20) had selected second-site revertants of *shp*1027 and *shp*1028 after random local mutagenesis with bisulfite. All revertants produced large, sharp-edged, rather clear plaques of the type shown in Fig. 3. We subsequently found that the pseudorevertant plaque morphology persisted when the mutant *ori* sequence was replaced by in vitro recombination with the wild-type sequence (Fig. 3, *shp*1035-sr1). Therefore, the pseudorevertants appeared to have mutations that suppressed the effect of the VP1 mutation independently of the initial *ori* substitution. Suppression of the plaque morphology defect was accompanied by an increase in viral yield measured after low-multiplicity infection of BSC-40 cells (Fig. 2).

To localize the suppressing mutations in the second-site revertants, we tested a series of in vitro recombinants and partial heteroduplexes for their plaque phenotypes. The second-site mutation mapped in the small *Hpa*II-*Eco*RI fragment (nucleotides 346 to 1,782) and in the larger of the two *Hpa*I fragments tested (nucleotides 3,733 to 499) (Fig. 4). These results localized the mutation to a segment which contains the leader sequence for late mRNA and the agnogene. This DNA segment was sequenced from each of six independently isolated pseudorevertants (Fig. 4). Five of the pseudorevertants were found to have a single guanine-cytosine to adenine-thymine transition in the agnogene sequence, and the sixth had two substitutions. (In all cases the entire agnogene was sequenced without detecting any other changes.) What is especially striking is that in every instance one of three serine codons near the beginning of the agnogene was changed to a codon for a hydrophobic amino acid, either leucine or phenylalanine.

To learn whether the agnogene mutation by itself had any detectable effect, we constructed a recombinant containing a small segment from *shp*1027-sr1 (including the agnogene), the remainder being derived from wild-type SV40. This was done by ligating the small *Hpa*II-*Hae*II fragment from the mutant (nucleotides 346 to 832) to the complementary fragment from the wild type. The plaques of this pure agnogene mutant (*lp*1036) resembled those of wild-type

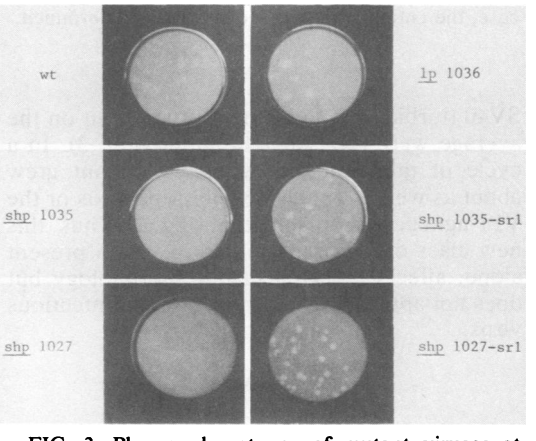

FIG. 3. Plaque phenotypes of mutant viruses at 37°C. wt, Wild-type SV40. *shp*1035 has a VP1 mutation only; *shp*1027 has VP1 and *ori* mutations; *shp*1027-sr1 has VP1, *ori*, and agnogene mutations; *shp*1035-sr1 has VP1 and agnogene mutations; and *lp*1036 has an agnogene mutation only.

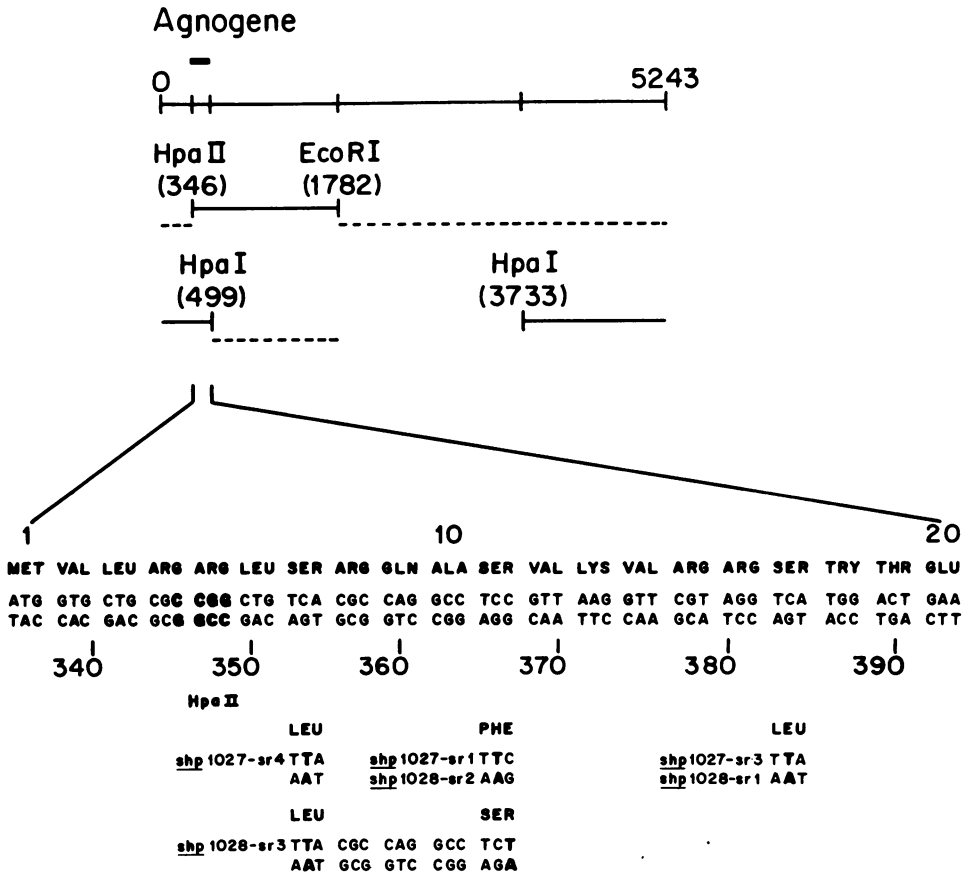

FIG. 4. Localization of the suppressing mutations in *shp*1027-sr1, *shp*1027-sr3, and *shp*1027-sr4. At the top of the figure is shown the location of the agnogene. Fragments shown as solid lines contain the suppressing mutations for each of the three revertants tested as determined by in vitro recombination or marker rescue experiments. Fragments shown as dashed lines do not contain the suppressing mutations. At the bottom of the figure is shown the nucleotide sequence of the altered region of the agnogene for the three *shp*1027 pseudorevertants used in the mapping experiments and for *shp*1028-sr1, *shp*1028-sr2, and *shp*1028-sr3. In each case, the entire agnogene sequence was determined.

SV40 (turbid with indistinct borders) but on the average were reproducibly larger (Fig. 3). In a cycle of infection the agnogene mutant grew about as well as either the wild-type virus or the VP1-agnogene double mutant (Fig. 2). Thus, this new class of agnogene mutation, when present alone, affects the viral plaque morphology but does not appreciably alter the yield of infectious virus.

DISCUSSION

In this communication, we describe the chance isolation of novel mutants of SV40 involving the genes for VP1 and the agnoprotein. The VP1 mutation, perhaps inadvertantly induced by bisulfite, led to a striking plaque phenotype, namely, small, clear, sharp-edged plaques. This mutation also caused a reduction in viral yield to about one-tenth that of wild-type SV40. When the VP1 mutant was subjected to random local mutagenesis by bisulfite, pseudorevertants could be readily selected based on a change in plaque morphology to large, clear, sharp-edged plaques. Such second-site revertants grew as well as wild-type virus, indicating suppression of the defect caused by the VP1 mutation, and they all had missense mutations in serine codons near the beginning of the agnogene, replacing the codon for serine with that for a hydrophobic amino acid. The simplest interpretation of these phenomena is that VP1 and the agnoprotein interact in a specific way, that the VP1 mutation causes a defect in this interaction, and that the suppressing mutation in the agnoprotein restores an effective interaction.

The fact that changes in serine codons of the agnogene were uniformly found suggests further that serine or phosphorylated serine residues affect the postulated VP1-agnoprotein interaction. (It was previously reported that the agnoprotein is phosphorylated in vivo [9].) Since mutants with the agnogene mutation alone grew normally, we must also postulate that the mutant agnoprotein interacts well with wild-type VP1.

Previously reported agnogene mutants have deletions of the protein-coding sequence that in some cases include the start codon, much of the gene, or both (14, 18, 21, 24). In spite of their lack of a functional agnogene, such mutants are viable, albeit defective, indicating that the agnoprotein is not essential for viral multiplication (2, 7, 11, 16, 23). Mutants of this type are transcomplementable (15), suggesting that their defect is due to a lack of the agnoprotein rather than to the effect of deleting part of the late mRNA leader sequence. Recently, A. Barkan and J. Mertz (personal communication) found that these agnogene deletion mutants are also suppressed by mutations in the VP1 gene. How this finding is related to the phenomena we report is not clear.

The postulated interaction between VP1 and the agnoprotein may be an early step in the assembly of SV40 virions and could also be involved in the suggested regulatory functions of the agnoprotein (1, 8). The mutants we describe should be useful in exploring these and other possibilities. More generally, the study of second-site revertants of other virion protein mutants may help elucidate the role of protein-protein interactions during the later stages of viral development.

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