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Physical and Genetic Characterization of Deletion Mutants of Simian Virus 40 Constructed In Vitro

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Mutants of simian virus 40 (SV40), with deletions ranging in size from fewer than 3 to 750 base pairs located throughout the SV40 genome, were obtained by infecting CV-1P cells with linear SV40 DNA and DNA of an appropriate helper virus. The linear DNA was obtained by complete cleavage of closed circular DNA with *Hae* II or *Bam*HI endonuclease or partial cleavage with either *Hae* III endonuclease or nuclease S1, followed, in some cases, by mild digestion with phage λ 5'-exonuclease. The following mutants with deletions in the late region of the SV40 genome were obtained and characterized. Ten, containing deletions at the *Hae* II endonuclease site (map location 0.83), define a new genetic complementation group, *E*, grow extremely slowly without helper virus, and cause alterations only in VP2. Two mutants with deletions in the region 0.92 to 0.945 affect both VP2 and VP3, demonstrating that VP3 shares sequences with the C-terminal portion of VP2. The mutant with a deletion at 0.93 is the first deletion mutant in the *D* complementation group and is also temperature sensitive; the mutant with a deletion at 0.94 is viable and grows normally. Three mutants with deletions at the *Eco*RI endonuclease site (0/1.0) and eleven with deletions at the *Bam*HI endonuclease site (0.15) fall into the *B/C* complementation group. Six additional mutants with deletions at the *Bam*HI endonuclease site are viable, growing more slowly than wild type. VP1 is the only polypeptide affected by mutants in the *B/C* group. A mutant with a deletion of the region 0.72 to 0.80 has a polar effect, failing to express the *E*, *D*, and *B/C* genes. Mutants with deletions in the early region (0.67 counterclockwise to 0.17) at 0.66 to 0.59, 0.48, 0.47, 0.33, and 0.285 to 0.205 are all members of the *A* complementation group. Thus, the *A* gene is the only viral gene in the early region whose expression is necessary for productive infection of permissive cells. Since mutants with deletions in the region 0.59 to 0.54 are viable, two separate regions are essential for expression of the gene *A* function: 0.66 to 0.59 and 0.54 to 0.21. Mutants with deletions at 0.21 and 0.18 are viable. Approximate map locations of SV40 genes and possible models for their regulation are discussed.

Deletions in the simian virus 40 (SV40) genome arise during propagation of the virus at high multiplicities of infection (56). Generally, the deletions are extensive, accompanied by compensating duplications, rearrangements (28, 47) and, occasionally, substitutions by cellular DNA sequences (23, 24). Initially, we intended to use such naturally arising deletion mutants to map the genetic organization of the SV40 chromosome, but the extensive alterations and the consequent multiplicity of genetic defects limited their usefulness (28).

An alternative approach for mapping SV40 genes was to use restriction and other endonucleases to construct deletions at various sites in the viral genome. *Hin*dIII (18) and *Eco*RII (30) endonucleases, enzymes that create cohesive termini at their cleavage sites, have been used to excise segments of the viral DNA. Alternatively, mutants lacking just a few to several hundred base pairs at virtually any site in the viral DNA can be generated by infecting cells with linear DNA made by cleaving wild-type circular DNA with restriction or other endonucleases; apparently, cells possess a mechanism for rejoining the ends of linear DNAs by a mechanism that causes deletion of a variable number of nucleotides at the site of the join (2). Mutants with deletions in essential functions

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require a complementing helper virus for growth (28), whereas mutants containing deletions in dispensible regions propagate without helpers (44).

Using cell-mediated cyclization of linear DNAs (2), mutants have been isolated with small deletions at or about the *Hae* II and *Bam*HI restriction sites (map positions 0.83 and 0.15, respectively) and at sites where S1 nuclease cleaves supercoiled and randomly nicked DNA (1, 45). Mutants with more extensive deletions in their DNA have been generated from linear DNA molecules produced by partial digestion with *Hae* III endonuclease, an enzyme that creates blunt ends at 19 sites in SV40 DNA (55). Here we report on the construction of the mutants, their physical and genetic characterization, and the effect of these deletions on virus-coded proteins. Based on these and other results, several deductions about the physical and genetic map of SV40 can be made.

MATERIALS AND METHODS

Virus and DNA. Our plaque-purified SV40 virus stock (WT830) of SV-S (50) was the parent for the mutants constructed with *Hae* II or *Bam*HI endonuclease and several produced by S1 nuclease cleavage. Other deletion mutants generated by S1 nuclease, or by partial *Hae* III endonuclease digestion, were derived from *Hpa* II endonuclease-resistant derivatives (*dl*-861 and *dl*-862) of WT830 (2). Temperature-sensitive mutants of SV40 used as helpers in growing the deletion mutants were generously provided by several individuals: *tsA*30 and *tsA*58 by Peter Tegtmeyer (51, 52), *tsD*101 by James Robb (39), and all the rest by Robert Martin (3). The viruses and their DNAs were obtained as previously described (28).

Purified virions were prepared from monolayer cultures of CV-1 cells (10-cm plates) infected with 10 ng of mutant DNA per plate. When 80 to 90% of the cells showed cytopathic effect, generally 10 to 30 days after infection depending on the mutant, the cells were harvested by scraping with a rubber policeman followed by brief centrifugation. The medium was aspirated and the cell pellets were frozen. Virions were purified by the method of Ozer (34) and dialyzed against two changes of 0.025 M Tris-chloride (pH 7.5)-0.1 M NaCl-0.1 mM EDTA. If analytical acrylamide gel electrophoresis revealed more than 10% nonvirion protein, the virions were further purified by centrifugation to equilibrium in CsCl (ρ = 1.33 gm/cm^3)-0.01 M sodium phosphate, pH 7.2.

Enzymes. Restriction endonucleases were prepared and used according to published methods: *Eco*RI (14, 32), *Hpa* II (43), *Hae* II (40), *Bam*HI (54), *Hin*dII and -III (46), *Hin*f (J. H. Middleton, Ph.D. thesis, University of North Carolina, Chapel Hill, 1973), and *Hae* III (31). *Mno* I, an isochizomer of *Hpa* II (R. Roberts, personal communication), was used in place of *Hpa* II in many experiments. S1 nuclease was from Miles Laboratories; linear molecules were generated from SV40 form I DNA according to Beard et al. (1), and deletions were mapped as described by Shenk et al. (45). Bacteriophage λ 5'-exonuclease, obtained according to Little et al. (25), was used to digest the 5' ends of linear SV40 DNAs as previously reported (2).

Plaque assays and complementation tests. The infectivities of SV40 virus and DNA were measured by plaque assay on CV-1P monolayers as described earlier (28). Where the rates of plaque development by various mutants were compared, the cultures were stained with crystal violet (15) and photographed, and the plaque diameters were measured.

Generally, complementation was assessed by simultaneous infection of CV-1P monolayers with DNA from the two mutants being tested (28). Although *tsD* virions do not complement other SV40 mutant virions in mixed infections, the use of DNA bypasses the "uncoating" block (39), and complementation occurs if the helper genome provides a wild-type gene *D* function (28). Complementation was scored as positive if the number of plaques counted on the 12th day after mixed infection was at least 10 times greater than the number of plaques produced by each infecting participant alone; generally, the enhancement was between 100- and 1,000-fold. With viable mutants (those producing tiny plaques late after infection), complementation was scored positive if co-infection with the helper DNA produced plaques of normal size and morphology.

Acrylamide and agarose gel electrophoresis of DNA. Restriction endonuclease-generated linear SV40 DNA was separated from circular DNA by one or two successive electrophoretic separations on 1 to 1.6% agarose gels in Tris-borate-EDTA buffer (89 mM Tris-89 mM boric acid-2.5 mM EDTA, pH 8.2). Restriction endonuclease cleavage patterns of the various DNAs were analyzed by electrophoresis in polyacrylamide disc gels (23 by 0.6 cm) as described by Carbon et al. (2) or in slab gels (14 cm by 14 cm by 1.6 mm) for 2.5 h at 120 V or 16 h at 40 V. The acrylamide gel concentrations are given in the figure legends, but in all cases the ratio of acrylamide to *N*,*N*'-methylenebisacrylamide was 20; after the electrophoresis, gels were stained with ethidium bromide (0.5 μg/ml) for 30 min and the DNA bands were visualized with a short-wavelength UV lamp.

Acrylamide gel electrophoresis of virion capsid proteins. Virions in 0.025 M Tris-chloride (pH 7.5)-0.1 M NaCl-0.1 mM EDTA were heated at 100°C for 5 min in a solution containing 0.5% sodium dodecyl sulfate (SDS), 0.5% 2-mercaptoethanol, 10% glycerol, and 0.03% bromophenol blue. Polyacrylamide gel electrophoresis was performed in vertical slabs (14 by 14 by 0.16 cm) according to Laemmli (17) and Maizel (26), except that gels were cast in 0.42 M rather than 0.375 M Tris-chloride. Gels were stained with 0.25% Coomassie brilliant blue. The gel concentrations are given in the figure legends.

Mapping the site and extent of deletions. *Hae* II and *Bam*HI endonucleases cleave SV40 DNA once, and because mutants generated by cleavage with these enzymes generally lacked the corresponding restriction site, that fixed the position of the modifi-

cation. This was confirmed by the S1 nuclease mapping procedure (45), using heteroduplexes produced from *Eco*RI endonuclease-generated linear DNA from the mutant and its parent. In one instance the boundaries of the deletion could be determined from the size of the fragments produced by the S1 nuclease cleavage of the heteroduplex (see results of mapping the *Hae* II endonuclease-generated mutant *dlE*1228, Fig. 3). Where deletions were not at restriction sites (those produced by S1 nuclease cleavage of SV40 DNA), they were located by the S1 nuclease mapping procedure (45), using heteroduplexes generated from *Eco*RI or *Bam*HI endonuclease-cleaved linear DNAs. The location and size of the modification could be confirmed by polyacrylamide gel electrophoresis of *Hin*dII + III, *Hin*f, or *Hae* III endonuclease digestion mixtures; the approximate size of the deletion or addition could be deduced from the mobility of the altered fragment.

Electron microscopy. Nucleic acid samples for electron microscopy were prepared, mounted, and shadowed as described by Davis et al. (6). The visualization of single-stranded DNA loops in heteroduplexes with *Escherichia coli* DNA-binding protein and its antibody (38) was done for us by Steven Reed according to J. Ferguson and R. Davis (personal communication).

RESULTS

General comments on construction of deletion mutants. Earlier work (2, 44) had established that small deletions ranging in size from a few to about 200 base pairs are introduced into SV40 DNA after endonucleolytic cleavage by a cell-mediated recircularization of the linear DNA. After cleavage with *Eco*RI or *Hpa* II endonuclease, mutants with deletions at map positions 0/1.0 and 0.74, respectively, were prepared (2). Now we report the construction and characterization of mutants with deletions that include the *Hae* II and *Bam*HI endonuclease cleavage sites at map positions 0.83 and 0.15, respectively, as well as mutants with small and extended deletions in the late and early regions of the genome resulting from scissions with S1 nuclease or *Hae* III endonuclease.

The following procedure, with minor variations, was used to generate the various classes of deletion mutants to be discussed. Full-length linear SV40 DNA was produced by extensive digestion of SV40 form I DNA with, for example, *Bam*HI endonuclease, followed by two successive electrophoretic separations in 1.6% agarose gels to eliminate uncleaved DNA; then varying amounts of nucleotides (0 to 200) were removed from the 5'-phosphoryl termini by brief digestion with λ 5'-exonuclease (25) as described previously (2). With *Hae* II endonuclease cleavage, the λ exonuclease digestion preceded the separation of linear molecules from contaminating supercoiled DNA. Deletions at other sites were obtained by digesting a mixture of SV40 (I) and (II) DNA with S1 nuclease in either 0.1 or 0.25 M Na^+, conditions known to cause scissions at nicks in SV40 (II) DNA (45) or within the regions at map positions 0.15 to 0.25 and 0.45 to 0.55 of supercoiled SV40 DNA (1). Mutants with extended deletions (350 to 750 base pairs) arose after partial digestion of DNA with *Hae* III endonuclease, an enzyme that cleaves SV40 DNA at 19 locations (55).

To clone and propagate deletion mutants, CV-1P cell monolayers were transfected with the purified linear DNA produced in the various endonuclease digestions with and without potential helper DNAs as described by Mertz and Berg (28). Mutants generated from *Hae* II endonuclease-cleaved DNA were propagated with *tsA*30 as helper; *Bam*HI endonuclease-generated viable deletion mutants were grown without a helper, whereas the nonviable mutants with deletions at that site were grown with *tsA*58. Mutants resulting from *Hae* III endonuclease cleavages within the early region were cloned with the late mutant *tsB*201, whereas mutants with deletions in the late region grew with *tsA*58 as helper. The mutants arising after S1 nuclease digestion were cloned with a late mutant lacking the *Eco*RI cleavage site *dlBC*864 (2) or one lacking the DNA segment from 0.74 to 1.0 on the map (M. Dieckmann and P. Berg, unpublished data). Viable deletion mutants generated by S1 nuclease were obtained without a helper genome.

Mutant genomes created with endonucleases that make a single scission in SV40 DNA (e.g., *Eco*RI, *Hpa* II, *Hae* II, and *Bam*HI) generally lack the restriction site, and therefore the modified DNA is readily separable from the helper DNAs by centrifugation in a CsCl-ethidium bromide (EtBr) density gradient after digestion with the appropriate enzyme; the mutant DNAs remain covalently circular, and the helper DNAs become linear. Where the deletions do not occur at unique restriction sequences (e.g., those made with S1 nuclease or *Hae* III endonuclease), this approach is not possible. To facilitate the separation of mutants of this type, a strain of SV40 that lacks the *Hpa* II endonuclease cleavage site at 0.74 (these modified strains of SV40, *dl*-861 and *dl*-862 [see reference 2], lack 53 and 32 base pairs, respectively) was used as the parent; consequently, the newly created mutants are resistant and the helper virus genomes are sensitive to cleavage by *Hpa* II endonuclease.

Table 1 lists the mutants described in this report. The experimental details of their physical and genetic characterization follow.

TABLE 1. *Summary of mapping data for SV40 deletion mutants*

Mutant no.	Endonuclease used to cleave DNA	Map location(s) included in deletion[a]	Base pairs deleted[b]
*dlE*1221	*Hae* II	0.83	−70
*E*1222		0.83	−<5
*E*1223		0.83	−10
*E*1224		0.83	−20
*E*1225		0.83	−10
*E*1226		0.83	−20
*E*1227		0.83	−20
*E*1228		0.795-0.835	−200
*E*1229		0.83	−20
*E*1230		0.83	−30
*dlBC*1231	*Bam* HI	0.15	−25
*BC*1232		0.15	−12
*BC*1233		0.15	−15
*BC*1235		0.15	−<3
*BC*1236		0.15	−10
*BC*1237		0.15	−5
*BC*1238		0.15	−15
*BC*1240		0.15	−28
*BC*1241		0.11 -0.16	−260
*BC*1242		0.15	−<3
*BC*1243		0.10 -0.16	−320
dl-1244	*Bam* HI	0.15	−<3
−1245		0.15	−<3
−1246		0.15	−15
−1247		0.15	−8
−1248		0.15	−10
−1249		0.15	−7
*dlA*1207	*Hae* III	0.205-0.285	−400
*A*1208		0.205-0.285	−400
*A*1209		0.59 -0.66	−350
*dlBCDE*1210		0.72 -0.80	−400
*BCDE*1211		0.80 -0.95	−750
*dlA*1201	S1	0.33	−43
*A*1202		0.47	−30
*A*1203		0.48	−150
*muA*1204		0.48	+100[c]
*dl(ts)D*1261		0.93	−70
dl-1262		0.94	−50
dl-1263		0.21	−50
dl-1264		0.57	−120
*dlA*1265		0.18	−50
*dlA*1266		0.48	−20

[a] Deletions include the listed map coordinate but, unless indicated, the exact limits of the deletion coordinates were not determined.

[b] Number of base pairs deleted was estimated from the change in electrophoretic mobility of appropriate restriction endonuclease fragments in acrylamide gels. When the analysis with two or more restriction enzymes was different, the values were averaged.

[c] Origin and type of the added DNA is not known.

Physical mapping of deletion mutants. (i) Mutants with deletions at the *Hae* II endonuclease cleavage site. Ten plaques were picked after CV-1P monolayers were infected at 41°C with *tsA*30 DNA and the *Hae* II endonuclease-cleaved, λ exonuclease-digested linear DNA. The mutant virus in each plaque was cloned through two successive plaque isolations as described by Mertz and Berg (28). Between 25 and 50% of the viral DNA recovered from CV-1 cells infected with each isolate (in the presence of *tsA*30) was not cleaved by prolonged incubation with *Hae* II endonuclease; the resistant DNA was separated from the *tsA*30 DNA by CsCl-

EtBr centrifugation. Digestion of the *Hae* II endonuclease-resistant DNAs with *Hin*dII + III endonucleases (see Fig. 1 for the sites of enzymatic cleavages) and electrophoresis in polyacrylamide gels revealed that nine of the ten isolates yielded *Hin*dII + III-D fragments that migrated more rapidly than the wild-type counterpart (Fig. 2 shows six of the mutant patterns); based on the mobility of DNA fragments of known lengths, we infer that the deletions at map position about 0.83 remove from <5 to approximately 200 base pairs.

Based on the following observations, the largest deletion (*dlE*1228) occurs between 0.795 and 0.835 ± 0.01 map units: (i) after digestion of *dlE*1228 DNA with *Hae* III endonuclease, the *Hae* III-H fragment, which begins at map coordinate 0.84, is still produced, but the *Hae* III-J fragment is missing (Fig. 3a and b), indicating that the end of the deletion is proximal to map coordinate 0.84; (ii) S1 nuclease produces a small fragment of 0.16 to 0.17 fractional SV40 DNA length (Fig. 3c and d) when it cleaves a heteroduplex molecule formed from *Eco*RI endonuclease-generated linear WT830 and *dlE*1228 DNAs, indicating that the deletion does not extend beyond map position 0.83 to 0.84 (0.16 to 0.17 map units counterclockwise from map position 0/1.0); (iii) after reaction of 50 heteroduplex molecules of the type mentioned above with DNA-binding protein and

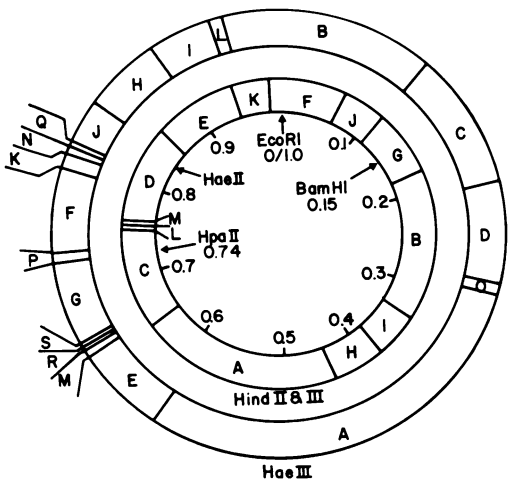

FIG. 1. *Restriction endonuclease cleavage sites in SV40 DNA. The sites of cleavage of SV40 DNA by Hae III (55) and HindII + III (5) restriction endonucleases are shown on the outer and inner rings, respectively. The single sites of cleavage by restriction endonucleases EcoRI (0/1.0; 32, 33), Hpa II (0.74; 42), Hae II (0.83; D. Charney, unpublished data), and BamHI (0.15; C. N. Cole, unpublished data) are indicated by arrows.*

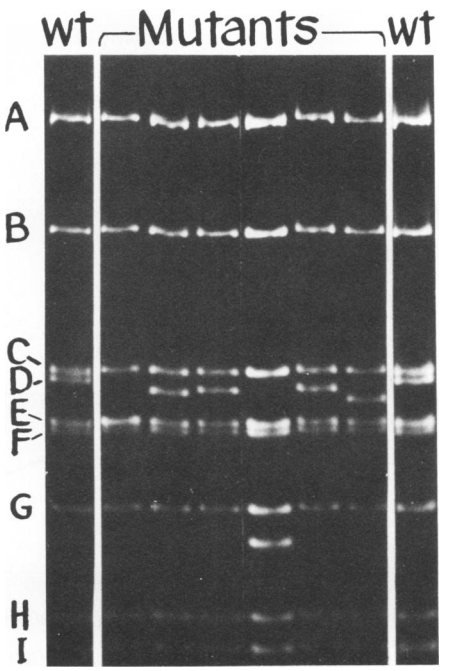

FIG. 2. *Polyacrylamide gel electrophoresis of DNA fragments produced by HindII + III endonuclease digestion of mutant DNAs with deletions at the Hae II endonuclease site (0.83). Purified mutant DNAs (0.2 μg) were digested with HindII + III endonucleases and electrophoresed in a 5% polyacrylamide slab gel. The left and right tracks are a digest of parental WT830 DNA. The six mutants are (left to right) dlE1221, dlE1224, dlE1225, dlE1228, dlE1229, and dlE1230.*

antibody to the binding protein (39), an aggregate centered at map coordinate 0.812 ± 0.007 (standard deviation) can be seen by electron microscopy (S. Reed, personal communication); (iv) the deletion size is estimated as 0.04 map unit based on altered mobility of the *Hin*dII + III-D fragment (Fig. 2) and the disappearance of the *Hae* III-J fragment (Fig. 3a).

(ii) Mutants with deletions at the *Bam*HI endonuclease cleavage site. CV-1P cells infected with *Bam*HI endonuclease-digested linear DNA in the absence or presence of *tsA*58 DNA produced plaques, representatives of which were cloned as mentioned above. Viral DNA from eleven helper-dependent and six helper-independent isolates was prepared from infected CV-1 cultures in the usual way (28). Digestion of most of the DNA samples with *Hin*dII + III endonucleases and electrophoresis on a 5% polyacrylamide slab gel revealed altered *Hin*dII + III-G fragments (Fig. 4). With several DNAs, however, the mobilities of the G fragments were indistinguishable from that of

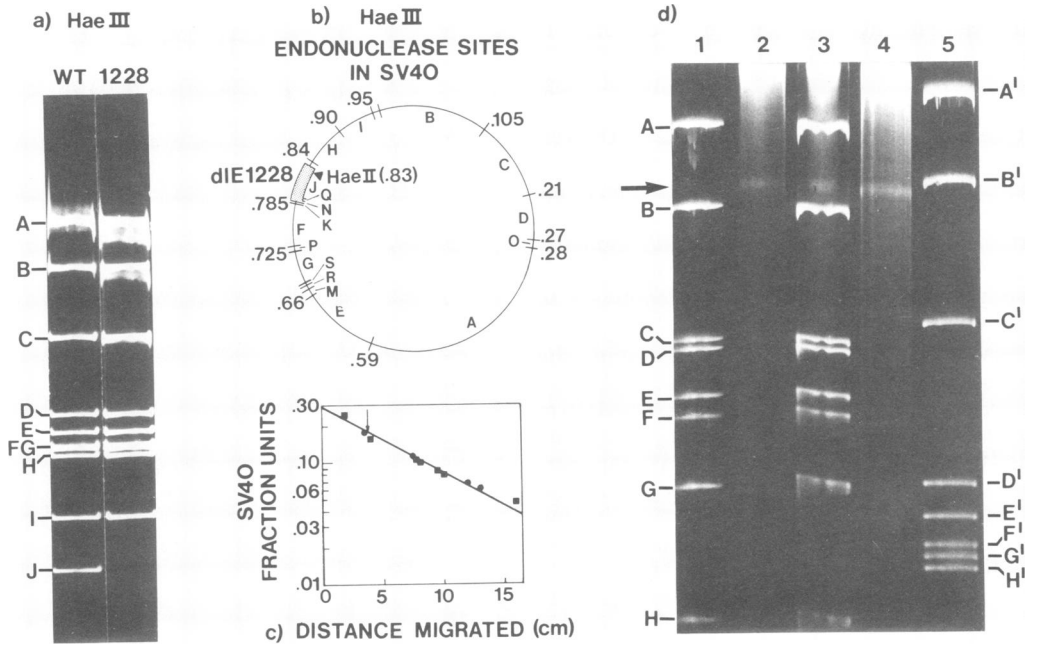

FIG. 3. *Location of the deletion in mutant dlE1228. (a) Mutant and wild-type DNA were digested with Hae III endonuclease and electrophoresed in a 3 to 7% linear polyacrylamide gradient slab gel. (b) Hae III endonuclease sites in SV40 DNA. (c) The size of the small fragment (arrow) was deduced from its position relative to the mobilities of fragments produced by HindII + III and Hae III endonucleases. (d) Polyacrylamide (5%) slab gel patterns of the S1 nuclease digest of heteroduplexes of EcoRI endonuclease-generated linear WT830 and dlE1228 DNAs. The position of the small fragment in tracks 2 to 4 is indicated by the arrow. As size markers, tracks 1 and 3 contain HindII + III endonuclease-digested SV40 DNA and track 5 contains Hae III endonuclease-digested SV40 DNA.*

the wild-type G fragment. In one (*dlBC*1243; Fig. 4a), the *Hin*dII + III-J and -G fragments were fused. Seven of the helper-dependent and four of the helper-independent isolates had altered *Hin*dII + III-G fragments, indicating deletions of 5 to 260 base pairs; mutant *dlBC*1243 appeared to have a deletion of 320 base pairs. Two helper-dependent (*dlBC*1235 and *dlBC*1242; Fig. 4b) and two helper-independent mutants (*dl*-1244 and *dl*-1245) yielded virtually normal-size *Hin*dII + III-G fragments. Mutants *dlBC*1235 and *dlBC*1242 had an alteration at the *Bam*HI endonuclease cleavage site since their DNAs were resistant to cleavage by that enzyme and, moreover, they were defective (i.e., helper dependent). Mutant *dl*-1244 was also sensitive to *Bam*HI endonuclease cleavage and yielded a normal *Hin*dII + III-G fragment; however, its deletion at map position 0.15 was confirmed by the fact that heteroduplexes prepared from *Eco*RI endonuclease-generated linear WT830 and *dl*-1244 DNAs were cleaved by S1 nuclease to produce 0.85 and 0.15 SV40 length fragments. Although mutant *dl*-1245 DNA was sensitive to *Bam*HI endonuclease and its heteroduplexes with wild-type DNA were not cleaved by S1 nuclease, we infer that it is a mutant, since its virions contained an appreciably shorter VP1 protein (see later data).

(iii) Mutants with alterations resulting from S1 nuclease cleavage. Mutants *dlA*1201 to -1204 were recovered from plaques after infection with linear DNA generated from WT830 by S1 nuclease digestion and a late mutant lacking the *Eco*RI restriction site (2). After purification by several successive plaque isolations, their viral DNA was isolated as described above and separated from the helper DNA by cleavage with *Eco*RI endonuclease (in this case, the helper was resistant to cleavage) and equilibrium centrifugation in CsCl-EtBr. Mutants *dl*-1261 to -1266 were prepared by S1 nuclease cleavage of circular DNA (containing about 30% nicked circular molecules) from the *Hpa* II endonuclease-resistant viable deletion mutant *dl*-861 (2). Although mutants *dl*-1261 to -1265 were initially grown with the helper mentioned above, they were later found not to need a helper for growth in CV-1 cells; mutant *dlA*1266, however, was defective and could not be propagated without a helper.

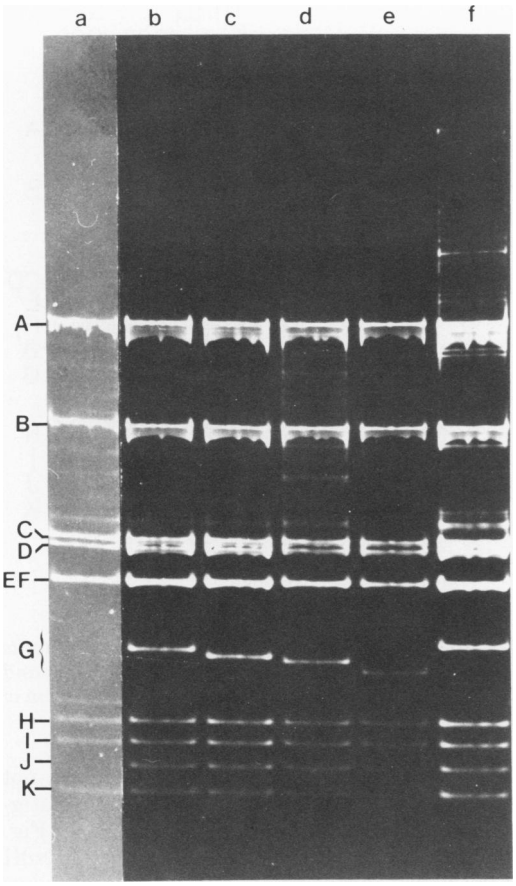

FIG. 4. *Polyacrylamide gel electrophoresis of DNA fragments produced by HindII + III endonuclease digestion of representative mutants at the BamHI endonuclease cleavage site (0.15). DNAs (0.2 μg) were digested with HindII + III endonuclease and electrophoresed in a 5% polyacrylamide slab gel. From left to right: (a) dlBC1243; (b) dlBC1242; (c) dlBC1236; (d) dlBC1233; (e) dlBC1231; (f) WT830.*

The map location of the alterations in each of these mutants was determined by a combination of methods: the S1 nuclease mapping method, using heteroduplex molecules produced from parental and mutant linear DNA strands cleaved with two different restriction endonucleases (45); and comparison of the electrophoretic mobility (size) of fragments produced by cleavage of parental and mutant DNAs with various restriction endonucleases (e.g., *Hin*dII + III, *Hin*f, or *Hae* III endonuclease). The map coordinates and size of the alterations are summarized in Table 1.

(iv) Mutants with deletions generated by *Hae* III endonuclease cleavages. *Hae* III endonuclease cleaves SV40 DNA at 19 locations, 4 times in the early region, 11 times in the late region, and 4 times in the vicinity of the origin of replication (47, 55); therefore, single but randomly located scissions yield a permuted population of full-length linear DNA, and multiple cleavages generate an assorted collection of smaller fragments. Although the termini are not cohesive (48), cell-mediated rejoining of ends produces both small and extended deletions at the cleavage sites.

Superhelical DNA of mutant *dl*-862, an *Hpa* II endonuclease-resistant derivative of WT830 (2), was digested with *Hae* III endonuclease under conditions that produce about 50% linear DNA, leaving the remainder as nicked and supercoiled circular DNA. After two successive electrophoretic separations in 1.6% agarose disc gels, the linear DNA of about 0.8 to 1.0 SV40 DNA length was used to infect CV-1P cell monolayers at 41°C by itself, with *tsB*201, or with *tsA*58 DNA. Virus from the plaques was purified by two successive plaque isolations with added helper virus; then viral DNA was isolated as described previously.

The *ts* helper DNA was separated from the mutant DNA by repeated digestions with *Hpa* II endonuclease and equilibrium centrifugation in a CsCl-EtBr gradient. Five of the helper-dependent deletion mutants were selected for analysis (see Table 1).

The electrophoretic mobility of mutant *dl*-1207 DNA in agarose gels indicates that it is 0.92 SV40 length. After digestion with *Hin*dII + III endonucleases and polyacrylamide gel electrophoresis, the *Hin*dII + III-B fragment was missing and a new fragment migrating between *Hin*dII + III-F and -G was found (Fig. 5); the increased mobility suggests that the deletion is about 0.08 SV40 length. Digestion of the same DNA with *Hae* III endonuclease and electrophoresis in a 4 to 10% linear gradient polyacrylamide gel revealed that the *Hae* III endonuclease A, C, and D fragments were missing, and a new fragment that migrated more slowly than the *Hae* III-A fragment appeared. We infer from this that the mutant's deletion removes a segment beginning within the *Hae* III-A fragment and ending within the *Hae* III-C fragment, thereby fusing *Hae* III-A and -C and removing the *Hae* III-D and -O fragments. Thus, the deletion in mutant *dl*-1207 (*dl*-1208 was identical in every respect) occurs between map coordinates 0.205 and 0.285.

Mutant *dl*-1209 DNA, freed from helper DNA as described above, was digested with *Hae* III endonuclease, and the fragment pattern was analyzed by gradient polyacrylamide gel electrophoresis (Fig. 6). Only the *Hae* III-E fragment was missing (of those larger than the 20-base-pair *Hae* III-N fragment [55]), suggesting that the deletion extends from 0.59 to 0.66 on

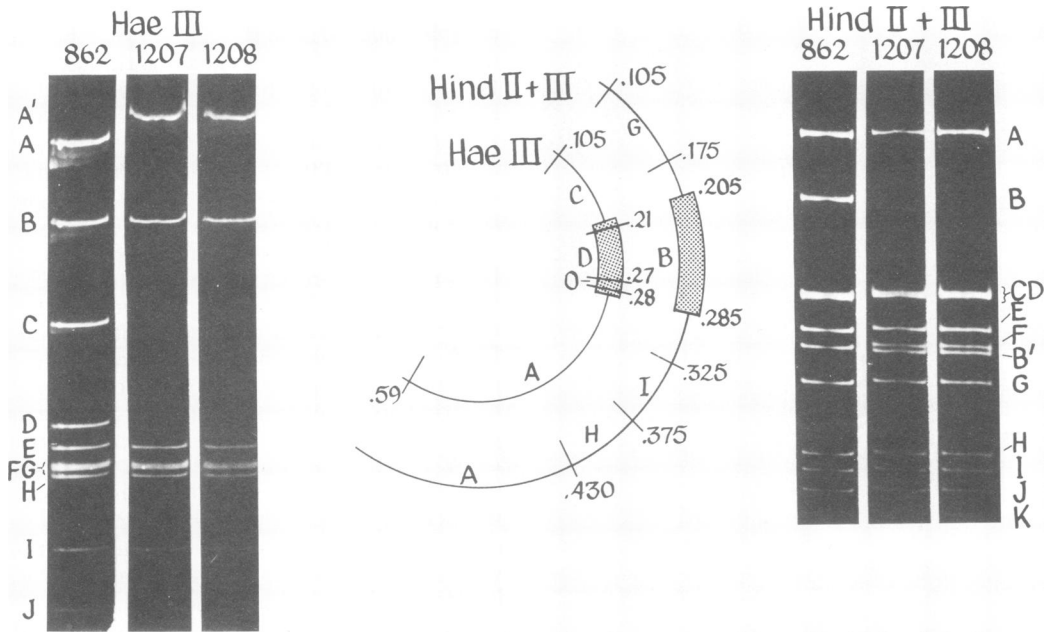

FIG. 5. *Mapping the deletion location in mutants dlA1207 and dlA1208. Purified DNAs of mutants dlA1207, dlA1208, and their parent, dl-862, were digested with HindII + III and Hae III endonucleases and electrophoresed in a 3 to 7% linear gradient polyacrylamide slab gel. The stippled arcs indicate the location of the deletion on the HindII + III and Hae III endonuclease cleavage maps for SV40 DNA.*

the map. *Hin*dII + III endonuclease digests of the mutant DNA lacked the *Hin*dII + III-A and -C fragments but contained a new fragment that was larger than *Hin*dII + III-A; this finding is explained if *dl*-1209 has a deletion including the *Hin*dII + III-A–C junction (map position 0.655), causing fusion of the remaining regions of the *Hin*dII + III-A and -C fragments (Fig. 6). Polyacrylamide electrophoresis of *Hin*f endonuclease digest of the same DNA shows that the *Hin*f-A and -D fragments were absent and a new fragment, larger than *Hin*f-A but 7% smaller than the combined length of *Hin*f-A and -D, was formed. Heteroduplex DNAs formed between *Eco*RI endonuclease-cleaved linear DNA from *dl*-1209 and its parent, *dl*-862, contained a 7% (±1%) deletion loop beginning at map position 0.59 (±0.01). From the nucleotide sequence (47) of the *Eco*RII-G fragment (0.635 to 0.70 on the SV40 map), the end of the deletion is likely to be at 0.66, i.e., within a 20-base-pair region between the *Hin*dII + III-A–C junction, which is missing, and the beginning of the *Hae* III-M fragment, which remains.

DNA of mutants *dl*-1210 and *dl*-1211 were separated from helper DNA by several cycles of digestion with *Hpa* II or *Mno* I endonuclease and equilibrium centrifugation in CsCl-EtBr. They were then digested with *Hae* III and *Hin*dII + III endonucleases and analyzed on linear gradient polyacrylamide gels (data not shown). Mutant *dl*-1210 was missing the contiguous *Hae* III fragments P, F, K, and N (see Fig. 1); no new fragments were visible. The *Hin*dII + III digest lacked the C and D fragments but contained a new fragment, approximately 0.12 SV40 length, which migrated slightly faster than the *Hin*dII + III-B fragment. Therefore, the new fragment was a fusion product formed from the undeleted regions of *Hin*dII + III-C and -D fragments, and the deletion must extend from 0.72 to 0.80 on the map. This analysis cannot determine whether this deletion is adjacent to or includes the original deletion of 32 base pairs at the *Hpa* II site in the parent, *dl*-862.

The *Hae* III digest of mutant *dl*-1211 was missing the contiguous *Hae*III-N, -Q, -J, -H, -I, and -L fragments (see Fig. 1) and contained no new fragments. A *Hin*dII + III digest of the same DNA shows that the *Hin*dII + III-D, -E, and -K fragments were absent and a new fragment, migrating between *Hin*dII + III fragments F and G, was present. This corresponds to a deletion of 15% of the SV40 genome extending from 0.80 to 0.95 on the map. The new fragment is a fusion product of the remaining portions of *Hin*dII + III fragments D and K.

Complementation behavior of defective deletion mutants. Complementation analysis

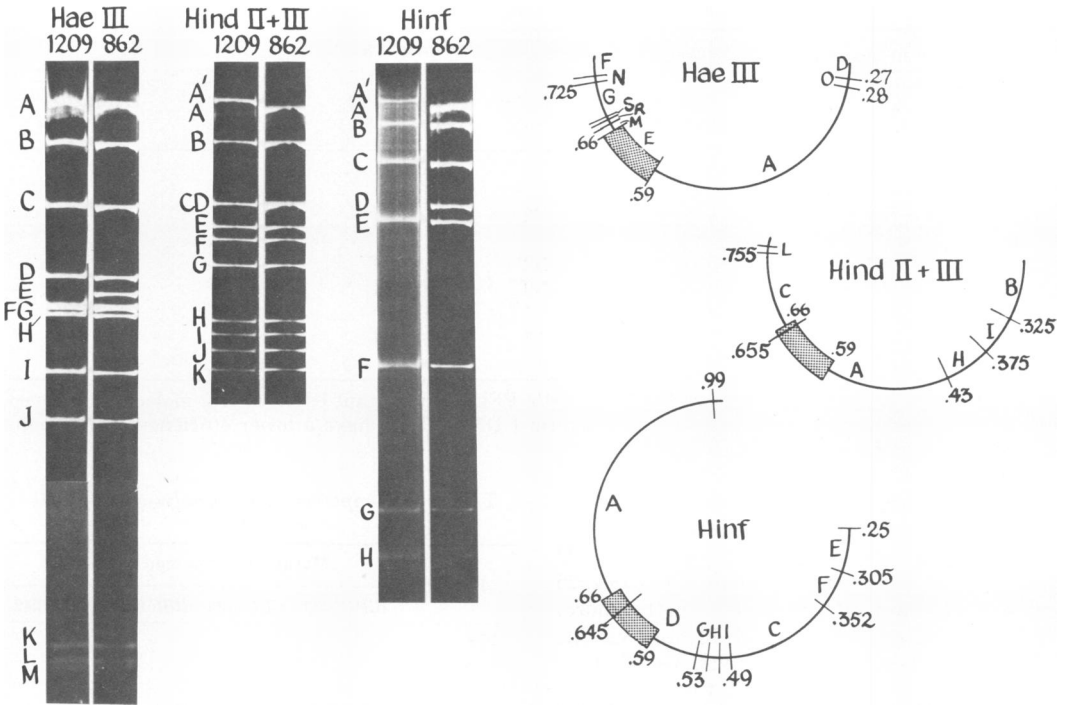

FIG. 6. *Mapping the location of the deletion in mutant dlA1209. Purified DNA of mutant dlA1209 and its parent, dl-862, were digested with Hae III, HindII + III, and Hinf endonucleases and electrophoresed on 7 to 12.5%, 3 to 7%, and 4 to 10% linear gradient polyacrylamide slab gels, respectively. The stippled arcs indicate the deletion location on the Hae III, HindII + III, and Hinf endonuclease cleavage maps for SV40 DNA.*

TABLE 2. *Complementation analysis of late mutants*[a]

Helper	Mutant/deletion map location				
	*dlE*1221 to 30	*dlBC*863 to 5	*dlD*1261	*dlBCDE*1210 *dlBCDE*1211	*dlBC*1241 *dlBC*1243
*tsA*30	+	+	+	+	+
*tsA*58	+	+	+	+	+
*tsB*201	+	−	+	−	−
*tsC*219	+	−	+	−	−
*tsBC*208	+	−	+	−	NT
*tsD*202	+	+	−	−	+
*dlE*1228	−	+	+	−	+
No helper	−	−	−	−	−

	*dlE*1225	*dlD*1261	*tsD*202
*tsD*101	+	+	+
*tsD*202	+	−	
*tsD*222	+	−	*NT*
*tsD*263	+	−	*NT*
tsD270	+	−	*NT*

[a] +, 10^5 to 5×10^6 PFU/μg of mutant DNA; −, $<10^3$ PFU/μg of mutant DNA; NT, not tested.

with the various deletion mutants and known *ts* mutants was carried out by infecting CV-1P monolayer cultures with their DNAs as described by Mertz and Berg (28).

(i) Late mutants. Mutant DNAs having a deletion about map position 0.83 (*dl*-1221 to 1230 in Table 1) complemented representatives of all known *ts* mutants (first column, Table 2), mutants with deletions in the early region (*dlA* mutants; Table 3) and, somewhat poorly, a deletion mutant (*dlBC*835) lacking the DNA segment between map coordinates 0.925 and

TABLE 3. *Complementation analysis of early mutants*[a]

Helper	Mutant/deletion map location					
	*dlA*1207 *dlA*1208 (0.205–0.285)	*dlA*1209 (0.59–0.66)	*dlA*1201 (0.33)	*dlA*1202 (0.48)	dlA1203 (0.47)	*dlA*1266 (0.47)
*tsA*30	–	–	–	NT	NT	NT
*tsA*58	–	–	–	–	–	–
*dlA*1209	–		NT	NT	NT	NT
*tsB*201	+	+	+	+	+	+
*tsC*219	+	+	NT	NT	NT	NT
*tsD*202	+	+	NT	NT	NT	NT
*dlE*1228	+	+	+*	+*	+*	NT
*dlBC*864	NT	NT	+*	NT	NT	+
No helper	–	–	–	–	–	–

[a] +, 10^5 to 5 × 10^6 PFU/μg of mutant DNA. +*, >10^4 PFU/μg of mutant DNA. Linear molecules, cleaved with *Eco*RI endonuclease, were used in place of form I DNA. These have a lower efficiency of infection. –, <10^3 PFU/μg of mutant DNA. NT, Not tested.

0.14 (30). Consequently, these mutants constitute a new complementation group, E. All *dlE* mutants complemented each of the known *tsD* mutants; Table 2 shows the result with *dlE*1225 and five different *tsD* mutants. Furthermore, unlike the *tsD* mutants (3), *dlE* mutant virions could complement *ts* mutant virions of other complementation groups. Mutations comprising the *E* complementation group also map differently than group *D* mutants; all group E mutations map in the *Hin*dII + III-D fragment (0.765 to 0.86), whereas all *tsD*-group mutations occur within the *Hin*dII + III-E fragment (0.86 to 0.945) (19–21).

Mutant *dl*-1261 (lacking 70 base pairs at map position 0.93) produced plaques on CV-1P cells incubated at 32 or 37°C but not at 41°C. Although *dlD*1261 virions did not complement other *ts* mutant virions at 41°C, a finding indicative of the *D* mutant phenotype, *dlD*1261 DNA readily complemented *tsA*, *tsB*, *tsC*, *tsBC*, and *dlE* mutant DNAs at the high temperature. Mutant *dlD*1261 is assigned to the *D* class, since it did not complement four of the five known *tsD* mutants (at 41°C); however, intracistronic complementation was observed between *tsD*101 and *dlD*1261 and also occurred between *tsD*101 and *tsD*202 (Table 2).

DNAs from three previously reported deletion mutant isolates (*dl*-863 to -865) that lack a small segment about the *Eco*RI restriction site (map position 0/1.0) complemented *tsA*-, *tsD*-, and *dlE*-group mutants, but not representatives of *tsB*, *tsC*, and *tsBC* mutants (Table 2). Thus, the mutants with deletions of the *Eco*RI restriction site are within the *B/C* complementation group.

Four representative defective mutants with deletions of the *Bam*HI endonuclease restriction site (map position 0.15) were tested for their complementation behavior (Table 4). Each of the four complemented *tsA*, *tsD*, and *dlE* mutants; two (*dlBC*1233 and *dlBC*1236) also complemented several different representatives of the *tsB*, *tsC*, and *tsBC* classes. However, the mutant lacking the *Eco*RI restriction site (*dlBC*865), which was not complemented by any *tsB*, *tsC*, or *tsBC* mutants (Table 2), was also not complemented by *dlBC*1233 and *dlBC*1236 (Table 4). Thus, *dlBC*1233 and *dlBC*1236 are probably *B/C* mutants that give intracistronic complementation with many *tsBC* mutants but not with the deletion mutant of that group. Two other mutants with deletions

TABLE 4. *Complementation analysis of* Bam*HI mutants*[a]

Helper	Mutant/deletion map location			
	*dlBC*1233	*dlBC*1236	*dlBC*1240	*dlBC*1242
*tsA*58	+	+	+	+
*tsD*202	+	+	+	+
*dlE*1288 (0.83)	+	+	+	+
*tsB*201	+	+	–	–
*tsB*204	+	+	–	–
*tsB*232	+	+	–	–
*tsB*228	+	+	–	–
*tsC*240	+	+	–	–
*tsC*244	+	+	–	–
*tsC*259	+	+	+	+
*tsBC*208	+	+	–	–
*tsBC*210	+	+	–	–
*tsBC*216	+	+	–	–
*dlBC*865 (0/1.0)	–	–	–	–
No helper	–	–	–	–

[a] +, 10^5 to 5 × 10^6 PFU/μg of mutant DNA; –, <10^3 PFU/μg of mutant DNA.

at map position 0.15 failed to complement *tsB*, *tsC* (there is weak complementation with *tsC*159), and *tsBC* mutants, confirming that these are in the *B/C* group. Both the mutants with large deletions about the *Bam*HI site (*dlBC*1241 and *dlBC*1243) failed to complement any group *B/C* mutant (Table 2).

Mutants *dl*-1210 and *dl*-1211, which lacked the regions 0.72 to 0.80 and 0.80 to 0.95, respectively, complemented *tsA* and *dlA* mutants but failed to produce plaques after co-infection with any *tsB*, *tsC*, *tsD*, or *dlE* mutant DNAs. These mutants are, therefore, *B/CDE* mutants.

(ii) **Early mutants.** Table 3 summarizes the results of the complementation analysis on representative mutants with alterations in the early region of SV40 DNA. Mutants *dlA*1207 and *dlA*1208, which lacked the map segments between 0.285 and 0.205, failed to complement *tsA* mutants. Mutants with small deletions at 0.33, 0.47, and 0.48 also fail to complement *tsA*58. The mutant *dlA*1209, which had a deletion between map positions 0.66 and 0.59, failed to complement the two *tsA* mutants tested. As found by others (3, 51), early SV40 deletion mutants failed to complement one another and therefore constitute a single genetic function.

Viable deletion mutants. Shenk et al. (44) have already described SV40 deletion mutants that grow without a helper virus. Analysis of these mutants suggests that there are at least three regions of the DNA that are dispensible for vegetative growth: map regions 0.17 to 0.175, 0.54 to 0.59, and 0.68 to 0.755. Besides *dl*-1264 and *dl*-1265, which fall into two of these regions, several other mutants in the present collection are able to grow without a helper virus.

The group with deletions about coordinate 0.83 that comprise the *E* complementation group (*dlE*1221 to 1230) produce tiny plaques only after 15 to 20 days, compared with 8 to 10 days for WT830 or *dl*-862 (Fig. 7); the relative difference in plaque morphology between mutants and wild type was the same at 32, 37, and 41°C. The reasons for the defectiveness of these virus isolates needs to be studied in more detail, but we suspect that it is due to the absence or alteration of the VP2 capsid protein (see below).

We have already mentioned that *dlD*1261, which had a deletion of 70 base pairs at map position 0.93, grew more slowly than WT830 at 37°C and was defective at 41°C (Fig. 7). Mutant *dl*-1262, which was adjacent to *dlD*1261 and was missing 50 base pairs at map location 0.94, was not distinguishable from its parent in its ability to produce plaques on CV-1P monolayer cultures (data not shown) at 32, 37, and 41°C, even though the deletion altered the structure of its VP2 and VP3 capsid proteins (see below).

Several of the mutants that had a deletion at map position 0.15 (*dl*-1244 to -1249) could grow without a helper virus. Their slow growth (Fig. 7) was probably attributable to the altered structure of their VP1 capsid protein (see below).

Although deletion of the segment between map coordinates 0.285 and 0.205 rendered SV40 defective, a deletion of about 50 base pairs at either map position 0.18 (*dl*-1265) or 0.21 (*dl*-1263) did not impair the virus's ability to multiply in CV-1P cells. Whether the region between 0.18 and 0.21 on the map is completely dispensible remains to be determined.

Altered capsid proteins from deletion mutants. The ability of several of the deletion mutants described above to grow without a helper virus has enabled us to examine the effect of these deletions on viral capsid polypeptides by using SDS-polyacrylamide gel electrophoresis.

Deletions about the *Hae* II restriction site (map position 0.83) affected only VP2 (Fig. 8). The electrophoretic mobility of virion VP2 was either unchanged (*dlE*1222, Fig. 8B) or somewhat faster (*dlE*1224, Fig. 8C; *dlE*1226, Fig. 8E), or the protein was completely absent (*dlE*1228, Fig. 8F; *dlE*1230, Fig. 8G). Neither VP1 nor VP3 appeared to be altered when compared with wild type (Fig. 8A). Thus, the lesion at map position 0.83 probably occurs within the structural gene for VP2.

Deletions in the region 0.93 to 0.94 affected both VP2 and VP3. The VP2 and VP3 proteins from *dlD*1261 (0.93) and *dl*-1262 (0.94) mutant virions migrated more rapidly than wild-type proteins (Fig. 9). The increased mobility of *dlD*1261 VP2 and VP3 proteins indicates that both proteins are about 6,000 daltons smaller; in the case of *dl*-1262 virions, the shortening of VP2 and VP3 was about 2,500 daltons. The major capsid protein, VP1, was unaffected in each mutant. (The band migrating slightly faster than VP2 is cellular actin.)

Deletions about the *Bam*HI endonuclease site (0.15) affected VP1 (Fig. 10). Viable mutants of this group had altered VP1 in their capsids; in various mutants, the changes ranged from 1,000 daltons (*dl*-1247, Fig. 10D) to 4,000 daltons (*dl*-1249, Fig. 10F). Whereas altered VP1 was detected primarily in capsids of viable mutants, some helper-dependent mutants with deletions in this region (*dlBC*1231, Fig. 10H) produced virions containing both wild-type and shortened VP1. However, most of these helper-dependent mutants had only normal-size VP1 both in the purified virions and in

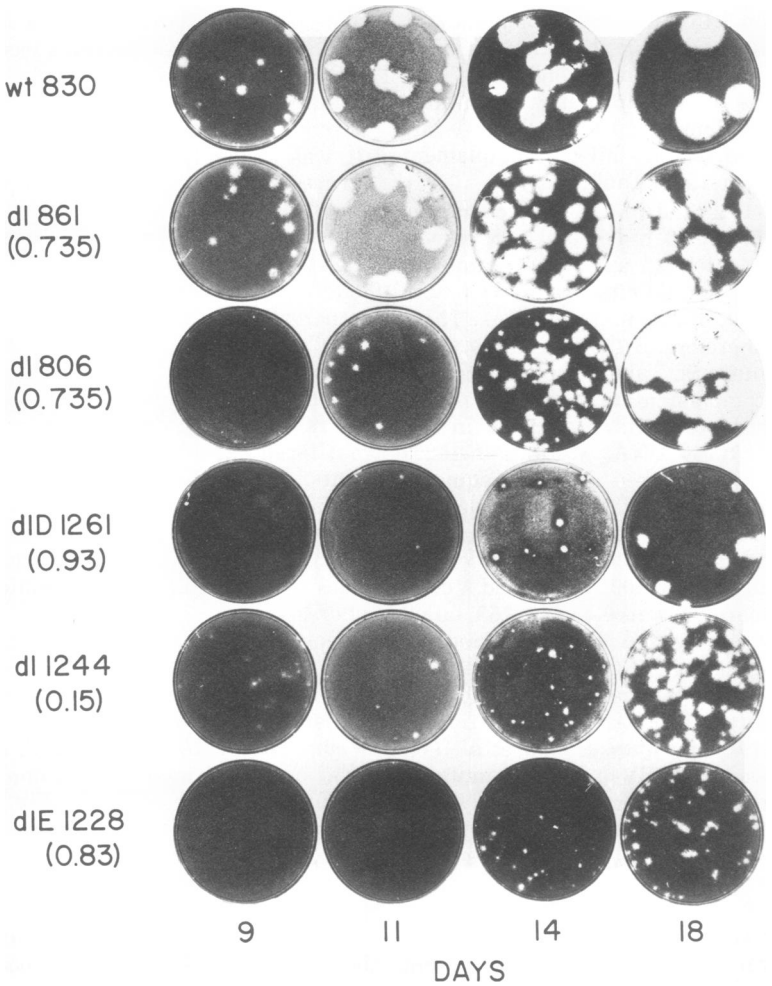

FIG. 7. *Comparison of plaque growth rates of SV40 viable deletion mutants. Plaque assays and staining of the plaques with crystal violet were performed as described in Materials and Methods.*

the cytoplasmic extracts of infected cells (data not shown). In these cases we could not determine whether the mutant VP1 migrated with wild-type VP1 or whether it was absent.

DISCUSSION

In this paper we have described the construction and characterization of a set of mutants of SV40 with deletions located throughout the viral genome. The map locations of these and the viable deletion mutants described by Shenk et al. (44) are shown in Fig. 11.

Late region. Complementation studies with *ts* mutants have already suggested that the region between map coordinates 0.945 and 0.16 contains the *B*, *C*, and *B/C* complementation groups (3, 9–21). Similar studies with mutants having deletions in this region (Tables 2 and 4) indicate that this region constitutes a single complementation group, the *B/C* group. J. E. Mertz (Ph.D. thesis, Stanford University, Stanford Calif., 1975) has already shown that intracistronic complementation within the *B/C* group is common.

The late region of SV40, whose mRNA maps clockwise from 0.76 to 0.17 (8, 27), is comprised of three genes, *B/C*, *E*, and *D*, coding for the major virion protein, VP1, and two minor virion proteins, VP2 and VP3, respectively.

(i) Coding region for VP1. VP1 is initiated within the *Hin*dII + III endonuclease K fragment, 15 nucleotides from the *Hin*dII + III-E/K boundary (12, 53), but the termination site is not known. The nucleotide sequence coding for the C-terminal end of VP1 (9) contains termina-

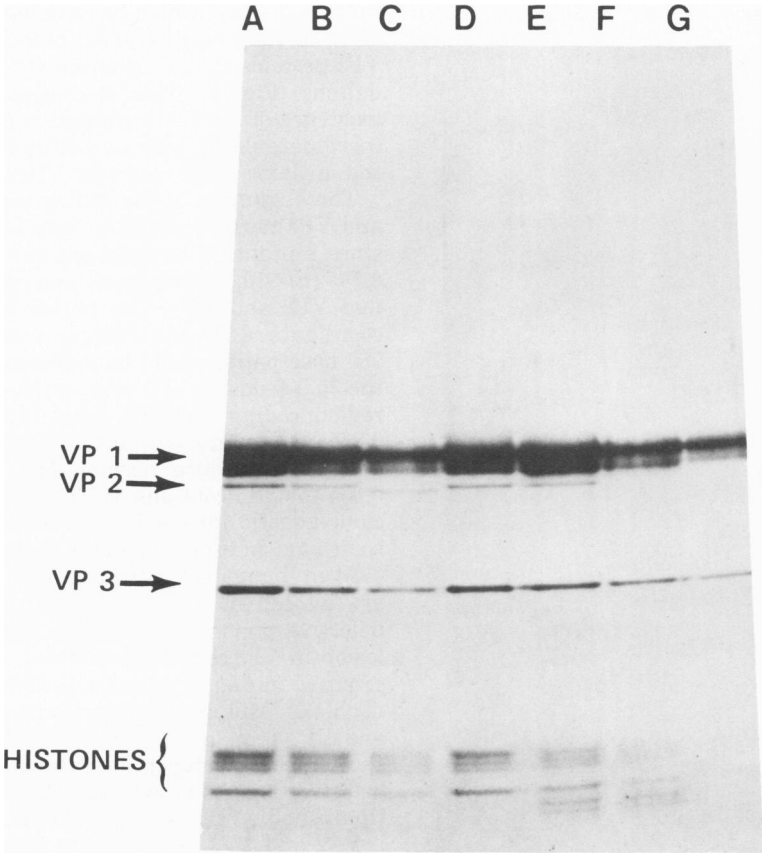

FIG. 8. *Polyacrylamide gel electropherogram of virion proteins of mutants with deletions about the Hae II endonuclease site (0.83). The separating gel was a 7.5 to 15% linear gradient; acrylamide-bisacrylamide ratio was 30:0.8. The stacking gel contained 5% acrylamide. (A) WT830; (B) dlE1222; (C) dlE1224; (D) dlE1225 (E) dlE1226; (F) dlE1228; (G) dlE1230.*

tion codons in all three phases. Only one of the phases would allow translation through the *Bam*HI endonuclease site (map position 0.15). Since mutants with deletions of this site make shorter VP1, translation must continue beyond 0.15 and probably terminates at a UGA codon 51 nucleotides away at map coordinate 0.16 (9).

If there are only 16 to 17 amino acids coded for beyond the *Bam*HI site, how could mutants with deletions of 3 to 10 base pairs at that site (*dl*-1245 and *dl*-1249) produce a VP1 protein that is about 35 amino acids shorter than wild-type protein? Possibly (i) the mutant protein has an incorrectly folded C-terminal region that is readily proteolyzed to a point proximal to the position at which the deletion occurs; (ii) the deletion or alteration of the C-terminal portion of VP1 prevents post-translational modification, e.g., glycosylation (T. Kempe and W. Konisberg, personal communication), thereby altering the electrophoretic mobility of the mutant VP1 in polyacrylamide gels; or (iii) translation of VP1 is normally read through the UGA triplet at map position 0.16 and terminates beyond that point.

(ii) Coding regions for VP2 and VP3. Mutants with deletions in the region bounded by map coordinates 0.755 and 0.835 constitute the *E* complementation group, whereas those that have deletions between 0.835 and 0.945 are classed as group *D* mutants. Curiously, whereas *tsD* mutants have been obtained, *tsE* mutants have never been described.

Mutant *dlD*1261 is, so far, the first SV40 deletion mutant having exclusively the group *D* phenotype. Lai and Nathans found (19–21), using marker rescue experiments, that all *tsD* mutants had lesions in the *Hin*dII + III endonuclease E fragment; the deletion in *dlD*1261 also occurs within the *E* fragment. Furthermore, just as with other group *D* mutants, *dlD*1261 virions are unable to complement any other mutants at the restrictive temperature, but *dlD*1261 DNA complements all other *ts* mu-

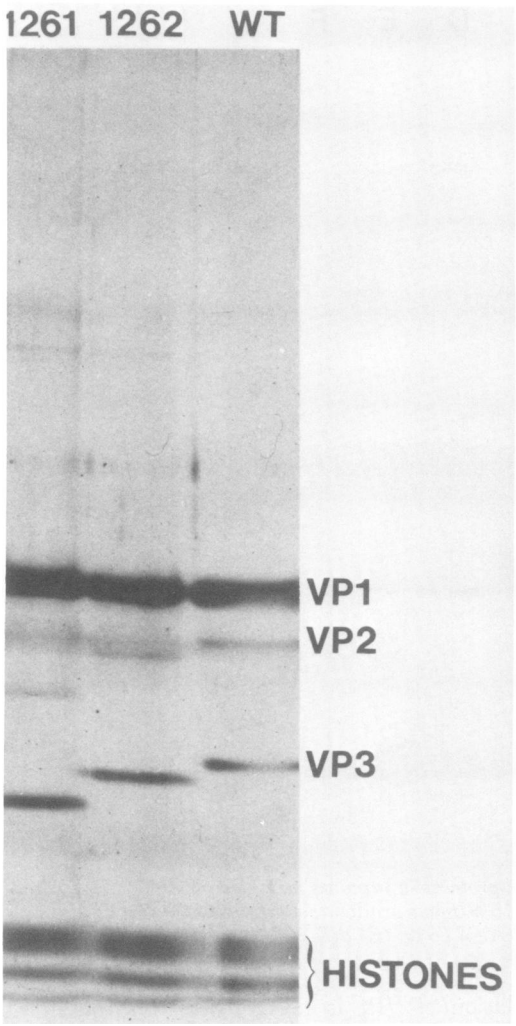

FIG. 9. *Polyacrylamide gel electropherogram of virion proteins of mutants with deletions in the region 0.92 to 0.945. The separating gel was a 7.5 to 15% linear gradient; acrylamide-bisacrylamide ratio was 30:0.8. The stacking gel contained 5% acrylamide.*

tants under the same conditions. This behavior follows from the putative defect of group *D* mutants, namely, faulty uncoating of the virion after infection (39).

VP3's tryptic peptides are a subset of the peptides produced from VP2 (G. Fey, personal communication). Since deletions within the region 0.795 to 0.835 affect only VP2 and deletions in the region 0.92 to 0.945 alter both VP2 and VP3, the common region must occur in the C-terminal two-thirds of the VP2 polypeptide.

The map position coding for the C terminus of VP2 and VP3 is not yet certain. However, it must be distal to map coordinate 0.94, since mutant *dl*-1262, which has a deletion of 50 base pairs at map position 0.94, produces VP2 and VP3 proteins that are shortened by about 2,500 daltons (Fig. 9). This is consistent with the existence of a UGA termination codon at map coordinate 0.945, two nucleotides proximal to the initiator AUG codon for VP1 (53).

The beginning of the coding region for VP2 and VP3 must be distal to map location 0.755, since mutants with deletions extending up to 0.755 (*dl*-810; 29) are viable and synthesize normal VP2 and VP3. The region between map coordinates 0.755 and 0.945, equivalent to about 950 base pairs, would be sufficient to code for the 35,000-dalton VP2 polypeptide (10, 13). The region coding for VP3 must begin distal to 0.835, as mutants with deletions between 0.795 and 0.835 produce normal VP3. The region 0.835 to 0.945 contains 580 base pairs, or sufficient genetic information to encode a protein somewhat in excess of 20,000 daltons.

Even though both VP2 and VP3 polypeptides are altered in mutant *dlD*1261 (Fig. 9), the defective phenotype probably arises from the lesion in VP3, since this mutant complements group *E* mutants, whose sole defect is in VP2 (Table 2). Mutant *dl*-1262 also has altered VP2 and VP3 proteins (Fig. 9), but, in spite of the shorter virion polypeptides, the mutant is nondefective and its growth parameters are indistinguishable from those of WT830.

VP2 and VP3 are probably translated independently of one another, since VP3 production is unaffected in mutants that fail to make VP2 or make an altered VP2. But the possibility that VP3 is derived from VP2 by proteolysis cannot be dismissed entirely. If there is independent expression of VP2 and VP3, it could be due to separate initiation and translation on the same mRNA or translation from two separate mRNA species. In vitro translation of polyoma virus mRNA's suggests that VP2 and VP3 are translated from 19S and 18S mRNA's respectively (T. Hunter, personal communication).

(iii) Control of late gene expression. The deletions in two mutants, *dlBCDE*1210, reported here, and *dlBCD*1003 (18), have a polar effect on the expression of the late genes distal to the site of the deletion. Mutant *dlBCDE*1210, which lacks the segment 0.72 to 0.80, expresses no late functions, even though the deletion does not affect the VP3 and VP1 coding regions. The polarity of this mutant could be explained in three ways. (i) The deleted region might contain the promoter for the synthesis of late mRNA. But the fact that there are SV40 RNA transcripts homologous to the 0.66 to 0.76 region of the late strand in the cytoplasm of SV40-

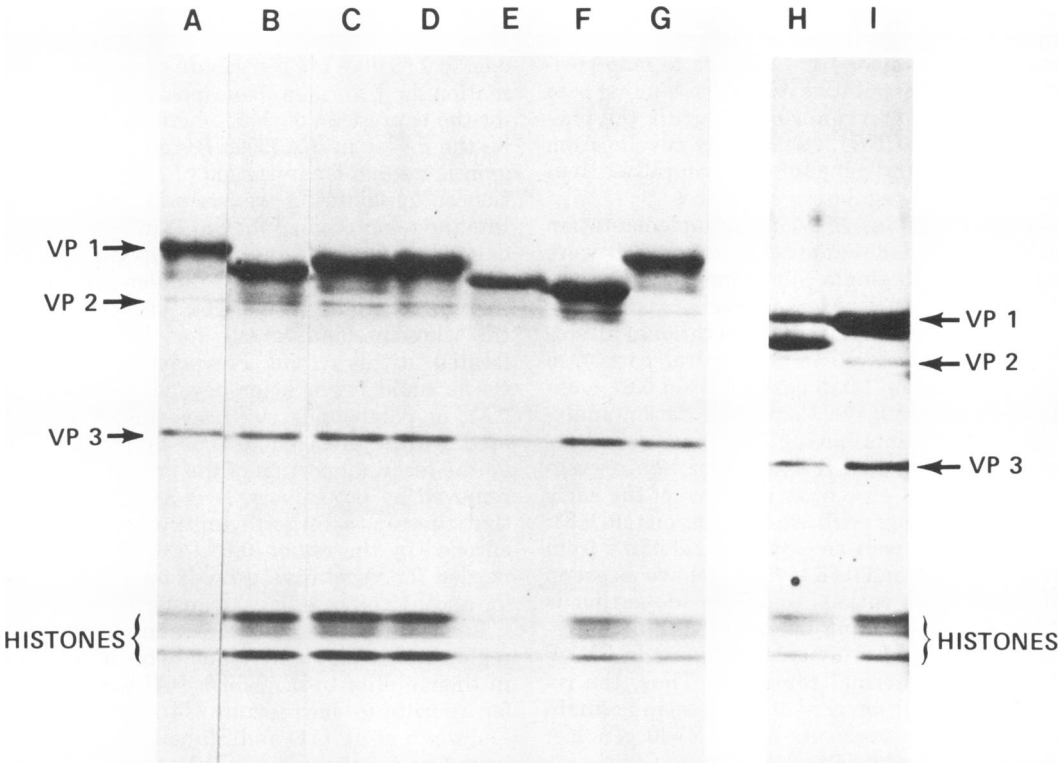

FIG. 10. *Polyacrylamide gel electropherogram of virion proteins of mutants with deletions about the BamHI endonuclease site (0.15). Acrylamide concentration in the separating gel was 15%; acrylamide-bisacrylamide ratio was 30:0.4. The stacking gel contained 5% acrylamide. (A) WT830; (B) dl-1244; (C) dl-1245; (D) dl-1247; (E) dl-1248; (F) dl-1249; (G) WT830; (H) dlBC1231; (I) WT830.*

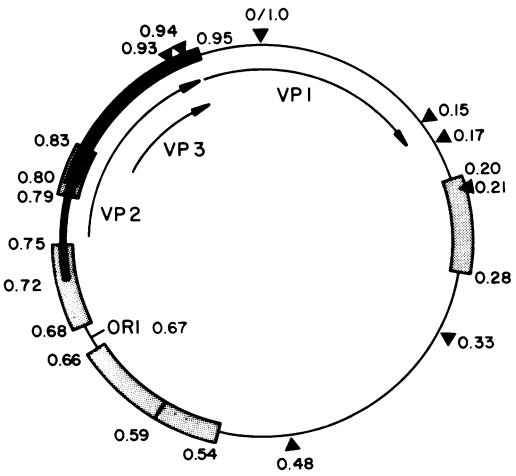

FIG. 11. *Map location of SV40 deletion mutants on the SV40 chromosome. All mutants described in this paper as well as those viable deletions described by Shenk et al. (44) are included. Single mutants with small deletions and sets of mutants with small deletions at a single site are indicated by ▼. Mutants with extended deletions and sets of small deletions spanning larger regions are indicated by solid or stippled bars.*

infected cells (7, 16) suggests that late mRNA synthesis is not initiated in the region 0.72 to 0.80. (ii) The deletion might create a sequence terminating transcription, thereby preventing expression of genes distal to the deletion. (iii) The deleted region could be needed for processing and/or transport of late mRNA. It is known that the 5′ end of the largest discrete species of cytoplasmic late mRNA (19S) maps at coordinate 0.765 ± 0.01 (8, 27), and it is possible that a specific cleavage in that region is essential for its production and/or transport; conceivably, that processing step could be crucial to the production or modification of other late mRNA species, e.g., the 16S RNA coding for VP1.

Mutant *dlBC*1003, a mutant obtained by Lai and Nathans (18), has a deletion of the entire *Hin*dII + III E fragment (0.86 to 0.945); it fails to complement either *tsD* or *tsB/C* mutants (22), but it was not tested for complementation of group *E* mutants. These workers have suggested that the deleted region might contain a promoter, a ribosome-binding site, or a processing sequence needed for the formation of the late 16S mRNA and VP1. Another mutant, *dlBCDE*1211, with a deletion from 0.80 to 0.95,

also fails to express any late genetic functions. Since the deletion begins in the *E* region, spans the entire *D* region, and extends to map position 0.95 (the putative ribosome-binding site and the amino terminus of VP1 [53]), the phenotype of *dlBCDE*1211 probably results from loss of structural gene information rather than from a loss of regulatory sequences.

Early region. Previous complementation studies indicated that all early *ts* mutants were contained in a single complementation group, the *A* group (3, 4). Marker rescue studies (19–21) established that the *tsA* mutational alterations were clustered in the central portion of the early region (map position from 0.67 counterclockwise to 0.17). Complementation analyses with mutants having deletions in this region show that gene *A* expression requires segments of DNA encompassing most of the early region. Mutants with small deletions at 0.33, 0.47, 0.48, as well as extended deletions from 0.285 to 0.205 and 0.66 to 0.59, behave as group *A* mutants. A curious finding made earlier is that mutants having deletions within the region 0.59 to 0.54 are viable and grow in CV1-P cells at near-normal rates (44). Thus, the regions essential for gene *A* expression occur in two separated segments of the SV40 genome, 0.66 to 0.59 and 0.54 to 0.205.

(i) Coding region for T antigen. The *A* gene codes for the SV40 T antigen or A protein (35–37, 41); based on its electrophoretic mobility, the molecular weight of T antigen is estimated as 95,000 (42). Shenk et al. (44) and P. Tegtmeyer (personal communication) have found that the T antigen produced by the viable deletion mutant *dl*-884 (lacking 184 base pairs; 0.54 to 0.57) co-migrates with wild-type T antigen. This suggests that the region coding for the T-antigen structure begins distal to map location 0.54. The T antigen of mutant *dl*-1263 (lacking 50 base pairs at map position 0.21) is apparently 6,000 daltons shorter than the wild-type T antigen, but the T antigen produced by mutant *dl*-1265 (lacking 50 base pairs at map position 0.18) is indistinguishable from the wild-type T antigen (P. Tegtmeyer and C. N. Cole, unpublished data). This suggests that the sequence coding for the C terminus of the T antigen probably occurs within the map region 0.21 to 0.17. The discrepancy between the reported size of T antigen, estimated from its electrophoretic mobility in SDS-polyacrylamide gels (95,000 daltons; 42) and the size dictated by the coding capacity of the DNA bounded by map coordinates 0.54 to 0.17 (75,000 daltons) is at present unreconciled. Post-translational modification or an unusual protein structure could account for the disagreement.

(ii) Control of early gene expression. Although the segment of the DNA at map position 0.66 to 0.59 does not contribute structural information for T antigen, it is apparently essential for the expression of that function. What could be the defect in *dlA*1209? Possibly (i) that region is needed for initiation of early transcription or for allowing transcription to continue into the region coding for the T antigen; (ii) the deleted region contains sequences that control early mRNA processing and/or transport, thereby controlling T-antigen synthesis; or (iii) the ribosome-binding site for the *A* gene is located in this region. Polypeptide chain synthesis could begin at or beyond map position 0.54, or polypeptide synthesis might be initiated within the 0.66 to 0.59 region and the amino terminal portion of the protein might be removed by proteolysis. It is also conceivable that there is a hitherto unrecognized protein encoded by the region 0.68 to 0.54 that is not needed for vegetative growth but is essential for another process, e.g., transformation.

Earlier, mutants with deletions in the map region 0.59 to 0.54 were found to be unimpaired in their ability to transform BALB/c 3T3 cells for growth in low serum (44). But recently Feunteun et al. (11) and Staneloni et al. (46a) have found that analogous deletions in polyoma virus DNA eliminate the ability of polyoma to transform BHK cells. On reexamining the transforming efficiency of *dl*-884, the mutant lacking 184 base pairs at map positions 0.57 to 0.54, di Mayorca and Berg (unpublished data) have found that it is only 2% as efficient as WT830 in transforming F2408 rat fibroblasts for growth in semisolid medium. Further studies are needed to determine whether this region is needed for transformation of some cells but not others, or whether it is the criterion of transformation that is the important parameter.

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