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DNA Sequence Alterations Responsible for the Synthesis of Thermosensitive VP1 in Temperature-Sensitive BC Mutants of Simian Virus 40

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The segment of simian virus 40 (SV40) genome which is recognized as the BC domain encodes for the COOH-terminal end of the SV40 major capsid protein VP1. Mutations in this domain lead to the synthesis of a thermosensitive VP1 which fails to assemble mature SV40 at the nonpermissive temperature. We determined the DNA sequences of eight BC mutants and compared them with the DNA sequences of wild-type SV40, polyomavirus, and BK virus. We found that BC11 and BC223 mutations result from changes in nucleotide residues 2367 (A to C) and 2084 (C to T), respectively. The others (i.e., BC208, BC214, BC216, BC217, BC248, and BC274) share the same point mutation at nucleotide 2354 (C to T). These mutations resulted in the following changes: Lys to Thr, His to Tyr, and Pro to Ser at VP1 amino acid residues 290, 196, and 286, respectively.

The simian virus 40 (SV40) genome consists of a covalently closed circular DNA duplex with a known primary sequence (6, 13). On the basis of complementation tests between temperature-sensitive and deletion mutants, the presence of six genes has been inferred (reviewed in reference 17). Two early genes encode for the small t and large T antigens, the latter of which is required for the initiation of viral DNA replication during lytic growth (17). The four late genes code for the agnoprotein and the capsid proteins VP1, VP2, and VP3 (17). The agnoprotein is encoded within the leader region of some of the mRNAs used for the synthesis of the virion proteins and has been implicated in expediting virion assembly (12a) and in the control of the expression of late genes (1, 7). The capsid proteins are involved in condensing the SV40 chromosomes to form the mature virion structure (17).

Complementation analysis has further revealed that mutations which occur in the gene coding for the major capsid protein VP1 can be subdivided into three groups: *ts*B, *ts*C, and *ts*BC (5, 9). All three groups of VP1 mutants show defects in virion assembly at 40°C. In *ts*C-infected cells the initiation of virion assembly is blocked; in *ts*B-infected cells semiassembled virions are produced; and in cells infected with BC mutants, except *ts*BC11, capsid-SV40 chromatin complexes accumulate (2–4, 12).

Using marker rescue analysis, Lai and Nathans demonstrated that the BC mutations are primarily clustered at the end of the VP1 gene of SV40 (9). They therefore mapped the *ts*BC11, *ts*BC208, *ts*BC214, *ts*BC216, *ts*BC217, *ts*BC248, and *ts*BC274 mutations to the *Hin* G fragment and the *ts*BC223 mutation to the *Hin* J fragment (9). The *ts*BC11 mutant was induced by nitrosoguanidine (16), which produces both transitions and transversions, whereas the other BC mutants were generated by hydroxylamine (5), which is known to cause C-to-T transitions in bacteria.

To delineate the amino acid residues responsible for the synthesis of thermosensitive VP1, we have determined the DNA sequence changes which occur in the BC region. Nucleotide sequence determination was performed by using M13 cloning (11) and the dideoxy sequencing techniques of Sanger et al. (14). We isolated the *ts*BC DNA by the method described by Hirt (8) and employed different strategies for determining the sequence of restriction fragments which contain the mutations. For BC208, BC214, BC216, BC217, BC248, and BC274 we isolated the DNA of each mutant and digested it with *Hin*cII. The *Hin* G fragment was purified by gel electrophoresis and blunt-end ligated to the *Hin*cII site of the M13mp10 vector. The resulting recombinant molecules were used to transform *Escherichia coli* JM103. Colonies were screened for colorless plaques which were used for the isolation of single-stranded DNA template. We annealed a DNA sequence complementary to a region immediately downstream from the insertion site (the universal primer) to the template and extended this primer by using the Klenow fragment of DNA polymerase I in the presence of deoxynucleoside triphosphates, the appropriate dideoxynucleoside triphosphates, and α^{32}P-labeled dATP. The analysis of the reaction products by gel electrophoresis and autoradiography revealed a single base change (C to T) at residue 2354 (Fig. 1) for all of the BC mutants listed above. We further confirmed this result by sequencing the complementary DNA strand. For this analysis *ts*BC DNA was cleaved with *Bam*HI and *Pst*I. The fragment which contained the *Hin* G segment was isolated by gel electrophoresis and ligated to the unique *Pst*I-*Bam*HI restriction sites of M13mp11 vector. Single-stranded DNA recombinant molecules were obtained and sequenced as described above. The autoradiograms of sequencing gels revealed a single nucleotide change from G to A (Fig. 1). This result agrees with that obtained for the coding strand.

We employed a similar strategy to deduce the nucleotide sequence of the mutated DNA fragment obtained from *ts*BC11. In this case we found that the mutation had occurred at a different site. Nucleotide 2367 was changed from A to C on the coding strand and from T to G on the complementary strand (Fig. 2).

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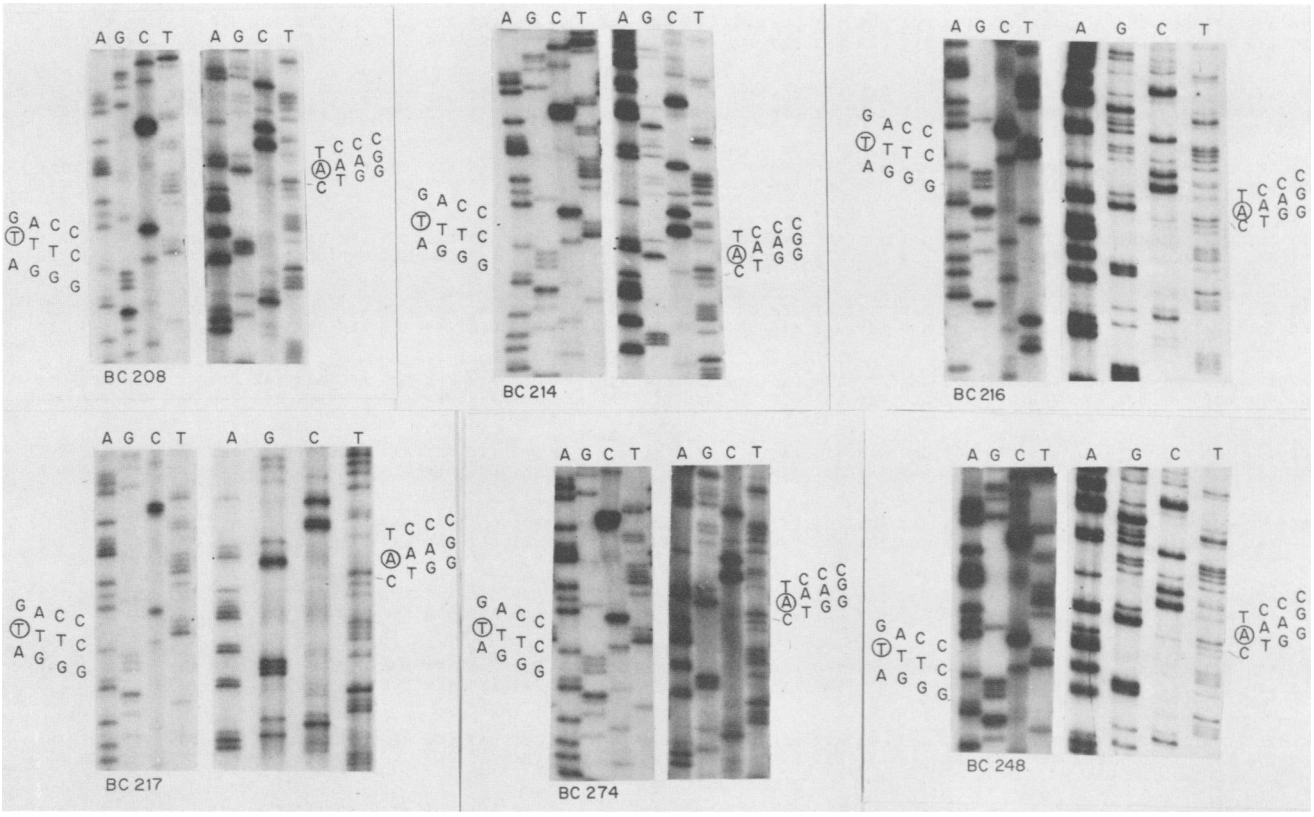

FIG. 1. Autoradiographs of sequencing gels of DNA fragments from *ts*BC208, *ts*BC214, *ts*BC216, *ts*BC217, *ts*BC248, and *ts*BC274 mutants. Panels show the changes which occur in the nucleotide sequence of the coding and complementary strands (shown on the left and right of each panel, respectively).

The *Hin* J fragment contains the mutation which confers temperature sensitivity to the *ts*BC223 strain (9). The site of this mutation was deduced by cloning the *Pst*I-*Bam*HI and *Hin* J fragments restricted from *ts*BC223 DNA in M13mp10 vector and by determining their sequences. We found that the residue 2084 on the coding strand was changed from C to T (Fig. 3). The nucleotide sequence change of the complementary strand (G to A) was also consistent with this result (Fig. 3).

We also determined the nucleotide sequence of the DNA of each mutant in the region flanking the *Bam*HI site by using the Maxam and Gilbert procedure (10). We found no

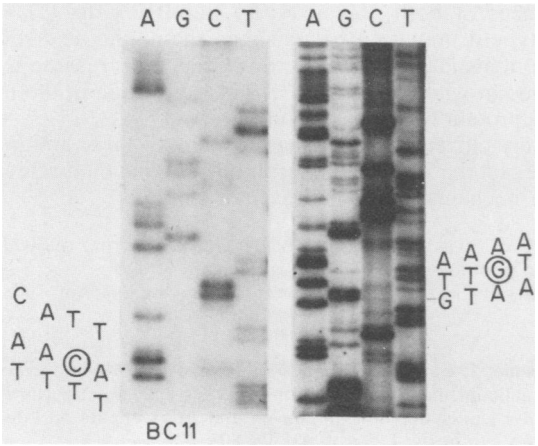

FIG. 2. Autoradiograph of sequencing gels of DNA fragments obtained from *ts*BC11. Left and right panels show the nucleotide changes which occur in the coding and complementary strands, respectively.

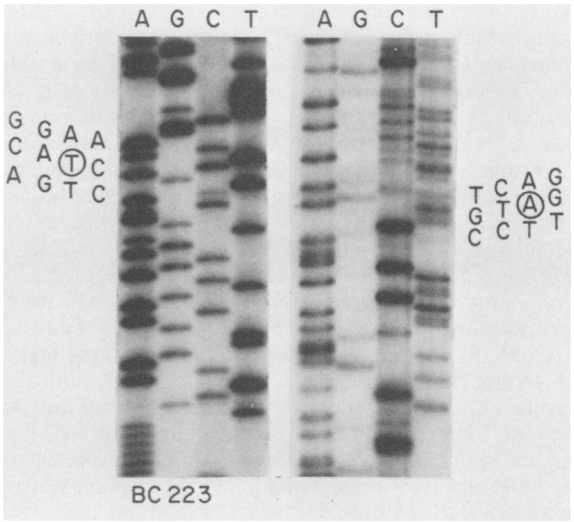

FIG. 3. Autoradiograph of sequencing gels of DNA fragments obtained from *ts*BC223. Left and right panels show the nucleotide changes which occur in the coding and complementary strands, respectively.

FIG. 4. DNA and amino acid sequences of wild-type VP1 and *ts*BC mutants. The DNA sequence has the same polarity as the late mRNA. The nucleotide and amino acid sequence numbers are shown above and at the right of each line, respectively (we have used the numbering system published in reference 15). The differences that exist in the DNA of the mutants, resulting in a different amino acid, are shown by the encircled numbers and are as follows: 1 (BC11), residue 2367 (A to C, Lys to Thr); 2 (BC223), residue 2084 (C to T, His to Tyr); 3 (BC208, BC214, BC216, BC217, BC248, BC274), residue 2354 (C to T, Pro to Ser).

additional alterations in the sequences corresponding to the VP1 COOH terminal end (data not shown).

Figure 4 shows our sequencing results for eight BC mutants which were previously characterized by Lai and Nathans (9) using marker rescue analyses. The ensemble of results demonstrates that BC mutations consist of a single nucleotide change in the VP1 gene. Our data demonstrate that there are only three mutants in the SV40 BC complementation group, because six of eight mutations occurred at the same position in the wild-type sequence and thus changed the VP1 polypeptide in an identical way. Although it is possible that BC208, BC214, BC216, BC217, BC248, and BC274 were not independent events but represent the progeny of a mutant that preexisted in the virus stock used by Chou and Martin (5), the fact that these mutants and BC223 and A209 (15) are the results of C-to-T transitions makes it more likely that each mutant arose as a result of the mutagenesis, i.e., that each mutant represents an independent event. This would imply that nucleotide 2354 is a super-hot spot. It may be relevant that the sequences at 2354, 2084, and 3539 consist of adjacent C residues upstream from an AT-rich region.

We deduce from our data that the nucleotide change at 2354 resulted in the exchange of proline to serine at amino acid residue 286. This residue is also proline in wild-type VP1 of polyomavirus, but it is alanine in the major capsid protein of BK virus (17). A second type of mutation in the BC domain was found in the BC11 strain in which lysine residue 290 was changed to threonine. Interestingly, this lysine residue has been preserved in the wild-type VP1 sequences of both polyomavirus and BK virus (17). The third type of mutation has changed the amino acid residue 196 from histidine to tyrosine in BC223. This residue is also histidine in wild-type BK VP1, but it is serine in the major capsid protein of polyomavirus (17).

We are currently examining the effect of these amino acid changes on the structure of VP1 and studying their relevance to the mechanism of SV40 assembly.

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