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Deletion of DNA Sequences in a Nononcogenic Variant of *Herpesvirus saimiri*

J. MICHAEL KOOMEY,[1]† CAREL MULDER,[1,2] ROBERT L. BURGHOFF,[3] BERNHARD FLECKENSTEIN,[4] AND RONALD C. DESROSIERS[3]*

Departments of Pharmacology[1] and Molecular Genetics and Microbiology,[2] University of Massachusetts Medical School, Worcester, Massachusetts 01605, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772,[3] and Institut für Klinische Virologie, Universität Erlangen-Nürnberg, D-8520 Erlangen, Federal Republic of Germany[4]

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The 110-kilobase-pair stretch of unique sequence DNA of *Herpesvirus saimiri* is flanked by highly repetitive DNA. Detailed restriction endonuclease mapping has localized the left junction of repetitive and unique DNA to a 100-base-pair region. *H. saimiri* 11att, a replication competent nononcogenic variant of strain 11, has a deletion of 2.3 kilobase pairs of sequence information that spans this left junction of repetitive and unique DNA.

Study of oncogenic transformation in model tumor virus systems has revealed the existence of transforming oncogenes with a host cell homolog (some retroviruses) (1), transforming genes without a host cell homolog (papovaviruses and adenoviruses) (11, 14), and integrative downstream promotion mechanisms (avian leukosis virus and mouse mammary tumor virus) (15, 18). Unlike these model tumor virus systems, little is known at a molecular level about any oncogenic transformation induced by a herpesvirus. Study of oncogenic transformation in herpesvirus systems may provide insight into additional mechanisms by which a virus can induce transformation of cells. For example, herpes simplex viruses and cytomegalovirus can apparently induce transformation of cells without viral genes being consistently retained (12, 17). *Herpesvirus saimiri* is a useful system for studying oncogenic transformation since diffuse lymphomas are induced rapidly and reproducibly in a variety of New World primates, large amounts of viral DNA remain stably associated with transformed and tumor cells, and *H. saimiri* produces lytic infection of some monolayer cell lines.

One approach toward understanding the molecular basis for the oncogenicity of *H. saimiri* that we are exploiting is the study of nononcogenic variants. Repeated passage of strain 11 at 39°C, followed by repeated plaque purification at 34°C, yielded a nononcogenic variant (11att) (19). This variant grows as well as parental strain 11 virus in permissive owl monkey kidney (OMK) and Vero cells in vitro. Strain 11att fails to induce lymphomas in cotton-topped marmosets (9, 19), white-lipped marmosets (8), common marmosets (21), and owl monkeys (L. Falk, unpublished data), species normally susceptible to the lymphoma-inducing capacity of the parental virus. Animals inoculated with strain 11att become infected, since virus can be recovered from peripheral lymphocytes long after experimental inoculation; infected animals, however, survive and do not develop signs of lymphoma or leukemia. In fact, common marmosets infected with strain 11att are protected from challenge by parental strain 11 (22).

* Corresponding author.
† Present address: Department of Medical Microbiology, Stanford University Medical School, Stanford, CA 94305.

Infectious virion DNA (M-DNA) contains an internal stretch of unique sequence DNA of low G+C content (L-DNA) flanked by repetitive DNA of high G+C content (H-DNA) (2). Virion DNA was prepared from strains 11 and 11att for comparative restriction endonuclease analysis. Digestion of virion DNA of both strains with *Kpn*I + *Sma*I, *Aos*I + *Sma*I, *Taq*I, *Hpa*II (Fig. 1), and other restriction endonucleases suggested a deletion of DNA sequences at or near the left junction of unique (L-) and repetitive (H-) DNA in strain 11att. *Sma*I cuts four times in each repeat unit of strain 11 H-DNA but does not cut within L-DNA (2). The *Kpn*I + *Sma*I fragment G (1.6 kilobase pairs [kbp]) derived from the left H/L-DNA junction of strain 11 was not detected in strain 11att; the adjacent *Kpn*I fragment B, although not apparently altered in size, was now the terminal L-DNA fragment, since digestion with and without *Sma*I revealed that the H-DNA was attached to it. When virion M-DNA was digested with restriction endonucleases that cleave L-DNA frequently, such as *Taq*I and *Hpa*II (Fig. 1), other changes were not apparent. These results confirmed that 11att was indeed derived from strain 11, since these enzymes readily distinguish different strains of *H. saimiri* (7, 10), and they also indicated that no other major changes occurred in the genome of strain 11att.

To map the limits of the deletion precisely, we first had to define the nature of the sequences at the left H/L-DNA junction. The H-DNA repeat unit is generally defined by *Taq*I, which cuts each H-DNA repeat unit once. We isolated a DNA fragment from strain 11 that spans the left H/L-DNA junction by cloning of a partial *Taq*I digest of virion DNA into the *Cla*I site of pBR322 (pTP8) (Fig. 2). Detailed restriction endonuclease maps of this fragment were derived and compared with H-DNA maps to determine the nature of repetitive H-DNA at the junction. Results from this comparison, as well as Southern blot hybridizations, have localized the left H/L-DNA junction of strain 11 to a 100-base-pair region between adjacent *Sma*I and *Taq*I sites (Fig. 2). This is an accurate reflection of virion DNA structure since comparative digestion of pTP8 and strain 11 M-DNA with several restriction endonucleases gave identically sized fragments that span this H/L-DNA junction (Fig. 2).

To determine the nucleotide sequences deleted in strain

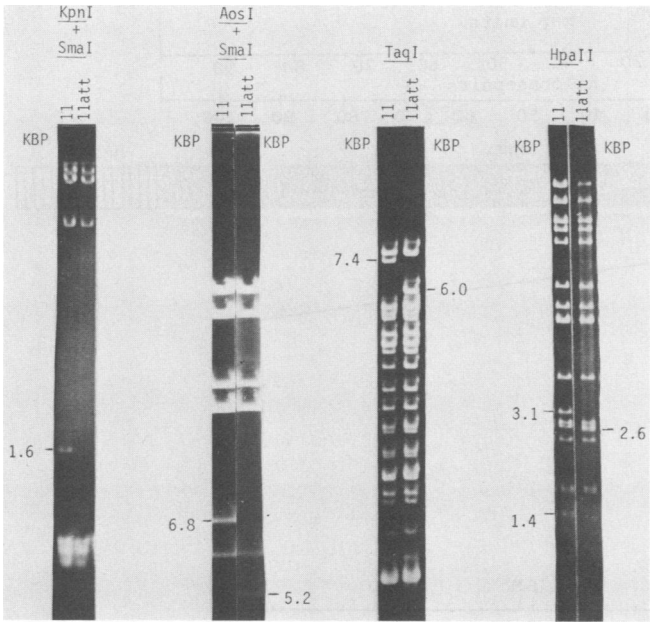

FIG. 1. Comparative restriction endonuclease analysis of *H. saimiri* 11 and 11att virion M-DNA. Virion M-DNA was prepared as previously described (2). After digestion with restriction endonucleases, DNA fragments were separated by electrophoresis through a 0.8% agarose gel, stained with ethidium bromide (1 μg/ml), and photographed over UV light. *Hin*dIII fragments of bacteriophage lambda DNA were used as size markers.

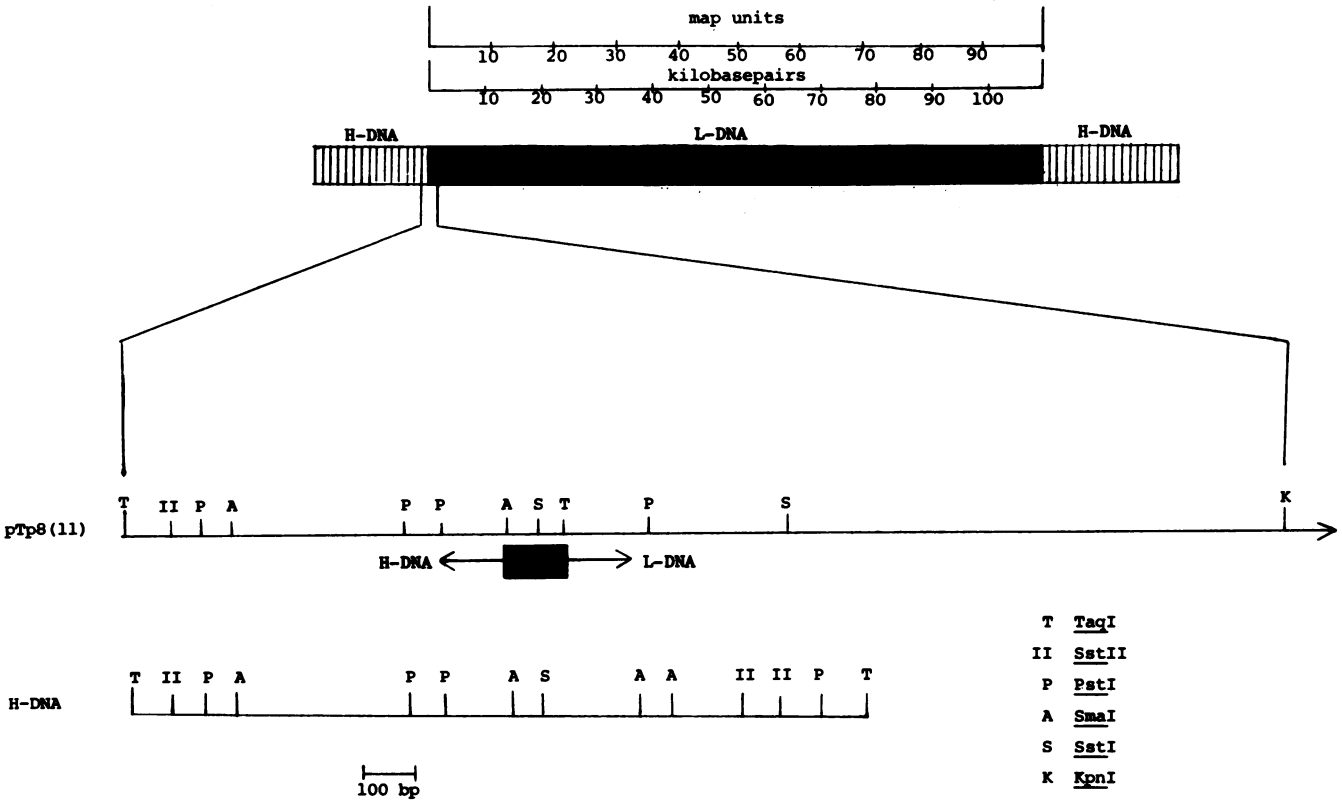

FIG. 2. Nature of H-DNA sequences at the left junction of H- and L-DNA. *H. saimiri* 11 M-DNA was partially digested with *Taq*I, such that digestion was ca. 50% complete. Digested DNA was layered over a 5 to 20% sucrose gradient in 10 mM Tris-hydrochloride (pH 7.5)–100 mM NaCl–1 mM EDTA and centrifuged for 15.5 h at 28,000 rpm at 4°C in a Beckman SW40 rotor. Twelve fractions were collected from the bottom of the tube; DNA from a single fraction, containing DNA primarily of 8 to 13 kbp, was precipitated with ethanol. Approximately 0.2 μg of this DNA was ligated with 0.2 μg of *Cla*I-cleaved pBR322 and transferred into competent *Escherichia coli* K-12 HB101. Colonies were screened for left-end L-DNA sequences by Grunstein-Hogness colony hybridization (13) with a ^{32}P-labeled *Taq*I 7.4-kbp fragment insert of pT7.4 (6) used as hybridization probe. One of these colonies, pTP8, containing 8.3 kbp of *H. saimiri* DNA, was selected for restriction endonuclease mapping. H-DNA repeat unit maps derived from reference 10 and H-DNA sequence data (W. Bear, E. Knust, B. Fleckenstein, and B. Barrell, unpublished data) are shown at the bottom. That pTP8 truly reflects the H/L-DNA junction was confirmed by appropriate comigrating fragments from strain 11 virion M-DNA with *Kpn*I + *Sma*I, *Sst*I, *Taq*I, and *Pst*I. Only one of two closely spaced *Sst*I sites in H-DNA is shown.

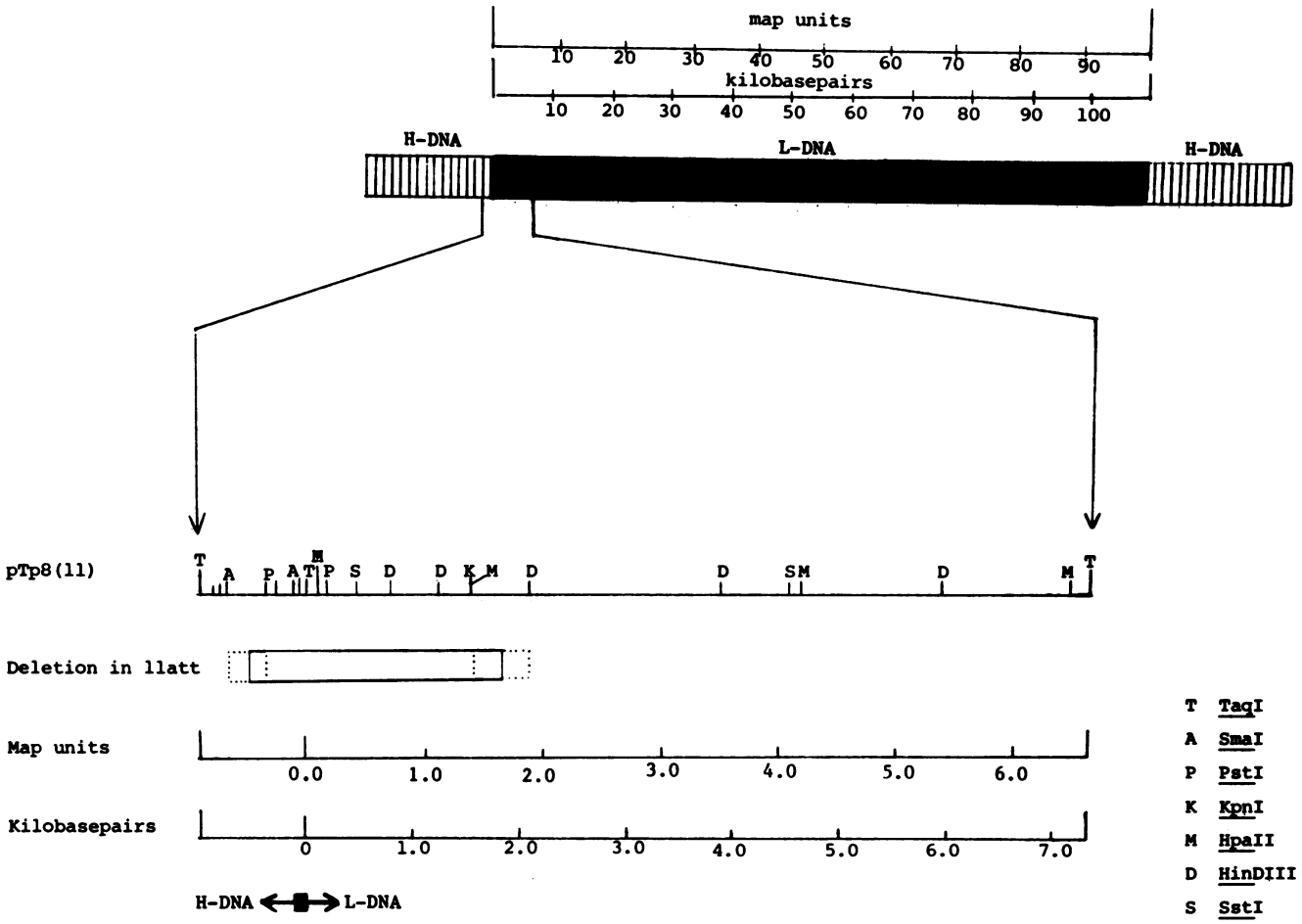

FIG. 3. The limits of the deletion in strain 11att were determined by restriction endonuclease mapping of pT6.0 DNA. The pT6.0 clone was derived by isolating the 6.0-kbp *Taq*I fragment of strain 11att from an agarose gel (see Fig. 1) and inserting it into the *Cla*I site of pBR322. The limits of the deletion are primarily defined by the presence of the *Sma*I site at −0.6 map units, the presence of the *Hin*dIII site at +1.9 map units, and the absence of intervening restriction endonuclease sites. The internal *Taq*I site of pTP8 is taken as 0.0 map units. The uncertainty in the limits of the deletion in 11att are indicated by the dotted line. Numerous *Hpa*II sites within the H-DNA region leftward of 0.0 map units are not indicated. Vertical lines corresponding to *Sst*II and some *Pst*I and *Sst*I sites are unlettered, but their position may be gleaned from inspection of Fig. 2. A more detailed map of 0 to 6.7 map units can be found in reference 6.

11att, we also cloned the H/L-DNA junction fragment of this variant strain. The 6.0-kbp *Taq*I fragment spanning the left junction of H- and L-DNA of strain 11att was cloned into the *Cla*I site of pBR322. DNA from this clone (pT6.0) was used for detailed restriction endonuclease analysis. The left boundary of the 11att deletion was defined by a *Sma*I site which was present in pT6.0, 11att DNA, and pTP8 and a *Pst*I site of pTP8 which was not present in pT6.0 or 11att DNA. The right boundary of the 11att deletion was defined by *Kpn*I and *Hpa*II sites which were not present in pT6.0 or 11att DNA and a *Hin*dIII site which was present in pT6.0 and 11att DNA (Fig. 3). This indicates that strain 11att is missing ca. 2.3 kbp of DNA sequence information that span the H/L-DNA junction as indicated in Fig. 3.

Are sequences at the left junction of H- and L-DNA of *H. saimiri* required for oncogenic transformation? Since strain 11att arose spontaneously in cell culture, there is no way of knowing at this time whether other undetected changes or point mutations may be responsible for or contribute to the loss of oncogenicity of strain 11att. It is remarkable, however, that another nononcogenic variant of *H. saimiri*, strain SMHIatt, has a deletion of about 7 kbp also at or near the left H/L-DNA junction (3). Analysis of constructed deletion mutants or viruses in which the deleted sequences have been reinstated should more definitively answer this question. The construction of engineered virus strains with defined deletions in this region has recently been described (6). Other findings can also be cited relevant to the possible involvement of this region in oncogenic transformation. DNA from this region of viral DNA is preserved in all tumor and transformed cell lines so far tested, even when other large regions of viral DNA are not retained (4, 16, 20). Also, some CG dinucleotides in this region remain specifically unmethylated in tumor biopsy specimens and in tumor cell lines, whereas most other CG dinucleotides are extensively methylated (5).

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