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Configuration of Latent Varicella-Zoster Virus DNA

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The configuration of latent varicella-zoster virus (VZV) DNA was analyzed by PCR. Template DNA for both internal and terminal VZV primers was present in a 1:1 ratio in ganglionic DNA, compared with a 15:1 ratio in DNA extracted from VZV virions, indicating that the VZV genomic termini are adjacent in latently infected human ganglia.

After primary infection, varicella-zoster virus (VZV) becomes latent in dorsal root ganglia at all levels of the neuraxis (14–16). Multiple regions of the VZV genome (16) are present during latency in humans; however, the configuration of latent VZV DNA is unknown.

Genomes extracted from VZV virions are 125-kbp linear double-stranded DNA molecules (13, 21), consisting of a unique long (U_L) region of 100 kbp and a unique short (U_S) region of 5.4 kbp. The U_S is bounded by repeat regions of 6.8 kbp and is found in an inverted orientation in 50% of genomes extracted from virions. The U_L is bounded by repeats of 88 bp and is found in an inverted orientation in 5% of genomes extracted from virions (reviewed in reference 4).

In this study, PCR amplification using two sets of primers was used to determine the configuration of latent VZV DNA. The terminal primers, T1 and T2 (Table 1), consisted of oligonucleotides (Operon, Inc., Alameda, Calif.) from the ends of the VZV genome (5) that prime DNA synthesis outward toward the genome termini (Fig. 1). The internal primers, I1 and I2 (Table 1), consisted of oligonucleotides from within the U_L region of the VZV genome (Fig. 1).

PCR analysis of pRLT DNA. The PCR products obtained after amplification of VZV-infected BSC-1 cell DNA with both the internal (I1 and I2) and terminal (T1 and T2) primers were ligated into *Hin*dIII-*Sac*I-digested cloning vector pT7BlueR (Novagen, Madison, Wis.) and *Eco*RI-digested pCRII (Invitrogen Corp.), respectively. The amplification products were then subcloned into PCRVector (Invitrogen Corp.), producing plasmid pRLT, which was sequenced (Sequenase; United States Biochemicals Corp., Cleveland, Ohio) to ensure that it contained one copy of template DNA for both the terminal and internal primers. DNA extracted from BSC-1 cells was digested with *Eco*RI and then spiked with *Eco*RI-digested pRLT DNA. PCR amplification was performed by using the internal and terminal PCR primers (0.25 μM) in the same reaction tube. PCR mixtures contained 1 μg of DNA in 50 mM KCl–10 mM Tris-HCl (pH 8.3)–200 μM each dATP, dCTP, dGTP, and dTTP, 1 mM $MgCl_2$, and 10% dimethyl sulfoxide. Reagents were heated to 95°C for 5 min before the addition of 2.5 U of *Taq* DNA polymerase (Promega, Madison, Wis.). After a further 10 min at 80°C, 34 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and polymerization at 72°C for 1 min was performed. The final cycle included a polymerization step at 72°C for 7 min. Amplified DNA fragments were separated by electrophoresis and detected by hybridization to ^{32}P-end-labeled oligonucleotide probes located internal to the amplified DNA segment. The amplification product was quantitated with a PhosphorImager, and a graph was constructed by plotting the $\log_{10}$ of the amount of amplified product against the $\log_{10}$ of the plasmid copy number (Fig. 2). A product was detectable after amplification of 10^2 copies of pRLT DNA with both the terminal and internal primers. Between 10^2 and 10^4 copies of plasmid DNA, increasing the copy number of plasmid DNA produced a proportional increase in the amount of product obtained (Fig. 2). Thereafter, increasing the plasmid copy number had little effect on the amount of product.

TABLE 1. Oligonucleotide primers

Primer	Sequence	Location[a]
T1	TTGTTGTTAATAAAAGCGGAACGG	124743
T2	AGTCTAGGCAATCGTAAGACTGT	401
I1	CACACACAATCGGATGTTGCTTA	75281
I2	CATCGCTTGAGCATAGTGGTGG	75614
T3	AGATCGGAAAACGGTGCTCATG	104400

[a] Location of the first 5′ base on the DNA sequence of the VZV genome (3).

PCR amplification of DNA extracted from human ganglia. PCR amplification (39 cycles) was performed on 1 μg of

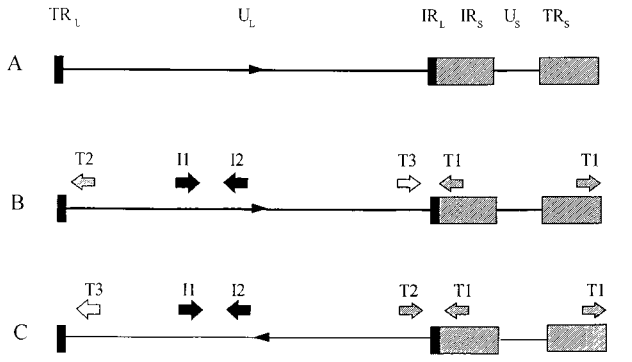

FIG. 1. The VZV genome, showing the U_L, U_S, TR_S, IR_S, TR_L and, IR_L regions (A), the positions of the internal (I1 and I2; black arrows) and terminal (T1 and T2; shaded arrows) PCR primers with the U_L in the standard orientation (B), and the positions of the internal (I1 and I2) and terminal (T1 and T2) primers with the U_L in the inverted orientation (C). The position of oligonucleotide primer T3 (white arrow) is also indicated.

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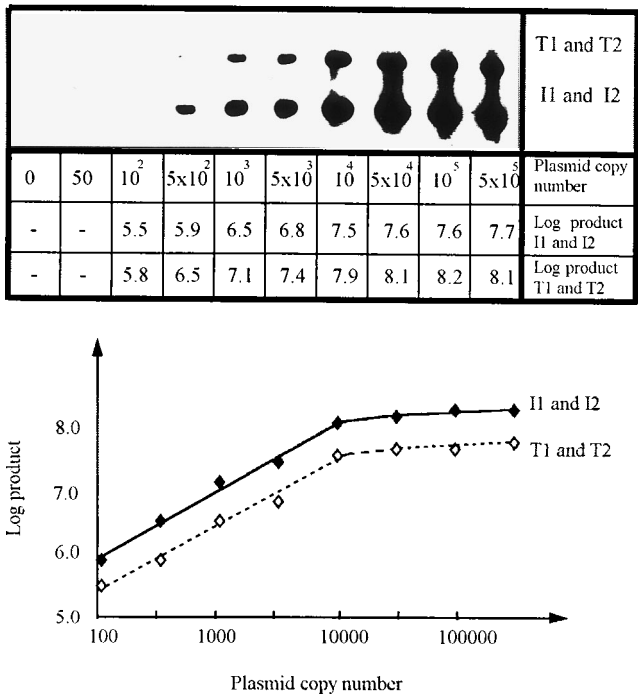

FIG. 2. PCR analysis of pRLT DNA. The terminal (T1 and T2) and internal (I1 and I2) primers were used to amplify 1 μg of BSC-1 DNA spiked with pRLT DNA. The PCR data and graph show the effect of increasing plasmid copy number on the amount of product obtained after 34 amplification cycles.

*Eco*RI-digested DNA extracted (16) from the trigeminal ganglia of 12 humans (Table 2), using the internal and terminal primers. In parallel, PCR amplification was performed on 1 μg of *Eco*RI-digested BSC-1 DNA-spiked with *Eco*RI-digested pRLT and VZV virion DNA. The amount of product was quantitated with a PhosphorImager (Fig. 3). A standard graph of the amount of product obtained after amplification of 10^2, 10^3, and 10^4 copies of pRLT DNA was constructed for each primer set (Fig. 3). The terminal and internal primers consistently amplified these copy numbers of plasmid DNA in a linear manner. The $\log_{10}$ of the amount of product obtained after amplification of each sample of ganglionic or virion DNA was compared with the standard graph to determine the number of copies of template DNA for each primer set (Table 3, experiment 1). The experiment was repeated three times. The products obtained after amplification of virion and ganglionic DNA with both primer sets were cloned and sequenced (data not shown). The sequences obtained were identical for both virion and ganglionic DNAs and were consistent with published VZV DNA sequences (5).

TABLE 2. Subject history[a]

Subject no.	Age (yr), sex[b]	Cause of death	No. of ganglia
1	64, F	Lung cancer	1
2	69, M	Chronic myelogenous leukemia	2
3	62, M	Cardiac arrest	1
4	63, M	Pulmonary obstruction	1
5	44, F	DIC[c]	2
6	67, M	Myocardial infarction	1
7	59, M	Myocardial infarction	2
8	73, M	Pseudoaneurysm	2
9	64, M	Respiratory failure	1
10	50, F	Respiratory failure	2
11	68, M	Pneumonia	1
12	80, F	Heart failure	2

[a] All subjects except 6 and 12 were VZV antibody positive.
[b] F, female; M, male.
[c] DIC, disseminated intravascular coagulation.

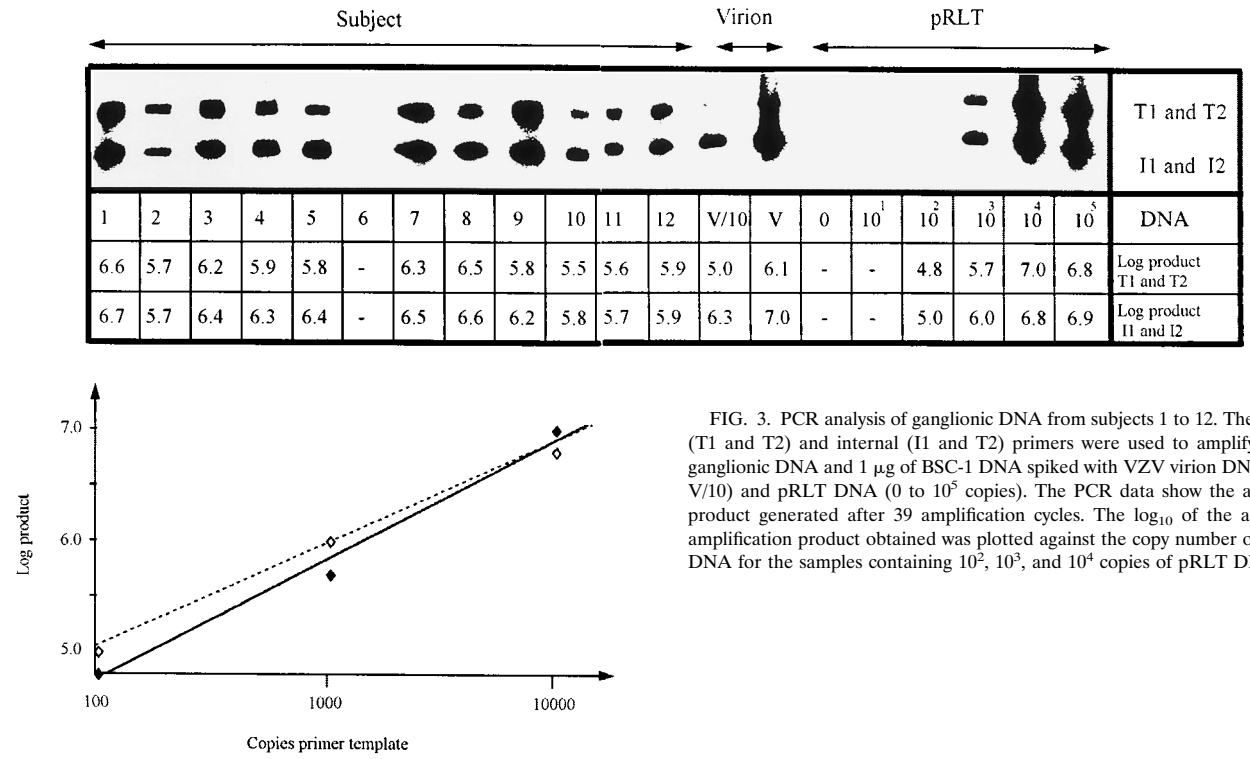

FIG. 3. PCR analysis of ganglionic DNA from subjects 1 to 12. The terminal (T1 and T2) and internal (I1 and T2) primers were used to amplify 1 μg of ganglionic DNA and 1 μg of BSC-1 DNA spiked with VZV virion DNA (V and V/10) and pRLT DNA (0 to 10^5 copies). The PCR data show the amount of product generated after 39 amplification cycles. The $\log_{10}$ of the amount of amplification product obtained was plotted against the copy number of plasmid DNA for the samples containing 10^2, 10^3, and 10^4 copies of pRLT DNA.

TABLE 3. PCR Amplification of ganglionic DNA[a]

Subject no. or source	Expt no.	No. of internal primer templates (I)	No. of terminal primer templates (T)	Ratio, I/T	Avg ratio
1	1	6,310 (6.7)[b]	6,310 (6.6)	1.00	
	2	5,495	7,244	0.76	0.86
	3	5,192	6,310	0.81	
2	1	562 (5.7)	794 (5.7)	0.71	
	2	1,047	891	1.18	0.91
	3	798	955	0.83	
3	1	2,512 (6.4)	2,512 (6.2)	1.00	
	2	2,042	1,000	2.04	1.16
	3	2,399	5,370	0.45	
4	1	2,238 (6.3)	1,259 (5.9)	1.78	
	2	1,472	1,858	0.79	1.07
	3	1,047	1,621	0.65	
5	1	2,512 (6.4)	1,122 (5.8)	2.24	
	2	2,399	5,370	0.45	1.17
	3	1,621	1,995	0.81	
6	1	0	0		
	2	0	0		
	3	0	0		
7	1	3,918 (6.5)	3,162 (6.3)	1.24	
	2	2,399	2,951	0.81	1.02
	3	3,411	3,333	1.02	
8	1	5,012 (6.6)	5,012 (6.5)	1.00	
	2	5,129	5,012	1.02	1.48
	3	9,247	3,819	2.42	
9	1	1,778 (6.2)	1,122 (5.8)	1.58	
	2	1,047	1,202	0.87	1.02
	3	1,472	2,388	0.62	
10	1	708 (5.8)	501 (5.5)	1.41	
	2	603	691	0.87	1.44
	3	2042	1,000	2.04	
11	1	562 (5.7)	631 (5.6)	0.89	
	2	1,472	2,388	0.62	0.90
	3	1,047	891	1.18	
12[c]	1	891 (5.9)	1,259 (5.9)	0.71	
	2	1,079	1,671	0.65	0.68
Virion	1 (10^3 copies)	2,239 (6.3)	158 (5.0)	14.17	
	2	5,495	269	20.43	15.03
	3	7,244	691	10.48	

[a] One microgram of ganglionic DNA was used for amplification.
[b] Values in parentheses are the $\log_{10}$ of the amount of product obtained after amplification. $\log_{10}$ values are given for the experiment described in Fig. 3.
[c] Amplification was performed only twice on DNA extracted from subject 12.

VZV DNA was detected with both primer sets in ganglionic DNA extracted from 11 of 12 subjects. Antibody to VZV was detected in 10 of 12 subjects (Table 2). Ganglionic DNA from subject 6 was negative for VZV DNA, and serum from this subject did not contain VZV antibodies, indicating that subject 6 had not experienced primary VZV infection. VZV sequences were detected in ganglionic DNA extracted from subject 12, whose serum did not contain VZV antibodies, consistent with the occasional lack of detectable antibody in elderly subjects with a history of varicella (22).

The copy number of template DNA in 1 μg of ganglionic DNA was ~500 to 9,000 for the internal primers and ~500 to 6,000 for the terminal primers (Table 3). The ratio of template copies for the internal and terminal primers in a sample of VZV DNA was used to determine its configuration. The internal primers would be expected to amplify 100% of genomes extracted from VZV virions, whereas the terminal primers would be expected to amplify only the ~5% of genomes containing an inverted U_L region (Fig. 1). Our results show that VZV virion DNA contains a ~15:1 ratio of templates for the internal and terminal primers, indicating that 6.7% of VZV genomes contain an inverted U_L region.

The average ratio of template copies in ganglionic DNA for the internal to terminal primers was determined for each subject and ranged from 0.68 to 1.48 (Table 3), compared with ~15 for virion DNA. Overall, in latently infected human ganglionic DNA, the mean ratio (±2× the standard error of the mean) of template DNA for the internal and terminal primers was 1.06 ± 0.15, indicating that the VZV genome termini are adjacent during latency. The herpes simplex virus type 1 (HSV-1) genome termini are also adjacent in DNA from ganglia of latently infected mice (7, 19, 20) and humans (7). Thus, latent VZV and HSV-1 DNAs appear to assume similar configurations.

The simplest genome configuration consistent with adjacent genome termini of latent VZV and HSV-1 DNA is a circle, consistent with the finding that latent HSV-1 DNA is extrachromosomal (17). The unpaired nucleotide at the terminus of many herpesvirus genomes, including those of VZV (5), HSV-1 (18), and simian varicella virus (2), may facilitate circularization, which also occurs during productive infection (1, 12). After circularization, it is possible that VZV undergoes limited DNA replication before establishing latency, producing concatemers of viral DNA which would also result in adjacent genome termini. All attempts to distinguish between circularization and concatemerization by fractionation of DNA prior to PCR were unsuccessful.

Although we cannot rule out the possibility of integration into the cellular chromosome, it is interesting that, like HSV-1 DNA, VZV DNA resides in nondividing neurons during latency (6, 8, 11), where extrachromosomal DNA can be retained. Additionally, on the basis of bacteriophage and proviral integration (reviewed in references 9 and 10), the most likely integration site would be the terminal repeat regions which would not result in adjacent genome termini.

Adjacent genome termini would also result if all latent VZV genomes were unit-length linear molecules containing an inverted U_L region. However, since oligonucleotides T1 and T3 (Fig. 1) would not be able to amplify DNA from linear VZV genomes containing an inverted U_L but did amplify ganglionic DNA with an efficiency similar to that of the internal primers (results not shown), such is not the case.

In summary, we have shown by PCR analysis that the termini of VZV DNA are adjacent in latently infected human ganglia, results which suggest an extrachromosomal, circular configuration for latent VZV DNA.

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