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*Xba*I, *Pst*I, and *Bgl*II Restriction Enzyme Maps of the Two Orientations of the Varicella-Zoster Virus Genome

A. M. DUMAS,[1]* J. L. M. C. GEELEN,[2] M. W. WESTSTRATE,[2] P. WERTHEIM,[2] AND J. VAN DER NOORDAA[2]

Department of Virology, Gemeentelijke Geneeskundige en Gezondheidsdienst, 3011 EN Rotterdam,[1] and Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, 1092 AD Amsterdam,[2] The Netherlands

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Cleavage of varicella-zoster virus DNA with the restriction endonucleases *Pst*I, *Xba*I, and *Bgl*II resulted in 18, 22, and 20 fragments, respectively. Based on the molecular weights and molarities of these fragments, a molecular weight of 84 × 10^6 could be calculated for the varicella-zoster virus genome. In both the *Xba*I and the *Bgl*II patterns, four 0.5 M fragments were identified. The arrangement of the fragments was determined by molecular hybridization techniques, and the terminal fragments were identified by λ exonuclease digestion. The 0.5 M fragments, of which two were located at the same terminus of the genome, contained repeated sequences: one terminally and one inverted internally. These results were in agreement with the existence of two equimolar subpopulations of the varicella-zoster virus genome, differing in the relative orientation of a short region of unique sequences. This region was bounded by the repeated sequences. From the molecular weights of the submolar fragments, a maximal molecular weight of 5 × 10^6 for the repeated region and a minimal molecular weight of 3.5 × 10^6 for the short unique sequence could be calculated.

Studies on the molecular biology of varicella-zoster virus (VZV), which is strongly cell associated, have long been hampered by the difficulty of isolating cell-free VZV in sufficient quantities. Ludwig et al. (10) obtained cell-free VZV by sonication of infected cells and estimated the guanine plus cytosine content (46%) of the DNA on the basis of buoyant density in CsCl (1.705 g/cm^3). The isolation of VZV DNA by Hirt extraction of infected cells has been described (12), and values of 92 × 10^6 and 110 × 10^6 have been reported for the molecular weight of the DNA, based on velocity sedimentation in neutral sucrose gradients (8, 12), Recently we described a method to isolate sufficient amounts of VZV by trypsin treatment of infected cultures (5). Isolation of the DNA resulted in a homogeneous preparation, which was infectious. The molecular weight of the VZV DNA was shown to be approximately 80 × 10^6 by electron microscopic measurements (5).

In this paper we describe the structural organization of the VZV genome and present physical maps for the restriction endonucleases *Pst*I, *Xba*I, and *Bgl*II.

MATERIALS AND METHODS

Cells. Human diploid embryonic lung cells were grown in Eagle minimal essential medium with Hanks salts, supplemented with 10% newborn calf serum and antibiotics (100 U of penicillin per ml and 100 μg of streptomycin per ml). For maintenance, Eagle minimal essential medium with Earle salts, 5% newborn calf serum, and antibiotics was used.

VZV. A VZV strain isolated from a patient with chicken pox was used. VZV was plaque purified by transfection of cells with VZV DNA by the calcium dimethyl sulfoxide method (6, 17). All DNA preparations were isolated from passage 6 of infected cells (ratio of infected to uninfected cells, 1:20).

Purification of VZV and DNA. VZV and DNA were isolated as previously described (5). About 1 or 2 μg of DNA was obtained per roller bottle (720 cm^2).

Enzymes and marker DNA. *Xba*I (*Xanthomonas badrii*) and *Bgl*II (*Bacillus globigii*) were isolated as described by Skare and Summers (14) with some modifications. *Pst*I (*Providencia stuartii*) was isolated as described by Crawford and Robbins (4). λ exonuclease was purchased from Biolabs. Bacteriophage λ DNA was isolated from *Escherichia coli* K-12 strain PC2169 as described by Wu et al. (19).

Restriction endonuclease digestion. The following buffers were used for the restriction endonucleases: for *Pst*I, 6 mM Tris-hydrochloride (pH 7.4)-6 mM $MgCl_2$-50 mM NaCl-6 mM β-mercaptoethanol; for *Xba*I, 6 mM Tris-hydrochloride (pH 7.9)-6 mM $MgCl_2$-150 mM NaCl-6 mM β-mercaptoethanol; and for *Bgl*II, 10 mM Tris-hydrochloride (pH 7.4)-6 mM KCl-10 mM $MgCl_2$-1 mM dithiothreitol. Each reaction mixture was incubated for 2 h at 37°C (*Xba*I and *Bgl*II) or 1 h at 30°C (*Pst*I) with sufficient enzyme to

produce a limit digest. The reaction was stopped by the addition of 0.1 volume of 0.1 M sodium-EDTA–50% sucrose–0.2% bromophenol blue (pH 7.6).

Agarose gel electrophoresis. DNA fragments were separated on horizontal 0.6% agarose slab gels. The gels were electrophoresed at room temperature for about 18 h at 1.5 V/cm, using the buffer system described by Aay and Borst (1) containing 1 μg of ethidium bromide per ml. The DNA fragments were visualized by illumination with a UV lamp (Sylvania type F8T$_3$-BLB) and photographed by using a Polaroid MP-3 camera with a Kodak 23 A filter.

Estimation of molecular weights and molarities. The molecular weights of the fragments were estimated in 0.6 or 0.3% agarose slab gels for fragments with molecular weights of less or more than 5×10^6, respectively. λ DNA fragments generated by digestion with the restriction endonuclease *Hin*dIII were used as molecular weight markers (2). Photographs of restriction enzyme patterns were scanned with a Beckman Acta II spectrophotometer equipped with a linear transport. The molar ratios were calculated by dividing the fragments' peak areas by their molecular weights.

λ exonuclease digestion. VZV DNA was incubated with λ exonuclease in 67 mM glycine (pH 9.4)–3 mM $MgCl_2$–3 mM β-mercaptoethanol for 30 min at 37°C. Before subsequent treatment with restriction endonuclease *Pst*I or *Bgl*II, the reaction mixture was incubated at 65°C for 5 min and the buffer adjusted to correspond to the second enzyme. Before treatment with *Xba*I, the DNA was precipitated overnight at −20°C with 2 volumes of ethanol and 0.1 volume of 2 M sodium acetate (pH 5.6) and dissolved in a small volume of *Xba*I buffer.

Blotting. DNA fragments were transferred to nitrocellulose filters essentially as described by Southern (15).

Cross-blot hybridization. To identify homology between different fragments, the Hutchison cross-blot technique was used (18). The DNA was labeled with α-^{32}P-labeled deoxyribonucleoside triphosphates (specific activity, 2,000 to 3,000 Ci/mmol) by nick translation as described by Rigby et al. (13) except that no DNase was added. [^{32}P]DNA was cleaved with the restriction endonuclease and electrophoresed. After electrophoresis the gel was autoradiographed to locate the bands, denatured, and neutralized as described by Southern (15). The radioactive DNA was transferred to a nitrocellulose sheet containing immobilized unlabeled DNA fragments perpendicular to the bands on the gel. Transfer was carried out overnight at 65°C in 5× SSC (1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate, pH 6.35)–0.1% sodium dodecyl sulfate–0.2% Ficoll–0.2% bovine serum albumin–0.2% polyvinylpyrrolidone (hybridization solution) in an oven. All the components of the system, except the gel, were preheated at 65°C. The nitrocellulose filter containing the unlabeled fragments was preincubated at 65°C for 1 h in hybridization solution. After transfer and hybridization, the nitrocellulose filter was washed as described by Jeffreys and Flavell (9).

Double digestion. Double digestion experiments were carried out by cross-blot hybridization of unlabeled, double-digested VZV DNA with ^{32}P-labeled *Xba*I or *Pst*I fragments.

RESULTS

Digestion of VZV DNA with the restriction enzymes *Pst*I, *Xba*I, and *Bgl*II resulted in 18, 22, and 20 fragments, respectively (Fig. 1). After digestion with *Xba*I or *Bgl*II, four 0.5 M fragments were found: *Xba*I-a, one of the comigrating *Xba*I fragments b and c (arbitrarily called *Xba*I-b), *Xba*I-d, and *Xba*I-i or *Bgl*II-c, one of the comigrating *Bgl*II fragments d and e (arbitrarily called *Bgl*II-e), *Bgl*II-g, and *Bgl*II-j. This indicated the existence of subpopulations of DNA molecules differing in the relative orientation of a part of the genome. No submolar bands were identified after *Pst*I digestion.

Based on the molarities and molecular weights of the fragments (Table 1), a molecular weight of 84×10^6 was calculated for the VZV genome, which was in good agreement with the molecular weight of 80×10^6 estimated by electron microscopic measurements (5).

Fragments containing repeats. Cross-blot hybridization of *Xba*I-cleaved ^{32}P-labeled VZV DNA with *Xba*I-cleaved unlabeled DNA showed that the four bands containing submolar fragments hybridized with one another and therefore shared sequences (Fig. 2). These findings indicated the existence of two orientations in the genome.

Figure 3 shows the cross-blot hybridization of *Xba*I-cleaved DNA and *Pst*I-digested [^{32}P]DNA. *Xba*I fragments d and i (both 0.5 M) hybridized only with *Pst*I-a (1 M), whereas *Xba*I fragments a and b (both 0.5 M) hybridized with both *Pst*I-a and *Pst*I-d (Fig. 3). As *Pst*I-a did not hybridize with other *Xba*I fragments, it could be concluded that the two pairs of 0.5 M *Xba*I fragments were adjacent to each other in opposite orientations on the DNA molecule (see Fig. 6). Based on their molecular weights, the only way to map these *Xba*I fragments was d-b and i-a. This was further confirmed by cleavage of *Pst*I-a with *Xba*I. This resulted in four fragments, 1, 2, 3, and 5 (Table 2), of which fragments 2 and 5 were identical with *Xba*I fragments d and i and 1 and 3 were recleavage products of *Xba*I fragments a and b (Fig. 4). Furthermore, the recleavage products of *Pst*I-a hybridized with all four 0.5 M *Xba*I fragments (Fig. 4) and therefore shared sequences.

Cross-blot hybridizations of *Bgl*II fragments with *Pst*I (Table 3)- and *Xba*I (Table 4)-cleaved VZV DNA showed that all of the bands containing 0.5 M *Bgl*II fragments hybridized with both *Pst*I-a and the 0.5 M *Xba*I fragments. The 0.5 M *Bgl*II fragments contained, therefore, the same redundant sequences as the 0.5 M *Xba*I fragments and *Pst*I-a. Based on their molecular weights, the only way to map these *Bgl*II fragments was e-g and j-c (see Fig. 6).

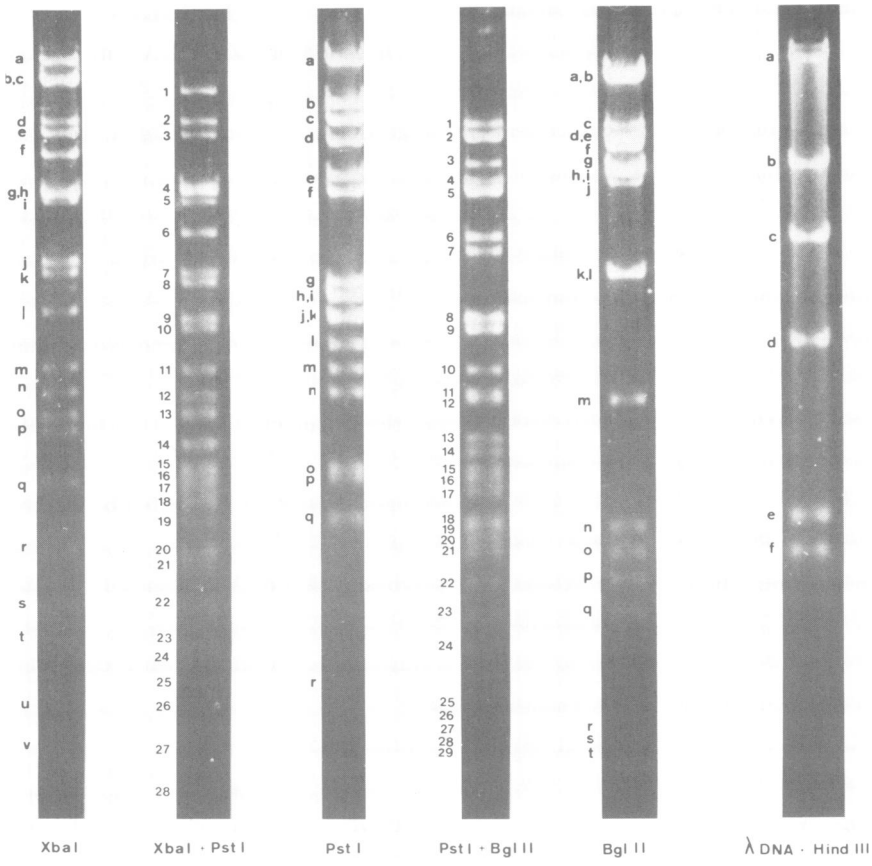

FIG. 1. *Restriction endonuclease profiles of VZV DNA. VZV DNA was cleaved with the restriction endonucleases shown and separated by electrophoresis for 18 h at 30 V on 0.6% agarose gels. HindIII fragments of λ DNA were coelectrophoresed as molecular weight markers.*

As *Xba*I and *Bgl*II digestions resulted in submolar fragments, there were no cleavage sites in the redundant sequences, and therefore a maximal molecular weight of 5×10^6 and a minimal molecular weight of 3.5×10^6 could be calculated for the repeated region and the short unique sequence, respectively. These molecular weights were in agreement with those estimated by electron microscopy for these parts of the genome (P. Sheldrick, A. M. Dumas and N. Berthelot, unpublished data), 3.6×10^6 for the unique sequence and 4.5×10^6 for the repeated region (terminally and internally inverted).

Cross-blot hybridization of *Pst*I-cleaved ^{32}P-labeled VZV DNA with unlabeled *Pst*I-cleaved DNA showed weak hybridization of *Pst*I-a with *Pst*I-m (data not shown). Also, some hybridization of *Xba*I-m (located at the same position on the genome as *Pst*I-m [see below]) with the comigrating *Xba*I fragments b and c and *Xba*I-d was found. These fragments therefore may have contained a common sequence.

Terminal fragments. To identify the terminal fragments, VZV DNA was incubated with λ exonuclease, and this was followed by cleavage with restriction endonucleases and agarose gel electrophoresis (Fig. 5). In the *Pst*I pattern, fragment m disappeared and band a decreased in intensity. Since these were the only fragments affected by λ exonuclease, fragment m must have been located at one site on the genome and fragment a must have been located at the other site.

In the *Xba*I pattern, fragments i and m disappeared and band d decreased in intensity. Cross-blot hybridization of the *Xba*I-cleaved VZV DNA and *Pst*I-digested DNA showed that *Pst*I-m only hybridized with *Xba*I-m (Fig. 3). Therefore, *Xba*I-m mapped at the same terminus of the genome as *Pst*I-m. The 0.5 M *Xba*I fragments i and d hybridized with *Pst*I-a and were therefore located at the same end of the DNA molecule as *Pst*I-a. These results were in agreement with double digestion data, which

TABLE 1. *Molecular weights and molar ratio values of fragments generated by cleavage of VZV DNA with XbaI, PstI, and BglII*

Endonuclease	Fragment	Mol wt (10^6)	Molar ratio	
*Xba*I	a	14.5	0.5	(0.45)[a]
	b,c	11.0	1.5	(1.44)
	d	8.5	0.5	(0.48)
	e	7.8	1	(1.00)
	f	6.8	1	(1.03)
	g,h	5.4	2	(2.67)
	i	5.1	0.5	
	j	3.9	1	(1.09)
	k	3.8	1	(0.88)
	l	3.2	1	(0.99)
	m	2.7	1	(1.06)
	n	2.5	1	(1.01)
	o	2.3	1	(0.95)
	p	2.2	1	(0.95)
	q	1.8	1	(1.08)
	r	1.5	1	(1.12)
	s	1.2	1	(1.08)
	t	1.0	1	(0.95)
	u	0.6	1	(ND[b])
	v	0.5	1	(ND)
*Pst*I	a	14.8	1	(1.18)
	b	10.4	1	(0.98)
	c	8.7	1	(1.00)
	d	7.4	1	(1.16)
	e	5.9	1	(1.04)
	f	5.4	1	(1.21)
	g	3.6	1	(1.09)
	h,i	3.4	2	(2.15)
	j,k	3.2	2	(2.01)
	l	2.9	1	(1.19)
	m	2.7	1	(0.94)
	n	2.5	1	(1.10)
	o	1.9	1	(0.92)
	p	1.8	1	(0.87)
	q	1.6	1	(0.89)
	r	0.7	1	(ND)
*Bgl*II	a,b	12.7	2	(2.12)
	c	8.2	0.5	(0.46)
	d,e	7.8	1.5	(1.43)
	f	7.2	1	(0.97)
	g	6.3	0.5	(2.57)
	h,i	6.2	2	
	j	5.9	0.5	(0.53)
	k,l	3.8	2	(2.18)
	m	2.5	1	(1.16)
	n	1.6	1	(0.96)
	o	1.4	1	(0.91)
	p	1.3	1	(0.86)
	q	1.1	1	(0.91)
	r	0.6	1	(ND)
	s	0.5	1	(ND)
	t	0.4	1	(ND)

[a] Mean values of three independent estimations.

[b] ND, Not determined (comigration with the bromophenol blue marker). Molarities were derived from their localizations in heterologous molar fragments.

showed that *Xba*I fragments i and d were not cleaved by *Pst*I and were recleavage products of *Pst*I-a (Table 2 and Fig. 4).

In the *Bgl*II pattern it was found that fragments q and j disappeared and the intensity of bands c and d,e (1.5 M) decreased. *Bgl*II-q hybridized with *Xba*I-m and *Pst*I-m (Tables 3 and 4) and was therefore located at the same end of the genome. *Bgl*II fragments c and j and one fragment of *Bgl*II fragments d and e hybridized with *Pst*I-a (Table 3) and were therefore located at the same site on the genome as *Pst*I-a. Although the intensity of the *Bgl*II-c band was decreased upon exonuclease incubation, it was not a terminal fragment, but was located adjacent to the terminal fragment *Bgl*II-j (see above).

Alignment of fragments. The fragments were aligned based on cross-hybridizations of single- and double-digested VZV DNA (Fig. 1, 3, and 4) and on the molecular weights of the fragments (Tables 1 and 5). The mapping was started with the terminal fragments containing repeats. The physical maps for *Xba*I, *Pst*I, and *Bgl*II fragments are shown in Fig. 6.

*Xba*I fragments a and b hybridized with *Pst*I fragments a and d (Fig. 3). Therefore, *Pst*I-d was adjacent to *Pst*I-a. *Pst*I-d hybridized also with *Xba*I fragments q and r (Fig. 3). Of these fragments only *Xba*I-q had a cleavage site for *Pst*I (Fig. 4), and *Xba*I-r was therefore situated within *Pst*I-d, adjacent to *Xba*I fragments a and b.

*Pst*I-d hybridized with one of the comigrating *Bgl*II fragments g, h, and i (Table 3). As the 0.5 M fragment *Bgl*II-g mapped entirely within *Pst*I-a, this hybridization must have been with one of the *Bgl*II fragments h and i (arbitrarily called *Bgl*II-h). *Bgl*II-h must have been adjacent to *Bgl*II fragment g and c and must have spanned the junction between *Pst*I fragments a and d. *Pst*I-d hybridized also with *Bgl*II fragments m, r, and t (Table 3), fragments that also hybridized with *Xba*I-r (Table 4). As *Bgl*II-m hybridized with both *Pst*I-h and *Pst*I-d (Table 3), *Bgl*II fragments r and t were located within *Pst*I-d and *Xba*I-r.

*Xba*I-q hybridized with *Pst*I fragments d and h (comigrating with *Pst*I-i) (Fig. 3), and therefore *Pst*I-h was adjacent to *Pst*I-d. Cleavage of the comigrating *Pst*I fragments h and i with *Xba*I yielded products with molecular weights of 2.9×10^6, 1.8×10^6, 1.6×10^6, and 0.5×10^6 (fragments 10, 15, 18, and 27, respectively) (Table 2). The only way to construct fragments with molecular weights of 3.2×10^6 from these digestion products was by combining fragments 15 and 18 or fragments 10 and 27. *Pst*I-h shared a

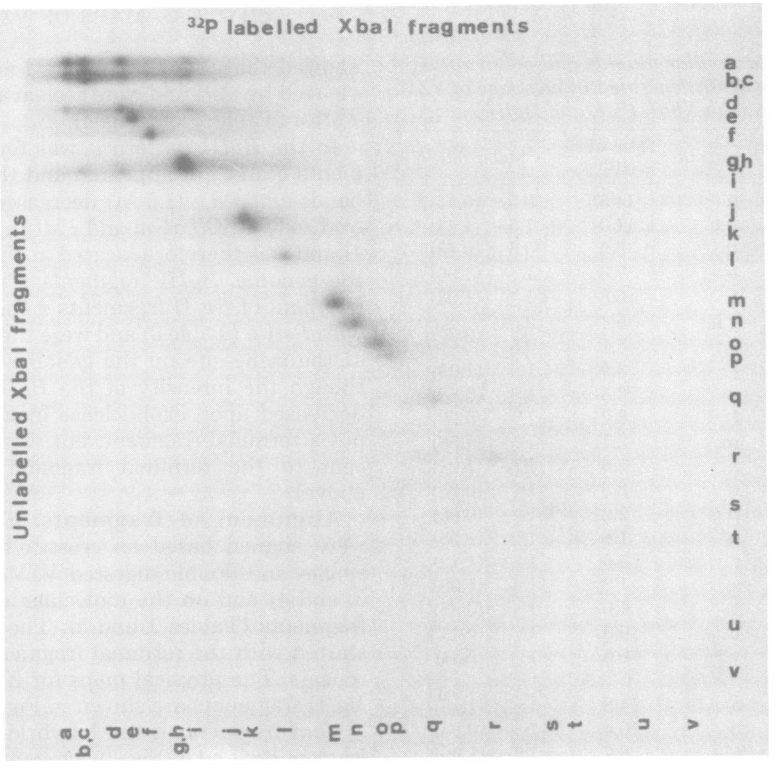

FIG. 2. *Cross-blot hybridization of* ^{32}*P-labeled XbaI fragments with unlabeled XbaI fragments.*

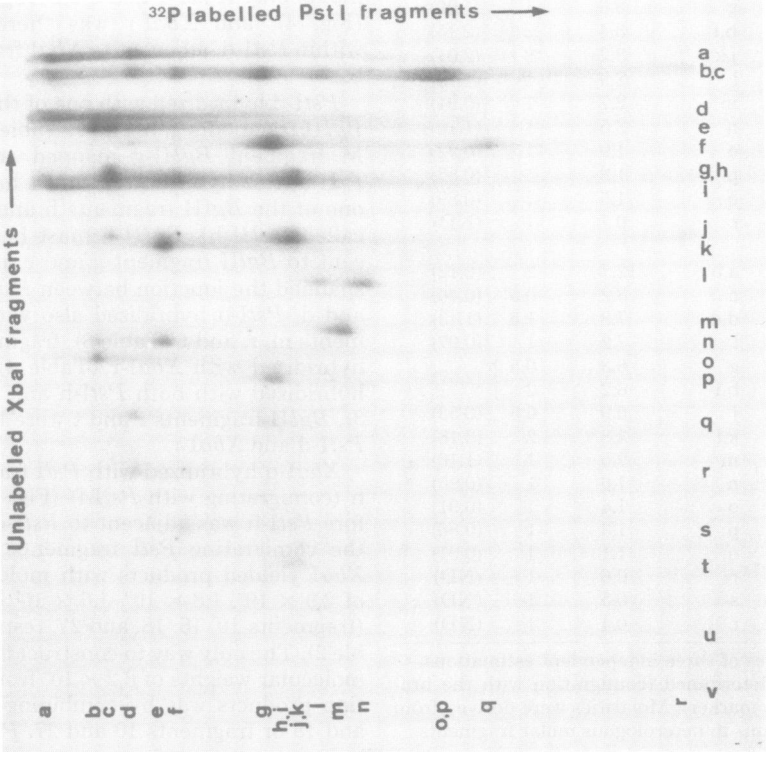

FIG. 3. *Cross-blot hybridization of* ^{32}*P-labeled PstI fragments with unlabeled XbaI fragments. A dark spot indicates homology between the fragments. Hybridization of PstI-b with XbaI fragments u and v was detected only after a longer exposure time.*

TABLE 2. *Hybridization of ^{32}P-labeled PstI fragments and unlabeled fragments generated by cleavage of VZV DNA with both PstI and XbaI*

PstI-XbaI fragment	Homology between *PstI-XbaI* fragment and *PstI* fragment:																	
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
1	+																	
2	+																	
3	+																	
4		+	+															
5	+																	
6				+														
7					+													
8							⊕[a]											
9										⊕								
10								+										
11													⊕					
12						+												
13		+									+							
14			+											+				
15									+						⊕			
16						+										⊕		
17					+													
18									+			+						
19																	⊕	
20				+														
21												+						
22						+												
23			+								+							
24		+																
25																		⊕
26		+																
27		+			+			+										
28														+				

[a] ⊕, *PstI* fragment not cleaved by *XbaI*.

sequence of molecular weight 0.5×10^6 with *XbaI*-q (data not shown) and one of molecular weight 2.9×10^6 with *XbaI*-f (Fig. 4 and Table 2). Thus, *XbaI*-q spanned the junction between *PstI* fragments d and h and *XbaI*-f was adjacent to *XbaI*-q.

BglII-m hybridized with *PstI* fragments d and h (Table 3) and therefore linked these two fragments. *PstI*-h hybridized with the comigrating *BglII* fragments g, h, and i (Table 3). As *BglII* fragments g and h already had been located, it was *BglII*-i which was adjacent to *BglII*-m.

XbaI-f and *BglII*-i hybridized only with each other (Table 4), and both reacted with *PstI*-q and *PstI* fragments h and i (Table 3 and Fig. 3). *PstI*-q had no cleavage site for *XbaI* (Table 2) and therefore mapped entirely within *XbaI*-f and was adjacent to *PstI*-h. Furthermore, *XbaI*-f shared a sequence of molecular weight 1.8×10^6 (*PstI-XbaI*-15) with *PstI*-i (Table 2, Fig. 4). As *PstI*-i, which therefore mapped adjacent to *PstI*-q, hybridized with *XbaI*-p (Fig. 3), *PstI*-i spanned the junction between *XbaI* fragments f and p. *PstI*-i hybridized also with *BglII* fragments i and f (Table 3) and thus linked these two fragments.

XbaI-p hybridized with *PstI*-i and hybridized weakly with *PstI*-n (Fig. 3), with which it had a sequence with a molecular weight of 0.4×10^6 in common (*PstI-XbaI*-28) (Table 2). *PstI*-n, which therefore was adjacent to *PstI*-i, shared the other recleavage product, with a molecular weight of 2.1×10^6 (*PstI-XbaI*-14 [Table 5]), with *XbaI*-l (Table 2, Fig. 4), which hybridized also with *PstI*-l (Fig. 3). Thus, *XbaI*-l was adjacent to *XbaI*-p and spanned the junction between *PstI* fragments l and n. Based on the hybridization of *PstI*-l with *XbaI*-c (Fig. 3), this fragment must have been adjacent to *XbaI*-l.

XbaI-c, comigrating with *XbaI*-b, hybridized with *PstI* fragments l, o, p, g, and f (Fig. 3). Of these fragments, only *PstI* fragments l and f were cleaved by *XbaI* (Table 2). Thus, *PstI* fragments o, p, and g mapped entirely within *XbaI*-c. *PstI* fragments o, p, and g hybridized with *BglII*-d (Table 3), which therefore was adjacent to *BglII*-f. As *PstI* fragments o and p had no cleavage site for *BglII* (data not shown), these fragments were located within *BglII*-d. However, we were unable to determine their exact arrangement.

XbaI-c hybridized with *BglII* fragments f and

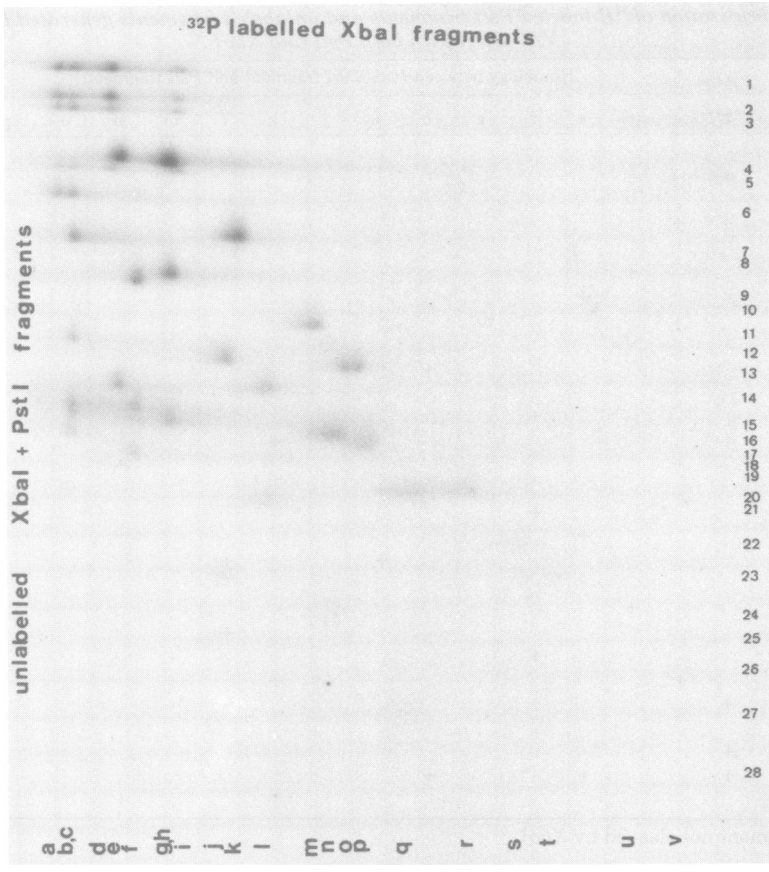

FIG. 4. *Cross-blot hybridization of ^{32}P-labeled XbaI fragments with unlabeled fragments generated by cleavage of VZV DNA with both PstI and XbaI. A dark spot indicates homology between the fragments. Hybridizations of XbaI-PstI fragments 26 (with XbaI-u), 27 (with XbaI fragments g, q, and v), and 28 (with XbaI-p) were detected only after a longer exposure time.*

d and one of the comigrating *Bgl*II fragments a and b (arbitrarily called *Bgl*II-a) (Table 4). *Pst*I-g, which mapped entirely within *Xba*I-c, was cleaved by *Bgl*II (Fig. 1). *Xba*I-c and *Pst*I-g therefore spanned the junction between *Bgl*II fragments d and a.

*Pst*I-f hybridized with *Xba*I-c, *Bgl*II-a (already located), *Xba*I-s, and one of the comigrating *Xba*I fragments g and h (Fig. 3). *Xba*I-s had no cleavage site for *Pst*I (Fig. 4) and therefore mapped entirely within *Pst*I-f. Incubation of the 2 M fragment *Xba*I-g,h by *Pst*I resulted in one uncleaved fragment (arbitrarily called *Xba*I-h) and one fragment (called *Xba*I-g) cleaved into three products, with molecular weights of 3.2×10^6 and 1.8×10^6 (Fig. 4) and 0.5×10^6 (data not shown). The fragment with a molecular weight of 1.8×10^6 (*Pst*I-*Xba*I-16) was shared with *Pst*I-f, and the fragment with a molecular weight of 3.2×10^6 was *Pst*I-j (comigrating with *Pst*I-k)

TABLE 3. *Hybridization of ^{32}P-labeled PstI fragments and unlabeled BglII fragments*

*Bgl*II fragment(s)	Homology between *Bgl*II fragment(s) and *Pst*I fragment(s):														
	a	b	c	d	e	f	g	h, i	j, k	l	m	n	o	p	q	r
a, b		+	+		+	+			+							
c	+															
d, e	+						+						+	+		
f								+		+		+				
g, h, i	+			+				+							+	
j	+															
k, l		+			+				+							+
m				+				+								
n											+					
o		+														
p			+													+
q											+					
r				+												
s					+											
t				+												

TABLE 4. *Hybridization of ^{32}P-labeled XbaI fragments and unlabeled BglII fragments*

*Bgl*II fragment(s)	Homology between *Bgl*II fragment(s) and *Xba*I fragment(s):																		
	a	b, c	d	e	f	g, h	i	j	k	l	m	n	o	p	q	r	s	t	u	v
a, b		+		+		+			+								+			
c	+	+	+				+													
d, e	+	+	+				+													
f										+				+						
g, h, i	+	+	+		+		+													
j	+	+	+				+													
k, l								+				+	+					+	+	+
m															+	+				
n											+									
o													+							
p								+												
q											+									
r																+				
s												+								
t																+				

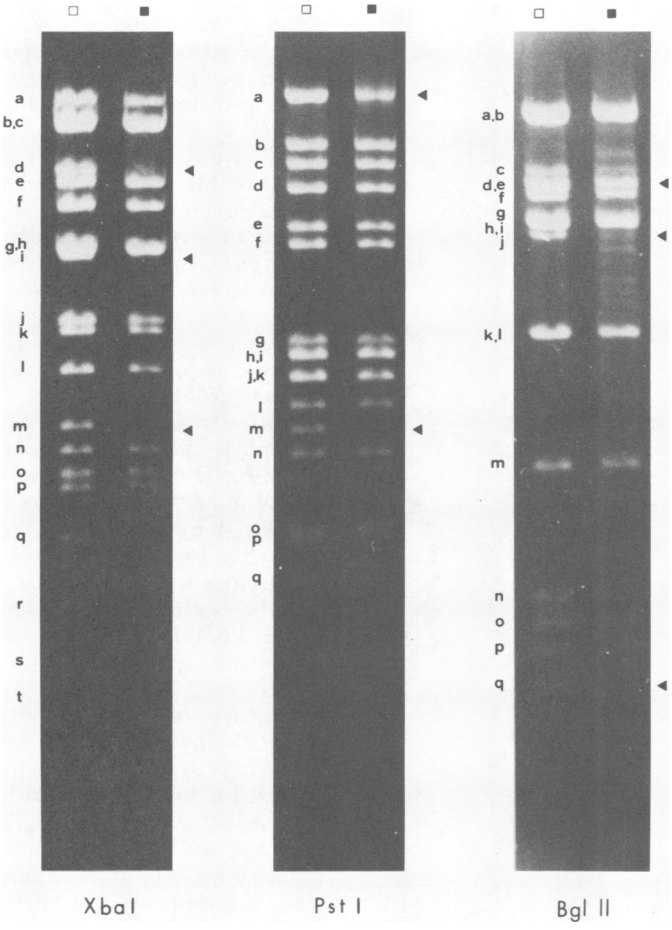

FIG. 5. *Identification of the terminal fragments of VZV DNA. VZV DNA was digested by λ exonuclease, and this was followed by cleavage with the restriction endonucleases PstI, XbaI, and BglII and analysis by electrophoresis on 0.6% agarose gels. Symbols: □, DNA not incubated with exonuclease; ■, DNA incubated with exonuclease; ◀, bands affected by exonuclease.*

(Table 2). *Pst*I-j was not cleaved by *Xba*I and was therefore located within *Xba*I-g, next to *Pst*I-f. The remaining sequence of *Xba*I-g (molecular weight, 0.5×10^6) must have been a recleavage product of a *Pst*I fragment, as the smallest *Pst*I fragment of VZV DNA had a molecular weight of 0.7×10^6. *Pst*I fragments b, e, and h had a recleavage product with a molecular weight of 0.5×10^6 (*Pst*I-*Xba*I-27, Table 2). *Pst*I-h had already been mapped, whereas *Pst*I-b hybridized with *Xba*I-v (molecular weight, 0.5×10^6). Thus, the remaining sequence of *Xba*I-g was shared with *Pst*I-e, which therefore was adjacent to *Pst*I-j.

TABLE 5. *Molecular weights of PstI-XbaI and PstI-BglII double digest products*

Endonucleases	Fragment	Mol wt (10^6)
*Pst*I-*Xba*I	1	10.2
	2	8.2
	3	7.6
	4	5.4
	5	5.1
	6	4.3
	7	3.7
	8	3.6
	9	3.1
	10	2.9
	11	2.7
	12	2.5
	13	2.3
	14	2.1
	15	1.8
	16	1.8
	17	1.7
	18	1.6
	19	1.6
	20	1.5
	21	1.3
	22	1.2
	23	1.0
	24	0.9
	25	0.7
	26	0.6
	27	0.5
	28	0.4
*Pst*I-*Bgl*II	1	8.2
	2	7.7
	3	6.5
	4	5.9
	5	5.4
	6	4.3
	7	4.0
	8	3.1
	9	2.9
	10	2.7
	11	2.5
	12	2.5
	13	2.1
	14	2.1
	15	1.9
	16	1.8
	17	1.7
	18	1.6
	19	1.5
	20	1.4
	21	1.4
	22	1.3
	23	1.1
	24	1.0
	25	0.6
	26	0.6
	27	0.5
	28	0.5
	29	0.4

*Pst*I-e hybridized with *Xba*I fragments k and n (Fig. 3). *Xba*I-k was not cleaved by *Pst*I (Fig. 4) and was therefore located completely within *Pst*I-e, adjacent to *Xba*I-n (cleaved by *Pst*I [Fig. 4]). As *Xba*I-n hybridized also with *Pst*I-b (Fig. 3), *Pst*I-b must have been adjacent to *Pst*I-e.

*Pst*I-e hybridized with *Bgl*II fragments a and s and one of the comigrating *Bgl*II fragments k and l (arbitrarily called k) (Table 3). Based on the hybridization of *Bgl*II-s with both *Xba*I-k and *Xba*I-n (Table 4), *Bgl*II-s spanned the junction between *Xba*I fragments k and n and mapped completely within *Pst*I-e and next to *Bgl*II-k.

*Pst*I-b hybridized with *Xba*I fragments e, n, and o (Fig. 3) and u and v (data not shown). *Xba*I fragments o, u, and v had no cleavage site for *Pst*I and were therefore located within *Pst*I-b. *Xba*I-o hybridized with *Bgl*II fragments k and o, whereas *Xba*I fragments u and v hybridized only with *Bgl*II-k (Table 4). Therefore, *Bgl*II-o was adjacent to *Bgl*II-k, and *Xba*I fragments u and v mapped entirely within *Bgl*II-k and between *Xba*I fragments n and o. Furthermore, *Xba*I-e was adjacent to *Xba*I-o. As *Xba*I fragments u and v had no cleavage site for both *Pst*I and *Bgl*II (data not shown), we were unable to determine their exact arrangement.

*Pst*I-b hybridized with *Bgl*II fragments k and o (already located) and b (Table 3). *Bgl*II-b therefore was adjacent to *Bgl*II-o. *Bgl*II-b hybridized with *Pst*I fragments b and c (Table 3) and *Xba*I fragments e and h (Table 4) and therefore spanned the junction between these fragments. *Xba*I-h was not cleaved by *Pst*I and was therefore situated completely within *Pst*I-c.

*Pst*I-c hybridized with *Bgl*II fragments p and b (Table 3) and *Xba*I fragments h and j (Fig. 3). *Xba*I-j hybridized also with *Pst*I-r (not cleaved by *Xba*I), *Pst*I-k, and *Bgl*II fragments p and l (Fig. 3 and Table 4). Thus, *Bgl*II-p had to be placed between *Bgl*II fragments b and l, *Xba*I-j was adjacent to *Xba*I-h, *Pst*I-r mapped entirely within *Xba*I-j, and *Pst*I-k was adjacent to *Pst*I-r. *Pst*I-r hybridized with *Bgl*II fragments l and p (Table 3) and therefore spanned the junction between these two fragments. *Pst*I-k hybridized with *Xba*I fragments j and t (Fig. 3). Recleavage of *Pst*I-k by *Xba*I resulted in two products: one

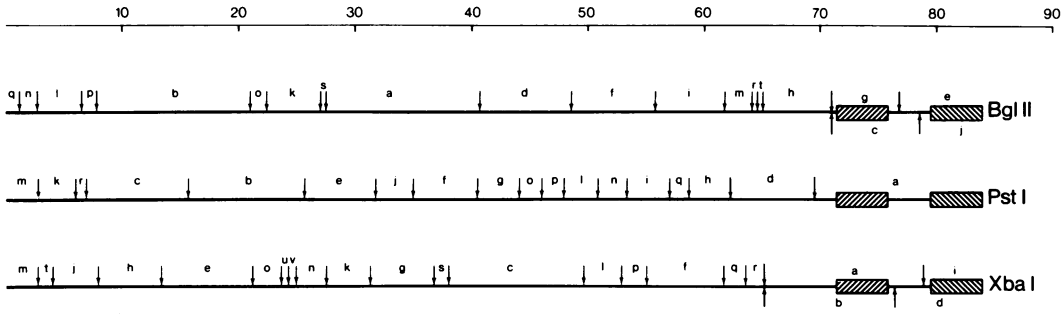

FIG. 6. *Physical maps for the PstI, XbaI, and BglII fragments of VZV DNA.*

fragment with a molecular weight of 2.3 × 10^6 (*Pst*I-*Xba*I-13) shared with *Xba*I-j and a sequence with a molecular weight of 1.0 × 10^6 which was *Xba*I-t (not cleaved by *Pst*I) (Fig. 4 and Table 2). Furthermore, *Pst*I-k was not cleaved by *Bgl*II (data not shown) and hybridized with *Bgl*II-l (Table 3). From these data it could be concluded that in this region of the genome, *Pst*I, *Xba*I, and *Bgl*II cleavage sites virtually coincided.

*Xba*I-m and *Pst*I-m, both terminal fragments, hybridized only with each other (Fig. 3). No cleavage site was detected for the other enzyme by double digestion (Table 2 and Fig. 4). Apparently, the cleavage sites for *Pst*I and *Xba*I virtually coincided, which was in agreement with the molecular weights of these fragments (Table 1). Therefore, *Xba*I-m and *Pst*I-m must have been adjacent to *Xba*I-t and *Pst*I-k, respectively, as this was the only area of the genome, where the *Xba*I and *Pst*I cleavage sites almost coincided.

*Bgl*II fragments n and q hybridized only with *Xba*I-m (Table 4) and *Pst*I-m (Table 3). As *Bgl*II-q was a terminal fragment, *Bgl*II-n had to be placed between *Bgl*II fragments l and q.

DISCUSSION

Cleavage of VZV DNA with the restriction enzymes *Pst*I, *Xba*I and *Bgl*II resulted in 18, 22, and 20 fragments, respectively. In the *Xba*I and *Bgl*II patterns, four 0.5 M fragments could be identified. The restriction enzyme patterns were very similar in different DNA preparations (previously mentioned by Oakes et al. [11]) and did not change after plaque purification (A. M. Dumas, unpublished data).

The presence of four 0.5 M fragments in both the *Bgl*II and the *Xba*I cleavage patterns indicated the existence of DNA molecules differing in the relative orientation of a short unique sequence which was delimited by redundant sequences. Examination by cross-blot hybridization showed that in both the *Xba*I and the *Bgl*II patterns, the two 0.5 M terminal fragments contained a sequence which was repeated internally in the two other 0.5 M fragments. Examination of self-annealed single-stranded VZV DNA by electron microscopy has shown a single-stranded loop (unique sequence) with a molecular weight of approximately 1.8 × 10^6 (3.6 × 10^6 for a double-stranded equivalence) contiguous to a double-stranded region with a molecular weight of approximately 4.5 × 10^6 (terminal and inverted internal repeat) (Sheldrick et al., unpublished data). This is in good agreement with the maximal molecular weight of 5 × 10^6 for the repeated region and the minimal molecular weight of 3.5 × 10^6 for the unique sequence, which could be calculated from the molecular weights of the submolar fragments. As the *Pst*I endonuclease had no cleavage site in the unique sequence, no submolar bands were found in this cleavage pattern. Based on these findings it can be concluded that the VZV genome shares important properties with the genomes of other herpesviruses (7) and most closely resembles those of pseudorabies virus (3, 16) and equine abortion virus (P. Sheldrick and N. Berthelot, personal communication).

The terminal fragments *Pst*I-m and *Xba*I-m weakly hybridized with *Pst*I-a and *Xba*I fragments b, c, and d respectively (data not shown). Therefore, the left end (as shown in Fig. 6) of the VZV genome may possess limited sequence homology to both arms of the inverted repeat. It is not known to what extent, if at all, VZV DNA is terminally repetitive.

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