JOURNAL OF VIROLOGY, May 1984, p. 615–618
0022-538X/84/050615-04$02.00/0

Vol. 50, No. 2

NOTES

Physical Maps of Varicella-Zoster Virus DNA Derived with 11 Restriction Enzymes

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Received 11 August 1983/Accepted 10 January 1984

Varicella-zoster virus DNA was digested with 11 restriction endonucleases, and the resulting fragments were separated on agarose gels. Terminal fragments were identified by lambda exonuclease digestion. Physical maps were then constructed using a combination of double restriction enzyme digestion and hybridization to cloned *Bam*HI fragments to place the remaining fragments in order.

The genome of varicella-zoster virus (VZV) consists of two covalently linked, unique sequences, a long unique sequence (U_L) and a short unique sequence (U_S). The terminus of U_S is repeated internally in an inverted form and separates U_L and U_S. The short DNA segment inverts with respect to the long, resulting in two isomeric forms of the VZV genome (5, 9–11, 19, 21). The molecular weight of VZV DNA has been estimated to be 80×10^6 to 86×10^6 (8–10, 19, 21). In this paper, we present the physical maps of VZV DNA (EF strain) derived with 11 restriction endonucleases: *Bam*HI, *Bgl*I, *Bgl*II, *Eco*RI, *Hpa*I, *Hin*dIII, *Pst*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I.

The EF strain of VZV was originally isolated from a patient with herpes zoster. The virus was propagated in Flow 5000 human embryo fibroblasts (Flow Laboratories, Inc., Rockville, Md.) as previously described (14). Viral DNA was purified by the methods of Straus et al. (19). The DNA was radiolabeled in vivo with carrier-free $^{32}P_i$ or in vitro with [^{32}P]dCTP (New England Nuclear Corp., Boston, Mass.) by nick translation (16). Specific activities were 0.5×10^5 to 1.6×10^5 cpm/μg and 0.5×10^8 to 1.5×10^8 cpm/μg, respectively.

Restriction endonucleases were obtained from Bethesda Research Laboratories, Gaithersburg, Md., or New England Biolabs, Beverly, Mass., and used according to the specifications of the supplier. Bacteriophage lambda DNA was digested with *Hin*dIII or *Eco*RI for use as a molecular weight marker. The samples were electrophoresed (16 to 17 h at 50 V) through 0.5 or 0.8% agarose (low electroendosmosis; Sigma Chemical Co., St. Louis, Mo.) with a Tris-borate buffer system in a Bethesda Research Laboratories H1 horizontal-gel apparatus. Fragments in unlabeled gels were stained with ethidium bromide and visualized with 254-nm UV transillumination. The ^{32}P-labeled gels were dried and autoradiographed with Kodak X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.).

The digestion fragments were labeled alphabetically in order of decreasing molecular weight. The molecular weight was determined by the mobility of lambda DNA fragments run as standards on the same gels. Fragment molarity was estimated by scanning the autoradiographs of ^{32}P-labeled VZV DNA gels with a Zenith soft-laser densitometer (Biomed Instruments, Inc., Chicago, Ill.) and calculating the area under each peak. The molecular weight of intact strain EF VZV DNA used in the molarity calculations was 86×10^6, as determined by electron microscopy (unpublished observations). Photographic negatives of ethidium bromide-stained gels were analyzed in similar fashion for comparison. Restriction enzymes *Bam*HI, *Bgl*I, *Hpa*I, *Hin*dIII, *Pst*I, *Sal*I, and *Xho*I produced only one- or two-molar fragments. *Sma*I produced molar or multimolar fragments. Restriction enzymes *Bgl*II, *Eco*RI, and *Xba*I all produced half- as well as one-molar fragments.

*Bam*HI VZV DNA fragments were cloned in plasmid vector pBR322 by standard techniques (3, 4, 7). Of the 26 detectable *Bam*HI fragments of VZV DNA, 21 were cloned in this manner. Each cloned viral DNA fragment comigrated with the identical fragment from a *Bam*HI digest of whole viral DNA. The cloned fragments were ^{32}P-labeled by nick translation and shown to be homologous to the identical whole VZV digest fragment by Southern blot hybridization (Table 1). Three of the cloned *Bam*HI fragments, B, K, and R, also hybridized to other viral DNA fragments, suggesting regions of shared homology.

Terminal fragments of VZV DNA were identified by digestion of whole VZV DNA with lambda exonuclease (New England Biolabs) before restriction endonuclease digestion. VZV DNA was digested with lambda exonuclease for 1 to 20 min at 30°C (12). The reaction was stopped by heating at 65°C for 5 min and followed with the appropriate restriction enzyme digestion. Lambda exonuclease was omitted from the control reactions. The terminal fragments of VZV DNA were the first to disappear under these conditions. As expected, some of the terminal fragments proved to be half molar.

Fragment linkage and the physical maps of the VZV genome were determined by a two-step process. First, Southern blots (17) were hybridized to radiolabeled cloned *Bam*HI VZV DNA fragments. Second, to confirm the hybridization results, the cloned *Bam*HI fragments were digested with a second restriction enzyme. The dried blots were preincubated in Denhardt solution (6) and hybridized to 0.3×10^6 to 1×10^6 cpm of a ^{32}P-labeled cloned *Bam*HI VZV DNA fragment in a buffer containing modified Denhardt solution in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–50% formamide–200 μg of yeast RNA per ml for 40 to 50 h at 45°C. *Bam*HI fragment A was eluted from an agarose gel (23) instead of being cloned as were the other

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TABLE 1. Hybridization of ^{32}P-labeled cloned *Bam*HI restriction fragments of VZV DNA to the fragments generated by cleavage of VZV DNA with *Bam*HI, *Bgl*I, *Hin*dIII, *Hpa*I, *Sal*I, *Xba*I, *Eco*RI, *Bgl*II, *Pst*I, *Xho*I, and *Sma*I

^{32}P-labeled *Bam*HI fragments	Restriction fragment(s) hybridizing with probe DNA[a]										
	*Bam*HI	*Bgl*I	*Hin*dIII	*Hpa*I	*Sal*I	*Xba*I	*Eco*RI	*Bgl*II	*Pst*I	*Xho*I	*Sma*I
A[b]	A	F/GLPRW	EGJSFND	GIK/L/MUVcef	AFGHO	ABEFG/HS	A/BG/H	A/B	EI	C/D/EJ/KM	A/BF
B	BS	AHL	HIGJKNPFO	A/BH	BE/FGJLMRSTU	ACDFI	A/B/CE/FIJ	C-J	AD	F/GKN/O	A/B[c]
C	C	CF/GM	A	C	C	EO	G/HN	A/BO	B	A/B	CS/T
D	D	A	FG	A/B	B	AB/CFQR	IK	G/HIMR	DH/I	EJ/K	A/BJ/K/L/MRU/Vb/c
E	E	D	KLM	FU/VZ/a	IK	JMT	C	K/LNP	J/KMR	A/B	N/OPQWYZ
F	F	C	A	D/EN/O	DO/P	EG/H	MPQ	A/B	C	A/B	GJ/K/L/MZb
G	G	A	FNQR	A/B	AB	F	KL	G/H/I	H/IQ	C/D/EI	J/K/L/M
H	H	B	BC/D	R/S	A	AB	BD	C/D/E/F	GP	A/B	DH/I
I	I	EJ	AE	N/OU/V	CF	K/LN	NO	A/BK/L	BE	A/BC/D	H/IS/T
J	J	KT/U	C/DNO	A/BHJR/S	QRSU	ACDI	AE/FJK	C/D/E/FG/H/IJ	A	C/DL	U/VW/XY/Zab/cd/e
K	KR	NO/PV	C/D	J/K[c]	JN	ABCDI	AE/FIJK	C/D/E/FG/H/IJ	A	C/D	EH/I
L	L	AI	B	K/L/M	A	K/LP		C/D/E/F	H/IN	A/B	N/OX/Y
M	M	H	B	V/WZ/a	A	BK/L	D	C/D/E/F	L	A/B	J/K/L/MU/V
N	N	E	A	OP/Q	C	OUV	N	K/LO	B	A/B	S/T
O	O	HI	B	Y/Z	A	K/L	D	C/D/E/F	LN		J/K/L/MW/X/Y/Z
P	P	BKNV	BC/D	JP/QR/Sa	AJN	ABDI	DJK	C/D/E/FJ	ALO	A/BF	H/I
Q	Q	T/U	K	K/L/MF/GH	O/P	G/HJ	C	A/BP	C	A/B	N/Ob/c
R	RK	NO/PV	C/D	JR/S	JN	ADCI	AE/FIJK	C/D/E/FG/H/IJ	A	C/D	E
U	U	A	BFN	A/B	A	FP	L		H/I		J/K/L/MN/Od/e
V	V	C	AK	D/E	O/P	G/H	C		C		J/K/L/M
W	W	B	C/D	I	A	B	BM				D
Z	Z	SH	IL	DY/Z	KMR	MBD	C				P

[a] Slash indicates that the precise fragment hybridizing is indeterminate.
[b] *Bam*HI fragment A DNA eluted from gel.
[c] Unidentified small fragment(s) also hybridized.

*Bam*HI fragments. It was then radiolabeled in vitro and used for hybridization. The blots were then washed in 2× SSC at 20°C heated at 65°C for 2 h in 6× SCC with 0.1% sodium dodecyl sulfate, washed with 2× SSC at 20°C again, air dried, and autoradiographed with a Du Pont Cronex intensifying screen (Du Pont Co., Wilmington, Del.) (22). Finally, the blots were rehybridized to ^{32}P-labeled whole VZV DNA to identify the fragments hybridizing to the cloned DNA probes. The results are shown in Table 1.

Physical maps of the VZV genome were derived from the hybridization results (Table 1) and the double digestion of the cloned *Bam*HI fragments of VZV DNA (data not shown). The terminal fragments served as a starting point, and adjacent fragments were determined by homology (linkage data) and size (double digestion). The maps are shown in Fig. 1. As expected from the model of the VZV genome (5, 9–11, 19, 21), the terminal fragment from U_S was homologous to the U_L-U_S junction fragment. *Bam*HI-B, the junction fragment, hybridized to itself and to *Bam*HI-S, a terminal fragment. Also, as expected, the junction fragment *Bam*HI-B hybridized to all four half-molar fragments produced by other restriction enzymes (e.g., *Xba*I fragments A, C, D, and I and *Eco*RI fragments A, E, F, and J). Some fragments (e.g., *Bam*HI-J) appeared twice, again as expected from their molarity and the inverted repeats characteristic of the VZV genome. The order of fragments mapping entirely within a *Bam*HI fragment (e.g., *Sal*I-H and *Sal*I-O or *Sal*I-Q, *Sal*I-S, and *Sal*I-U) could not be determined.

In some cases, the hybridization data appear to be partially inconsistent with the maps. Hybridization to fragments which clearly mapped elsewhere were considered to be spurious or anomalous. *Bam*HI fragments A, B, P, and Z consistently produced these anomalous results. *Bam*HI-A probably was contaminated with other viral sequences, since it was uncloned and purified from gels. The other fragments (*Bam*HI-B, *Bam*HI-P, and *Bam*HI-Z), however, were cloned. *Bam*HI fragments P and Z hybridized to U_S and inverted repeat regions of the VZV genome, respectively, although hybridization to *Bam*HI fragments mapping in these areas could not be detected. Anomalous hybridization to *Bam*HI-B tended to cluster around that region of the genome map covered by *Bam*HI-D and *Bam*HI-G, but scattered hybridization was also seen elsewhere.

Some of the inconsistencies between the published maps and ours may be a function of virus strain (20). Others are due to arbitrary fragment assignments. For example, the positions of *Bgl*II-H and *Bgl*II-I, *Pst*I-O and *Pst*I-P, and *Xba*I-C and *Xba*I-B are all interchanged from the positions reported by Dumas et al. (9). Still others are due to fragment nomenclature. We included *Hin*dIII fragments H and I when naming the *Hin*dIII fragments but did not include them in the map because they were not invariably detectable in the gels. These same two *Hin*dIII fragments were also observed by Richards et al. (15) and Zweerink et al. (24). When *Hin*dIII nomenclature changes are taken into account, our *Hin*dIII map is identical to that of Ecker and Hyman (10). Similarly, *Sal*I fragments E, L, and M were named but not included in the *Sal*I map, as they too were not present in all gels.

Four small *Bam*HI fragments were not cloned. However, we were able to map one of these fragments, *Bam*HI-S, because it is the terminal fragment with homology to *Bam*HI-B. The remaining three small *Bam*HI fragments, T, X, and Y, make up less than 2.4% of the genome and were not included in the map.

These data confirm the model of the VZV genome and the maps presented by Dumas et al. (9), Ecker and Hyman (10), Straus et al. (19, 21), and Davison and Scott (5). The structure of the VZV genome is similar to those of pseudora-

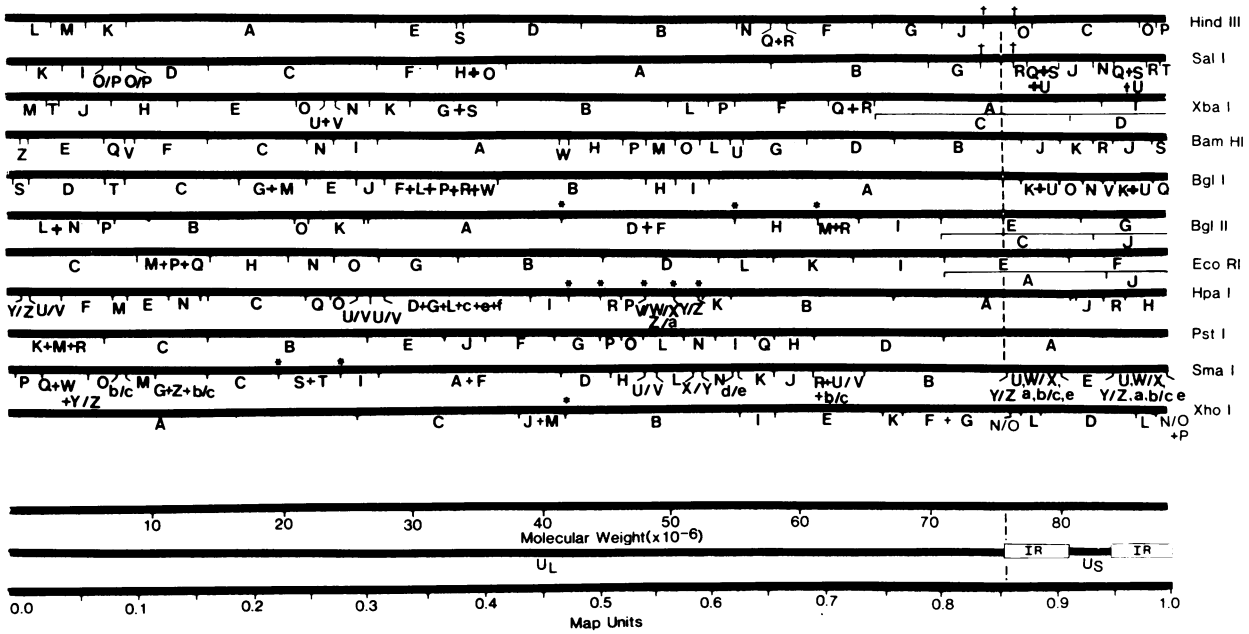

FIG. 1. Restriction endonuclease cleavage maps of VZV DNA (strain EF). The fragments are named alphabetically in order of decreasing molecular weight. The lower three horizontal lines indicate molecular weight, genome segments (U_L, IR [inverted repeat], U_S), and map units, respectively. The dashed vertical line represents the boundary between U_L and IR. *, Imprecisely mapped restriction endonuclease cleavage site; †, fragment suggested by a discrepancy between the estimated molecular weight of the *Bam*HI B fragment and the sum of the molecular weights of fragments released from *Bam*HI-B by digestion with a second enzyme. The molecular weight discrepancy is probably due to overestimation of the molecular weights of all of the larger fragments.

bies virus (2, 18) and equine herpesvirus types 1 and 3 (1, 13). The sizes of the short DNA segment and the inverted repeats (in VZV strain EF) can be estimated from the physical maps and the hybridization data. The inverted repeat is between 4.7×10^6 and 4.9×10^6 daltons (including the inverted repeat sequences in the *Bam*HI K and R fragments), whereas the whole invertible short DNA segment (from the *Bam*HI map) is ca. 13×10^6 daltons. This is in close agreement with the 12.5×10^6 to 13×10^6 daltons estimated by other investigators (5, 9–11, 21). These physical maps of the VZV genome should provide a framework for future studies of VZV.

This study was supported by the Veterans Administration and by Public Health Service grant AI13219 from the National Institutes of Health. We also received support from Public Health Service training grants CA09129 and AI00495 from the National Institutes of Health as well as from Washington University Basic Cancer Center grant CA16217.

We thank Joel Martin and Eileen Harrahill for their technical assistance and Karen Janek for typing the manuscript.

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