JOURNAL OF VIROLOGY, Mar. 1988, p. 1097–1100
0022-538X/88/031097-04$02.00/0

Vol. 62, No. 3

Variation of R1 Repeated Sequence Present in Open Reading Frame 11 of Varicella-Zoster Virus Strains

HISASHI KINOSHITA,[1] RYO HONDO,[2] FUMIAKI TAGUCHI,[1] AND YOSHIAKI YOGO[3]*

Department of Microbiology, School of Hygienic Sciences, Kitasato University, Sagamihara, Kanagawa 228,[1] and Department of Pathology[2] and Department of Viral Infection,[3] the Institute of Medical Science, the University of Tokyo, Shirokanedai 4-6-1, Minato-ku, Tokyo 108, Japan

Received 3 September 1987/Accepted 30 November 1987

We molecularly cloned the tandem direct reiteration (R1) present in open reading frame (ORF) 11 from three independent strains of varicella-zoster virus. Comparison of the R1 sequences among varicella-zoster virus strains revealed that, although the portion of R1 near the 5′ terminus of ORF 11 was conserved among strains, the 3′-terminal portion varied remarkably. This variation was due to the different arrangement of two elements (*A* and *B*) and a segment produced by fusion of *A* and *B* and to a single-base change in the *A* element. Since the difference in the size of R1 among strains was a multiple of 3 base pairs, the variation in R1 caused no frame shift in ORF 11.

Varicella-zoster virus (VZV) is a human herpesvirus which belongs to the *Alphaherpesvirinae* family (10). The genome of VZV is a linear double-stranded DNA of about 80 $\times 10^6$ daltons in size (3). The arrangement of the genome is illustrated in Fig. 1A. The complete nucleotide sequence of a VZV strain (Dumas) has recently been reported by Davison and Scott (2).

With restriction endonucleases, several laboratories have made an attempt to distinguish the VZV strains (4, 6, 9, 12, 13, 18, 19, 21). It has turned out, however, that VZV strains can rarely be distinguished with respect to the presence or absence of restriction sites. The difference which is usually detected among VZV strains is the size variation in a few restriction fragments containing particular regions on the VZV genome. Four such regions have been found so far, and the approximate positions of these regions have been mapped on the VZV genome (6, 19) (Fig. 1B).

Recently, two variable regions of VZV, I and III, have been compared in DNA sequence among strains (1, 8). The results of these studies have shown that direct reiterations R2 and R4 of the VZV genome (2) (Fig. 1B) are responsible for strain differences in variable regions I and III, respectively, and that R2 and R4 vary among strains in the copy numbers of repeating units. Fine mapping and sequence determination of other variable regions (II and IV) have not yet been reported. However, since these variable regions approximately coincide on the map with direct repeats R3 and R5 (Fig. 1B), R3 and R5 probably differ in the copy number of repeating units among VZV strains.

A region containing the R1 direct repeat (2) has not yet been recognized to vary among VZV strains (Fig. 1B). It is conceivable, however, that the restriction endonuclease analysis used was not sensitive enough to detect the size difference of R1. In this study, we have molecularly cloned and sequenced the R1s of three VZV strains. Comparison of R1 among these three VZV strains and Dumas strain, for which the complete nucleotide sequence has been determined (2), has revealed that R1 represents the fifth region that is variable among strains. Furthermore, we have examined the stability of R1 during in vitro passage of VZV and during molecular cloning of R1.

All VZV strains were propagated in human embryonic lung cells as described previously (6). Viral DNA was extracted from purified virions as described elsewhere (R. Hondo, Y. Yogo, T. Kurata, and Y. Aoyama, Jpn. J. Exp. Med., in press). Viral DNA was cleaved with *Eco*RI, and the resultant fragments were cloned with plasmid vector pAT153. In the initial experiment, a recombinant plasmid containing *Eco*RI-C was selected for size and then confirmed by blot hybridization (17). In the subsequent experiments, recombinant plasmids were selected by colony hybridization with the cloned *Eco*RI C fragment. We obtained two, three, and two clones containing *Eco*RI-C from three VZV strains, H-S1, H-N3, and YS, at passages 41, 17, and 7, respectively (Table 1). Appropriate fragments containing R1 (Fig. 1C) were isolated from each clone, subcloned in M13 mp18 or mp19, and sequenced by dideoxy chain termination (15).

The structure of R1 was identical in independent clones derived from the same virus strains but was remarkably different among the three strains examined and Dumas strain (Fig. 2). As described previously, R1 of Dumas strain is a complex reiteration containing four elements: an 18-base-pair (bp) sequence (*A*), a 15-bp sequence (*B*), a variant of *A* with a single-base change, and a 15-bp sequence produced by fusion of *A* and *B* (2). R1 of H-S1 consisted of only *A* and *B*. R1 of H-N3 consisted of the same four elements present in Dumas. R1 of YS contained *A*, *B*, the fused sequence, and a new variant of *A* different from the variant in Dumas R1. The arrangement of these elements in the left-hand portion of R1 was conserved among these strains but was remarkably divergent in the right-hand portion. In addition, the total length of R1 differed significantly among strains (303 bp for Dumas, 243 bp for H-S1, 192 bp for H-N3, and 258 bp for YS). Thus, insofar as examined, R1 of each virus strain has a unique repeated structure.

In the above experiment, two or three clones containing R1 were isolated from each VZV strain. The results of analyses with these sister clones suggested that the detected difference of R1 in strains was not caused by molecular cloning of fragments containing R1. This was further examined by direct comparison of R1-containing fragments derived from viral DNAs. As described above, because the size difference of R1 among strains is small, 15 to 66 bp, we used a relatively short fragment containing R1 to facilitate

* Corresponding author.

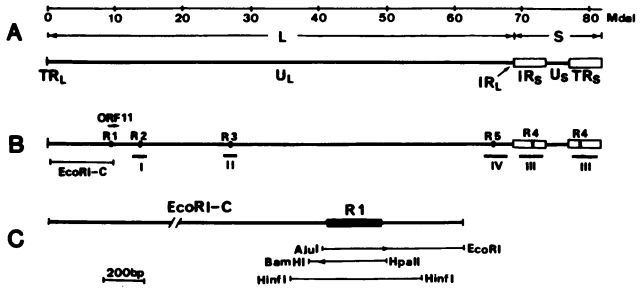

FIG. 1. Arrangement of VZV genome and map location of DNA fragments used in this study. (A) The linear double-stranded DNA of VZV genome consists of two covalently linked segments of long (L) and short (S) lengths (for a review, see reference 2). The L segment is composed of a long unique sequence (U_L) flanked by short inverted repeats (TR_L and IR_L), and the S segment is composed of a short unique sequence (U_S) flanked by inverted repeats (TR_S and IR_S). A scale is shown above the VZV genome (in 10^6 daltons [Mdal]). (B) On the VZV genome are located five tandem direct reiterations (R1 to R5) (2), four regions that are variable among strains (I to IV) (6, 19), ORF 11 (2), and the *Eco*RI C fragment (7). We tentatively designated a reiteration between nucleotides 102,020 and 102,219 (2) as R5, since only this reiteration has not been named of five direct reiterations of VZV (2). (C) The *Eco*RI C fragment is expanded to illustrate the strategy used for sequencing R1. *Alu*I-*Eco*RI and *Bam*HI-*Hpa*II fragments shown (the *Hpa*II terminus had been converted to a blunt end with the Klenow fragment) were subcloned in M13 mp18 or mp19. Single-stranded DNAs were sequenced by chain termination method (15) in the directions indicated by the arrowheads. The location of the *Hin*fI fragment, which was used as probe in blot hybridization, is also shown.

the detection of the difference of R1. *Hin*fI fragments containing R1 (Fig. 1C), 700 to 800 bp in size, were excised from viral DNAs of H-S1, H-N3, and YS, electrophoresed in a 1.5% agarose gel in parallel with corresponding *Hin*fI fragments derived from cloned *Eco*RI C fragments, and detected by blot hybridization (17) (Fig. 3). In each strain, the *Hin*fI fragments derived from viral DNA and from cloned *Eco*RI C fragments comigrated. The *Hin*fI fragments from viral DNA differed significantly among different strains, as did those from cloned *Eco*RI C fragments. Thus, molecular cloning does not appear to introduce variation in the structure of R1. It is not known, however, whether the structure of R1 within a recombinant plasmid shows variation upon extensive passage of *Escherichia coli* carrying the recombinant plasmid containing R1.

TABLE 1. Origin and passage numbers of VZV strains used in this study[a]

Virus strain[b]	Origin	Passage no.	
		Molecular cloning	Blot hybridization
H-S1	Vesicle (varicella)	17, 41	41
H-N3	Vesicle (zoster)	17	9
YS*	Vesicle (zoster)	7	4
YG*	Spinal ganglion 7		7
18-1	Vesicle (zoster)		5
43-1†	Vesicle (zoster)		4
43-2†	Vesicle (zoster)		4
47-1‡	Vesicle (zoster)		4
47-2‡	Vesicle (zoster)		4
102-1	Vesicle (zoster)		4
135-1	Vesicle (zoster)		2
T80-1	Vesicle (zoster)		3
T90-1	Vesicle (zoster)		3
ONI	Vesicle (zoster)		5

[a] Origin of YS and YG was previously described in detail (16).
[b] VZV strains derived from the same individuals are indicated by identical symbols.

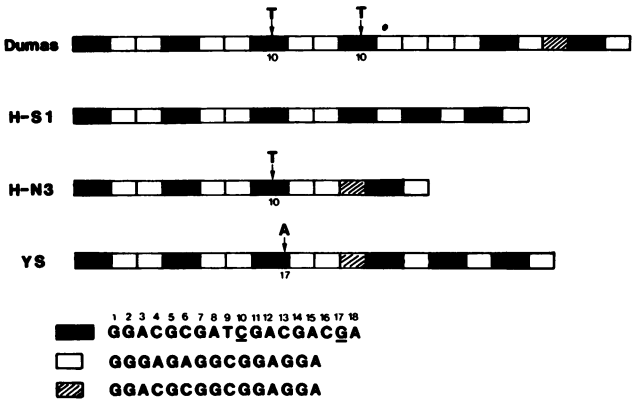

FIG. 2. Comparison of R1 among various VZV strains. R1 of Dumas strain (2) and of the three strains examined in this study are schematically represented (for H-S1, the major species of R1 is shown). The direction of ORF 11 which contains R1 is shown from left to right. The repeating elements of 18 bp (*A*) (▬) and 15 bp (*B*) (▭) (2) and a 15-bp element, which consists of a left-hand portion of *A* (6 bp) and a right-hand portion of *B* (9 bp) (▨), are shown. The sequences of these elements are given at the bottom of the figure. The underlined bases indicate those that are variable among VZV strains. Arrows and bases above the boxes denote single-base replacements at sites indicated by numbers below the boxes.

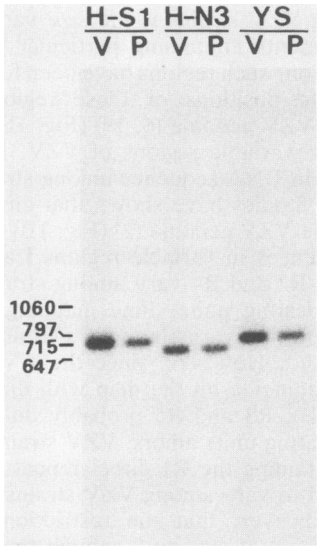

FIG. 3. Size comparison of R1-containing fragments derived from viral DNAs and from cloned *Eco*RI C fragments. Viral DNAs extracted from infected cells (14) (V) and cloned *Eco*RI C fragments (P) were digested with *Hin*fI and electrophoresed in a 1.5% agarose gel. DNA fragments separated in the gel were transferred to a nitrocellulose filter and hybridized to a ^{32}P-labeled *Hin*fI fragment containing R1 (Fig. 1C). The probe was derived from the cloned *Eco*RI C fragment of H-S1. The filter was autoradiographed for 3 h. Some *Eco*T22I (*Ava*III) fragments of λ DNA, electrophoresed in parallel and visualized with ethidium bromide staining, are given on the left of the photograph as size markers. The numbers to the left of the gel indicate the sizes (in base pairs).

To examine the stability of R1 during passage of VZV in vitro, we analyzed a large number of independent clones containing R1 obtained from virus stocks at two different passage levels. We obtained 14 and 9 clones containing R1, including those characterized in the initial experiment, from virus stocks of H-S1 at passages 14 and 41, respectively. Analysis of these clones for the R1 sequence revealed that R1 in 12 and 8 clones at passages 14 and 41, respectively, were identical with the R1 of H-S1 analyzed in the initial experiment (Fig. 2). However, we detected two variants of R1: one lacking two sets of *A* plus *B* elements in the right-hand portion and one containing an additional set of *A* plus *B* element in the left-hand portion. The former was found at each passage level, but the latter was only at passage 14. The identity of R1 in most clones derived from H-S1 at passages 14 and 41 suggests that R1 undergoes little rearrangement during the passage of viruses in vitro. The minor species of R1 may have been generated during viral transmission between human hosts rather than during the in vitro passage of H-S1.

Tentative comparison of R1 was made of VZV strains derived from different individuals and from the same individuals. The DNAs of 14 strains shown in Table 1 were digested with *Hin*fI and electrophoresed in an agarose gel, and fragments containing R1 were detected by blot hybridization (17) (Fig. 4). In addition to H-S1, H-N3, and YS, some strains derived from different individuals could be distinguished by the mobilities of R1-containing fragments (e.g., 18-1 and 43-1, and 102-1 and 135-1), but some were hardly differentiated (e.g., 135-1 and T80-1, and T90-1 and ONI). VZV strains derived from the same individual (YS and YG) could be distinguished by a small but significant difference in the migration of R1-containing fragments. We have previously presented evidence that YS and YG are genetically unrelated (6). In each of the other cases in which two isolates derived from the same individuals were compared (43-1 and 43-2, and 47-1 and 47-2), two R1-containing fragments essentially comigrated (Fig. 4).

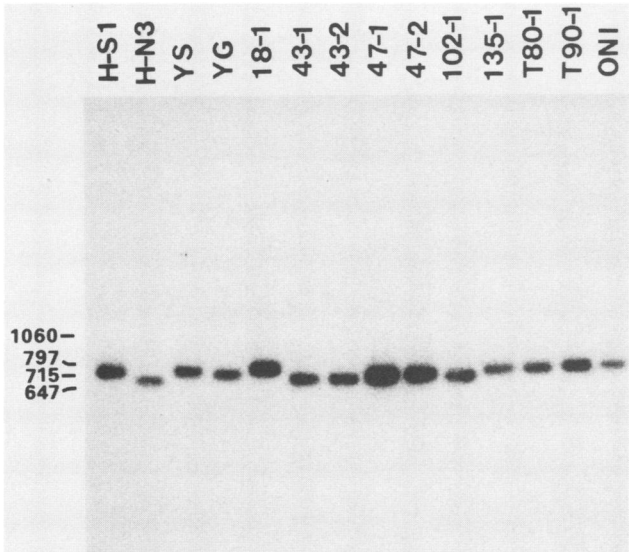

FIG. 4. Variation of R1 among various VZV strains. Viral DNAs of 14 VZV strains, indicated on the top of the photograph, were digested with *Hin*fI, electrophoresed in a 1.5% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized and autoradiographed as described in the legend for Fig. 3. Table 1 indicates the origin and passage number of VZV strains used in this experiment. The numbers to the left of the gel indicate the sizes (in base pairs).

Since R1 of Dumas strain constitutes part of ORF 11 (2), we examined the effect of the variation of R1 in strains upon the amino acid sequences of ORF 11-encoded protein. The length of R1 varied from strain to strain, but the size differences in R1 between Dumas strain and the strains we used, including minor species of R1 found in H-S1, were multiples of 3 bp without exception. Therefore, the change in R1 does not introduce any alteration in the amino acid sequence encoded by the region subsequent to R1. As indicated from the nucleotide sequence of R1 (Fig. 2), the left-hand portion of R1-encoded peptides (50 amino acids) was almost identical among strains, although the right-hand portion varied extensively. The hydrophilicity of the R1-encoded peptide due to abundance in asparaginic and glutamic acids is also observed in our strains. The conservation of the basal structure of ORF 11-encoded protein among strains suggests that ORF 11-encoded protein plays an important function in the life cycle of VZV. In this respect, it is of interest to note that ORF 11 shares significant homology with herpes simplex virus type 1 ORF A whose product modulates α-*trans*-inducing factor-dependent activation of α genes (11).

In most epidemiological studies of VZV, discrimination of clinical isolates of VZV has been made with respect to the size difference of variable regions (5, 6, 20). In addition to variable regions which have been previously used (5, 6, 20), R1 may be useful for discrimination of VZV strains. Because difference in R1 between VZV strains is a complex rearrangement of *A* and *B* elements, including point mutations, comparison of R1 segments can distinguish pairs of isolates which are hardly distinguished by the copy number of repeating units in other variable regions. However, since the size difference among VZV strains is generally subtle (Fig. 4), analysis of R1 for strain discrimination must be performed at the level of nucleotide sequence. Sequence analysis of R1 of various VZV strains is now in progress in our laboratories.

LITERATURE CITED

1. **Casey, T. A., W. T. Ruyechan, M. N. Flora, W. Reinhold, S. E. Straus, and J. Hay.** 1985. Fine mapping and sequencing of a variable segment in the inverted repeat region of varicella-zoster virus DNA. J. Virol. **54**:639–642.
2. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67**:1759–1816.
3. **Dumas, A. M., J. L. M. C. Geelen, W. Maris, and J. van der Noordaa.** 1980. Infectivity and molecular weight of varicella-zoster virus DNA. J. Gen. Virol. **47**:233–235.
4. **Hayakawa, Y., S. Torigoe, K. Shiraki, K. Yamanishi, and M. Takahashi.** 1984. Biologic and biophysical markers of a live varicella vaccine strain (Oka): identification of clinical isolates from vaccine recipients. J. Infect. Dis. **149**:956–963.
5. **Hayakawa, Y., T. Yamamoto, K. Yamanishi, and M. Takahashi.** 1986. Analysis of varicella-zoster virus DNAs of clinical isolates by endonuclease *Hpa*I. J. Gen. Virol. **67**:1817–1829.
6. **Hondo, R., Y. Yogo, T. Kurata, and Y. Aoyama.** 1987. Genome variation among varicella-zoster virus isolates derived from different individuals and from the same individuals. Arch. Virol. **93**:1–12.
7. **Kinchington, P. R., W. C. Reinhold, T. A. Casey, S. E. Straus, J. Hay, and W. T. Ruyechan.** 1985. Inversion and circularization of the varicella-zoster virus genome. J. Virol. **56**:194–200.
8. **Kinchington, P. R., J. Remenick, J. M. Ostrove, S. E. Straus, W. T. Ruyechan, and J. Hay.** 1986. Putative glycoprotein gene of varicella-zoster virus with variable copy numbers of a 42-base-pair repeat sequence has homology to herpes simplex virus

glycoprotein C. J. Virol. **59**:660–668.
9. **Martin, J. H., D. E. Dohner, W. J. Wellinghoff, and L. D. Gelb.** 1982. Restriction endonuclease analysis of varicella-zoster vaccine virus and wild-type DNAs. J. Med. Virol. **9**:69–76.
10. **Matthews, R. E. F.** 1982. Classification and nomenclature of viruses. Intervirology **17**:1–199.
11. **McKnight, J. L. C., P. E. Pellett, F. J. Jenkins, and B. Roizman.** 1987. Characterization and nucleotide sequence of two herpes simplex virus 1 genes whose products modulate α-*trans*-inducing factor-dependent activation of α genes. J. Virol. **61**:992–1001.
12. **Oakes, J. E., J. P. Iltis, R. W. Hyman, and F. Rapp.** 1977. Analysis by restriction enzyme cleavage of human varicella-zoster virus DNAs. Virology **82**:353–361.
13. **Richards, J. C., R. W. Hyman, and F. Rapp.** 1979. Analysis of the DNAs from seven varicella-zoster virus isolates. J. Virol. **32**:812–821.
14. **Rosenthal, L. J., D. B. Crutchfield, P. J. Panitz, and D. J. Clanton.** 1983. Isolation of human cytomegalovirus DNA from infected cells. Intervirology **19**:113–120.
15. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
16. **Shibuta, H., T. Ishikawa, R. Hondo, Y. Aoyama, K. Kurata, and M. Matumoto.** 1974. Varicella virus isolation from spinal ganglion. Arch. Gesamte Virusforsch. **45**:382–385.
17. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
18. **Straus, S. E., H. S. Aulakh, W. T. Ruyechan, J. Hay, T. A. Casey, G. F. Vande Woude, J. Owens, and H. A. Smith.** 1981. Structure of varicella-zoster virus DNA. J. Virol. **40**:516–525.
19. **Straus, S. E., J. Hay, H. Smith, and J. Owens.** 1983. Genome differences among varicella-zoster virus isolates. J. Gen. Virol. **64**:1031–1041.
20. **Straus, S. E., W. Reinhold, H. A. Smith, W. T. Ruyechan, D. K. Henderson, R. M. Blaese, and J. Hay.** 1984. Endonuclease analysis of viral DNA from varicella and subsequent zoster infections in the same patient. New Engl. J. Med. **311**:1362–1364.
21. **Zweerink, H. J., D. H. Morton, L. W. Stanton, and B. J. Neff.** 1981. Restriction endonuclease analysis of the DNA from varicella-zoster virus: stability of the DNA after passage in vitro. J. Gen. Virol. **55**:207–211.