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Transcription Mapping of the Varicella-Zoster Virus Genome

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RNA was isolated from varicella-zoster virus-infected Flow 5000 cells (diploid fibroblasts) at late times after infection. With the use of overlapping DNA probes representing all regions of the varicella-zoster genome, an extensive Northern blot analysis of the RNA was carried out. The analysis revealed at least 58 discrete transcripts ranging in size from approximately 0.8 to 6.5 kilobases. RNAs were found to be homologous to all probes used except for those mapping at approximately map unit 0.3, where no RNA transcripts could be detected. Comparison of the sizes and locations of RNA transcripts mapping in the right-hand ends of the varicella-zoster virus and the herpes simplex virus DNAs shows a number of striking analogies, suggesting their similar genomic organization.

Varicella-zoster virus (VZV), a herpesvirus, is the causative agent of both chicken pox (varicella) and shingles (zoster). The virus contains a linear, double-stranded DNA genome of approximately 80 × 10^6 daltons (125,000 base pairs) (9). Research into the molecular biology of VZV has been hampered by the difficulty in obtaining substantial amounts of cell-free virus for analysis or for characterization of events in the viral replicative cycle. Nonetheless, purification of VZV DNA from virions or nucleocapsids isolated from infected tissue-culture cells has permitted preparation of recombinant libraries of genomic fragments and construction of multiple restriction endonuclease maps (9, 10, 21, 24, 28), as well as detailed analyses of the structure of the viral genome (10, 11, 27). VZV DNA can be considered to contain two segments, a long unique region (U_L) and a short unique region (U_S), both bounded by terminal repetitions (TR_S, IR_S, TR_L, IR_L). Presumably, the U_S region inverts during DNA replication, resulting in two isomeric forms of VZV DNA (10, 11, 27). Recently, Davison (5) and Kinchington (personal communication) have shown that the VZV U_L segment is inverted in approximately 5% of the genomes. Portions of the VZV genome have been sequenced, but little is known of the mapping of individual VZV gene products.

Other biochemical studies have shown that the VZV virion contains more than 30 structural proteins, including six to seven glycoproteins (13, 15, 16, 18, 25, 26, 31). Ellis et al. have recently determined that one viral envelope glycoprotein is encoded by sequences within the U_S region (12). Other viral proteins (including the DNA-binding proteins, thymidine kinase and DNA polymerase) have been identified, but their coding locations are unknown (20, 23, 32). In all, over 40 VZV proteins have been identified in infected cells.

As an approach to further defining the genetic map and identifying the gene products of VZV, we have prepared a preliminary viral transcript map. By using Northern blot analysis, we have mapped 58 virus-coded transcripts along the genome. These messenger RNA (mRNA) transcripts range in size from 0.8 to 6.5 kilobases (kb). The genomic positions and relative sizes of a number of these transcripts appear to be similar to those seen in herpes simplex-infected cells and, by analogy, allow us to surmise which of the gene products might be encoded by some of these transcripts.

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MATERIALS AND METHODS

Cells and virus. VZV strain Ellen (ATCC VR-586) was grown in flasks and roller bottles of Flow 5000 cells (whole human fetal cells, passaged 12 to 18 times; Flow Laboratories, McLean, Va.) and passaged or harvested when about 80% of the cells exhibited cytopathic changes, as previously described (27).

Cloning, preparation, and labeling of VZV DNA. VZV DNA purified from viral nucleocapsids was cleaved with restriction endonucleases, ligated into λgt WES · λB, subcloned into pBR322 or pUC8 plasmids, and grown in HB101 or JM83, as described in previous studies (19, 20, 27, 29). Restriction endonucleases and T4 ligase were purchased from Bethesda Research Laboratories (BRL), Gaithersburg, Md., and used as described in the instructions of the manufacturer. A large amount of plasmid DNA was prepared as previously described (2). Some VZV DNA restriction fragments were recovered from agarose gels and purified on Nacs-prepac columns (BRL). Cloned or recovered DNA fragments were labeled in vitro by nick translation (kits purchased from BRL) with [α-^{32}P]dCTP (specific activity, >3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) to specific activities greater than 10^8 dpm/μg.

VZV RNA. Flow 5000 cells growing in roller bottles (850 cm^2) were infected as previously described (15). When 80 to 90% of the cells showed characteristic viral cytopathic changes, they were placed on ice and washed three times in ice-cold phosphate-buffered saline. The cells were lysed in situ with 4 M guanidinium-thiocyanate, layered onto a 5.7 M CsCl cushion, and centrifuged in an SW41 rotor at 33,000 rpm for 25 h (3, 14). The supernatant was decanted, and the RNA pellet was then suspended in 360 μl of 4 M guanidinium-thiocyanate without Sarkosyl and precipitated by the addition of 40 μl of 2 M potassium acetate (pH 5) and 1 ml of 100% ethanol. After being incubated overnight at −20°C, the sample was spun in a microcentrifuge at 13,800 rpm for 5 min at 4°C. The RNA pellet was washed twice in 70% ethanol–30 mM NaCl and dried for 60 s in a vacuum

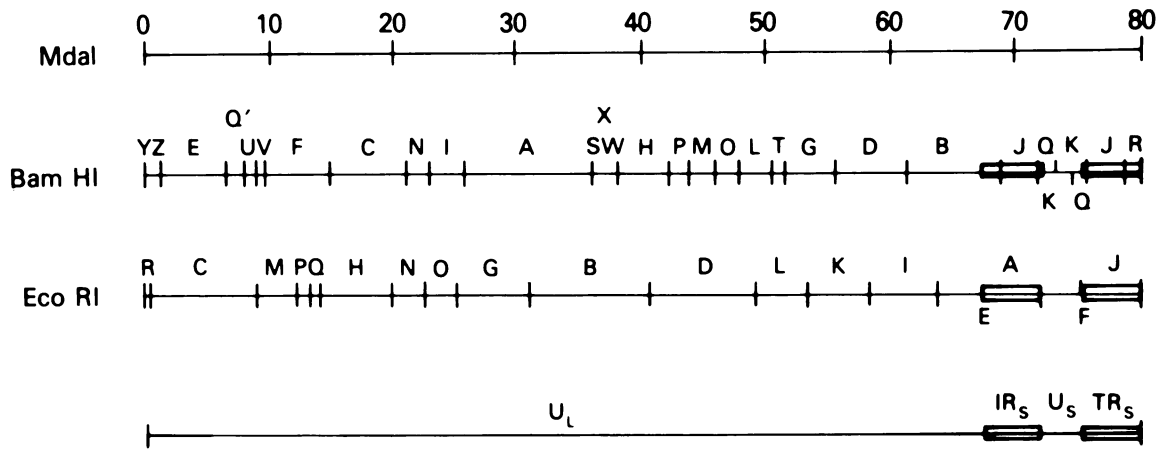

FIG. 1. *Bam*HI and *Eco*RI restriction endonuclease maps of the varicella-zoster virus genome. The boxed areas represent the internal and terminal repeats (IRs and TRs). The U_L is bounded by 88.5-base-pair repeats.

centrifuge. The RNA was suspended in 200 μl of distilled water and shaken with an equal volume of phenol for 5 min. One-half volume of a mixture of chloroform and isoamyl alcohol (24:1) was added, and the sample was shaken for an additional 5 min. This mixture was spun in the microcentrifuge for 2 min, and the aqueous phase was then transferred to a fresh tube. Twenty microliters of 2 M potassium acetate (pH 5) and 500 μl of 100% ethanol were added, and the RNA was precipitated overnight at −20°C. The RNA was sedimented for 5 min at 4°C in a microcentrifuge and washed twice for 30 min in 70% ethanol–30 mM NaCl. The pellet was dried and suspended in distilled water at a concentration of about 1 mg/ml. Aliquots of the RNA were stored under ethanol at −20°C.

Northern blot analysis. Aliquots of cellular RNA were heated at 60°C for 10 min in 50% formamide–6% formaldehyde–1× electrophoresis buffer (20 mM morpholinopropanesulfonic acid [MOPS], 5 mM sodium acetate, 1 mM EDTA [pH 7.0]). Samples (about 5 μg per lane) were electrophoresed at 35 mA for 16 h through 6% formaldehyde–1.5% agarose horizontal slab gels (22). The gel was transferred overnight to water-soaked nitrocellulose (BA85; Schleicher & Schuell, Inc., Keene, N.H.) by using 20× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) as a transfer medium. The filter was cut into strips and baked for 2 h at 80°C under vacuum. Prehybridization was carried out at 42°C for 6 h in 5× SSC–4×

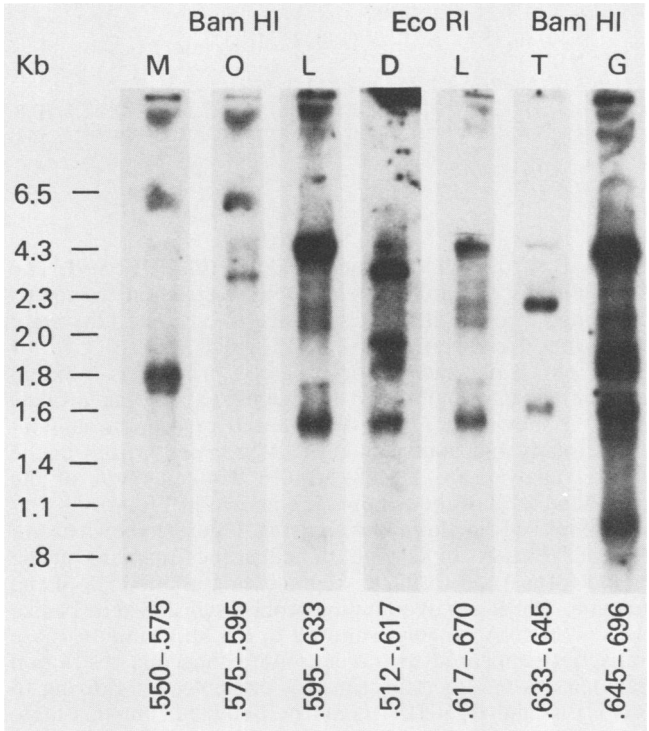

FIG. 2. Northern hybridization analysis of transcripts in VZV-infected Flow 5000 cells. The cloned *Bam*HI and *Eco*RI DNA probes used span the region between map units 0.550 and 0.696. The size markers in kilobases (kb) are listed on the left. Autoradiograph exposure time was 16 h.

TABLE 1. Sizes of VZV transcripts mapping in the right-hand 20% of the genome

Probe[a]	Transcript of the following size (in kilobases)[a]											
	4.3	3.7	2.6	2.3	2.05	1.95	1.85	1.8	1.65	1.4	1.1	0.8
*Eco*RI-A	+	+	+	+	+	+	+	+	+	+	+	+
*Sal*I-F	+			+		+	+					+
*Sal*I-T	+											
*Sal*I-S	+											
*Sal*I-Q	+											
*Sal*I-P	+				+			+		+		
*Sal*I-K		+	+		+							
*Sal*I-I		+	+		+			+				
*Bam*HI-J	+							+		+		
*Bam*HI-K		+	+		+			+	+	+		
*Sma*I-BB	+											
*Sma*I-Z	+											
*Sma*I-CC	+											
*Sma*I-R	+											
*Sma*I-W	+											
*Sma*I-P	+							+		+		
*Sma*I-U			+					+		+		
*Sma*I-AA			+					+			+	

[a] *Sal*I and *Sma*I restriction fragments are listed according to their genomic position, from left to right as determined from restriction mapping (6,23; W. Reinhold, unpublished observations). +, Presence of transcript of the indicated size.

TABLE 2. Sizes of VZV transcripts representing the entire genome

Probe	Transcript size (kilobases)[a]
*Bam*I-Q	3.1, 1.8
*Bam*I-U	3.1, (2.3), 1.7, 1.4
*Bam*I-V	3.9, 2.3, (1.7)
*Bam*I-F	ND[b]
*Bam*I-C	(6.5), 3.6, 1.75, (1.45)
*Bam*I-N	3.6, (2.0), (1.75), 1.45
*Bam*I-I	0
*Bam*I-A	4.1, 3.6, 2.2, 1.8, (1.5), (1.3), (1.1)
*Bam*I-S	4.1, 3.0
*Bam*I-X	4.1, 3.0
*Bam*I-W	4.1, (3.0), (2.0), (1.5)
*Bam*I-H	(4.8), 4.1, 2.5, 1.9, (1.8), (1.75), 1.5
*Bam*I-P	2.5, 1.85, 1.8
*Bam*I-M	6.5, (4.3), 1.85, 1.80
*Bam*I-O	6.5, 3.3, (1.75), (1.70)
*Bam*I-L	6.5, (4.6), 3.8, (3.3), (2.4), (1.7), 1.45
*Bam*I-T	4.6, 2.4, 1.7
*Bam*I-G	4.6, 2.4, 2.15, 2.1, (1.9), 1.8, (1.3), 1.1
*Bam*I-D	4.1, 3.6, (3.2), 2.8, (2.4), (2.1), 1.9, (1.6), 1.5
*Bam*I-B	(4.3), (3.6), (3.2), 2.3, 1.95, 1.85, (1.3), (0.8)
*Bam*I-J	4.3, (1.8), (1.4)
*Bam*I-Q	ND
*Bam*I-K	3.7, (2.6), 2.05, (1.8), 1.65
*Bam*I-R	ND
*Eco*RI-R	ND
*Eco*RI-C	(3.1), 2.3, 1.8, 1.4
*Eco*RI-M	(3.9), 3.2, 2.3
*Eco*RI-P	2.5, (2.1), (1.95), 1.75
*Eco*RI-Q	1.45
*Eco*RI-H	6.5, (3.6), 1.75, (1.45)
*Eco*RI-N	3.6
*Eco*RI-O	0
*Eco*RI-G	4.1, 3.0, (2.6), 2.2, 1.8, 1.5, 1.3, 1.1
*Eco*RI-B	4.1, 3.0, (2.3), 1.9, 1.5
*Eco*RI-D	6.5, (3.8), 3.3, 2.5, 1.85, 1.8, 1.45
*Eco*RI-L	6.5, 4.6, (3.8), 3.3, 2.3, (1.8), 1.7, (1.1)
*Eco*RI-K	ND
*Eco*RI-I	3.6, 2.8, 2.2, 2.1, (1.9), 1.5, 1.3, (1.1)
*Eco*RI-A	4.3, 3.7, 2.6, 2.3, 2.05, 1.95, 1.85, 1.8, 1.65, (1.4), (1.1), 0.8

[a] Parentheses indicate a low level of transcript (i.e., 1+).
[b] ND, Not done.

Denhardt solution (0.08% bovine serum albumin, 0.08% Ficoll, 0.08% polyvinylpyrrolidinone)–50 mM potassium phosphate (pH 7.0)–50% formamide–0.1% sodium dodecyl sulfate. The nitrocellulose strips were placed into sealable bags and hybridized in prehybridization buffer containing heat-denatured DNA probes labeled in vitro with ^{32}P at 42°C. After 40 h, the filters were rinsed three times in 2× SSC–25 mM potassium phosphate (pH 7)–0.1% sodium dodecyl sulfate at room temperature and then washed twice in 0.1× SSC with 0.1% sodium dodecyl sulfate at 50°C for 15 min each. The filters were dried and autoradiographed on Kodak XAR-5 film in casettes fitted with Dupont Lightning-Plus intensifying screens for 2 to 16 h. RNA sizes were estimated by comparisons with the mobility of 18S and 28S ribosomal RNA and ^{32}P-end-labeled *Hin*dIII and φX174 *Hae*III digests analyzed under denaturing conditions on formaldehyde-agarose gels as described above.

RESULTS

Preparation of VZV probes. The use of overlapping VZV DNA restriction fragments has enabled us to carry out a detailed Northern blot analysis of many of the transcripts encoded by the VZV genome. *Eco*RI, *Bam*HI, *Sal*I, and *Sma*I digests of viral DNA or DNA restriction fragments previously cloned in bacteriophage λ vectors (28) were subcloned into either pBR322, pBR325, pUC8, or BPV · pML1 plasmid vectors, thereby providing an extensive library of small VZV fragments for use as probes. Figure 1 shows the *Eco*RI and *Bam*HI restriction endonuclease maps and many of the VZV fragments used in the general analysis (6, 11, 28). (More detailed maps of the right-hand 20% of the genome are shown in Fig. 3).

Examples of Northern blot analyses. Figure 2 shows examples of Northern blots with cloned probes mapping in the region from 0.550 to 0.696, representing about 15% of the genome. The seven overlapping probes hybridized to 14 size classes of RNA ranging from 0.9 to 6.5 kb. Some of these transcripts appeared to traverse more than one restriction fragment. A 6.5-kb transcript, for example, hybridizing to *Bam*HI-M and *Bam*HI-O could be detected, but this large transcript was not consistently observed in all hybridizations in which these probes were used. It is not clear whether this reflected different levels of gene expression or simply degradation of the large transcript. The *Bam*HI-M probe also hybridized to two transcripts of approximately 1.85 and 1.80

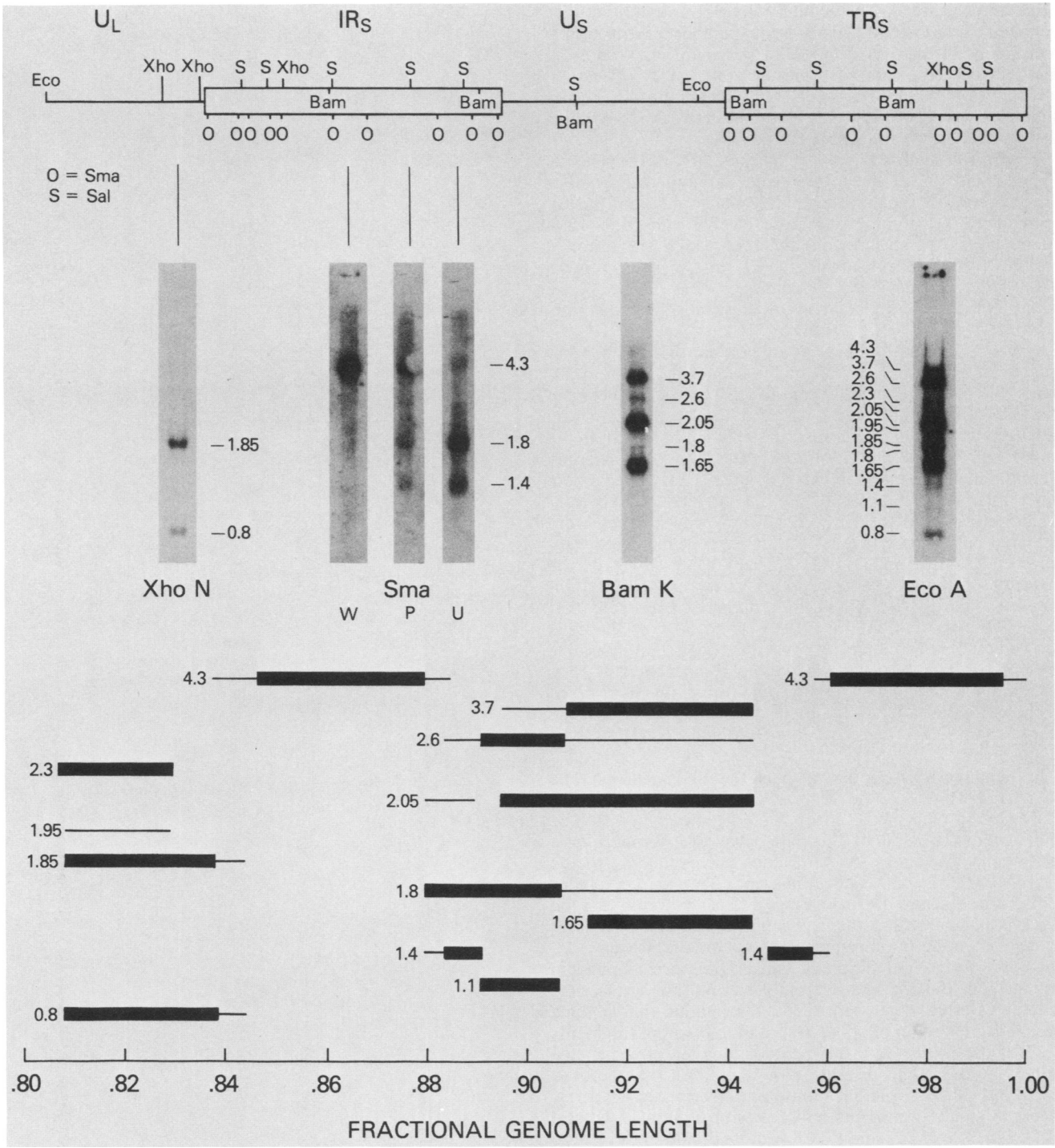

FIG. 3. Northern hybridization analysis of the right-hand 20% of the VZV genome. The upper portion shows a restriction map of the region with the following sites: *Eco*RI (Eco), *Xho*I (Xho), *Bam*HI (Bam), *Sal*I (S), and *Sma*I (O). The middle portion shows representative Northern hybridizations to demonstrate the transcripts present. In the lower portion the data from this figure and Table 1 are combined to generate the transcription map. Bold lines show the main body of the transcript; thin lines represent regions where the DNA probe detected low levels (1+) of hybridization. Autoradiograph exposure times ranged from 3 to 20 h.

kb; the larger transcript was more abundant, while the smaller transcript appeared to continue into *Bam*HI-L. Another transcript, which was about 3.3 kb in size, appeared to hybridize to both *Bam*HI-O and *Bam*HI-L, although the intensity of hybridization with the *Bam*HI-O probe (as reflected by the autoradiogram) seems much greater. These results suggest the presence of an mRNA whose major body is in *Bam*HI-O, while a small portion of this RNA may either begin or terminate in the *Bam*HI-L fragment. *Bam*HI-L hybridizes to seven or eight transcripts of different sizes;

three of these are also detected by *Bam*HI-T, and others are detected by *Bam*HI-O. This large number of transcripts hybridizing to the 3.6-kb *Bam*HI-L fragment may represent multiple overlapping transcripts or common 3′ coterminal transcriptional stops as seen very commonly in herpes simplex virus (HSV). The rest of Fig. 2 shows the transcripts that overlap in this region of the genome.

Transcripts encoded by the right-hand 20% of the genome. Figure 3 and Table 1 show the results of detailed analyses of the region of the genome extending rightward from the U_L segment to the genome terminus. Twelve transcripts have been mapped to this region, eight of which are encoded entirely by sequences within the U_S or the short repeats (IRs, TRs). By using 19 overlapping probes (Table 2), we could locate the ends of many of these mRNA transcripts reasonably well.

Using as a probe the recombinant *Eco*RI A (16.3-kb) fragment, which includes all of the sequences in the right-hand 20% of the genome (except for 530 base pairs between the end of *Eco*RI-A in the U_S and the beginning of the repeats), we detected 12 transcripts ranging in size from 0.8 to 4.3 kb (Fig. 3). The 3.7-, 2.05-, and 1.65-kb transcripts were the most abundant in extracts of cells harvested at late times after infection. While the 2.6- and the 1.1-kb transcripts mapping in the U_S were always present, they were detected only at low levels. Within the repeats, three transcripts (4.3, 1.8, and 1.4 kb in size) were detected; the quantity of the 4.3-kb transcripts varied greatly and increased when the RNA had been prepared from cells that were harvested earlier (i.e., when 40 to 50% of the cells showed cytopathic changes). Four transcripts that hybridize to *Eco*RI-A have been mapped within U_L and are 2.3, 1.95, 1.85, and 0.8 kb in size; the latter two transcripts hybridized to the gel-purified DNA fragment *Xho*I-N as well as to the recombinant DNA fragments *Bam*HI-B and *Eco*RI-I (Tables 1 and 2).

Preliminary transcription map of the VZV genome. The data from Tables 1 and 2 were combined to generate a transcription map of the entire VZV genome (Fig. 4). The map incorporates all of the data from the Northern blot analyses, applying 1+ to 4+ values corresponding to the relative intensity of each transcript. The map excludes transcripts that are 1+ in intensity. The main body of the RNA is placed within the restriction fragments or part of overlapping restriction fragments that contain at least a 2+ intensity. Fifty-eight different transcripts were mapped in this fashion, ranging in size from 0.8 to 6.5 kb. Transcripts seem to derive from sequences throughout the genome except in the region of about map unit 0.3, where fragments *Eco*RI-O and *Bam*HI-I are located. Neither of these two clones hybridized to discrete transcripts. All other probes tested hybridized to either multiple transcripts or, in a few cases, to single transcripts only, as with *Eco*RI-Q, -N, and -O (Table 2). pBR322 and PUC8 vectors did not hybridize to VZV-infected cell RNA, and VZV-cloned probes representing 20% of the genome did not hybridize to uninfected flow 5000 cell RNA (data not shown).

DISCUSSION

We have detected at least 58 apparently unique transcripts that are encoded by genomic sequences spanning nearly all regions of the genome. Other transcripts were detected, but because they were in relatively low abundance, we could not accurately assess their nature and position. All of the transcripts detected represent size classes of RNA because we

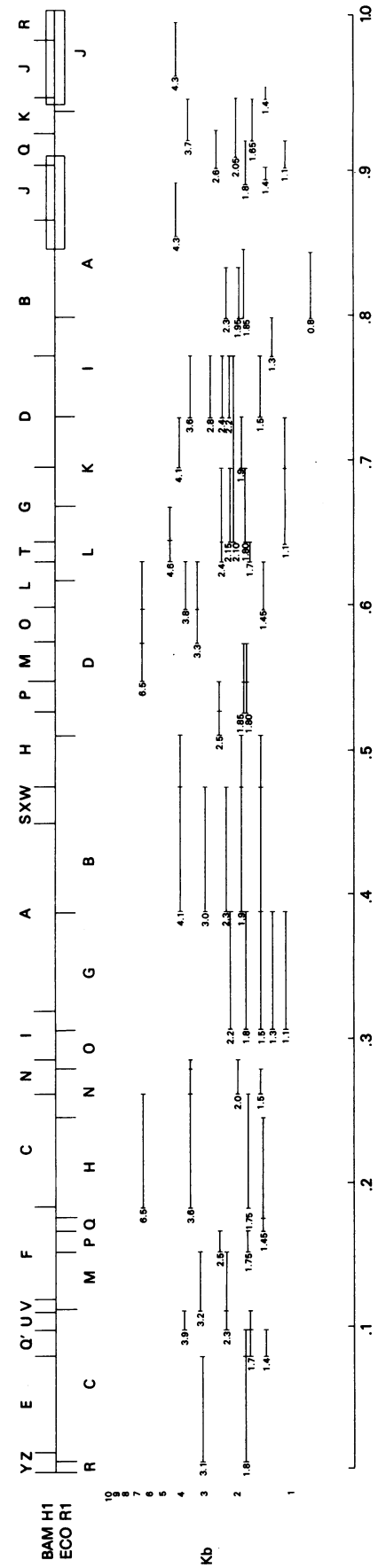

FIG. 4. Transcript map of the entire VZV genome. The data from Tables 1 and 2 were combined to generate a transcript map of the genome.

were unable in some cases to distinguish multiple RNA species of the same size mapping in the same region. The transcripts are all relatively abundant ones, but we cannot as yet determine which represent the products of immediate early (α), early (β), or late (γ) genes because of the difficulty in obtaining synchronous infections with VZV. Many of the VZV transcripts detected probably represent late RNAs since the cells were harvested when showing 80% cytopathic changes. When the best available techniques are used, titers of cell-free VZV approach 10^4 PFU/ml; this titer is inadequate to simultaneously infect large numbers of cells, which would be required to determine the kinetics of RNA synthesis. Therefore, all of the RNA transcripts characterized in this report were prepared from cultures in which VZV-infected cells were added to an established monolayer of uninfected Flow 5000 cells, resulting in a largely asynchronous infection. Attempts to synchronize the infection by using drugs like cycloheximide and acyclovir or to harvest cells for RNA preparations at different times postinfection led to changes in the intensity of specific bands; however, interpretation of data from such experiments is complicated by the constant presence of a background image reflecting the entire population of mRNA transcripts, which is presumably from the input inoculum of infected cells.

Among the approximately 40 VZV-coded proteins, only one has been definitely mapped. A 70,000- to 74,000-dalton protein, which, when glycosylated, migrates at 92,000 daltons, has been mapped within the right side of U_S (map unit 0.94) (12). The DNA sequence analysis of this region of the genome done by Davison predicts an open reading frame that could code for a transcript sufficient to generate a protein of this size. We detected a 3.7-kb transcript that hybridizes to the right-hand end of the U_S and, consistent with the findings of Ellis and Davison, it may code this 92,000-dalton glycoprotein (4, 12). Monoclonal antibodies directed against this protein have the ability to neutralize the virus in a complement-dependent manner, implying that this is an envelope glycoprotein that may be important in immunity to, and recovery from, disease (17, 18).

Analysis of the Davison VZV DNA sequence for the entire right-hand part of VZV DNA demonstrated open reading frames with coding capacities of 11,000, 39,000, 44,000, and 70,000 (74,000) daltons for the U_S and 140,000, 30,000, and 20,000 daltons in the repeats (4). Consistent with these predictions, we have identified six transcripts in the U_S and four in the repeats. Preliminary experiments involving hybrid selection with DNA fragments from this region of the genome and in vitro translation of the selected RNA transcripts have revealed proteins of the approximate sizes predicted from the sequence data (J. Ostrove, unpublished observation).

It has been reported that VZV and HSV show some DNA homology under low-stringency hybridization conditions (8). At the transcriptional level, there is a striking similarity between VZV and HSV in the region of the repeats. There may be additional similarities in other regions of the genome, but this remains to be firmly established. We determined that VZV codes for a 4.3-kb transcript, while HSV is known to code for a 4.2-kb transcript mapping in IRs and TRs (1). The HSV transcript codes for the immediate early ICP4 (175-kilodalton) DNA-binding protein. The equivalent VZV DNA sequence contains an open reading frame that can code for a polypeptide of approximately 140,000 daltons (7). Other regions of the genome appear to share DNA sequence relatedness and also to code for transcripts that are similar in size to HSV transcripts, but characterization of the proteins they encode will be necessary before any further comparisons can be made (7, 30).

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