Molecular Cloning of the Varicella-Zoster Virus Genome and Derivation of Six Restriction Endonuclease Maps

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(Accepted 13 May 1983)

SUMMARY

KpnI and SstI fragments representing 96% of the varicella-zoster virus genome, including the termini, were cloned in plasmid vector pAT153. The clones were used to derive maps of virion DNA for SstI, KpnI, XhoI, PvuII, EcoRI and SalI by molecular hybridization and restriction endonuclease digestion.

Varicella-zoster virus (VZV) is one of five herpesviruses which infect man, and is the cause of chickenpox and shingles. The linear duplex virion DNA molecule comprises two covalently linked segments, L [66 to 70 megadaltons (mdal) and S (12.6 to 13.5 mdal)]. L consists of a unique sequence (UL), and S of a unique sequence (US) bounded by inverted repeats (IRs and TRs) (Dumas et al., 1981; Ecker & Hyman, 1982; Straus et al., 1982). VZV DNA populations contain equimolar amounts of two arrangements of the genome as a result of inversion of S relative to L. Restriction endonuclease maps of VZV DNA have been published for BglII, XbaI and PstI (Dumas et al., 1981) and EcoRI (Straus et al., 1982). The latter authors also reported the cloning in bacteriophage lambda of EcoRI fragments representing 95% of the VZV genome, the genome termini remaining uncloned. Ecker & Hyman (1982) have cloned EcoRI and HindIII fragments in bacterial plasmids, excepting the genome termini, and derived incomplete restriction maps of VZV DNA for these two endonucleases. In this communication we describe the cloning in a bacterial plasmid of 96% of the VZV genome, including the termini, and the derivation of five new restriction endonuclease maps.

The VZV strain used by Dumas et al. (1981) was supplied at passage 8 by Dr J. L. M. C. Geelen, Amsterdam, and virus was grown using a line of human foetal lung cells established by Dr B. Carritt, Glasgow, and purified as described by Dumas et al. (1980). DNA for cloning was kindly supplied by Dr Geelen, who had isolated the DNA from cells infected with the same VZV strain at passage 6. DNA for clone analysis was prepared by phenol extraction of sucrose-banded virions isolated from infected cells at passages 13 to 20.

To construct recombinant plasmids, pAT153 vector DNA (Twigg & Sherratt, 1980) was linearized with PstI, while VZV DNA was cleaved with KpnI or SstI. Otsuka's method (1981), employing terminal deoxynucleotidyl transferase, was used to add homopolymer 'tails' of deoxyguanosine residues to PstI sites and deoxycytidine residues to KpnI or SstI sites. 'Tailed' pAT153 (100 ng) was annealed with 'tailed' VZV DNA fragments (100 ng) in 0.05 ml of 0.01 M-Tris–HCl pH 7.6, 0.1 M-NaCl, 0.001 M-EDTA by heating to 70 °C and cooling slowly to room temperature. Competent Escherichia coli K12 strain HB101 cells (Boyer & Roulland-Dussoix, 1969) were transformed by the annealed DNA essentially as described by Cohen et al. (1972), and colonies were grown on agar plates containing L-broth (0.17 M-NaCl, 10 g/l Difco Bactotryptone, 5 g/l yeast extract) and 10 µg/ml tetracycline hydrochloride. Recombinant plasmids were harvested from minicultures by the method described by Holmes & Quigley (1981) and sizes of inserts were ascertained by cleavage with KpnI or SstI. In addition, ligation of VZV BglII fragments into the BglII site of plasmid pKC7 (Rao & Rogers, 1979) generated several BglII clones. Those colonies chosen to form the clone library were colony-purified once and bacterial stocks were stored at −20 °C in 1% Difco Bactopeptone, 40% glycerol. All

Key words: VZV/cloning/restriction maps
Fig. 1. Restriction endonuclease maps of VZV DNA. Maps for SstI, KpnI, XhoI, PvuII, EcoRI and SalI are aligned accurately with previously published maps for BglII, XbaI and PstI (Dumas et al., 1981). The upper scale shows molecular size in megadaltons and the lower scale shows fractional genome length. Alternative fragments at the joint between L and S and at the S terminus, due to inversion of S, are indicated.

experiments involving growth of live bacteria subsequent to transformation were done under conditions of good microbiological practice, as advised by the Genetic Manipulation Advisory Group and the local safety committee.

The cloning method involving 'tailing' offers three advantageous features. First, all the transformed colonies analysed possessed recombinant plasmids. Second, the genome termini were cloned. Third, PstI and KpnI or SstI sites bounding the insert were regenerated, enabling the insert to be excised precisely. Plasmids containing a genome terminus lacked a KpnI or SstI site at the junction between the terminus and vector sequences.

Bulk amounts of plasmid DNA were prepared by inoculation of 800 ml of L-broth with a 10 ml overnight starter culture in L-broth containing 10 μg/ml tetracycline hydrochloride (KpnI and SstI clones) or 100 μg/ml ampicillin (BglII clones). Bacteria were grown with vigorous aeration overnight at 37 °C, harvested by centrifugation and lysed using lysozyme treatment and Nonidet P40 extraction by the method of Komano & Sinsheimer (1968). Plasmid DNA was isolated essentially as described by Clewell & Helinski (1970) and purified by isopycnic banding on caesium chloride–ethidium bromide gradients.
A number of experiments were done in order to characterize the clones. The VZV KpnI map shown in Fig. 1 was derived from the results of molecular hybridization and restriction endonuclease cleavage experiments using virion DNA fragments. The KpnI and BglII clones were then characterized by hybridization to nitrocellulose blot strips of PstI-digested virion DNA and by cleavage with PstI. The SstI map shown in Fig. 1 was derived using the KpnI clones, and SstI clones were characterized by hybridization to blot strips of BglII, XbaI and PstI digests of virion DNA and by cleavage with PstI. The fully analysed clone library contains BglII fragment d, SstI c to j, and KpnI a to d and f to v. We isolated no clones of KpnI e even though several clones of fragments of equal size (KpnI f and g) were identified. Straus et al. (1982) found sequence rearrangements in EcoRI clones originating from this region of the genome.

Fig. 1 shows the maps, and Fig. 2 a sketch of restriction endonuclease digest profiles. Data for previously published maps are included in the figures. All the fragments were unambiguously ordered except SalI h and j, and EcoRI h, n and p. The three EcoRI fragments are shown in Fig. 1 in the order reported by Straus et al. (1982), and in all other respects we have confirmed their map.

Three conclusions may be drawn from the maps. First, the arrangement of restriction sites in S implies that the molecular weights of TRs/IRs and Us are 4.6 to 5.1 and 3.4 to 4.0 mdal respectively. Straus et al. (1982) reported sizes for these regions of 3.4 \( \pm \) 0.3 and 5.8 \( \pm \) 0.9 mdal respectively. The apparent discrepancy may be due to the use of different virus strains. Second, we have confirmed the presence of an EcoRI site close to the L terminus, as suspected by Ecker & Hyman (1982) and Straus et al. (1982): our current sequence data place it 440 base pairs (bp) from the terminus. Third, if a sequence is precisely repeated in direct orientation at the termini of the VZV genome (terminal redundancy), it is not greater than 20 bp in size. This was deduced from the presence of an XhoI site which our current sequence data place only 20 bp from the L terminus, whereas the XhoI site delimiting XhoI m is 1200 bp from the S terminus. The presence
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of an EcoRI site 440 bp from the L terminus and the absence of an EcoRI site in TRs/IRs rules out a terminal redundancy larger than 440 bp. Therefore, the terminal redundancy does not contain an XhoI site, and, if it is present, cannot be larger than 20 bp. Therefore, the terminal redundancy does not contain an ZhoI site, and, if it is present, cannot be larger than 20 bp. This is in contrast to the genome of herpes simplex virus type 1, which has a terminal redundancy of approx. 400 bp (Davison & Wilkie, 1981).

We are grateful to Professor J. H. Subak-Sharpe and Dr F. Rixon for critical reading of the manuscript. Data in support of the maps were made available to the reviewers.

REFERENCES


(Received 10 March 1983)