Volume 6 Number 4 April 1979 Nucleic Acids Research

Origin of two different classes of defective HSV-1 Angelotti DNA

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Received 21 December 1978

ABSTRACT

During serial passages of Herpes simplex virus (HSV) at high multiplicity of infection, virions containing defective viral DNA accumulate in the progeny. The defective DNA molecules are made up by repeats of restricted portions of the standard viral genome. Two different classes of defective DNA derived from HSV-1 Angelotti (ANG) in independent series of high MOI-passages were studied. The nucleotide sequences contained in the defective DNA were localized on the parental viral genome. One of the two classes contained sequences from non-contiguous sites mapping in unique and in redundant regions of the parental DNA, whereas the second class apparently originates from the S-terminal redundant region of the parental DNA. The localization of defective DNA sequences was complicated by the fact that there exists sequence homology between the S-terminal redundancy and various unique DNA sequences in the L-segment of the HSV-1 ANG genome.

INTRODUCTION

Defective herpes simplex virus (HSV) particles accumulate in the virus offspring during serial virus passages at high MOI. The defective DNA has approximately the same molecular weight as standard viral DNA (100 x 10^6 dalton), however, does not contain the complete viral genetic information. The defective DNA molecules are made up by repeats of restricted portions of viral DNA sequences[1-9].

HSV-1 DNA is composed of a large segment (L) and a small segment (S) accounting for 82% and 18%, respectively, of the total genome. Both L and S are flanked by inverted redundant regions. L and S are joined end to end in each of the four possible orientations giving rise to four isomers which have been shown to occur in standard DNA populations at equimolar ratios[10-16].

Frenkel et al.[4] reported that serial propagation of HSV-1 JUSTIN results in the generation of a class of defective DNA that is uniform with regard to its resistance to the restriction endonuclease HindIII and its sensitivity to the restriction endonuclease EcoRI and which has a higher buoyant density than standard viral DNA. The defective HSV-1 JUSTIN DNA originates from contiguous DNA sequences of the HSV-1 genome from the S-terminal redundancy[6]. Similar data have been obtained for a defective DNA derived from HSV-1 strain Patton[9].

In previous studies[5,7] we have shown that HSV-1 ANG is capable of generating different classes of defective particles in independent series of high MOI virus passages. In one series defective virus particles contained a single class of DNA molecules of approximately standard DNA density (dDNA1) which was completely resistant to the restriction endonucleases EcoRI, HindIII, HpaI and according to recently obtained data (Kaerner unpublished results) also to XbaI, all of which degrade standard viral DNA. In another series together with this defective DNA species an additional class of defective HSV ANG DNA (dDNA2) developed that was resistant to HindIII and HpaI but sensitive to EcoRI and had a higher density than standard DNA.

In the present study the DNA sequences that make up both classes of defective DNA molecules were localized on the standard HSV-1 ANG genome. It could be shown that dDNA1 maps in noncontiguous regions of the parental viral DNA, whereas dDNA2 apparently is made up by repetitions of a presumably coherent region comprising the S-terminus of the standard viral genome.

The mapping which was performed using the blot hybridization procedure[17,18] turned out to be complicated because it was found that part of the S-terminal redundant sequences are repeated internally in different regions of the L-segment of the standard genome.

MATERIALS AND METHODS

Virus strain. HSV strain ANG[19] was kindly provided by Dr. Munk and has been classified as HSV subtype 1[5,20]. The virus was

propagated on African green monkey kidney cells (RC-37 Rita, Italdiagnostics, Rome, Italy) as described[5].

Isolation of HSV-1 ANG standard and defective DNA has been described in detail elsewhere[5,7]. EcoRI and HindIII resistant HSV ANG dDNA1 was isolated from a high MOI passage (HP)[5] virus stock containing 50% dDNA1 particles. The mixture of standard DNA and dDNA1 was digested with EcoRI and the uncleaved dDNA1 molecules were separated from standard DNA fragments by sucrose gradient centrifugation. HindIII resistant and EcoRI sensitive HSV ANG dDNA2 was prepared in two steps from HP11[7] virus particles containing about 15% dDNA1 and 15% dDNA2. First, HindIII resistant DNA was isolated. In a second step the HindIII resistant DNA was digested with EcoRI. The resulting fragments of dDNA2 were separated from uncleaved dDNA1 molecules in sucrose gradients.

Restriction endonucleases. HindIII and HpaI were prepared following a modified procedure[21] described by Philippsen et al.[22] EcoRI according to Thomas and Davis[23]. Restriction of HSV DNA and agarose gel electrophoresis has been described previously[5].

^{32}P labeling of viral DNA in vitro was performed by nick-translation according to Rigby et al.[24]. The labeled DNA had a specific activity of about 2 x 10^8 ^{32}P-CPM/µg.

Blot hybridization of ^{32}P-labeled viral DNA to restriction fragment patterns of HSV-1 ANG DNA on nitrocellulose filter strips was performed following the techniques of Southern[17] and Kelly et al.[18]. ^{32}P DNA bands on the filters were monitored by autoradiography using X-ray films (Agfa-Gevaert, Curix RP1).

RESULTS AND DISCUSSION

The localization of DNA sequences within the standard HSV-1 ANG genome was rendered possible by the construction of maps of restriction endonuclease cleavage sites on the HSV-1 DNA. Fig. 1 shows the physical maps of HSV-1 ANG for the HindIII and HpaI restriction endonucleases[25] which are rather consistent with corresponding maps constructed by other authors[10,11,16,26]. One peculiar feature of HSV-1 ANG DNA which is relevant for the interpretation of the results of this study is the following:

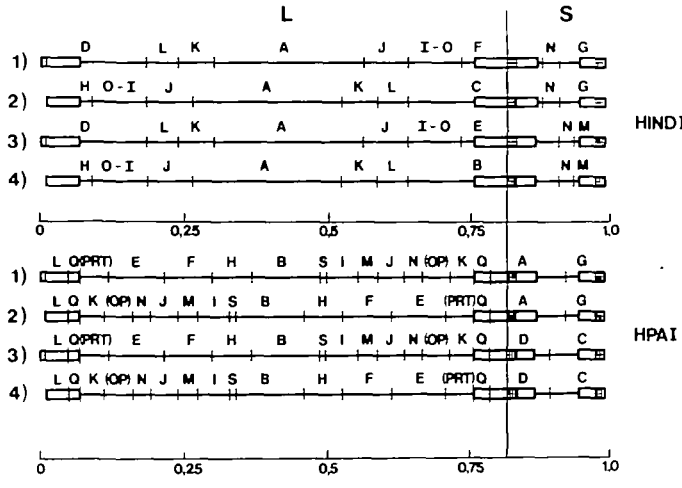

FIGURE 1

Scale maps of HindIII and HpaI endonuclease cleavage sites on HSV-1 ANG DNA[25] The designation of the DNA fragments is according to Wilkie[10] and to Buchman et al.[26] (HpaI map) and to Skare and Summers[11] (HindIII map). The joint of the L- and S-segment of the genome is marked by a vertical line.

The S-segment of the genome displays a discrete size heterogeneity due to multiple additions of a 500 - 550 base pairs DNA sequence to the S-terminal redundancy. As a consequence the S-terminal HindIII fragments G and M and the HpaI fragment G appear as series of bands in agarose gels equidistant in MW by 0.33 - 0.35 x 10^6 dalton.

In Fig. 2 a and c the blot hybridization of ^{32}P-labeled dDNA1 and 2, respectively, to HpaI standard DNA fragment patterns is illustrated. As a control complete standard DNA fragment patterns were made visible by blot hybridization with ^{32}P-labeled standard DNA and are shown in Fig. 2 b and e. Referring to the HpaI cleavage site map shown in Fig. 1 the results can be summarized as follows: (i) dDNA1 and dDNA2 both contain nucleotide sequences stemming from the S-terminal redundant region of the parental genome in that they hybridize to the S-terminal HpaI fragments C and G and the joint fragments A and D. (ii)

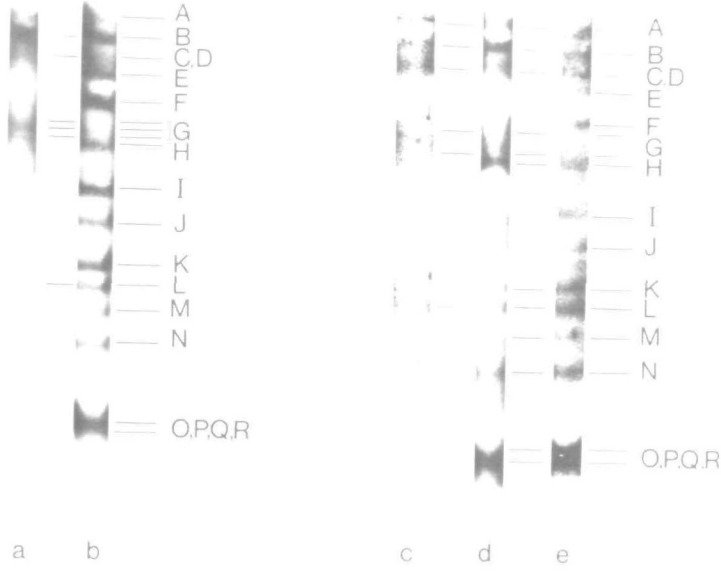

FIGURE 2

Blot hybridization of ^{32}P-labeled dDNA1 (a), dDNA2 (c) and the EcoRI restriction fragment K of HSV-1 ANG DNA (d) to unlabeled HpaI restriction fragments of HSV-1 ANG standard DNA on nitrocellulose filter strips monitored by autoradiography. The positions of the unlabeled DNA fragments were determined by hybridizing ^{32}P-labeled standard DNA to the fragment patterns on parallel filter strips (b and e).

Both, dDNA1 and 2 hybridize to fragment HpaI B which is located in the middle of the L-segment. Apparently dDNA1 displays considerable more sequence homology to this genome region than dDNA2. (iii) To a minor degree dDNA1 and dDNA2 also hybridize to the HpaI fragment L. This finding can be explained by the fact that the S-terminal and the L-terminal redundancies of HSV-1 DNA have a small DNA sequence in common[27,28,29]. The surprizing finding that both dDNA1 and dDNA2 display sequence homology to HpaI B could mean that both of them originated from non-contiguous sites of the parental viral genome. If, on the other hand, there would be sequence homology between the S-terminal redun-

dancy and HpaI B none of the defective DNA classes necessarily had picked up sequences from the central region of the L-segment. In order to detect a possible sequence homology between the S-terminal redundancy and the middle of the L-segment the following experiment was performed. The S-terminal EcoRI fragment K which is known to comprise exclusively sequences of the S-terminal redundancy[11] was recovered from EcoRI digests of HSV-1 ANG DNA electrophoretically separated on agarose gels. For further purification the probe was re-electrophorezed on agarose gels. Following ^{32}P-labeling by nick-translation the material was blot hybridized to HpaI restriction fragment patterns on cellulose nitrate filters. The autoradiography of a representative filter is shown in Fig. 2 d. As expected the EcoRI fragment K hybridizes to the HpaI fragments A, C, D, G, and L. The pronounced hybridization of EcoRI K to the fragment HpaI B strongly suggests sequence homology between the S-terminal redundancy to the middle of the L-segment of the HSV-1 ANG genome. In addition hybridization is observed to the fragments HpaI K, M, N, O, P, Q and R. At the moment we cannot decide whether the latter finding is due to true sequence homology or to residual contaminations of the EcoRI K probe with sequences from different regions of the standard genome[10].

The above results do not allow to conclude unambiguously whether either class of dDNA actually contains DNA sequences mapping in fragment HpaI B. There are two lines of evidence, however, which suggest that dDNA1 in fact contains non-contiguous sequences of the parental genome mapping in the unique region of the L-segment and in the S-terminal redundancy whereas dDNA2 likely contains sequences of the S-terminal redundancy exclusively, similar to the defective HSV-1 JUSTIN DNA described by Frenkel et al.[4]. (i) dDNA1 has approximately the same buoyant density as standard viral DNA[5], in contrast to dDNA2, which has a higher density as demonstrated in Fig. 3. This indicates that dDNA2 clearly must be composed predominantly of sequences localized in the S-terminal redundancy which are known to have a higher CG contents than the average of standard viral DNA[15,30].

(ii) Recent studies in our laboratory revealed that dDNA1 has a total sequence complexity of 11 x 10^6 dalton and that

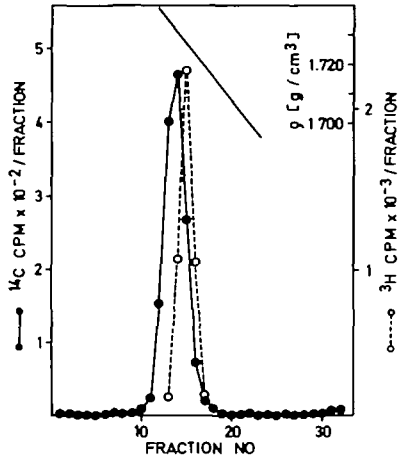

FIGURE 3

CsCl equilibrium density gradient centrifugation of ^{3}H-labeled HSV-1 ANG dDNA2 and ^{14}C-labeled standard DNA. The average density of the gradient was adjusted to 1.725 g/cm^3. The gradient was run for 48 1 at 20°C and 38.000 RPM in a Spinco Ti50 fixed angle rotor.

dDNA1 sequences accounting for 9 x 10^6 dalton are unique and a 2 x 10^6 dalton sequence portion is redundant on the parental viral genome[8]. In case dDNA1 originated from a contiguous site of the parental genome it should comprise nearly the total of the unique sequences of the S-segment and hence should display significant homology to the HindIII fragment N (Fig. 1). Blot hybridization of ^{32}P-labeled dDNA1 to the HindIII restriction fragment pattern of standard DNA (Fig. 4) demonstrates that there is no detectable homology of dDNA1 to this fragment. We conclude from the above data that dDNA1 did arise from non-contiguous sites of the parental viral genome. In contrast dDNA2 stems from the S-terminal region of the standard DNA exclusively and hybridizes to the fragment HpaI B (Fig. 2 c) only by virtue of sequence homology between HpaI B and the S-terminal redundancy of the HSV-1 ANG genome (Fig. 2 d).

In order to demonstrate that dDNA2 in fact is made up by repetitions of the S-terminal redundant region of HSV ANG stan-

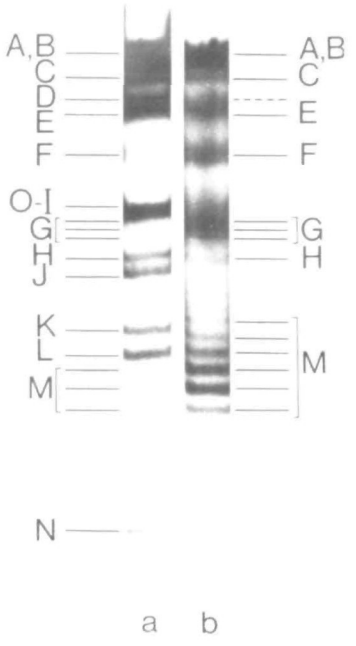

FIGURE 4

Blot hybridization of ^{32}P-labeled dDNA1 to unlabeled HindIII restriction fragments of HSV-1 ANG standard DNA monitored by autoradiography (b). The positions of the unlabeled standard DNA fragments were localized by hybridization of ^{32}P-labeled standard DNA to a parallel HindIII restriction fragment pattern (a).

dard DNA, HP11 HSV ANG DNA containing 15% each of dDNA1 and 2 was cleaved with EcoRI. Uncleaved dDNA1 and the generated fragments of standard DNA and dDNA2 were electrophoretically separated on agarose gels. The resulting fragment pattern is shown in Fig. 5 a together with the EcoRI restriction fragment pattern of standard DNA (Fig. 5 b). The S-terminus of standard DNA is represented in both patterns by fragment K (MW 3,8 x 10^6 dalton[11,25]) as judged by the physical map of EcoRI restriction sites of HSV-1 established by Skare and Summers[11] and own results[25]. Starting with this fragment a series of DNA fragments is visible in the pattern of Fig. 5 a displaying a regular in molecular weight. This fragment series corresponds to a similar,

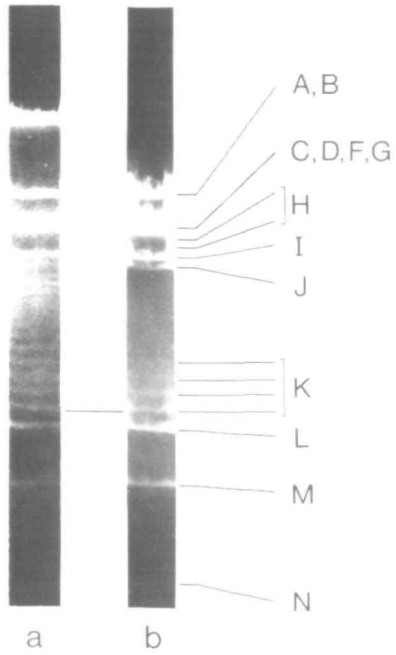

FIGURE 5

Ethidium bromide-stained EcoRI restriction fragments of HSV-1 ANG standard DNA (b) and of a mixture of standard DNA, dDNA1 and 2 (a) separated on a 0.5% agarose gel.

less pronounced, series of fragment bands found in the EcoRI restriction pattern of standard DNA in Fig. 5 b which have been shown to represent the S-terminal EcoRI fragment K plus multiple addition of a 500 - 550 base pairs sequence[25].

The following experiment revealed that the extended K band series in the pattern of Fig. 5 a does not reflect regular sequence additions to the S-terminal redundancy of standard DNA exclusively but originated to a substantial extent from the EcoRI cleavage of dDNA2. A mixture of dDNA1 and 2 was digested with EcoRI. The fragments of dDNA2 were electrophoretically separated from the uncleaved dDNA1. The resulting restriction pattern (Fig. 6 a) displays similar series of DNA fragments as

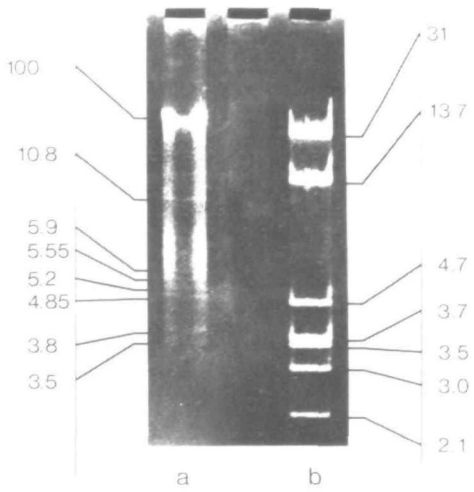

FIGURE 6

Ethidium bromide-stained EcoRI restriction fragments of a mixture of dDNA1 and 2 (a) and of bacteriophage λ DNA plus uncleaved λ DNA (MW 31 x 10^6 dalton) (b) serving as MW. markers[23]. The numbers on the right and the left hand scale mean MW. x 10^6 dalton of the individual fragments.

the K band series observed in the patterns of Fig. 5 a and b. The MW difference of the individual DNA fragment was determined to account for about 500 - 550 base pairs from a calibration gel using the known molecular weights of bacteriophage λ DNA and λ DNA EcoRI restriction fragments (Fig. 6 b). The results strongly suggest that dDNA2 contains repetitions of the S-terminal redundant region of standard DNA represented by the total of sequences of the EcoRI fragment K. This assumption is further supported by the fact that dDNA2 is resistant to the restriction endonuclease KpnI (Kaerner, unpublished results), which cleaves HSV-1 standard DNA at a site located within the unique part of the S-segment close to the S-terminal redundancy[31]. In contrast dDNA1 does not contain the EcoRI restriction site creating the S-terminal standard DNA fragment K suggesting that this class of defective HSV-1 ANG DNA does not contain the total of EcoRI K.

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