Analysis of Sequences of Simian Adenovirus SA7 (C8) DNA in Transformed Rat Cells and Hamster Tumour Cells

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(Accepted 6 January 1983)

SUMMARY

Different methods of molecular hybridization were used to study DNA sequences of the highly oncogenic simian adenovirus SA7 (C8) present in the genomes of two transformed rat cell lines and in cells from three hamster tumours induced by adenovirus SA7. The entire DNA or the left-hand terminal SalI C fragment (19.5% of the genome) were employed. All cell lines retained an intact left-hand region of the SA7 genome (0 to 12.4 map units). The blot hybridization technique failed to detect any site specificity of integration of SA7 DNA into the cell genome. In all cell lines the expression of the BglII D fragment (1.8 to 10 map units) of SA7 DNA was observed. As judged by the patterns of integration of virus sequences into the cell genome, the highly oncogenic simian adenovirus SA7 (C8) is similar to the non-oncogenic human adenoviruses of group C, and is different from the highly oncogenic human adenovirus type 12.

The transformation of rodent cells by human adenoviruses is associated with integration of the adenovirus DNA. In rodent cells transformed by the non-oncogenic adenoviruses Ad2 and Ad5 a small number of copies of the left-hand part of the viral genome are integrated (Sambrook et al., 1974; Gallimore et al., 1974). However, cells transformed by highly oncogenic human adenovirus Ad12 contain as a rule many copies of the entire viral genome (Doerfler et al., 1980; Stabel et al., 1980). It is tempting to explain these differences in the pattern of integration by a difference in oncogenicity but, unfortunately, Ad12 is the only well-studied representative of the highly oncogenic type of adenovirus. Therefore, we investigated the patterns of integration of another highly oncogenic adenovirus, the simian adenovirus SA7, in transformed and tumour cell lines.

Adenovirus SA7 (C8) was grown in African green monkey kidney cells (Gavrilov et al., 1966). WAG rat cells were transformed by the calcium technique of Graham & Van der Eb (1973). The tumours were produced by treatment of 1-day-old hamsters by transfection of 3 to 5 μg of SA7 DNA or an equimolar amount of its fragment, or by injection of 10⁸ p.f.u. of SA7 virus subcutaneously in the dorsal surface of the neck. DNA was isolated from transformed and tumour cells according to Kraiselburd et al. (1975). Preparations of ³H-labelled cytoplasmic RNA were obtained using a modification of the technique of Flint et al. (1975). SA7 DNA and SA7 DNA fragments were labelled by the nick translation procedure (Maniatis et al., 1975). The number of copies of viral DNA fragments present in the cell genome was calculated on the basis of kinetic reassociation analysis according to Gelb et al. (1971), using plots of the fraction of single-stranded fragments (1/fss) against hybridization time. Blot hybridization using various restriction endonucleases was carried out according to Southern (1975). RNA–DNA hybridization was carried out according to Astrin (1978).

Transformation foci appearing in flasks of primary cultures of rat kidney cells exposed to adenovirus SA7 or its DNA gave rise to the cell lines PK-2 and 412 respectively. Cell lines 150, D-2 and 1020 were derived from hamster tumours induced by SA7 virus, its DNA, and the left-hand SalI C fragment of SA7 DNA (Ponomareva et al., 1981) respectively.
Short communication

Fig. 1. (a) Detection of fragments of DNA containing SA7 sequences after hydrolysis of transformed and tumour cell DNA with endonuclease BglII. The products of digestion of cell DNA and calf thymus DNA + SA7 DNA (control) were fractionated, transferred to nitrocellulose filters and hybridized with ³²P-labelled SA7 DNA. Lane 1, SA7 DNA; lane 2, 1020 cell line DNA; lane 3, 412 cell line DNA; lane 4, D-2 cell line DNA; lane 5, PK-2 cell line DNA; lane 6, 150 cell line DNA. The figures on the extreme left indicate the sizes of the virion DNA fragments, in megadaltons. (b) Detection of DNA fragments containing SA7 sequences after hydrolysis of transformed and tumour cell DNA with endonuclease BamHI. The products of digestion of cell DNA and calf thymus DNA + SA7 DNA (control) were fractionated, transferred to nitrocellulose filters and hybridized with ³²P-labelled SA7 DNA. Lane 1, SA7 DNA; lane 2, 1020 cell line DNA; lane 3, 412 cell line DNA; lane 4, D-2 cell line DNA; lane 5, PK-2 cell line DNA; lane 6, 150 cell line DNA. The figures on the extreme left indicate the sizes of the virion DNA fragments in megadaltons. BglII and BamHI restriction maps of SA7 (C8) DNA are shown at the bottom [based on the data of Naroditsky et al. (1978) and Zavizion et al. (1979)].

Table 1. SA7 DNA sequences in the genome of transformed and tumour cells

<table>
<thead>
<tr>
<th>Sall/BglII fragments of SA7 DNA</th>
<th>Coordinates on physical map of SA7 genome (%)</th>
<th>No. of copies (equivalents) of fragment per diploid cell genome in DNA of cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>62-100</td>
<td>PK-2 0.5 412 150 D-2 10-2 4-0 6-0 1-5 2-5</td>
</tr>
<tr>
<td>B</td>
<td>37-62</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0-19</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>27-37</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>19-24</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>24-27</td>
<td></td>
</tr>
</tbody>
</table>

* - indicates the presence of 0.4 copies or less of the viral fragments in the cell genome.

The number of DNA copies of each of the SA7 DNA fragments present in the cell genome, calculated on the basis of the reassociation rate increase, are presented in Table 1.

The arrangement of integrated SA7 DNA in the genomes of five transformed and tumour cell lines was determined by cleavage of the entire intracellular DNA with various restriction endonucleases and subsequent analysis.
Fragments with the same molecular weight as the internal SA7 DNA fragments were detected after cleavage of PK-2 and 412 cell line DNAs with endonuclease BgII (Fig. 1a), transfer to nitrocellulose filter and hybridization with \(^{32}\)P-labelled SA7 DNA. In cell DNA no fragments were found with the same molecular weight as the virion DNA BgII B and F terminal fragments. The data obtained point to the integration of the intact viral genome into the genome of these cells. For cell line 412 this was also confirmed using BamHI (Fig. 1b). Minor bands on Fig. 1(a, b) seem to correspond to the terminal viral fragments linked to 412 cell DNA. The absence of major bands corresponding to the terminal fragments indicates that different copies of SA7 DNA are integrated at different sites of the cell genome. BamHI analysis of PK-2 cells (Fig. 1b) shows only one virus-specific internal fragment BamHI D in the autoradiogram after blot hybridization, and two bands in the high molecular weight zone. This may be related to partial methylation of the BamHI sites of SA7 DNA in the PK-2 cell genome (N. M. Chaplygina, unpublished results).

After hydrolysis by BgII (Fig. 1a), virus-specific fragments with molecular weights equal to that of SA7 fragments D and E were detected in the DNA of lines 150, D-2 and 1020, as well as several additional virus-specific bands, whose interpretation required hydrolysis by other restriction endonucleases. Analysis of cell line 150 using BamHI (Fig. 1b) indicated the presence in it of a fragment with a molecular weight equal to that of BamHI A (62 to 94 map units). The presence of the intact right-hand part of the SA7 genome in this cell line was also confirmed by using endonucleases XbaI and HindIII (data not shown).

In the D-2 and 1020 cell lines, only the left-hand part of the SA7 genome (SalI C fragment of SA7 DNA, Table 1) is present. This is because in D-2 (SA7-induced hamster tumour) most of the SA7 genome is deleted, whereas the 1020 line is derived from a hamster tumour induced by transfection of the left-hand SalI C terminal fragment of SA7 DNA. Digestion with BamHI (Fig. 1b), whose site is absent in the integrated viral DNA, indicates the number of integration sites of SA7 DNA fragments in the cell genome. By analysing the data shown in Fig. 1(b), the number of sites of integration of viral sequences in the genome of D-2 and 1020 cells can be estimated as 2 to 3. The number of integration sites was confirmed by analysis of these lines using HindIII and EcoRI (data not shown), whose sites are also absent in the DNA of lines 1020 and D-2.

On the whole, the patterns of integration in the five lines studied are as follows. In the two in vitro transformed PK-2 and 412 rat cell lines, the entire viral genome persists. In the 150 and D-2 tumour cell lines, a portion of the SA7 genome is missing. In the 150 line, intact left- and right-hand parts of the SA7 genome are present, while the middle part of SA7 DNA is either lost or rearranged. In the D-2 line about 12% of the SA7 DNA sequence from its left-hand end are present, integrated at 2 or 3 sites in the cellular genome. In the hamster tumour (cell line 1020 induced by SalI C fragment of SA7 DNA) no significant deletion of these adenovirus sequences was observed. They were integrated at 2 or 3 sites in the cell genome.

The expression of virus sequences in the cell genomes was studied by RNA–DNA hybridization on nitrocellulose filters. \(^{3}H\)-labelled RNA isolated from the transformed and tumour cells was hybridized with BgII fragments of SA7 DNA transferred to nitrocellulose after electrophoresis in agarose gel (Fig. 2). Labelled RNA of all the five lines was, in turn, hybridized with BgII D fragment of SA7 DNA (1-8 to 10 map units). No hybridization with any other BgII fragments of SA7 DNA was observed, using labelled RNA from the 412 or D-2 cell lines. It cannot be ruled out that in the 412 cells the absence of hybridization with other fragments of SA7 DNA is due to an insufficient amount of labelled RNA participating in the hybridization (Fig. 2). \(^{3}H\)-labelled RNA isolated from the 1020 cell line hybridized also with BgII fragments E and F and labelled RNA from the 150 line with BgII fragments C, E and F; RNA from PK-2 was additionally hybridized with BgII fragments B, C and F.

The study of the DNA of the two transformed and three tumour cell lines induced by the highly oncogenic simian adenovirus SA7, its DNA and its left-hand terminal fragment showed that the left part of the SA7 genome (BgII D, E and F fragments, 0 to 12-4 map units) is present in the DNA of all the cell lines tested. The sequences of this region remain intact and the greater part of these are expressed as RNA. Considering these data in conjunction with transformation
Fig. 2. Hybridization of $^3$H-labelled RNA from transformed and tumour cells with *Bg*II fragments of SA7 DNA. After hybridization with $6 \times 10^6$ to $10 \times 10^6$ ct/min $^3$H-labelled RNA and washing, the filters were cut into pieces and their radioactivity was measured. (a) PK-2 cell line DNA; (b) 412 cell line DNA; (c) 150 cell line DNA; (d) D-2 cell line DNA; (e) 1020 cell line DNA. At the bottom, the physical map of SA7 DNA obtained by digestion with *Bg*II (Zavizion et al., 1979) is shown.

of cells by the left-hand terminal *Sal I* C fragment of SA7 DNA (19% of the genome) alone (line 1020), we can locate the oncogene(s) of SA7 DNA in the left-hand terminal part of the genome, where it has been previously located in the genome of human adenoviruses (Weinberg, 1980).

In all the cell lines analysed in this work, SA7 DNA was integrated into the cell genome. The number of integration sites is about the same as the number of copies of integrated virus DNA fragments. Blot hybridization did not detect any site specificity of integration of SA7 DNA sequences into the cell genome.

The small number of copies of integrated SA7 DNA fragments in the genome of transformed or tumour cells, as well as the absence of a portion of the SA7 genome in tumour cells suggests that the integration of the highly oncogenic adenovirus SA7 DNA into the cell genome is similar to that of the non-oncogenic human adenoviruses of group C.

Doerfler et al. (1980) suggested that the difference in DNA persistence patterns between Ad12-transformed cells and Ad2- and Ad5-transformed cells may be explained by differences in permissiveness. It has been reported that rat cells are semi-permissive for Ad2 (Gallimore, 1974)
and that hamster cells are totally non-permissive for Ad12 (Fanning & Doerfler, 1976). The hypothesis of Doerfler et al. (1980) is that in a semi-permissive system there is a strong selective force directed against the persistence of entire Ad2 and Ad5 genome in the cell, since subsequent replication of the viral genome might kill the cell. In Ad12-infected rodent cells, on the other hand, persistence and integration of the intact viral DNA will not necessarily have a deleterious effect on cell survival.

Rat and hamster cells may be considered to be non-permissive for SA7 virus, since infectious virus is not produced by them (Alstein et al., 1967). However, it cannot be ruled out that disadvantages in integration of entire viral genomes are related not to permissiveness, but only to the replication and partial expression of viral DNA in the particular cell types. Under conditions of defective viral infection, the presence of an entire viral genome may be deleterious for transformed cells. It should be noted that although no production of infectious SA7 virus is observed in the rat or hamster cells, SA7 DNA is replicated in the hamster cells (Ogino & Takahashi, 1970). No studies have so far been reported for the state of SA7 DNA replication in rat cells. Probably, the difference in permissiveness of rat and hamster cells may account for the integration of intact viral genomes into the two transformed rat cell lines tested (PK-2 and 412) and the deletion of a part of the viral sequences into the two hamster tumour cell lines tested (150 and D-2). Ultimate elucidation of the relationships between the integration of adenovirus DNA, on one hand, and the ability of a host cell to sustain the replication of viral DNA, on the other, will require an analysis of more rat and hamster SA7-transformed cell lines.

We thank A. V. Gudkov for advice and helpful discussions and M. A. Lipman for translating the article into English.

REFERENCES


*(Received 29 September 1982)*