The Mechanism of
Viral Carcinogenesis by DNA Mammalian Viruses: DNA-DNA
Homology Relationships Among the ‘Weakly’ Oncogenic
Human Adenoviruses

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SUMMARY

Hybridization reactions between the DNAs of the six members of the
‘weakly’ oncogenic adenovirus group (types 3, 7, 11, 14, 16 and 21) show
that they are closely related to each other, sharing 70 to 100 % of their nu-
cleotide sequences. The ‘weakly’ oncogenic adenoviruses are but distantly
related to ‘strongly’ oncogenic types 12 and 18, showing only 11 to 22 %
homology. Thus, two groups of oncogenic adenoviruses exist, differing
remarkably in nucleotide sequence as well as in base composition and degree
of oncogenicity.

INTRODUCTION

The 31 human adenoviruses (Ad) have been characterized by chemical and physical
properties (Piña & Green, 1965; Green & Piña, unpublished data) and may arbitrarily
be divided into three groups according to DNA base composition: (1) those with a
low G+C content, 48 to 49 %, which include ‘strongly’ oncogenic Ad 12 (Trentin,
Yabe & Taylor, 1962), Ad 18 (Huebner, Rowe & Lane, 1962), and Ad 31 (Pereira,
Pereira & Clarke, 1965); (2) those with a high G+C content, 55 to 61 %, which in-
clude non-oncogenic Ad 1, 2, 4, 5, 6, 8, 9, 10, 13, 15, 17, 19, 20, and 22 to 30; and
(3) those with an intermediate G+C content, 50 to 52 %, which include Ad 3, 7, 11,
14, 16, and 21. (Ad 8, included initially in the third group, was later found to be
wrongly typed.) Mainly on the basis of the similarity in G+C content between
‘weakly’ oncogenic Ad 7 (Girardi, Hilleman & Zwickey, 1964) and the other five
members of this intermediate group, it was predicted (Piña & Green, 1965; Green
& Piña, unpublished data) that Ad 3, 11, 14, 16, and 21 would also be oncogenic.
Subsequently, the results of tests in newborn hamsters demonstrated that Ad 3
(Huebner et al. 1965; Green, Piña, Tockstein & Thornton, to be published), Ad 14, 16,
and 21 (Green, Piña, Tockstein & Thornton, to be published) also produced tumours
in a small proportion of animals after a relatively long period of time, and therefore
may also be classified as ‘weakly’ oncogenic.

The ‘weakly’ oncogenic adenoviruses in the intermediate G+C group are alike also
in several other chemical and biological properties. (1) The buoyant densities of their
DNAs are higher than would be predicted from their base composition as calculated

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from $T_m$ values (Piña & Green, 1965; Green & Piña, unpublished); direct base composition analyses of the DNAs of these viruses indicate that the lower values of G+C content (50 to 52%) obtained from $T_m$ measurements are correct (Green et al. 1967). (2) A common cross-reacting tumour antigen is present in tumours induced in hamsters by injection of purified preparations of the 'weakly' oncogenic adenoviruses (Huebner et al. 1965; Huebner, personal communication); this tumour antigen differs from the common cross-reacting tumour antigen(s) induced by 'highly' oncogenic adenoviruses (Huebner et al. 1963; Huebner et al. 1965; Huebner, personal communication). (3) The 'weakly' oncogenic types Ad 3, 7, 11, 14, 16, and 21 belong to one group with similar haemagglutination properties (the non-oncogenic types Ad 20, 25, and 28 also are included in this group); Ad 12 and 18 comprise another haemagglutination group (Pereira et al. 1963).

'Strongly' oncogenic Ad 12 and 18 were shown previously to be closely related genetically (Lacy & Green, 1964) as determined by DNA-DNA homology measurements (79% homology), but distantly related (Lacy & Green, 1965) to 'weakly' oncogenic Ad 7 (10 to 20% homology). In this paper, we examine the relationship of the five additional members of the proposed 'weakly' oncogenic group, Ad 3, 11, 14, 16, and 21: (1) to each other, (2) to 'highly' oncogenic Ad 12 and 18, and (3) to non-oncogenic Ad 2 and 4.

**METHODS**

Viral DNA was prepared and labelled as previously described (Green & Piña, 1964; Lacy & Green, 1964; Lacy & Green, 1965). The DNA agar technique (Bolton & McCarthy, 1963; McCarthy & Bolton, 1962) was used with slight modifications (Lacy & Green, 1964, 1965) to measure the extent of homology between pairs of adenovirus DNAs. DNA agar preparations containing denatured DNA of a given adenovirus type were incubated at 60° with an equal volume of 2 $\times$ SSC (SSC = 0.15 M-NaCl + 0.015 M-sodium citrate) containing radioactive, denatured fragments of DNA of the same adenovirus type (homologous reaction) or of another adenovirus type (heterologous reaction). The ratio of embedded to free, labelled DNA was 10:1 or greater in nearly all of the experiments (Lacy & Green, 1964). After 16 hr incubation the DNA agar preparations were washed five to ten times by mixing with 8 to 10 ml. amounts of 2 $\times$ SSC in 15 ml. centrifuge tubes for 5 min. at 60°. After centrifugation at 2000 rev./min. for 5 min. in an International Centrifuge Model PR-2 the eluates were collected. The unbound labelled DNA was removed by this procedure. The DNA agar was washed 5 to 10 times more with 8 to 10 ml. amounts of 0.01 $\times$ SSC at 75°; the contents of the tubes were mixed each time for 5 min. and centrifuged as described above. These eluates contained the hybridized radioactive DNA. One hundred $\mu$g. of carrier DNA plus trichloracetic acid to a final concentration of 0.3 M were added to each eluate. The DNA was collected on a Schleicher and Schuell membrane filter and sufficient counts recorded in a Packard Scintillation Counter to give < 5% error. The degree of homology was measured by the percentage of the total radioactive DNA fragments bound to the DNA in the agar (amount present in the second set of eluates) relative to that obtained in the homologous reaction (equated to 100%).
RESULTS

DNA hybrids were defined as those duplex structures formed by and surviving overnight incubation in 2×SSC at 60°C (Hoyer, McCarthy & Bolton, 1964). DNA homology here refers to the possession by DNA molecules of nucleotide sequences sufficiently similar to be detected as DNA-DNA hybrids. However, as pointed out elsewhere (McLaren & Walker, 1965), while the degree of binding between samples of DNA from different organisms reflects similarity in base sequences, there is no information on how divergent in base sequence two samples of DNA must be before binding no longer occurs.

Table 1. Homology among DNAs of 'weakly' oncogenic adenoviruses

<table>
<thead>
<tr>
<th>Group no</th>
<th>Hybrid pair of adenovirus types*†</th>
<th>Labelled DNA bound relative to homologous reaction</th>
<th>No of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ad 3×Ad 7</td>
<td>102±8</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Ad 3×Ad 11</td>
<td>79±19</td>
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<td>3</td>
<td>Ad 3×Ad 14</td>
<td>70±10</td>
<td>11</td>
</tr>
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<td>4</td>
<td>Ad 3×Ad 16</td>
<td>100±4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Ad 3×Ad 21</td>
<td>81±12</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Ad 7×Ad 11</td>
<td>80±12</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Ad 7×Ad 14</td>
<td>80±9</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>Ad 7×Ad 16</td>
<td>81±15</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>Ad 7×Ad 21</td>
<td>98±19</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>Ad 11×Ad 14</td>
<td>94±10</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>Ad 11×Ad 16</td>
<td>91±19</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>Ad 11×Ad 21</td>
<td>83±17</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>Ad 14×Ad 16</td>
<td>86±11</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>Ad 14×Ad 21</td>
<td>74±18</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>Ad 16×Ad 21</td>
<td>92±12</td>
<td>7</td>
</tr>
</tbody>
</table>

* Seed cultures for the adenoviruses used in the experiments described here were obtained from the following sources: Ad 3 (GB), Ad 7 (GOMEN and S-1058 strains), Ad 11 (SLOBITSKY strain), Ad 14 (DE Witt strain), Ad 16 (strain CH 79) and Ad 21 (strain AV-1645) from the American Type Culture Collection; Ad 7 (PINCKNEY strain) from A. J. Girardi and R. J. Huebner; Ad 7 (strain c 14500) from B. Forsyth; Ad 7 (CHAMPAGNE and GRIDBRI strain) from A. J. Girardi; Ad 3 (strain 15520) and Ad 16 (strain 586386) from R. J. Huebner.

† Portions of DNA agar (0.1 to 0.9 g, containing 1.4 to 20.7 µg DNA) were incubated with labelled DNA fragments in 0.1 to 0.9 ml of 0.3 M-NaCl + 0.03 M-sodium citrate. The range of radioactivities of the viral DNA fragments used in these experiments was:

- Group 1, 860 to 1850 counts/min.
- Group 3, 740 to 4390 counts/min.
- Group 5, 300 to 1630 counts/min.
- Group 7, 260 to 3080 counts/min.
- Group 9, 250 to 1500 counts/min.
- Group 11, 225 to 900 counts/min.
- Group 13, 300 to 1500 counts/min.
- Group 15, 750 to 2080 counts/min.

- Group 2, 560 to 1080 counts/min.
- Group 4, 1220 to 1760 counts/min.
- Group 6, 300 to 750 counts/min.
- Group 8, 1160 to 1860 counts/min.
- Group 10, 300 to 790 counts/min.
- Group 12, 170 to 370 counts/min.
- Group 14, 260 to 1370 counts/min.

The extent of nucleotide sequence homology among the DNAs of Ad 3, 7, 11, 14, 16, and 21 was determined by hybridization reactions (Table 1). In all these experiments, the results of reciprocal reactions (e.g. Ad 3 DNA in agar×added labelled Ad 7 DNA, and vice versa) were compared with those values obtained in the homologous reactions included in every experiment. The relative binding of the DNAs of
these viruses ranged from 70% in the case of hybridization between the DNAs of Ad 3 and 14 to 102% between those of Ad 3 and 7. The extensive genetic homology among members of this group was clear from the average degree of hybridization of the DNA of any one virus type with that of each of the other five members studied (Table 1). Of the six members of the group studied here, only Ad 11 has yet to be shown to be oncogenic.

Hybridization reactions between the DNAs of ‘highly’ oncogenic Ad 12 and 18 and members of the ‘weakly’ oncogenic adenovirus group gave 11 to 22% homology between these two groups of viruses (Table 2).

<table>
<thead>
<tr>
<th>Group no</th>
<th>Hybrid pair of adenovirus types*†</th>
<th>Labelled DNA bound relative to homologous reaction (% ± S.D.)</th>
<th>No of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ad 12 × Ad 3</td>
<td>13 ± 5</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Ad 12 × Ad 7</td>
<td>15 ± 4</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>Ad 12 × Ad 11</td>
<td>11 ± 3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Ad 12 × Ad 14</td>
<td>15 ± 7</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Ad 12 × Ad 16</td>
<td>13 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Ad 12 × Ad 21</td>
<td>14 ± 4</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Ad 18 × Ad 3</td>
<td>20 ± 3</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Ad 18 × Ad 7</td>
<td>16 ± 5</td>
<td>42</td>
</tr>
<tr>
<td>9</td>
<td>Ad 18 × Ad 11</td>
<td>13 ± 9</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>Ad 18 × Ad 14</td>
<td>19 ± 9</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>Ad 18 × Ad 16</td>
<td>13 ± 9</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>Ad 18 × Ad 21</td>
<td>23 ± 2</td>
<td>5</td>
</tr>
</tbody>
</table>

* Seed cultures of Ad 12 (HUE) and Ad 18 (PBC) were supplied by R. J. Huebner. Origin of the other adenovirus types are described in the footnote (*) to Table 1.
† Portions of the DNA-agar (0.1 to 0.6 g, containing 1.3 to 12.9 μg DNA) were incubated with labelled DNA fragments in 0.1 to 0.6 ml. of 0.3 M-NaCl + 0.03 M-sodium citrate. The range of radioactivities of the viral DNA fragments used in these experiments is as follows:

Group 1, 300 to 2250 counts/min. Group 2, 170 to 5850 counts/min.
Group 3, 2290 to 2420 counts/min. Group 4, 270 to 2850 counts/min.
Group 5, 1550 to 2320 counts/min. Group 6, 250 to 1880 counts/min.
Group 7, 780 to 1490 counts/min. Group 8, 350 to 2290 counts/min.
Group 9, 720 counts/min. Group 10, 690 to 1350 counts/min.
Group 11, 1110 to 2200 counts/min. Group 12, 340 to 760 counts/min.

Between 22 and 44% nucleotide sequence homology was observed between the DNAs of the ‘weakly’ oncogenic adenovirus types and that of Ad 2, and 33 to 51% between the DNAs of these same types and that of Ad 4 (Table 3). Thus the ‘weakly’ oncogenic adenoviruses appeared more closely related to these non-oncogenic virus types than to the ‘strongly’ oncogenic ones.

**DISCUSSION**

The extensive (70 to 100%) homology observed among the DNAs of the viruses in the intermediate G + C group (50 to 52% G + C content), Ad 3, 7, 11, 14, 16 and 21, correlated well with their grouping according to other parameters such as tumour antigen production and results of haemagglutination tests. The low degree of nucleo-
'Weakly' oncogenic human adenoviruses

Table 3. Homology between DNAs of 'weakly' oncogenic and non-oncogenic adenoviruses

<table>
<thead>
<tr>
<th>Group no</th>
<th>Hybrid pair of adenovirus types*†</th>
<th>Labelled DNA bound relative to homologous reaction (%)†+s.d.</th>
<th>No of reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ad2×Ad3</td>
<td>27±6</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Ad2×Ad7</td>
<td>30±8</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>Ad2×Ad11</td>
<td>36±12</td>
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<tr>
<td>4</td>
<td>Ad2×Ad14</td>
<td>22±3</td>
<td>4</td>
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<td>5</td>
<td>Ad2×Ad16</td>
<td>44±8</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Ad2×Ad21</td>
<td>35±11</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Ad4×Ad3</td>
<td>46±8</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Ad4×Ad7</td>
<td>51±11</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>Ad4×Ad11</td>
<td>76±10</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>Ad4×Ad14</td>
<td>33±4</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>Ad4×Ad16</td>
<td>50±12</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>Ad4×Ad21</td>
<td>46±11</td>
<td>8</td>
</tr>
</tbody>
</table>

* Seed cultures of Ad 2 (strain 38-2) were obtained from R. J. Huebner, and of Ad 4 (strain 88578-111) from R. Chanock. Origin of the other adenovirus types are described in the footnote (*) to Table 1.

† Portions of DNA agar (0.1 to 0.7 g., containing 1.1 to 25.9 µg. DNA) were incubated with labelled DNA fragments in 0.1 to 0.7 ml. of 0.3 M-NaCl + 0.03 M-sodium citrate. The range of radioactivities of the viral DNA fragments used in these experiments is as follows:

- Group 1, 425 to 1220 counts/min.
- Group 2, 225 to 3740 counts/min.
- Group 3, 230 to 2840 counts/min.
- Group 4, 350 to 5200 counts/min.
- Group 5, 1750 to 2810 counts/min.
- Group 6, 500 to 3690 counts/min.
- Group 7, 1060 to 1780 counts/min.
- Group 8, 350 to 1650 counts/min.
- Group 9, 100 to 850 counts/min.
- Group 10, 180 to 1760 counts/min.
- Group 11, 825 to 2590 counts/min.
- Group 12, 580 to 1320 counts/min.

Tide sequence homology (11 to 22 %) observed between the DNAs of Ad 3, 7, 11, 14, 16, and 21 and those of Ad 12 and 18, suggests that these 'weakly' oncogenic viruses and the 'strongly' oncogenic ones, Ad 12 and 18, may differ in 80 to 90 % of their nucleotide sequences. Since tumour production may be due to specific nucleotide sequences held in common by these two oncogenic groups and since the proportion of the total genome associated with carcinogenesis is not known, the small amount of hybridization observed between the DNAs of the 'weakly' and 'strongly' oncogenic viruses does not eliminate the possession by all these types of a common 'carcinogenic nucleotide sequence'. Adenovirus DNAs have molecular weights of 20 to 25 million (Green et al. 1967), thus there is enough genetic information in the adenovirus genome to specify at least 25 polypeptides. The 11 to 22 % of nucleotide sequences shared by both 'weakly' and 'strongly' oncogenic adenovirus types could represent from three to six cistrons which could specify the characteristics common to these adenoviruses and their oncogenic capacity. On the other hand, the region(s) of DNA responsible for tumour production may be different in the 'weakly' and 'strongly' oncogenic viruses.

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REFERENCES


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