Isolation and Phenotypic Characterization of Human Adenovirus Type 2 Temperature-Sensitive Mutants

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SUMMARY

Thirty-nine temperature-sensitive (ts) mutants that fail to grow at 39.5 °C but develop normally at 33 °C have been isolated from a nitrous-acid-treated stock of a wild-type strain of type 2 human adenovirus. The frequency of ts mutants among the surviving viruses was about 10%. Complementation tests in doubly infected cell cultures at restrictive temperature permitted the assignment of 19 of these mutants to 11 complementation groups. They were characterized phenotypically according to their soluble capsid antigen production quantified by two-dimensional immunoelectrophoresis, virus DNA synthesis, as measured by alkaline sucrose gradient sedimentation of 34S DNA, and virion morphogenesis, as analysed by electron microscopy of cell sections. Two complementation groups were defective for DNA synthesis, four for soluble hexon production and two groups for total penton (penton base+fibre), while one group revealed no fibre production. Two complementation groups presented a normal antigen pattern, but the particles exhibited altered morphology as observed in cell sections.

INTRODUCTION

Animal viruses and particularly adenoviruses are considered, with reason, to be invaluable tools in investigating the regulation of replication and transcription of DNA, the translation of mRNA's, and malignant transformation in eukaryotic systems. Essential to these investigations are viruses deficient in different properties. Host-range, conditionally lethal temperature-sensitive and deletion mutants have therefore been isolated.

Temperature-sensitive mutants of human adenovirus type 2 (Begin & Weber, 1975; Kathmann et al. 1976), type 5 (Williams et al. 1971; Ensinger & Ginsberg, 1972), type 7 (Estes & Butel, 1977), type 12 (Shiroki et al. 1972; Ledinko, 1974) and type 31 (Suzuki et al. 1974) have been selected in several laboratories. Their properties have been reviewed recently (Ginsberg & Young, 1976, 1977).

Thirty-nine temperature-sensitive (ts) mutants of human adenovirus type 2 (H 2) have been isolated in our laboratory. Twenty-five have been characterized and 19 of them have been assigned to 11 groups of complementation. Some biochemical and immunological characteristics are reported in the present study. Certain of these ts mutants, such as penton-defective mutants, or a DNA-negative mutant which maintains a tight inhibition of host DNA synthesis at restrictive temperature, have not been reported so far for adenovirus type 2.

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METHODS

**Virus and cells.** Human adenovirus type 2 (H 2) wild type (WT) originally obtained from Dr J. F. Williams (Carnegie-Mellon Institute, Pittsburgh, U.S.A.) was grown on KB cells cultured in suspension (2 to 2.5 x 10⁶ cells/ml) in Eagle’s minimal essential medium supplemented with 5% horse serum.

Virus was titrated by the plaque assay or by the fluorescent focus unit assay (Philipson et al. 1968) on HeLa cells at 37 °C. The WT virus to be used for mutagenesis underwent two cycles of plaque purification at 37 °C.

Human adenovirus type 5 (H 5) ts mutant 36 was kindly supplied by Dr J. F. Williams. H 5 ts-125, originally isolated by Dr H. S. Ginsberg was obtained from Dr W. C. Russell.

**Mutagenesis of adenovirus 2.** Nitrous acid was used as the mutagen (Williams et al. 1971). Plaque-purified WT stock (0.1 ml) with a titre of 6 x 10⁹ plaque-forming units p.f.u./ml was diluted with 1.9 ml of 0.7 M-NaNO₂ in 1 M-sodium acetate buffer, pH 4.6, at room temperature for 8 min. The reaction was stopped by addition of four volumes of cold 1 M-tris-HCl buffer, pH 7.9, and the final mixture diluted a further 50 times with Eagle’s medium. Samples thus diluted were frozen and stored at −70 °C until used.

**Isolation of ts mutants.** The mutagenized stock was diluted to give 30 to 40 plaques/plate and plated on HeLa cell monolayers at 33 °C. After 14 days of incubation at 33 °C, the plaques which appeared were removed with a Pasteur pipette and resuspended in 1 ml tris-saline (0.15 M-NaCl, 0.01 M-tris-HCl, pH 7.5). Virus was released by three cycles of freezing and thawing and the resulting virus isolate was tested for plaque formation on HeLa cell monolayers at 33 °C and 39.5 °C. Those virus isolates which gave a 33/39.5 °C p.f.u. ratio > 100 were plaque-purified twice more and tested again for plaquing ratio before a final working mutant stock was prepared at 33 °C. According to the proposed nomenclature for adenovirus mutants (Ginsberg et al. 1973) the block numbers 101 to 200 have been allotted to these ts mutants.

**Complementation tests.** HeLa cell monolayers were doubly infected at an input multiplicity of 5 p.f.u. of the two ts mutants/cell. After 2 h of adsorption at 33 °C, unadsorbed virus was rinsed off and the cells treated for 30 min with an antiserum against whole type 2 adenovirus, at a dilution of 1:100/1:200. The cells were rinsed again and further incubated at 39.5 °C for an additional 40 h. Control cultures were singly infected in parallel with 10 p.f.u. of each mutant/cell. At the end of the incubation period, the cells were scraped off the dishes into the culture medium, disrupted by three cycles of freezing and thawing and virus titrated using the fluorescent focus assay at 33 °C. Complementation index was given as the ratio of yield of the double infection to that of the higher of the two single infections at 39.5 °C, expressed as p.f.u. or f.f.u./ml. Complementation was considered as positive when this complementation index was at least 10 (Williams et al. 1971).

**Antisera.** Whole adenovirus type 2 (Ad 2) virion antiserum was obtained as follows: 0.5 ml adenovirus particles suspension purified by two CsCl bandings (Green & Piña, 1963), was mixed with 0.5 ml complete Freund’s adjuvant (Difco Lab., Mich.) and injected intradermally into a rabbit in twenty 0.05 ml portions. Three weeks later, 0.25 ml adenovirus suspension in 0.25 ml complete Freund’s adjuvant was injected intramuscularly, and the animal was bled 10 days after this last injection.

Antisera against purified hexon, penton, fibre and virion polypeptide IX were also prepared by immunizing rabbits in a similar way. Hexon, penton and fibre antigens were purified by a four-step procedure previously described (Boulanger & Puvion, 1973). Ad 2 polypeptide IX was purified according to the procedure of Everitt et al. (1973).
Crossed immunoelectrophoresis. An adaptation of the procedure described by Weeke (1973) was employed. First dimension: 5 to 20 μl samples were applied in wells punched in agarose gel (four wells on a 90 x 110 x 1.5 mm glass plate, corresponding to 15 ml 1% agarose). Electrophoresis was carried out at 10 V/cm for 70 min in agarose gel buffered with barbitone, pH 8.6, ionic strength 0.02. The electrophoresis tank was refrigerated at 15 °C by tap-water circulation. After the run, the agarose gel was divided into four slabs, each of them corresponding to one well. Each of the four first-dimension gel slabs was then transferred to a glass plate (100 x 100 x 1.5 mm) along one edge, and the remaining part of the plate was filled with antibody-containing agarose (50 to 200 μl antiserum mixed with 12 ml 1% agarose solution maintained in a 52 °C water bath). Second dimension: 16 h, 3 V/cm, 15 °C, in antibody-containing agarose buffered at pH 8.6. After electrophoresis in the second dimension, the gel was washed in saline, pressed, dried, stained with Coomassie brilliant blue R-250 (0.5% in 50% ethanol-10% acetic acid) and destained in 10% acetic acid-50% ethanol.

Quantitative estimation of the soluble adenovirus antigens was made either by measuring the area enclosed in the precipitate (expressed as height x width at half-height) or by drawing the precipitate outline on transfer paper, cutting out the drawing, and weighing it. The antiserum was calibrated for each adenovirus antigen by running purified hexon, penton and fibre solutions separately (Martin et al. 1975). Their protein contents were determined by the method of Lowry et al. (1951).

DNA analysis. KB cells in suspension culture were infected with WT or ts mutants and labelled with 3H-thymidine (2 μCi/ml, 25 Ci/mmol) at 16 to 24 h post infection (p.i.) at 39.5 °C. Control cultures at 33 °C were labelled from 40 to 48 h p.i. Cells were harvested, resuspended in tris-saline and loaded on top of a 5 to 20% alkaline sucrose gradient made in 0.3 M-NaOH, 0.7 M-NaCl, 1 mM-Na EDTA overlayered with 0.2 ml of 0.5 M-NaOH, 0.05 M-Na EDTA. After standing for 16 h at 4 °C, the gradients were centrifuged for 5 h at 35000 rev/min and 4 °C in a SW 41 rotor. Fractions were collected dropwise from the bottom and assayed for acid-precipitable radioactivity.

Electron microscopy. Cell samples were harvested 36 h after infection at 39.5 °C, fixed with 2.5% glutaraldehyde in 0.1 M-cacodylate buffer, pH 7.2 and post-fixed in 1% osmium tetroxide. After dehydration in an alcohol gradient, the samples were embedded in Araldite and sectioned. Sections were stained with lead citrate and examined in an Hitachi HU-12 electron microscope.

RESULTS

Isolation of ts mutants

The stock of type 2 adenovirus mutagenized by 0.7 m-nitrous acid was screened for ts mutants. From 400 plaques isolated at random at 33 °C, 39 mutants were isolated without selection pressure, i.e. a frequency of 10%. The 33/39.5 °C plaque-forming ratio ranged from 102 to 105, whereas the ratio for the WT varied from 1.5 to 2. The tightness of the mutants was estimated by comparing the yields from single growth cycles at 33 and 39.5 °C in cells infected at an m.o.i. of 10 p.f.u./cell. Mutants with a minimal 33/39.5 °C yield ratio lower than 102 were considered as leaky. As summarized in Table 4, only a few mutants were found to be leaky (H 2 ts 101 and 112).

Synthesis of virus structural antigens

Two-dimensional immunoelectrophoretic analysis of WT-infected HeLa cell extracts using an antiserum against whole virion reveals five main precipitate peaks which have been
previously identified and quantified (Martin et al. 1975). Peak 1 corresponds to hexon antigen, peak 2 to free penton base, peak 3 corresponds to the fibre antigenic determinants of the complete penton, and peak 4 to the penton base antigenic determinants of the complete penton; peak 5 has been identified as free fibre antigen (Fig. 1a).

The capacity of the ts mutants to induce the synthesis of immunologically active capsid antigens was tested at both permissive and restrictive temperatures by the two-dimensional immunoelectrophoretic technique. Three principal types of two-dimensional antigenic patterns were reproducibly observed: (i) a drastic reduction or a disappearance of one or several structural antigens; (ii) a homogeneous reduction of all the virus antigens; and (iii) an antigenic pattern similar to that of the WT. Among the ts mutants of the first serological class, some were defective in fibre production at 39.5 °C and showed no free fibres and no complete penton (ts 115, Fig. 1b), while others were reduced in penton (penton base + fibre) production (ts 104, Fig. 1c) and still others in hexon synthesis (Fig. 1d to f). Significant differences were visible in the antigenic pattern of the hexon-defective ts mutants, and three subclasses of patterns were distinguishable: (i) in hexon-defective mutants of subclass I (H 2 ts 118 and ts 121) hexon was produced in minute amounts, non-quantifiable (Fig. 1d); (ii) in subclass II (H 2 ts 102, ts 110, ts 117 and ts 122) the hexon production was 15 to 20% of the normal rate (Fig. 1e); (iii) in subclass III (H 2 ts 106, ts 108 and ts 119) the hexon production was 30 to 40% of the normal synthesis, but still attained the level of complete penton antigen (Fig. 1f). These hexon-defective mutants appear to belong to four different complementation groups designated as A, B, H and K (see Tables 3 and 4).

**Virus DNA synthesis**

The analysis of virus DNA synthesis in cells infected (m.o.i. = 25) by the ts mutants at both permissive (33 °C) and restrictive (39.5 °C) temperatures was carried out by centrifugation of 3H-thymidine-labelled infected cell DNA in alkaline sucrose gradients. The velocity sedimentation pattern obtained with ts mutants (Fig. 2b) was compared with the WT pattern obtained at the same temperature (Fig. 2a): the synthesis of 34S virus DNA was drastically reduced at 39.5 °C for three ts mutants which appeared, therefore, to be DNA-negative: H 2 ts 105, 111 and 114. During infection with ts 105 and 114, host DNA synthesis was only slightly depressed at the non-permissive temperature, whereas this synthesis was strongly altered during infection with ts 111 at 39.5 °C. Experiments, to be published elsewhere, have shown that these mutants are not decapsidation mutants.

**Virus capsid morphogenesis**

The assembly of virus was studied by electron microscopy of cells infected with ts mutants at the restrictive temperature under identical m.o.i. conditions (25 p.f.u./cell). Virus particles were analysed with respect to their number and morphology in cell sections. Four different types of intranuclear particle patterns were observed: (i) a total absence of detectable virus particles; (ii) less than 50 particles per nucleus; (iii) the number of intranuclear particles ranging from 100 to 500; (iv) more than 1000 particles per section. Fig. 3b shows part of a section of HeLa cell infected with ts 104, exhibiting more than 1000 particles per section. Most of these particles presented an irregular contour but seemed to contain a densely-stained internal core. As already shown by immunoelectrophoresis, ts 104 presented an abnormal pattern of antigens, containing no visible penton and fibre cross-reactivity. In contrast, most of the intranuclear virus particles of ts 112, which showed a normal pattern of synthesis of soluble antigens, exhibited an altered structure: they appeared
Ad 2 ts mutants

Fig. 1. Serological analysis of lysates of cells infected with adenovirus 2 WT or ts mutants at 39.5 °C. Six x 10^6 HeLa cells were disrupted in 100 μl of hypotonic buffer and 10 μl of cell lysate loaded in the well; 100 μl of antiserum against whole Ad 2 virion was added to agarose gel in the second dimension (plates a, b, d to f). On plate (c), 5 μl of cell extract was reacted against 50 μl of antiserum. (a) WT; (b) fibre-defective ts 115; (c) penton-defective ts 104; (d) hexon-negative ts 121; (e) hexon-defective ts 102; (f) hexon-defective ts 106.
Fig. 2. Synthesis of virus DNA analysed by velocity sedimentation in alkaline sucrose gradient. HeLa cells infected with WT or ts mutants were labelled with 3H-thymidine. (a) WT; (b) profile representative of ts 105 and 114. Fraction 15 corresponds to the position of 34S virion marker DNA. ○—○, 33 °C; ●—●, 39.5 °C.

devoid of densely-stained core, suggesting a thermosensitive lesion in a late function required for virus maturation (Fig. 3 c).

In some cases dense amorphous material was visible in the cytoplasm surrounding the nucleus. These dense perinuclear inclusions were observed in infection with hexon-defective mutants showing no (or only a few) intranuclear particles, such as ts 110, 118, 121 and 123. Fig. 3 d presents the dense inclusions induced by H 2 ts 118, which was reminiscent of the intracytoplasmic lesion reported for two hexon-defective adenovirus mutants H 2 ts 3 (Weber et al. 1977) and H 7 ts 19 (Estes & Butel, 1977).

Complementation

Complementation tests were carried out to group the ts mutants, and particularly those which presented the same phenotype, such as the DNA-negative mutants, the hexon-defective, penton- and fibre-defective mutants. The 19 mutants studied here fell into 11 groups of complementation. The complementation between the DNA-negative mutants was determined by two different methods: (i) comparison of the virus yields in single and
Fig. 3. Virion morphogenesis – electron microscopy of cells infected at 39.5 °C with (a) WT, 
(b) ts 104, (c) ts 112 and (d) ts 118. Many particles in (b) and (c) appear abnormal in morphology, 
lacking their densely stained inner core. In (d) electron-dense material is visible surrounding the 
nucleus. Magnifications: (a)–(c) ×80000; (d) ×10500.
Table 1. Complementation between DNA-negative ts mutants of type 2 adenovirus (H 2)*

<table>
<thead>
<tr>
<th>Mutants</th>
<th>ts 105</th>
<th>ts 111</th>
<th>ts 114</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts 105</td>
<td>(9 × 10⁶)</td>
<td>83 (7.5 × 10⁶)</td>
<td>1.05 (9.6 × 10⁶)</td>
</tr>
<tr>
<td>ts 111</td>
<td>—</td>
<td>(6 × 10⁹)</td>
<td>65 (3.9 × 10⁹)</td>
</tr>
<tr>
<td>ts 114</td>
<td>—</td>
<td>—</td>
<td>(3.3 × 10⁹)</td>
</tr>
</tbody>
</table>

Virus yield†

<table>
<thead>
<tr>
<th>Mutants</th>
<th>ts 105</th>
<th>ts 111</th>
<th>ts 114</th>
</tr>
</thead>
<tbody>
<tr>
<td>ts 105</td>
<td>(21650)</td>
<td>7.1 (153840)</td>
<td>0.7 (15550)</td>
</tr>
<tr>
<td>ts 111</td>
<td>—</td>
<td>(4220)</td>
<td>14:1 (105500)</td>
</tr>
<tr>
<td>ts 114</td>
<td>—</td>
<td>—</td>
<td>(7460)</td>
</tr>
</tbody>
</table>

Virus DNA synthesis‡

* Complementation tests were performed as described in Methods.
† Values in table are complementation indices (CI), calculated from the virus yield, as explained in Methods. Complementation was considered as positive when the CI was ≥ 10. Values in parentheses are yields of infection expressed as p.f.u./ml.
‡ KB cells were infected at an input multiplicity of 25 p.f.u./cell with each mutant and labelled with 10 µCi/ml of ³H-thymidine from 16 to 24 h p.i. The virus DNA was analysed as in Fig. 2. Values in the table are ratios of label in 34S DNA peak of the double infection to that of the higher of the two single infections at 39.5 °C, expressed as total cT/min. The leakiness of ts 105 explains the value of 7.1 for the CI calculated from the rates of DNA synthesis.

Table 2. Complementation indices between DNA-negative ts mutants of human type 5 (H 5) and type 2 (H 2) adenovirus*

<table>
<thead>
<tr>
<th>Mutants</th>
<th>H 5 ts 36</th>
<th>H 5 ts 125</th>
<th>H 2 ts 111</th>
<th>H 2 ts 114</th>
<th>H 2 ts 105</th>
</tr>
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<tbody>
<tr>
<td>H 5 ts 36</td>
<td>.</td>
<td>300</td>
<td>400</td>
<td>4'2</td>
<td>5'4</td>
</tr>
<tr>
<td>H 5 ts 125</td>
<td>.</td>
<td>.</td>
<td>50</td>
<td>600</td>
<td>120</td>
</tr>
<tr>
<td>H 2 ts 111</td>
<td>.</td>
<td>.</td>
<td>300</td>
<td>400</td>
<td>.</td>
</tr>
<tr>
<td>H 2 ts 114</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>4'o</td>
<td>.</td>
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<tr>
<td>H 2 ts 105</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

* Values in table are complementation indices calculated from the virus yields, as described in Methods.

Table 3. Complementation between hexon-defective ts mutants of adenovirus 2*

<table>
<thead>
<tr>
<th>Mutants</th>
<th>102</th>
<th>106</th>
<th>108</th>
<th>110</th>
<th>117</th>
<th>118</th>
<th>121</th>
<th>122</th>
<th>123</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>.</td>
<td>14</td>
<td>10</td>
<td>1'6</td>
<td>0'4</td>
<td>5200</td>
<td>46</td>
<td>1'2</td>
<td>0'4</td>
</tr>
<tr>
<td>106</td>
<td>.</td>
<td>.</td>
<td>4</td>
<td>12</td>
<td>10</td>
<td>56</td>
<td>27</td>
<td>1'1</td>
<td>NT†</td>
</tr>
<tr>
<td>108</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>19</td>
<td>18</td>
<td>1500</td>
<td>58</td>
<td>10</td>
<td>NT</td>
</tr>
<tr>
<td>110</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>5'3</td>
<td>600</td>
<td>20</td>
<td>1'0</td>
<td>1'0</td>
</tr>
<tr>
<td>117</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>1000</td>
<td>320</td>
<td>4'5</td>
<td>1'3</td>
</tr>
<tr>
<td>118</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>410</td>
<td>75</td>
<td>1700</td>
</tr>
<tr>
<td>121</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>122</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>NT</td>
</tr>
<tr>
<td>123</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.‡</td>
</tr>
</tbody>
</table>

* Values in table are complementation indices, calculated from the virus yields, as described in Methods.
† NT = not tested.
‡ ts 123 which did not complement both ts 102 and ts 121 appears as a possible double mutant.

double infections at 39.5 °C as indicated in Methods; (ii) comparison of the amount of label in the peak of 34S DNA in single and double infections at restrictive temperature. In the second method the complementation index was the ratio of virus DNA synthesis of the double infection to virus DNA synthesis of the higher of the two infections at 39.5 °C. Complementation was considered as positive with respect to DNA synthesis when the ratio was higher than 2. The results were concordant for both kinds of analysis. As shown in
Table 4. Properties of ts mutants of human adenovirus type 2 at the restrictive temperature (39.5 °C)

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Complementation group</th>
<th>Virus DNA synthesis</th>
<th>Inhibition of host DNA synthesis</th>
<th>Soluble antigens*</th>
<th>Penton base</th>
<th>Fibre</th>
<th>Virion morphogenesis (Intranuclear particles)</th>
<th>Average no. Morphology of particles†</th>
<th>Ratio of yields of yields 33/39.5°C‡</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Class I</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 105</td>
<td>D</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
<td>N+A</td>
<td>3.2 × 10^4</td>
<td></td>
</tr>
<tr>
<td>ts 114</td>
<td>D</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50</td>
<td>N+A</td>
<td>4.0 × 10^2</td>
<td></td>
</tr>
<tr>
<td>ts 111</td>
<td>G</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50</td>
<td>N+A</td>
<td>1.5 × 10^2</td>
<td></td>
</tr>
<tr>
<td>Class II§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H (−)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 118</td>
<td>H</td>
<td>+</td>
<td>+</td>
<td>− (0.0)</td>
<td>+</td>
<td>0</td>
<td>5.9 × 10^4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 121</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>− (0.0)</td>
<td>+</td>
<td>50</td>
<td>N</td>
<td>1.1 × 10^3</td>
<td></td>
</tr>
<tr>
<td>ts 102</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>± (0.18)</td>
<td>+</td>
<td>0</td>
<td>8.4 × 10^3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 110</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>± (0.19)</td>
<td>+</td>
<td>0</td>
<td>3.1 × 10^3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 117</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>± (0.19)</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>4.1 × 10^6</td>
<td></td>
</tr>
<tr>
<td>ts 122</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>± (0.15)</td>
<td>+</td>
<td>0</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 106</td>
<td>K</td>
<td>+</td>
<td>+</td>
<td>+ (0.33)</td>
<td>+</td>
<td>50</td>
<td>A</td>
<td>5.0 × 10^2</td>
<td></td>
</tr>
<tr>
<td>ts 108</td>
<td>K</td>
<td>+</td>
<td>+</td>
<td>+ (0.38)</td>
<td>+</td>
<td>500</td>
<td>N+A</td>
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<tr>
<td>ts 119</td>
<td>K</td>
<td>+</td>
<td>+</td>
<td>+ (0.31)</td>
<td>+</td>
<td>500</td>
<td>N</td>
<td>9.0 × 10^2</td>
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</tr>
<tr>
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<td>ND</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
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</tr>
<tr>
<td>ts 120</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>1.3 × 10^3</td>
<td></td>
</tr>
<tr>
<td>ts 123</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>0</td>
<td>3.0 × 10^3</td>
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<tr>
<td>ts 124</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ts 126</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>4.0 × 10^2</td>
<td></td>
</tr>
<tr>
<td>P (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ts 103</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>ts 104</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>ND</td>
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</tr>
<tr>
<td>ts 116</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>1000</td>
<td>N+A</td>
<td>1.0 × 10^6</td>
</tr>
<tr>
<td>F (−)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>ts 115</td>
<td>E</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>−</td>
<td>1000</td>
<td>A</td>
<td>2.0 × 10^3</td>
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<tr>
<td>ts 125</td>
<td>E</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1000</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>N+A</td>
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<tr>
<td>ts 107</td>
<td>J</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>500</td>
<td>N+A</td>
<td>1.7 × 10^3</td>
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<tr>
<td>ts 112</td>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1000</td>
<td>N+A</td>
<td>2.7 × 10^4</td>
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</table>

* Soluble antigens: +++, amount of antigen identical to wild-type (WT) production; +, amount of antigen reduced by comparison with WT; ±, minute amount of antigen; −, no antigen detectable or measurable. Values in brackets are percents of WT hexon production at 39.5 °C, quantified by two-dimensional immunoelectrophoresis.

† Morphology of intranuclear particles visible on cell section: N, normal aspect; A, altered morphology.

‡ Minimal ratio of yields from cultures infected with a m.o.i. of 10 f.f.u./cell at both temperatures and harvested at 36 h (39.5 °C) and 92 h (33 °C) p.i.

§ Symbols: H (−), hexon-minus; P (−), penton-minus; F (−), fibre-minus mutants.

|| ND = not determined.

Table 1, two of our three DNA negative ts mutants of adenovirus 2 belonged to the same complementation groups, whereas ts 111 was in a different group. These three DNA-negative ts mutants were tested with the adenovirus type 5 ts mutants 36 and 125. Ts 111 was complemented by both H 5 ts mutants and was therefore different from them. In contrast, there was a very low complementation efficacy between H 5 ts 36 and H 2 ts 105 or 114, suggesting that the same function was mutated for these three mutants (Table 2).
Nine hexon-minus mutants were analysed by complementation and four complementation groups emerged from the crosses (Table 3). *Ts* 102, 110, 117 and 122 which produced minute amounts of hexon antigen, belonged to the same group (B), whereas *ts* 106 and 108, producing appreciable, although reduced, quantities of hexon, were in another group (K). The *ts* 118 (H) and *ts* 121 (A) which complemented each other and the other hexon-defective *ts* mutants appeared in two different groups: both of them failed to synthesize hexon antigen (Fig. 1d).

**DISCUSSION**

We have presented here the phenotypic characterization of 25 *ts* mutants of human adenovirus type 2, among which 19 could be assigned to 11 complementation groups. They were analysed with respect to their synthesis of virus DNA, soluble antigens and capsid morphogenesis. Their biological properties are summarized in Table 4. The interest of the serological characterization is obviously limited: most of the *ts* mutants appeared abnormal in production of soluble capsid antigen(s), but some of them exhibited a normal two-dimensional immunoelectrophoretic pattern. However, these antigens were normally produced in excess by cells infected with adenoviruses and some change in the over-production of the capsid antigens did not necessarily signify that the thermosensitive lesion was located on this particular antigen. Similarly a normal pattern of antigens does not imply a functional integrity of these antigens. In addition, since labile capsid intermediates could fall apart during extraction (Edvardsson et al. 1976), virion morphogenesis was analysed by electron microscopy of virus particles in cell sections. According to the soluble antigen pattern in two-dimensional immunoelectrophoresis, three serological classes could be discerned. In class I, there was a global and homogeneous reduction of all the virus antigens. In class II, there was a drastic reduction of one (or several) soluble antigen(s). In class III, the antigenic pattern was similar to that of WT.

The first class contained three DNA-negative mutants, whose minor antigen production represented either a certain degree of leakiness of the system, or the result of transcription and translation of the parental genomes. Biochemical analysis of these three early mutants, H 2 *ts* 105, 111 and 114, arranged in two complementation groups (Table 1), confirmed previous findings that the onset of adenovirus DNA replication was not required to shut-off of host DNA synthesis (Ensinger & Ginsberg, 1972). H 2 *ts* 105 and H 2 *ts* 114, partially depressed host DNA synthesis at restrictive temperature, while the H 2 *ts* 111 maintained this host DNA synthesis shut-off at 39.5 °C to the same extent as WT (to be published). This suggested that virus DNA replication and cellular DNA synthesis inhibition were independent and dissociable functions. However, it must be considered that host DNA synthesis inhibition may simply be due to the leakiness of all the DNA-negative *ts* mutants examined to date.

Our three mutants H 2 *ts* 105, 111 and 114 were complemented by H 5 *ts* 125 efficiently, suggesting that their mutated functions were different from that involved in H 5 *ts* 125 (Ginsberg & Young, 1977). By contrast, H 5 *ts* 36 complemented poorly both *ts* 105 and *ts* 114 (Table 2), suggesting a lesion of a similar function. However, since H 5 *ts* 36 inhibits almost totally the host DNA synthesis at 39.5 °C (Wilkie et al. 1973), which was not the case for either H 2 *ts* 105 or H 2 *ts* 114, it is possible that their lesions are in fact different. Other DNA-negative mutants of Ad 2 have been isolated and characterized recently (Kathmann et al. 1976): H 2 *ts* 206 is complemented by H 5 *ts* 125 and H 5 *ts* 36 with a high efficiency, and does not inhibit the host DNA synthesis. Maintenance of H 2 *ts* 206 for 4.5 h at 32.5 °C results in normal synthesis of virus DNA during the subsequent
incubation phase at 39.5 °C, whereas the synthesis of H 2 ts 105 and H 2 ts 114 DNA is
inhibited upon shift-up, whatever the time of incubation at permissive temperature.
H 2 ts 206 appears therefore as an earlier mutant than H 2 ts 105 and H 2 ts 114.

In the serological class II, the hexon-defective mutants could be arranged in four comple-
mentationation groups, and divided into three serological subclasses, according to their
immunological patterns. In the light of recent observations (Ginsberg & Young, 1977), it
was scarcely surprising that, using a complementation index of greater than or equal to 10,
four complementation groups were found for the hexon-defective ts mutants. Hexon being
a multimeric protein, intracistronic complementation are possible and have been suggested
(Williams et al. 1974). The low complementation efficacy between mutants of groups B
and K appears to support this hypothesis. Preliminary biochemical data suggested that
ts 121 (A) was mutated on the hexon polypeptide gene, whereas ts 118 (H) was altered on the
hexon assembly (unpublished results). The other hexon-defective mutants of both groups
B and K, which complemented poorly (ts 102, 110, 117 and 122, and ts 106, 108 and 119)
might be tentatively gathered in a unique group. However, the properties of the mutants
of groups B and K were significantly different (Table 4), which justifies the arrangement
in two separate groups for the moment.

Among the other late ts mutants of the serological class II, H 2 ts 104 and H 2 ts 115,
which were respectively penton-defective and fibre-defective, assembled their capsids at
restrictive temperature (Table 4). This phenomenon was reminiscent of fibre-minus mutants
of phage-φ 29, which can assemble without their head-fibres (Reilly et al. 1977), and
suggested that the penton-fibre structure was not indispensable for adenovirus assembly.
Fibre-defective ts mutants of adenovirus 5 also assemble capsids (Edvardsson et al. 1978).

The serological class III mutants contained three mutants with normal patterns of
antigens, and abundant intranuclear particles of both normal and abnormal features
(Table 4). These mutants, H 2 ts 101, ts 107 and ts 112, appeared to be altered in a late
function required for capsid assembly and/or virus maturation. This was the case for
H 2 ts 112 which has been found to be blocked at a stage of assembly preceding the encap-
sidation of virus DNA; this mutant accumulates light intermediate particles of density
1.315 (D'Halluin et al. 1978).

As already mentioned (Russell et al. 1972), one of the main interests in these ts mutants
of adenovirus type 2, a representative of Rosen's subgroup III (Rosen, 1960), is the possi-
bility of preparing major or minor virus components not normally accessible to investiga-
tion: the fibre-defective mutant H 2 ts 115 is thus used in our laboratory for isolation of
penton base which accumulates in the absence of fibre and of antigen IIIa (Everitt et al.
1973) which is usually masked by complete penton in two-dimensional immunoelectro-
phoretic patterns.

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G. R. MARTIN AND OTHERS

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