Polyoma Virus Basic Proteins

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SUMMARY

Polyoma virus particles contain three small basic polypeptides. These appear to be host cell histones which can be synthesized in mouse cells before infection. Comparison of peptides of polypeptides derived from [35S]-methionine labelled virus with those from mouse cell histones showed correspondence of two major and several minor peptides. It is concluded that these polypeptides are therefore not virus coded.

INTRODUCTION

The virus particles of polyoma and SV40 have been studied extensively. In both viruses a DNA molecule of $3 \times 10^6$ molecular weight is enclosed in a capsid made up of several different polypeptides. Up to six components are observed when the polypeptides are separated according to size by gel electrophoresis (Murakami et al. 1968; Fine, Mass & Murakami, 1968; Kass, 1970; Hirt & Gesteland, 1971; Girard, Marty & Suarez, 1970; Estes, Huang & Pagano, 1971). Depending on the exact molecular weights found for the individual polypeptides the total molecular weight of polypeptide varies from 140,000 to 180,000. Since the DNA of the virus could code for only 200,000 daltons of polypeptide, the component proteins of the virus particle would saturate three quarters of the virus genome. This seems a very high figure compared with other viruses and raises the possibility that this may be an overestimate for one or both of two reasons. First, the polypeptides may not all be entirely different, some perhaps being derived from parts of others, for example, as a result of cleavage by proteolytic enzymes. Secondly, some of the polypeptides may be coded for by the host cell and not by the virus. In this paper the second possibility has been examined for some of the minor components of the polyoma virus particle.

METHODS

Cells. Mouse embryo secondary cultures were grown in high serum medium in 80 oz. roller bottles or in 90 mm. plastic Petri dishes (NUNC).

Media. Cells were grown in Dubecco's modification of Eagle's medium E4 supplemented with 10% calf serum (high serum medium) or 0.5% calf serum (low serum medium). E4 containing one fifth the concentration of lysine (E4-L), or one fifth the concentration of methionine (E4-M) was used for incorporation of radioactive amino acids.

Virus. A small plaque strain of polyoma virus was used (Crawford, Crawford & Watson, 1962).

Radioactive compounds. DL-lysine-4, 5-[3H]-n. (10-8 C/m-mole), and L-lysine-[14C]-U (312 mc/m-mole) were obtained from the Radiochemical Centre, Amersham, [35S]-
methionine (approximately 20 c/m-mole) was prepared by a modification of the method described by Sanger, Bretscher & Hocquard (1964). *Escherichia coli* B was grown in 10⁻⁶ M sulphate plus 2 mc/ml. carrier free [³⁵S]-sulphate (from the Radiochemical Centre, Amersham), hydrolysed with 6 N-hydrochloric acid, and methionine isolated by paper chromatography.

Proteins. Ovalbumin and DNase I were obtained from Sigma Chemical Company. RNase A and Trypsin-TPCK from Worthington Biochemical Corporation. Calf thymus histone was prepared by the method of Johns. *et al.* (1961).

Other materials. Acrylamide (electrophoresis grade), NN'-methylenebisacrylamide, and NNN'N'-tetramethylethylenediamine were obtained from Eastman Kodak. Guanidine-HCl was purified by the method of Nozaki & Tanford (1967). Bio-Gel A-5M was obtained from Calbiochem Ltd. Thin layer chromatography plates (TLC-plates pre-coated CEL 300) and pre-coated plastic sheets (MN-polygram CEL 300) were obtained from Camlab. Urea solutions were deionised with ‘Amberlite’ mono-bed resin MB-1 analytical grade, obtained from BDH Chemicals Ltd. NCS solubilizer was obtained from Amersham–Searle.

Preparations and purification of radioactive virus

Infection of cells

Roller bottles. Virus was prepared from 3-day-old secondary mouse embryo cells grown in high serum medium in 8 oz. roller bottles. The medium was removed from the cells, the monolayer washed once with phosphate-buffered saline PBS (Dulbecco & Vogt, 1954) and the cells infected with polyoma virus in 10 ml. PBS at a m.o.i. of 20. After 90 min. at 37° the unabsorbed virus was removed and replaced by E4-L containing 10 % calf serum. [³H]-lysine (0.25 μc/bottle) was then added, the bottles gassed with CO₂ and rotated at 37° for 5 to 6 days.

90 mm. plastic Petri dishes. Three-day-old secondary mouse embryo cells were transferred to low serum medium on 90 mm. plastic Petri dishes by the method of Fried & Pitts (1968). After 4 days the medium was removed, the cells washed with PBS and infected with polyoma virus in 1.0 ml. PBS at a m.o.i. of 50. After 90 min. at 37° the unabsorbed virus was removed and replaced by E4-M medium containing 10 % calf serum. After 16 hr at 37° [³⁵S]-methionine (50 μc/plate) was added and the plates were left at 37° in a humidified CO₂ incubator for 6 days.

Pre-label experiment. Three-day-old secondary mouse embryo cells on 90 mm. plastic Petri dishes were transferred to E4 medium without lysine supplemented with 0.5 % calf serum by the method of Fried & Pitts (1968); [³H]-lysine (50 μc/plate) was added and the plates incubated for 2 days at 37°. The radioactive medium was removed, the plates washed with PBS, fresh low serum medium with lysine added, and the plates incubated for a further 2 days at 37°. The medium was removed, the cells washed with PBS, and infected with polyoma virus in 1.0 ml. PBS at a m.o.i. of 50. After 90 min. at 37° the unabsorbed virus was removed and replaced by high serum medium. The cells were harvested after 6 days at 37°.

Isolation of radioactive virus from infected cells

Polyoma virus was isolated from infected cells by the method described by Crawford (1969). Virus was purified by two cycles of equilibrium centrifugation in RbCl density gradients. Where indicated the virus was treated with DNase (100 μg./ml.) for 1 hr at 37° and then with RNase A (100 μg./ml.) for a further hour at 37° between the two cycles of
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The incubation mixtures were layered on to 4 to 20% sucrose gradients containing tris buffer (0.05 M, pH 8.0). The gradients were spun at 30,000 rev./min. for 30 min., the bands collected, and again banded on RbCl density gradients.

Virus dissociation.

With sodium dodecyl sulphate (SDS): Preparations of purified, radioactive polyoma virus were dissociated by heating for 8.5 min. at 80°C in sodium carbonate buffer (0.05 M, pH 10.5) containing 2% SDS and 1% mercaptoethanol. Other conditions used were heating for 2 hr at 37°C in 0.01 M-tris buffer, pH 7.0 containing 2% SDS and 1% mercaptoethanol, or heating for 3 hr at 37°C in 0.1 M-tris buffer, pH 8.6 containing 2% SDS and 1% mercaptoethanol and carboxymethylated by incubating for a further hour at 37°C with 0.3 M-iodoacetamide.

After dissociation the preparations were dialysed three times against 11. 0.1% SDS, 0.1% mercaptoethanol and 0.01 M-phosphate buffer, pH 7.0.

With urea: Preparations of purified radioactive virus were dissociated at pH 3.8 by heating for 10 min. at 80°C in 0.9 N-acetic acid containing 8 M-urea and 1% mercaptoethanol.

Separation of polypeptides

On SDS-polyacrylamide gels: polyacrylamide gels were prepared by mixing together acrylamide and NN'-methylenebisacrylamide and polymerising with NNN'N'-tetramethylethlenediamine and ammonium persulphate in the presence of SDS. The final concentrations were: acrylamide 10% (w/v), NN'-methylenebisacrylamide 0.26% (w/v), NNN'N'-tetramethylethlenediamine 0.06% (w/v), SDS 0.1%, ammonium persulphate 0.08% (w/v) and 0.1 M-phosphate buffer, pH 7.0. The gels were polymerised in 16 x 0.8 cm. glass tubes and pre-electrophoresed for 2 to 4 hr at 8 to 10 mA/tube in 0.075 M-phosphate buffer, pH 7.0 containing 0.1% SDS. Samples for electrophoresis were made 25% with glycerol, bromophenol blue was added, the solutions mixed thoroughly, and layered on top of the gels. The reservoirs were filled with fresh buffer and electrophoresed at 15 v for 2 hr and then 40 v (8 to 10 mA/tube) for 18 to 20 hr.

On urea gels: 7.5% acrylamide gels were prepared as described above. The final concentrations used to form the gels were acrylamide 7.5% (w/v), NN'-methylenebisacrylamide 0.2% (w/v), urea 2.5 or 6.0 M, NNN'N'-tetramethylethlenediamine 0.4% (w/v), 0.9 N-acetic acid, ammonium persulphate 0.4% (w/v). The gels were pre-electrophoresed with 0.9 N-acetic acid containing 2.5 or 6.0 M-urea until the current fell to a constant low level. Samples for electrophoresis were prepared as described above, except that basic fuchsin was the marker dye. The electrophoresis buffer was 0.9 N-acetic acid (Panyim & Chalkley, 1969).

Gel containing radioactively labelled polypeptides were extruded in 1 mm. sections through a fine wire mesh in a gel fractionator of the type described by Maizel (1966) and collected in scintillation vials. Each fraction was treated as follows: dried at 37°C overnight, 0.5 ml. of 90% NCS (Amersham–Searle) added, heated in a sealed vial at 50°C overnight, scintillation mixture (10 ml.) containing 0.5% PPO and 0.03% POPOP in toluene added, and after 24 hr in the dark counted in a Packard scintillation spectrometer (Zaitlin & Harharasubramanian, 1970). Using this method more than 75% of the radioactivity applied to the gels was recovered.

Background levels of radioactivity were determined for each gel on gel sections taken just in front of the dye markers. Settings for double isotope counting on the same samples were obtained using the channels ratio method (Hendler, 1964). The spill-over of [14C] or [35S] counts into the [3H] channel was determined for these settings by fractionating and counting
similar gels containing either\textsuperscript{35}S-methionine or \textsuperscript{14}C-lysine labelled histones, electrophoresed as described above.

\textbf{Isolation of histones.} Three day old secondary mouse embryo cells were transferred to low serum medium by the method of Fried & Pitts (1968). After 4 days at 37\textdegree \textsuperscript{C} the medium was replaced with E4-L or E4-M containing 10 \% calf serum and the cells incubated at 37\textdegree \textsuperscript{C} for a further 7 to 9 hr. \textsuperscript{14}C-lysine, \textsuperscript{3}H-lysine (2 \muCi/plate) or \textsuperscript{35}S-methionine (25 \muCi/plate) was added and the cells incubated for a further 40 hr. The radioactive medium was removed, the cells washed with 0.13 M-NaCl, 0.005 M-KCl, 0.001 M-MgCl\textsubscript{2}, suspended in the same solution, and sedimented (750g for 5 min. at 4\textdegree \textsuperscript{C}). The pellet was resuspended in a solution of 0.01 M-KCl, 0.75 M-MgCl\textsubscript{2}, 0.01 M-phosphate buffer, pH 6.8, the suspension was allowed to stand for 10 min. at 4\textdegree \textsuperscript{C} and then the cells were broken with a Dounce type homogenizer. The suspension was adjusted to 0.25 M-sucrose, the nuclei sedimented (750g for 5 min. at 4\textdegree \textsuperscript{C}), resuspended in a solution of 0.25 M-sucrose, 0.01 M-KCl, 0.75 M-MgCl\textsubscript{2}, 0.01 M-phosphate buffer, pH 6.3, 0.3 \%(v/v) Triton X100 and sedimented. This was repeated and then the nuclei were extracted at 4\textdegree \textsuperscript{C} successively with 0.14 M-NaCl, 0.01 M-phosphate buffer, pH 6.3; 0.08 M-NaCl, 0.02 M-EDTA, 0.001 M-MgCl\textsubscript{2}, 0.1 M-tris buffer, pH 8.0; 0.25 N-H\textsubscript{2}SO\textsubscript{4}; 0.4 N-H\textsubscript{2}SO\textsubscript{4}.

The acid extracts were combined, spun at 20,000g at 4\textdegree \textsuperscript{C}, and the clear supernatant removed. Ethanol (10 vol.) was added and the suspension left at \textminus 20\textdegree \textsuperscript{C} overnight to precipitate the histones. The histones were collected by centrifugation at 4\textdegree \textsuperscript{C}, washed with 90 \% ethanol, followed by ethanol, and dried in a vacuum desiccator over KOH pellets. The histones were either dissolved in 0.01 M-phosphate buffer, pH 7.0, 2 \% SDS, 1 \% mercaptoethanol, heated at 37\textdegree \textsuperscript{C} for 2 hr and dialysed against 0.01 M-phosphate buffer, pH 7.0 containing 0.1 \% SDS and 0.1 \% mercaptoethanol or dissolved in 0.9 N-acetic acid containing 0.1 \% mercaptoethanol.

\textbf{Separation of polypeptides on guanidine agarose columns.} \textsuperscript{35}S-methionine labelled polyoma virus was dissociated by heating at 80\textdegree \textsuperscript{C} for 10 min. in 0.1 M-tris buffer, pH 8.6 containing 6 M-guanidinium chloride and 0.1 M-mercaptopoethanol. After 4 hr at room temperature the pH was adjusted to 6.5 with dilute phosphoric acid and sucrose (10 \%) blue dextran 2000 (0.3 \%) and DNP-alanine (0.2 \%) were added.

A 6 \% agarose column, pre-equilibrated with the eluting solvent, 0.1 M-phosphate buffer, pH 6.5 containing 6 M-guanidinium chloride and 0.1 M-mercaptopoethanol, after 4 hr at room temperature the pH was adjusted to 6.5 with dilute phosphoric acid and sucrose (10 \%) blue dextran 2000 (0.3 \%) and DNP-alanine (0.2 \%) were added.

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Production and separations of tryptic peptides. Samples were oxidized with performic acid, lyophilized twice, suspended in 0.05 M-ammonium carbonate, pH 9.0 and trypsinized overnight at 37\textdegree (50 \mu g. TPCK-trypsin per sample). The samples were again lyophilized twice, suspended in 10 \% isopropanol and electrophoresed for 2 hr on cellulose coated polygram.
sheets in pyridine acetate buffer (0.5% pyridine, 5% acetic acid) at pH 3.5. Radioactivity was located by radioautography on Kodirex KD-54T film. Each band was then scraped off, eluted with 10% pyridine, lyophilized and transferred to cellulose TLC plates. After chromatography in n-butanol:pyridine:acetic acid:water (150:120:30:120) (Burns & Turner, 1967) the peptides were again located, eluted and electrophoresed as described above but in pyridine acetate at pH 6.5. Alternatively two other chromatographic systems were used, isobutanol:pyridine:water (35:35:30), and isoamylalcohol:pyridine:water:ethanol:acetic acid (35:35:30:10:2.5) (Burns & Turner, 1967).

Electron microscopy. Samples of virus DNA were examined by the direct lysis method, a modification of the technique of Kleinschmidt & Zahn (1959) and the formamide method of Lee, Davis & Davidson (1970) developed in conjunction with Dr E. A. C. Follett, Institute of Virology, Glasgow. Virus samples from equilibrium density gradients were dialysed into 0.05 M-tris buffer, pH 8.0 and SDS added to a final concentration of 5% in a final volume of 3 to 5 μl. Lysis occurred almost immediately, releasing the DNA from the virus particles. After 1 min. at 20° the following were added in order to the final concentration stated, cytochrome (100 μg./ml.), tris buffer (0.01 M, pH 7.5), EDTA (0.01 M) and formamide (40%, v/v) in a volume of 50 μl. This solution was spread slowly down a clean glass ramp on to a freshly prepared hypophase of 0.01 M-tris buffer, pH 7.5 containing EDTA (mM) and 10% formamide. The film was picked up after 1 min. on to carbon coated platinum mounts and dehydrated with two changes of absolute alcohol. After rotary shadowing with platinum:palladium (80:20) at an angle of 14°, the mounts were examined in a Siemens Elmskop 101 at 40 kV. Micrographs were taken at an instrumental magnification of 10,000 and 20,000× and enlarged photographically to 60,000×.

The DNA molecules were examined to determine their configuration and classified as circular (supercoiled or open) virus DNA, or linear (host) DNA. The lengths of representative open circular and linear molecules from each fraction were also determined by measuring their contours either on the prints, or on the image displayed from the plate in an enlarger, with a map measurer.

RESULTS

Separation of virus polypeptides by gel electrophoresis

A typical separation of polypeptides from polyoma virus by SDS phosphate gel electrophoresis is shown in Fig. 1. Separation here is primarily dependent on size. Lysine labelled virus gave rise to six peaks with a small amount of material, assumed to be aggregates, migrating more slowly than the main capsid polypeptide (1). Methionine labelled virus showed the same peaks except that polypeptide 5 was absent. Additional purification of the virus by treatment with DNase, RNase and sucrose gradient centrifugation between the two cycles of RbCl equilibrium density gradient centrifugation did not affect the pattern observed. The same pattern was obtained whether the virus was dissociated with SDS plus mercaptoethanol or dissociated and then carboxymethylated. Polypeptides 4, 5 and 6 have molecular weights between 15,000 and 20,000 by comparison with known proteins. Fig. 2 shows a similar gel of a mixture of [3H]-lysine labelled virus and [14C]-lysine labelled histones from mouse embryo cells. DNA synthesis had been stimulated in the latter by adding serum to serum depleted cultures before adding the radioactive lysine. Polypeptides from the histones migrated with virus polypeptides 4, 5 and 6. A minor histone component migrated with polypeptide 3 and there was also a more slowly migrating histone which did not correspond to any virus polypeptide.

The migration of virus polypeptides and mouse cell histones on acid urea gels where
Fig. 1. Two preparations of polyoma virus, one labelled with $[^3H]$-lysine and one with $[^35S]$-methionine, were mixed and dissociated by heating at 80° for 8·5 min. in sodium carbonate buffer (0·05 M, pH 10·5) containing 2 % SDS and 1 % mercaptoethanol. The mixture of polypeptides was dialysed, electrophoresed on a 10 % polyacrylamide SDS gel and the gel fractionated and counted as described in Methods. These virus preparations had been treated with RNase and DNase and purified by an additional sucrose density gradient centrifugation step as described in Methods.


Fig. 2. Histones labelled with $[^14C]$-lysine were extracted from secondary mouse embryo cells and treated with SDS and mercaptoethanol as described in Methods. The histones were mixed with $[^3H]$-lysine labelled polyoma virus that had been dissociated and dialysed as described in the legend to Fig. 1. The mixture was electrophoresed on a 10 % polyacrylamide SDS gel and the gel fractionated and counted as described in Methods.

Fig. 3. Histones labelled with [14C]-lysine were extracted from secondary mouse embryo cells and dissolved in 0.9 M acetic acid and 0.1% mercaptoethanol as described in Methods. The histones were mixed with [3H]-lysine labelled polyoma virus dissociated by heating at 80°C for 10 min. in 8 M urea, 1% mercaptoethanol at pH 3.8. The mixture was electrophoresed on a 7.5% polyacrylamide gel containing 6 M urea and the gel was fractionated and counted as described in Methods.

--- [3H]-virus, --- [14C]-histone.

Separation depends on charge as well as size is shown in Fig. 3. The resolution of the various polypeptides is less clear than on SDS gels but again there is a strong similarity between the most rapidly migrating polypeptides from the two sources. Therefore the similarity of these virus derived polypeptides and cell histones extends to both size and charge. In addition the histones include more slowly migrating components which do not seem to correspond to virus polypeptides.

Pre-labelling experiments

Mouse embryo cells were labelled for two days with lysine, chased with non-radioactive lysine for 2 days and then infected with polyoma virus. The virus produced by these cells contained radioactive lysine but the distribution of radioactivity in the various polypeptides was different from that obtained by labelling after infection (Fig. 4 and Table I). Some radioactivity appears in polypeptide I of the virus showing that, as usual with animal cells, the chase was not completely effective. If we assume that the label in polypeptide I is due to the inefficiency of the chase plus protein turnover, then we can calculate the amount of radioactivity expected in other polypeptides from the same source. The amount of radioactivity in the small polypeptides is greatly in excess of that expected from the inefficiency of the chase plus protein turnover. The excess of label in the small polypeptides must indicate either that they are made before infection, or that for some unexplained reason, they are preferentially labelled by incorporation of amino acids from polypeptides made before infection and subsequently broken down and reutilized.
Fig. 4. Polyoma virus was isolated from mouse embryo cells labelled with [3H]-lysine before (b) or after (a) infection. The virus was dissociated by heating at 80° for 8.5 min. in 0.05 M-sodium carbonate buffer, pH 10.5, containing 2% SDS and 1% mercaptoethanol. The polypeptides were dialysed, electrophoresed on 10% polyacrylamide SDS gels and the gels fractionated and counted as described in Methods. Total amounts of the radioactivity in the fractions indicated are summarized in Table 1.

**Virus fractionation experiments**

It is now necessary to exclude two possible explanations which could account for the finding of host derived polypeptides in virus preparations without implying that they are part of the virus. First, that these polypeptides are associated with DNA free empty particles or secondly, that the host polypeptides are carried only by pseudovirions, particles which contain host DNA and not virus DNA (Michel, Hirt & Weil, 1967; Winocour, 1967). Empty particles can be separated physically from full particles and, in the preparations used in the experiments described above, had been removed almost entirely during purification. However, it has not been possible to obtain polyoma preparations entirely free of pseudovirions, although this had been done with SV40 (Levine & Teresky, 1970).

Although virions cannot be obtained completely free of pseudovirions, some separation can be obtained by equilibrium density gradient centrifugation. Fractions of virus of different density were obtained and examined by electron microscopy to determine what fraction

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Percentage of total radioactivity</th>
<th>Cells labelled before infection</th>
<th>Cells labelled after infection</th>
<th>Expected</th>
<th>Observed</th>
<th>Expected</th>
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<tr>
<td></td>
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<td>Cells labelled before infection</td>
<td>Cells labelled after infection</td>
<td>Expected</td>
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<tr>
<td>1</td>
<td>17.3</td>
<td>60.7</td>
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<td>(2316)</td>
<td>2316</td>
<td>(1)</td>
</tr>
<tr>
<td>3</td>
<td>4.8</td>
<td>3.7</td>
<td></td>
<td>142</td>
<td>645</td>
<td>4.5</td>
</tr>
<tr>
<td>4, 5, 6</td>
<td>61.3</td>
<td>14.6</td>
<td></td>
<td>560</td>
<td>8190</td>
<td>14.6</td>
</tr>
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Data from Fig. 4. Radioactivity in the fractions shown in Fig. 4 expressed as percentage of the total radioactivity recovered from each gel. In the case of the virus from cells labelled before infection the amount of radioactivity expected in each peak has been calculated assuming the distribution of radioactivity would be the same as from the virus grown in cells labelled after infection, normalized on polypeptide 1 and expressed as counts/min.
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Fig. 5. [3H]-lysine labelled polyoma virus, extracted from infected secondary mouse embryo cells, was purified by equilibrium density gradient centrifugation on RbCl gradients. Full and empty particles, and the region of intermediate density between the two bands were collected separately. These were centrifuged to equilibrium again in RbCl density gradients, the bands fractionated by collecting drops from the bottom of the tubes and the radioactivity of an aliquot from each fraction determined. The results are shown in the top half of the figure; density increases from right to left. The fractions were grouped as indicated and dialysed against 0.05 M tris buffer, pH 8.0 at 4°C to remove RbCl. Samples were taken and dissociated by heating at 80°C for 8.5 min. in 0.05 M-sodium carbonate buffer, pH 10.5 containing 2% SDS and 1% mercaptoethanol. The mixture of polypeptides was dialysed, electrophoresed on 10% polyacrylamide SDS gels, the gels fractionated and counted as described in Methods. The percentage of the total radioactivity found in polypeptides 4, 5 and 6 was determined for each fraction. Samples of the same fractions were lysed with SDS and the DNA examined by electron microscopy as described in Methods to determine the percentage of linear and circular DNA. The results obtained are shown in the bottom part of the figure. The lengths of the linear DNAs were also measured, and since they did not vary significantly from fraction 1 to fraction 6 of the full particles the number of molecules of each type can be directly related to DNA mass. The percentage of internal proteins ○—○, the percentage of linear DNA □—□ and the percentage of circular DNA △—△.

of DNA was linear, i.e. host, as opposed to circular and therefore viral. The particles from the same fractions were dissociated and the percentage of the total radioactivity in the small polypeptides determined (Fig. 5). These polypeptides are confined to the DNA-containing particles and absent from those empty particles which are completely devoid of DNA particles (fractions 19 to 27) showing that the first possible explanation is not tenable.

If the small polypeptides were associated only with the host DNA carried by pseudovirions, and not with virions, the percentage radioactivity in them would be expected to parallel the percentage of linear DNA and, as shown in Fig. 5, it does not. The total amount of polypeptides 4, 5 and 6 in the virus increased with increasing density, whereas the fraction of linear DNA fell. Each pseudovirion would have to contain increasing amounts of these polypeptides as the density increased if these polypeptides were to be contributed entirely
Fig. 6. [35S]-methionine labelled polyoma virus was dissociated by heating in 0.1 M-tris buffer, pH 8.6 containing 6 M guanidinium chloride and 0.1 M-mercaptoethanol. The pH was adjusted to 6.5 with dilute phosphoric acid and the polypeptides were separated on a 6% agarose column and the radioactivity of each fraction determined. The arrows show the void volume as indicated by blue dextran 2000 on the left, and the position of DNP-alanine on the right. Acid soluble polypeptides were extracted from the fractions indicated as described in Methods.

by pseudovirions. The presence of additional protein would cause a decrease in their density, in fact the opposite of what was observed. Both these arguments make it more likely that most of polypeptides 4, 5 and 6 is contributed by virions. Pseudovirions may also contain them, but since they are a minority of the particles they can only contribute a small fraction of the total.

**Tryptic fingerprints**

The general similarity of some of the mouse cell histones and the basic polypeptides from the virus need not mean that they are identical polypeptides, although this would be the simplest explanation for the results of the prelabelling experiment. The pattern of peptides obtained by digestion of polypeptides with trypsin depends on the amino acid sequence of the polypeptide. Therefore, if similar peptides are obtained from polypeptides from different sources, this is good evidence for their having similar amino acid sequences. Trypsin cuts the polypeptide at lysine and arginine residues, so that each tryptic peptide has a carboxy terminal lysine or arginine, except for the carboxy terminal peptide of the original polypeptide.

Acid-soluble polypeptides from methionine labelled polyoma virus were isolated by gel filtration on agarose (Fig. 6). About 70% of the radioactivity in this fraction corresponded with histones (Fig. 7). These virus polypeptides were oxidized with performic acid and digested with trypsin in parallel with methionine labelled histones from secondary mouse embryo cells. The resultant peptides were then separated by electrophoresis and chromatography and compared with each other. The acid-soluble polypeptides both from virus and cell contain very little methionine, consistent with their being histones, and this has the advantage of giving very simple fingerprints, each polypeptide probably containing two or less methionine residues and giving rise to the corresponding number of tryptic peptides. The two major and
several minor tryptic peptides from the mouse cell histones and from the acid-soluble virus polypeptides showed identity through electrophoresis at pH 3.5 and chromatography (Fig. 8). Due to the effect of different loading on the mobility of peptides in the electrophoretic separation some of the corresponding peptides were found in adjacent rather than parallel bands, e.g. those in 4 v and 5 m. For the two major peptides this identity was confirmed by electrophoresis at pH 6.5, and chromatography in a different solvent (isobutanol:pyridine:water). The tryptic peptides which corresponded included the major peptides from both sources. Minor peptides which did not correspond were present in the virus derived material, probably due to small amounts of other proteins extracted by acid in the fractionation procedure. Additional peptides were present in the histone fingerprints and it is assumed that these are derived from other histones, for example, those migrating more slowly in Fig. 2, 3 and 7.

It may be argued that the correspondence of the tryptic peptides is not of any significance since they might be only di- or tripeptides and could easily occur by chance in any polypeptides compared. Peptides larger than this would be increasingly unlikely to correspond fortuitously. Chromatography of the major peptides on Sephadex G10 and G25 showed that they were both longer than octapeptides since they eluted before an angiotensin marker.

**DISCUSSION**

The polypeptide compositions of the polyoma virus particles found by different groups are now in good general agreement. The main capsid protein comprises about three quarters of the total protein and has a molecular weight of between 40,000 and 50,000. The number and size of the minor components vary rather more, but most authors have found a group of small polypeptides with molecular weights less than 20,000. The evidence presented here
Fig. 8 (a)

Fig. 8 (b)
that these polypeptides correspond to a class of histones acquired from the host cell comes from three sets of experiments. First, the two groups of polypeptides are generally similar in size and charge, as shown by their migration on SDS and acid urea polyacrylamide gels. Secondly, radioactive lysine incorporated into protein before infection was transferred to some polypeptides of the virus particle much more efficiently than to others. Polypeptides 4, 5 and 6 were relatively over fourteen times more heavily labelled than polypeptide 1, the main capsid protein. This is consistent with polypeptides 4, 5 and 6 being made before infection and later incorporated intact into virus particles without breakdown and resynthesis. The origin of polypeptide 3 is less clear, partly because it was present in small and rather variable amounts from 2 to 4% of the total lysine radioactivity. Its specific activity was much higher in virus from cells labelled before infection than after infection. The most straightforward interpretation would be that polypeptide 3 is, like 4, 5 and 6, acquired from the host but that its behaviour in prelabelling experiments is to some extent obscured by other material migrating in the same position on the gels. For example, if polypeptide 3 were behaving like polypeptides 4, 5 and 6 but 4% of the coat protein had been broken down to material migrating with polypeptide 3, then the ratio of observed to expected radioactivity would have been changed from over 14 (the same as 4, 5 and 6) to 4:5 (the figure actually observed, Table 1). As shown in Fig. 2 a minor histone component migrates with virus polypeptide 3 and is therefore a possible candidate for incorporation into the virus. Clearly further experiments will be needed to decide whether this polypeptide is derived from the host or is virus coded.

In the third set of experiments tryptic peptides were studied to give information on the amino acid sequence of the polypeptides. Polypeptides 4 and 6 appear to contain methionine and the information derived from the study of tryptic peptides refers only to these and not to polypeptide 5. The identity of two major and several minor tryptic peptides from a mixture of acid soluble virus polypeptides with those from cellular histones confirms that the sequence of the relevant polypeptides is similar, at least around the methionine residues. The major methionine containing tryptic peptides were found to be more than eight residues in length and therefore comprise a significant fraction of the polypeptides from which they come. From the histone amino acid sequences which have been published it can be seen that they would give rise to methionine containing tryptic peptides 14 or 15 residues in length (De Lange et al. 1969; Iwai, Ishikawa & Hayashi, 1970), representing more than 10% of the total histone sequence. The properties of the tryptic peptides studied here would be consistent with their also being about this size.

If it is accepted that the small basic polypeptides observed in virus preparations correspond to a class of host cell histones, there is still the possibility that they are carried by empty particles or pseudovirions rather than by virions. Empty particles are easily excluded, since they could be separated physically from full particles and were shown not to contain any significant amount of the relevant polypeptides. The amount of the basic polypeptides in fractions of full particles of different density correlated well with the fraction of the DNA which was circular and derived from virions. This strongly suggests that virions do contain

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Fig. 8. Radioautograms of peptides run on TLC.
Tryptic peptides from virus-derived acid soluble polypeptides and from cell histones were electrophoresed at pH 3.5. The bands, numbered 1 to 7 from origin to cathode, were eluted separately and rerun on TLC in n-butanol:pyridine:acetic acid:water (Fig. 8a) or isoamylalcohol:pyridine:water:ethanol:acetic acid (Fig. 8b). Major bands showing correspondence of virus derived (v) and cell derived (m) peptides are indicated by large arrows and minor peptides by small arrows. Each of the figures is a composite of radioautograms from two TLC plates.
these polypeptides. Particles containing linear DNA (pseudovirions) may also contain these polypeptides. Since pseudovirions comprise less than one third of the virus particles they could in any case contribute only a minority of the polypeptides studied. In the case of SV40, a virus physically very similar to polyoma virus, preparations free of pseudovirions can be obtained and these contain about the same proportion of small polypeptides as the preparations of polyoma virus studied here (Levine & Teresky, 1970; Estes, Huang & Pagano, 1971). Taken together all this evidence would seem to rule out pseudovirions as a major source of the small polypeptides.

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REFERENCES


Polyoma virus basic proteins


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