Simultaneous Production of Mouse Endogenous Virus and Rous Sarcoma Virus by Schmidt–Ruppin Virus Infected Mouse Cells

By MOSHE KOTLER, ISRAELA LERER, ZOHAR BEN MOYAL AND GAD SPIRA*

Laboratory for Molecular Virology and *Chanock Center for Virology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

(Accepted 2 February 1978)

SUMMARY

Schmidt–Ruppin Rous sarcoma virus infected chick cells injected into newborn C3H/f mice gave rise to tumours at the site of inoculation. These tumours were transplantable in adult C3H/f mice and were able to induce tumours in the wing of adult Leghorn chickens. Tumour cells from the 18th passage in mice were used to establish a cell line in tissue culture (C3HSR). These cells released C-type virus particles that produced foci and were able to propagate in chick cells. Cloning of the C3HSR cells demonstrated that the same cell expressed both avian and murine antigens. Mouse cells infected with virus released by C3HSR cells produced murine leukaemia virus-like particles as revealed by the reverse XC syncytial test and by immunofluorescence tests.

INTRODUCTION

Infection of mammalian cells both in vivo and in vitro with avian sarcoma virus (ASV) in most cases produces no progeny virus. The possibility that infection of mammalian cells is followed by activation of leukaemogenic-like viruses of mammalian origin was supported by Svec and his co-workers (1974). They showed that myeloid leukaemias developed in Sprague Dawley rats inoculated with ASV, in addition to the sarcomatous tumours. The rat sarcoma cells were found to produce virus which induced sarcomas in chickens, while the rat leukaemic cells produced particles which were able to induce leukaemias in rats and also a few tumours in chickens. However, the leukaemogenic agent released after injection of ASV was not characterized and its origin is not clear.

Spontaneous production of virus early in life by certain mouse strains is genetically controlled as is the inducibility of endogenous viruses from mouse cells in tissue culture by halogenated pyrimidine (Lowry et al. 1971; Rowe, 1972; Rowe & Hartley, 1972; Aaronson & Stephenson, 1973; Stephenson et al. 1973).

The present study demonstrates that inoculation of Schmidt–Ruppin virus-infected chick cells into C3H/f newborn mice gives rise to mouse tumour cells capable of expressing antigens of both avian sarcoma and mouse leukaemia viruses and of producing C-type particles. The nature of the released virus is discussed.
METHODS

Animals. C3H/1f mice were obtained from the Weizmann Institute, Rehovot. New inbred Leghorn chickens were obtained from the Hebrew University, Jerusalem.

Cells and viruses. All cells were grown in monolayers using Eagle’s basal medium (G-13, Grand Island Biological Company) supplemented with 5% foetal calf serum and 10% tryptose phosphate broth (Difco); this was designated growth medium. Primary and secondary cultures from mouse embryos were prepared by the same technique used to prepare chick embryo fibroblasts (CEF). These procedures as well as passaging and infection of cells were previously described (Kotler, 1971; Kotler et al. 1972). The Harvey strain of mouse leukaemia virus (H-MLV), the mouse leukaemia sarcoma complex MSV(H-MLV), and the B77 strain of avian sarcoma virus (ASV) were obtained from Professor H. M. Temin (University of Wisconsin). Schmidt–Ruppin strain Rous sarcoma virus subgroup D (SRV-D) was kindly supplied by Dr R. A. Weiss (Imperial Cancer Research Fund Laboratories, London). Avian myeloblastosis virus (AMV) and feline leukaemia virus (FeLV) were kindly supplied by Dr J. Gruber (Office of Program Resources and Logistics, National Cancer Institute, Bethesda, Maryland). The Moloney MLV (M-MLV) was grown on clone 1 cells (NIH Swiss derived cell line) kindly supplied by Dr A. Panet (The Hebrew University, Jerusalem, Israel).

Purification of virus. Culture fluids were harvested twice a day, pooled, clarified and then centrifuged at 25000 rev/min for 50 min in the R-30 Beckman rotor to sediment the virus particles. Pellets were resuspended in growth medium and centrifuged through 15% (w/v) sucrose on a 65% (w/v) sucrose cushion prepared in TNE buffer (0.01 M-tris-HCl, 0.001 M-EDTA, 0.1 M-NaCl, pH 8.1) for 1 h at 45000 rev/min in the Beckman SW 50-1 rotor at 4 °C. Virus collected dropwise from the interphase was diluted and layered on to linear 15 to 65% (w/v) sucrose gradients and centrifuged for 3 h at 38000 rev/min in an SW 41 rotor. Fractions were collected dropwise from the bottom of the tube.

Simultaneous detection test. Unlabelled medium was harvested from C3HSR cell cultures (see below) and the particles purified as described above. The particles banding at a density of 1.16 g/ml were collected, treated with 0.02% Nonidet P-40 (NP-40), and incubated in vitro for 10 min with the three deoxynucleoside triphosphates and 3H-TTP (sp. act. 50 Ci/mmol; The Radiochemical Centre, Amersham, U.K.) as described by Kotler et al. (1972). The RNA genomes on which DNA molecules were synthesized were extracted by treatment with 5% Sarcosyl (Sigma Chemical Co.) in the presence of 2% diethyl polycarbonate (Baycovan, Bayer, Leverkusen, Germany) and 100 μg/ml of yeast RNA, and layered on to 15 to 30% sucrose gradients in TNE buffer. The gradients were centrifuged in the Beckman SW 50-1 rotor for 100 min at 45000 rev/min at 4 °C (Spiegelman et al. 1970).

Reverse XC cell test. Cell cultures to be tested for the production of murine leukaemia viruses were removed from the plates with 0.25% (w/v) trypsin (Difco), counted and the desired number of cells in 0.5 ml of growth medium was plated over a layer of 1 x 10^6 XC cells in 5 cm Petri dishes (Nunc, Denmark). After 1 h of incubation, the XC cultures were overlaid with growth medium and incubated for 4 days at 37 °C. The cells were then fixed with methanol, stained with gentian violet solution and the syncytia were counted microscopically (Niwa et al. 1973).

Antisera. Antisera against disrupted SRV-D and FeLV were prepared in rabbits as follows: the virus was lysed with NP-40 followed by ether extraction and aeration with nitrogen. Antisera were obtained by injecting rabbits four times at 10-day intervals. The virus preparation (150 to 200 μg) was emulsified with Freund’s complete adjuvant prior to immunization.
Avian viruses produced by infected mouse cells

Antiserum to the 30000 mol. wt. protein of M-MLV (anti-M-MLV p30) was prepared in rabbits as previously described (Spira et al. 1974).

Preparation of cell extracts for complement fixation. Confluent cell monolayers were washed twice with PBS, pH 7.2. The cells were removed with a rubber policeman, washed and a 20% (v/v) suspension was prepared in PBS. The cell suspension was frozen and thawed twice; sonicated for 45 s in a water bath sonicator and centrifuged at 25000 g for 45 min at 4 °C in the R-30 Beckman rotor. The supernatant fluid was tested by complement fixation (CF) for the presence of virus antigen(s) (Spira et al. 1974). Two units of complement and four units of antiserum were employed, and fixation was carried out overnight at 4 °C. The antigens (cell extracts) in the CF test were standardized against anti-SRV-D, anti-FeLV and anti-M-MLV p30 sera. The protein concentration was determined by the Lowry (Lowry et al. 1951) method.

Immunofluorescence. The indirect immunofluorescent method described by Hilgers et al. (1972) was used. In brief, cells were removed with trypsin, washed twice in PBS and placed on coated slides. The cells were fixed with acetone for 15 min, air dried and stored at -70 °C until used. Rabbit anti-SRV-D and anti-M-MLV p30 sera were used. Fluorescein-conjugated goat anti-rabbit globulin (Meloy Laboratories, Inc., Springfield, Va) was used for the detection of specific antigens.

Polyacrylamide gel electrophoresis. Electrophoresis was done in 7.5% acrylamide gels (10 × 0.6 cm), in the presence of 0.1% sodium dodecyl sulphate (SDS) in 0.1 M-phosphate buffer, pH 7.2. Purified virus samples from sucrose gradients were dissociated by boiling for 3 min at 100 °C in the presence of 0.5% SDS, 0.1% mercaptoethanol and 0.5 M-urea. The radioactivity in 1 to 2 mm gel slices was determined. Bovine serum albumin, chymotrypsinogen and lysozyme with mol. wt. of 68000, 25700 and 14300 respectively were used as markers.

RESULTS

Establishment of the cell line

Subcutaneous injections of 1 × 10⁶ CEF transformed by SRV-D into newborn C3H/f mice gave rise to tumours after approx. 60 days in about 40% of the mice (7 out of 19 mice survived for more than 2 weeks after the injection). The tumours were cut up with sterile scissors into pieces of about 0.1 mm³, suspended in phosphate buffered saline and injected subcutaneously into C3H/f adult mice at 10 to 15 day intervals. After 18 passages in mice, tumour cells were transplanted into the wing web of five adult Leghorn chickens and gave rise to tumours after 4 to 6 weeks in all of the inoculated chickens.

Tumour cells, after 18 transplantation passages in C3H/f mice, were also used to establish the C3HSR cell line. The cells were transferred at weekly intervals at a density of 4 × 10⁶ cells per 5 cm Petri dish. Experiments described in this paper were done in C3HSR cells at passage levels 8 to 32. The karyotypes of the C3HSR, C3H/f and chick cells were analysed. It was found that the C3HSR karyotype is similar to that of C3H/f mouse cells. There was no indication of chick chromosomes in the C3HSR cells.

Expression of virus antigens in C3HSR cells

To determine whether C3HSR cells contain virus antigens of both the ASV group and mouse leukaemia virus, the cells were tested by the complement fixation test. Table 1 shows the CF titres and the protein concentration of cell extracts at the highest dilution used in the CF tests. Embryonic C3H/f cell extracts did not react with anti-SRV-D serum although low titres were obtained using anti-M-MLV p30 serum. C3H/f cells infected with
Table 1. C3HSR cells tested by complement fixation*

<table>
<thead>
<tr>
<th>Cell extract</th>
<th>Anti-SRV-D</th>
<th>Anti-M-MLVp30</th>
<th>Anti-FeLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3HSR</td>
<td>16-32 †</td>
<td>32-64</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>(75) ‡</td>
<td>(60)</td>
<td>(60)</td>
</tr>
<tr>
<td>C3H/f</td>
<td>0</td>
<td>16-32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>(&gt;475)</td>
<td>(180)</td>
<td>(118)</td>
</tr>
<tr>
<td>C3H(H-MLV)§</td>
<td>8</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>(1000)</td>
<td>(31)</td>
<td>(31)</td>
</tr>
<tr>
<td>CEF</td>
<td>16-32</td>
<td>&gt;0</td>
<td>&gt;0</td>
</tr>
<tr>
<td></td>
<td>(150)</td>
<td>(440)</td>
<td>(440)</td>
</tr>
<tr>
<td>Ch (SR)II</td>
<td>128</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(40)</td>
<td>(&gt;640)</td>
<td>(640)</td>
</tr>
</tbody>
</table>

* Two units of complement and four units of antiserum were employed. Fixation was carried out at 4 °C for 18 h.
† CF titre.
‡ Protein concentration (µg/ml) of cell extracts.
§ C3H/f cells infected with H-MLV.
|| CEF infected with SRV-D.

H-MLV [C3H(H-MLV)] had a titre of 256 when tested against both anti-M-MLV p30 and anti-FeLV sera. Uninfected chick cells and chick cells infected with SRV-D gave comparable results with their respective antisera (anti-SRV-D). The positive results obtained with normal chick cells may be due to the fact that chick cells positive for the avian retrovirus group specific antigen (gs +) were used. C3HSR cell extracts gave positive results with all three antisera used in the test, indicating that these cells expressed both avian and mammalian virus antigens.

C3HSR cells were cloned to determine whether both ASV and MLV antigens are expressed in the same cell. Three different C3HSR clones were subcloned and one subclone from each was tested by the indirect immunofluorescence technique. Immunofluorescence was obtained with each subclone against anti-AMV serum diluted 1/200 to 1/400 and against anti-M-MLV p30 serum diluted 1/200. There was no reaction with normal rabbit serum diluted 1/25. Clone I mouse cells productively infected with M-MLV were used as a control. Immunofluorescence was only obtained against anti-M-MLV p30 serum at a dilution of 1/800. CEF infected with B77 virus reacted only with anti-AMV serum diluted 1/200.

Production of type-C particles by the C3HSR cells

Studies on virus production by ASV-transformed mammalian cells have given diverse results (Gelderblom et al. 1970; Simkovic, 1972). In most cases the ASV-transformed mammalian cells do not produce virus although each one of the transformed cells contains the virus genome (Altaner & Temin, 1970; Kotler, 1971). However, it was found that tumours produced in vivo by injection of ASV into rats were able to produce virus particles (Altaner & Svec, 1966; Simkovic, 1972; Svec et al. 1974). Since the C3HSR cells expressed virus-specific antigens related to both SRV-D and M-MLV, it was of interest to determine whether this cell line also produces virus particles.

C3HSR cells in growth medium were labelled with 3H-glucosamine. The media were harvested twice a day and analysed under conditions which permit the isolation of C-type particles (see Methods). Labelled particles from C3HSR cell cultures banded at a density
Avian viruses produced by infected mouse cells

Fig. 1. Sucrose gradient and polyacrylamide gel analysis of SRV-D from (a) CEF, (b) M-MLV from clone 1 mouse cells and (c) virus from C3HSR cells. Each culture was labelled for 3 days with 5 µCi/ml of 3H-glucosamine (sp. act. 10 to 25 Ci/mmol; The Radiochemical Centre, Amersham) in 10 cm Petri dishes (Nunc). The viruses were centrifuged in sucrose gradients (a, b, c) as described in Methods. The radioactivity (●—●) in each fraction and the density (○—○) in certain fractions from the gradient were determined. Samples from each of the purified virus samples were treated with SDS, 2-mercaptoethanol and urea and analysed in polyacrylamide gels (a1, b1, c1). 125I-labelled M-MLV p30 antigen (□—□) was used as a marker.

of 1.16 g/ml (Fig. 1 c) which is the density of SRV-D grown in chick cells (Fig. 1 a) and M-MLV grown in mouse clone 1 cells (Fig. 1 b). Similar results were also obtained with C3HSR cells labelled with 3H-uridine. It should be noted that the virus band obtained from C3HSR cells (Fig. 1 c) is wider than that obtained with SRV-D (Fig. 1 a) or M-MLV (Fig. 1 b) suggesting that the C3HSR virus population is not homogeneous.

In addition, 3H-uridine-labelled particles from C3HSR cells with a density of 1.16 g/ml were found to contain 70S RNA similar to that of SRV-D (not shown). The particles released by C3HSR cells were further tested for the presence of the reverse transcriptase and the ability of the enzyme to synthesize DNA strands on 70S RNA (simultaneous detection test). Some of the radioactively labelled DNA molecules, synthesized in vitro by the reverse transcriptase present in the particles, also had a sedimentation coefficient of 70S (Fig. 2). In the presence of RNase, this complex was not detected (not shown). These results indicated...
that the C3HSR cells produced particles resembling retroviruses with a density of 1.16 g/ml, a 70S RNA genome, and the specific reverse transcriptase enzyme which synthesizes DNA on the RNA template.

The glycoproteins of the virus(es) produced by C3HSR cells

Since the glycoproteins of the avian and the murine C-type particles differ in their mol. wt. [84,000 for avian (gp84) and 70,000 for the murine viruses (gp70)], the glycoproteins of the virus particles produced by C3HSR cells were compared with the glycoproteins of SRV-D grown in CEF and M-MLV grown in clone 1 cells. The 3H-glucosamine labelled virus particles, purified in sucrose gradients (Fig. 1a, b, c) were collected and further analysed by polyacrylamide gel electrophoresis. The profiles obtained with SRV-D gp84 (Fig. 1a) and M-MLV gp70 (Fig. 1b) are similar to those described before (Bolognesi et al. 1974). The virus obtained from C3HSR cells showed both gp84 and gp70 (Fig. 1c) and an additional peak with a mol. wt. of 42,000. The nature of the latter glycoprotein is not yet clear. However, it may include the 38,000 glycoprotein described in ASV and the 45,000 glycoprotein described in murine C-type particles (Bolognesi et al. 1974). The ratio between gp84 of SRV-D and gp70 of M-MLV is almost equal. The ratio of the high mol. wt. glycoproteins (gp84, gp70) to the gp42 is surprising and differs from the ratios obtained with SRV-D gp84 and gp38 and M-MLV gp70 and gp45.

We do not yet know whether C3HSR cells produce viruses coated simultaneously by glycoproteins of both avian and murine origin or whether two different virus populations coated with different glycoproteins are produced by this cell line.
Table 2. Interference induced by SRV-D after super-infection of CEF with C3HSR virus*

<table>
<thead>
<tr>
<th>Initial infection</th>
<th>Challenge infection</th>
<th>Titre (f.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock infected SRV-D</td>
<td>SRV-D</td>
<td>5×10³</td>
</tr>
<tr>
<td>u.v.-inactivated SRV-D</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>u.v.-inactivated SRV-D</td>
<td>SRV-D</td>
<td>2.4×10⁴</td>
</tr>
<tr>
<td>u.v.-inactivated SRV-D</td>
<td>C3HSR</td>
<td>1.5×10⁴</td>
</tr>
<tr>
<td>Mock infected</td>
<td>C3HSR</td>
<td>2.3×10⁵</td>
</tr>
<tr>
<td>Mock infected SRV-D</td>
<td>B77</td>
<td>3.8×10⁵</td>
</tr>
<tr>
<td>u.v.-inactivated SRV-D</td>
<td>B77</td>
<td>5×10⁵</td>
</tr>
</tbody>
</table>

* Secondary CEF were infected for 2 h with 0.5 ml of SRV-D which had been exposed to u.v. light for 15 min at a distance of 20 cm from a Germicidal 15 W u.v. lamp (General Electric). Titre before inactivation was 10⁶ f.f.u./ml. The u.v.-irradiated virus was removed and the cultures were re-infected with SRV-D, C3HSR (grown in CEF) or B77 (subgroup C) virus in the usual way and overlaid with agar. Foci were counted 5 to 6 days later.

Table 3. Detection of cells producing endogenous murine virus by the reverse XC test*

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of syncytial plaques per 1 × 10⁶ cells</th>
<th>1 × 10⁷ cells</th>
<th>% syncytia formation</th>
<th>% of cells producing virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H[MSV(H-MLV)]†</td>
<td>646</td>
<td>120</td>
<td>69.6</td>
<td>100</td>
</tr>
<tr>
<td>C3H/f</td>
<td>10</td>
<td>0</td>
<td>0.9</td>
<td>1:2</td>
</tr>
<tr>
<td>C3HSR</td>
<td>80</td>
<td>4</td>
<td>7.6</td>
<td>10:6</td>
</tr>
<tr>
<td>C3H(C3HSR)‡</td>
<td>320</td>
<td>85</td>
<td>36.9</td>
<td>52:8</td>
</tr>
<tr>
<td>Ch(C3HSR)§</td>
<td>8</td>
<td>0</td>
<td>0.7</td>
<td>1:0</td>
</tr>
<tr>
<td>Ch(SR)‖</td>
<td>6</td>
<td>0</td>
<td>0.5</td>
<td>0:7</td>
</tr>
</tbody>
</table>

* The reverse XC cell test was performed as described in Methods. Syncytia containing more than five nuclei were counted microscopically.
† C3H/f cells chronically infected with MSV(H-MLV).
‡ C3H/f cells infected with virus released from C3HSR cells.
§ CEF infected with virus released from C3HSR cells.
‖ CEF infected with SRV-D.

The biological activity of the virus(es) released by the C3HSR cells

Medium from C3HSR cell cultures was clarified and 0.5 ml was used to infect CEF or C3H/f mouse embryonic cultures. After 10 to 13 days of incubation at 37 °C, foci of transformed cells ranging from 2 to 15 f.f.u./ml appeared in 12 CEF cultures. To demonstrate that the virus released by the C3HSR cells can propagate in permissive cells, clarified medium from the transformed CEF cultures was used to further infect CEF secondary cultures. At first passage 2×10⁶ f.f.u./ml were obtained within 10 days and at second passage 4×10⁴ f.f.u./ml were obtained within 7 days. Thus the titre of the virus increased with passage, indicating that the C3HSR cells produce transforming virus.

The ASV released by C3HSR cells was further characterized by the interference test (Table 2). Secondary chick cultures were mock infected or infected for 2 h with 0.5 ml of u.v.-inactivated SRV-D (from the same stock originally used to infect the C3H/f mice). The virus was then removed and the cultures were re-infected with SRV-D, C3HSR virus or B77 virus in the usual way. Interference was obtained to about the same extent with the original SRV-D and the virus released from the C3HSR cells. No interference occurred with B77 virus from subgroup C. This shows that the released virus envelope protein is similar to that of the original virus used to induce the tumours.

Several morphological foci appeared 14 days after inoculation of C3H/f mouse cell
cultures with C3HSR virus, but these foci disappeared after passage of the cells. It is possible that these are abortively transformed cells as was the case with rat cells infected with the B77 strain of RSV (Kotler, 1971; Varmus et al. 1973; Boettiger, 1974a, b).

To determine whether the C3HSR cells produce non-transforming MLV-like particles, we used the reverse XC syncytial test. This test has been shown to detect murine leukaemia virus-producing cells (Klement et al. 1969; Niwa et al. 1973). Table 3 shows that 10.6% of the C3HSR cells produced murine leukaemia virus (MLV)-like particles as revealed by the formation of syncytia in XC cells. Assuming that 100% of the C3H/f cells chronically infected with MSV(H-MLV) produced virus, the efficiency of our test was only 69.6%. The uninfected C3H/f cells used as a control produced about 1.2% syncytia on XC cells which is the background of the test. The MLV-like particles produced by the C3HSR cells were found to be infectious for C3H/f embryonic cells in tissue culture since 52.8% of the cells produced MLV-like particles. Attempts to characterize the host range of the MLV-like particles by the reverse XC test produced negative results using NIH-Swiss, BALB/c or C57/Bl mouse cells. A similar phenomenon was reported by Rapp & Nowinski (1976) who showed that viruses produced by a non-transformed C3H cell line were not detectable by the XC plaque test. Uninfected chick cells, or chick cells infected with SRV-D, or with virus released by C3HSR cells did not produce a significant level of syncytia on XC cells. It should, however, be noted that in preliminary tests, NIH-Swiss mouse cells infected with MLV-like particles gave positive immunofluorescence against M-MLV p30 antiserum after two further passages of the infected cells in culture.

**DISCUSSION**

The relationship between ASV and mammalian retroviruses gives rise to a number of questions. Can recombinants be formed between these two groups of viruses and is it possible that phenotypic mixing takes place between ASV and mammalian viruses? To answer these questions we looked for a cell culture system in which ASV and MLV can be produced simultaneously. The present paper describes such a system.

Inoculation of SRV-D-transformed chick cells into C3H/f newborn mice produces tumours that are transplantable in mice. Transplantation of tumour cells into the wing web of chickens also produces tumours that are presumably induced by SRV-D particles released from the tumour cells. A cell line (C3HSR) was established in tissue culture from one of these tumours that had been passaged 18 times in mice. Karyotype analysis revealed that C3HSR cells were of mouse origin.

Complement fixation studies were performed with antisera against M-MLV p30 antigen, FeLV and SRV-D viruses. Examination of three different subclones of C3HSR cells by immunofluorescence showed that both avian and mammalian retrovirus antigens are produced by the same cell. Virus isolates from C3HSR cultures propagate and produce foci on chick cells. These virus particles are also infectious in C3H/f and NIH-Swiss mouse cells (as revealed by the reverse XC and immunofluorescence tests, respectively) indicating an endogenous virus that is most probably N-tropic. The virus which is infectious for CEF increases in titre with subsequent passage in chick cells. The progeny have envelope properties similar to the SRV-D that originally produced the tumours, as revealed by the interference test.

A similar system previously described is that of B77 virus-infected rat cells in tissue culture that do not produce virus particles (Altaner & Temin, 1970; Kotler, 1971). However, the same virus induced tumours in vivo that were found to produce virus (Altaner & Svec, 1966; Svec et al. 1974). Weiss & Wong (1977) were able to demonstrate efficient phenotypic
Avian viruses produced by infected mouse cells

mixing but no recombinant formation between these viruses by mixed infection of avian cells with mammalian and avian retroviruses.

The titre of the virus produced by C3HSR cells, as measured by the focus formation test or XC test, was very low. These results do not correlate with the amount of virus labelled by $^3$H-glucosamine (Fig. 1 c), indicating that the number of particles produced by the C3HSR cell line is much higher than indicated by infectivity tests. It is possible that C3HSR virus contains a mosaic of glycoproteins of both avian and mammalian viruses that may interfere with infection of both CEF and C3H/f cells. However, at present it is impossible to exclude other possibilities, namely that C3HSR produces two different virus populations and perhaps also viruses that could not be detected by the infectivity tests used. Immunoprecipitation with specific antibody against glycoprotein of SRV-D and M-MLV is presently being done to answer this question.

The glycoprotein profiles of the C3HSR virus(es) (Fig. 1) revealed two peaks that co-electrophorese with gp84 of the avian and gp70 of the mammalian viruses. These two peaks appear in about equal amounts. Of special interest is the appearance of glycoprotein in the 42 000 region. The nature of this glycoprotein and its relatively high concentration is difficult to explain. This may be a structural glycoprotein that is included in large amounts during the assembly of the C3HSR virus together with the gp70 and gp84. However, it is also possible that this small glycoprotein is a cleavage product of one or both of the high mol. wt. glycoproteins.

A tempting explanation for the results observed with the analysis of the glycoproteins is that a recombinant virus has been produced. This recombinant would have a different glycoprotein pattern and would have the biological property of being able to infect both mammalian and avian cells.

We wish to thank Professor Yechiel Becker for his advice, assistance and support throughout this study and Dr Robin Weiss for helpful and fruitful discussion. This research was supported by a grant from the United States-Israel Binational Science Foundation (BSF) and by the Ministry of Health, Jerusalem, Israel.

REFERENCES


(Received 3 June 1977)