Quantification of Newly Synthesized Virus RNA in Moloney Murine Leukaemia Virus-infected Cells

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SUMMARY

A technique for the isolation and characterization of newly transcribed murine leukaemia virus RNA in chronically infected cells has been developed. Cellular RNA was pulse labelled with 3H-uridine and virus-specific sequences were annealed with an excess of mercurated complementary DNA. Based on the affinity between mercurated cDNA and sulphydryl-Sepharose, the hybrid was specifically selected by affinity column chromatography. The specificity of this method was dependent on the purity of the cDNA and it was necessary to remove non-viral sequences from the cDNA in order to isolate virus-specific RNA. Between 0.5 and 0.8% of the labelled RNA in Moloney MuLV-infected rat cells and 1.5% of the labelled RNA in Moloney MuLV-infected NIH Swiss mouse cells were virus-specific. Using this methodology, the effect of the cell cycle on the transcriptional activity of proviral genes was investigated. Cultures of Moloney MuLV-infected rat cells arrested in G0 phase of the cell cycle released reduced quantities of virus, but continued to synthesize virus RNA. The pools of virus RNA and p30 antigen in the G0-arrested cells equalled the pools in actively dividing cells. These results suggested that post-transcriptional events controlled virus production in the G0-arrested cells.

INTRODUCTION

Cells chronically infected with retroviruses appear to contain several copies of proviral genes integrated into the host chromosome. At least some of these genes are active templates for the transcription of virus RNA. The steady-state amount of virus RNA in these cells ranges from 0.1 to 1.0% of the total cellular RNA, as measured by kinetic hybridization of total RNA to a radioactive complementary DNA probe (Fan, 1978). Since this procedure measures only the pool of virus RNA in the cell, it provides no information concerning the kinetics of RNA synthesis. In recent years, several procedures have been developed to measure newly synthesized virus RNA by the hybridization of pulse labelled cellular RNA to unlabelled cDNA followed by specific selection of the hybrids formed (Parsons et al. 1973; Coffin et al. 1974; Jacquet et al. 1974; Bishop et al. 1976; Fan, 1977; Shih et al. 1977; Parsons et al. 1978; Bromley et al. 1979). Most of these procedures have the disadvantage of requiring ribonuclease treatment to reduce the background of non-specific hybridization or pre-selection of the RNA on the basis of size or poly(A) content.

We have developed a method to isolate and quantify the amount of newly synthesized virus RNA present in cells which does not employ ribonuclease. This method is based on the ability of mercurated polynucleotides to be selectively and reversibly bound by SH (sulphhydril)-Sepharose (Dale et al. 1973, 1975; Dale & Ward, 1975; Nguyen-Huu et al. 1978).
Thus, mercurated virus cDNA was hybridized with radioactively labelled cellular RNA and the virus RNA which hybridized was isolated on columns of SH-Sepharose. Using this novel methodology, we investigated the metabolism of virus RNA in Moloney murine leukaemia virus (MuLV)-infected mouse and rat cells.

Synchronization of MuLV-infected cells at the G₀ phase of the cell cycle by serum depletion has been shown to result in inhibition of virus production (Paskind et al. 1975; Panet & Cedar, 1977). Since the primary level of control for many cellular genes has been attributed to transcription, it was of interest to compare the transcriptional activity of the proviral genes in G₀ phase cells to that of dividing cells which produce large amounts of virus. We found that the amounts of virus-specific RNA synthesized in G₀-arrested and in growing cells were comparable, suggesting that the block in virus production was post-transcriptional.

**METHODS**

**Materials.** Deoxynucleotide triphosphates and polynucleotides were purchased from PL-Biochemicals (Milwaukee, Wisconsin, U.S.A.). ²H-uridine and ³H-thymidine were obtained from the Nuclear Research Center, Negev, Israel, and ³H-dTTP was from the Radiochemical Centre, Amersham, U.K. Ribonucleases A and T₁ were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) and Proteinase K was obtained from Merck (Darmstadt, Germany). Pancreatic deoxyribonuclease (DNase I) was the product of Worthington Biochemical Co. (Freehold, New Jersey, U.S.A.). ³H-thymidine-labelled SV40 DNA was the kind gift of Dr Roy Barzili. Sulphydryl-Sepharose was prepared from Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by Cuatrecasas (1970) and contained 0.78 μmol of sulphydryl groups per ml of packed Sepharose.

**Cells and viruses.** The NIH Swiss mouse cell line infected at endpoint dilution with Moloney-MuLV (M-MuLV; Clone 1 cells) was described by Fan & Paskind (1974). Normal rat kidney cells (NRK) and NRK cells infected at endpoint dilution with M-MuLV and later cloned were the kind gift of Dr Ellen Rothenberg. Cells were grown in Dulbecco's modified Eagle's medium and 10% calf serum, as previously described (Panet & Cedar, 1977). M-MuLV was isolated from the growth medium of Clone 1 cells and purified by sucrose gradient centrifugation as described (Fan & Baltimore, 1973).

**Preparation of cDNA.** Endogenous reactions (10 ml) contained 50 mM-tris-HCl (pH 8.3), 15 mM-MgCl₂, 60 mM-NaCl, 10 mM-dithiothreitol, 50 μg/ml actinomycin D, 0.0125% Triton X-100, 1 mM each of dTTP, dGTP, dCTP and dATP and 1 mg/ml purified M-MuLV. To estimate the efficiency of cDNA synthesis, radiolabelled cDNA was synthesized in a parallel reaction containing, in addition to the above components, ³H-dTTP (167 μCi/ml). The reactions were terminated by the addition of SDS to 0.5%, EDTA to 25 mM and 200 μg/ml Pronase. After 30 min at room temperature the reaction mixtures were extracted twice with phenol:chloroform (1:1) and once with chloroform:isoamyl alcohol (1:24), and precipitated three times by adding 2 vol. of ethanol. The nucleic acids were suspended in 10% piperidine and incubated at 50 °C for 18 h to hydrolyse the RNA. The DNA was lyophilized three times and resuspended in distilled water.

**Mercuration of polynucleotides.** RNA or DNA was suspended in 1.0 ml of 10 mM-mercuric acetate, 20 mM-sodium acetate, pH 6.0, and incubated for 20 h at 50 °C. Mercuration was stopped by the addition of 0.1 ml of 10 × TNE (TNE = 0.1 M-NaCl, 1 mM-EDTA, 20 mM-tris-HCl, pH 7.4), followed by dialysis against TNE, TNE containing 10⁻⁶ M-β-mercaptoethanol and, finally, TNE diluted 1:10. The mercurated polynucleotides were lyophilized and resuspended in distilled water.

**Preparation of RNAs.** Radioactively labelled 60 to 70S M-MuLV RNA and unlabelled virus RNA were isolated from purified virus as described (Fan & Baltimore, 1973). Labelled cellular RNA was obtained from cells grown in the presence of ²H-uridine (20 to 50 μCi/ml).
Virus RNA synthesis in MuLV-infected cells

The cells were trypsinized and counted, and the RNA was extracted according to the procedure of Wang et al. (1977). Briefly, cells were lysed in the presence of 0.5% SDS and proteinase K, and the DNA was sheared by passage through a 20-gauge needle. The nucleic acids were extracted three times with phenol:chloroform, once with chloroform:isoamyl alcohol and precipitated with ethanol. DNA in the samples was removed by DNase I treatment followed by phenol:chloroform extraction and ethanol precipitation. RNA from cells labelled for 21 to 24 h had specific activities of 0.2 to 1.6 × 10^6 cts/min/μg, while RNA from cells labelled for 4 h had specific activities of 0.3 to 1.5 × 10^6 cts/min/μg.

Cytoplasmic rat liver RNA was purified from cytoplasmic extracts prepared as described previously (Panet & Cedar, 1977), followed by phenol:chloroform extractions.

Hybridization conditions. Mixtures (20 to 40 μl) contained 3H-RNA (pre-heated for 3 min at 97 °C), mercured cDNA, 5 μg rat liver RNA, 50% formamide, 0.5 M-NaCl, 25 mM-Hepes buffer (pH 6.8) and 0.5 mM-EDTA. Hybridization was performed under paraffin oil for 20 h at 40 °C.

Chromatography on sulphydryl-Sepharose. Samples were diluted into 0.5 ml TNE, applied to SH-Sepharose columns (1.5 ml) and incubated for 10 min at room temperature. The columns were successively washed with TNE (10 ml), 2 M-NaCl in TNE (5 ml) and TNE (5 ml) to elute the unbound material. The bound material was eluted with 0.2 M-β-mercaptoethanol in TNE (5 ml) after 10 min incubation in the column. All the samples were precipitated with 10% trichloroacetic acid in the presence of 1 mg/ml bovine serum albumin and the precipitates collected on glass fibre filters. Radioactivity was determined by liquid scintillation counting. The percent radioactivity bound was calculated by dividing the radioactivity eluted in the presence of β-mercaptoethanol by the total radioactivity recovered from the column. Routinely, over 95% of the radioactivity applied was recovered from these columns.

Selection of virus-specific cDNA. Unlabelled cDNA was hybridized to a 100-fold excess of mercured rat liver RNA in 0.5 M-NaCl, 0.1 mM-EDTA, 10 mM-tris-HCl, pH 7.5, for 24 h at 70 °C. In parallel, 3H-labelled cDNA was similarly hybridized to the mercured rat RNA and was carried through the succeeding steps to monitor the extent of hybridization and recovery of the cDNA. The cDNA which hybridized to the mercured rat RNA (cell-specific) was separated from the unhybridized cDNA (virus-specific) by chromatography on an SH-Sepharose column. The cDNA fractions were recovered by ethanol precipitation in the presence of 200 μg/ml yeast RNA and the RNA was hydrolysed by exposure to 10% piperidine for 18 h at 50 °C. The cDNA was lyophilized three times and resuspended in distilled water. In a typical cDNA preparation, 10 to 15% were cell-specific sequences.

Virus detection. Virus production was determined by quantifying the amount of virus released into growth medium in 24 h by measuring reverse transcriptase activity in the pellet after centrifugation for 1 h at 105,000 g. Poly(rA) and oligo(dT) were used as the template and primer for the enzyme (Panet & Berliner, 1978). One unit is the amount of enzyme needed to catalyse the incorporation of one pmol of 3H-dTMP in 1 min.

Virus production was also determined by measuring the amount of 3H-uridine labelled virus released from the cells during a 4 h labelling period. The growth medium of the labelled cells was collected and after the removal of cell debris, the virus particles were collected by centrifugation for 1 h at 105,000 g. The pellet was resuspended in a Dounce homogenizer, sonicated and banded in a 4.5 ml gradient of 25 to 45% sucrose in TNE for 3 h at 35,000 rev/min in a Beckman SW 50.1 rotor. The virus band was located in a parallel gradient which contained unlabelled, purified virus, and analysed for exogenous reverse transcriptase activity. The amount of 3H-virus in each gradient was determined for each sample by measuring the acid-insoluble radioactivity of the fractions (Fan & Baltimore, 1973).
RESULTS

Mercuration of polynucleotides and affinity chromatography on SH-Sepharose

The isolation of specific RNA species from total cellular RNA requires a technique which is highly specific with a low background. We describe here the use of affinity chromatography on SH-Sepharose to specifically select hybrids of mercurated cDNA and radiolabelled virus RNA. Initially, \(^{3}\text{H}\)-SV40 DNA was used as a prototype to determine conditions for mercuration and chromatography on SH-Sepharose. A typical chromatographic profile of mercurated \(^{3}\text{H}\)-DNA is illustrated in Fig. 1. Over 95% of the mercurated DNA bound to the column and was eluted with \(\beta\)-mercaptoethanol. Under the same conditions, only 0.02% of unmercurated \(^{3}\text{H}\)-DNA bound to the column (Fig. 1).

Mercuration conditions were varied in order to achieve maximal binding of the mercurated DNA without damaging the annealing properties of the DNA. Variables which were tested included incubation time, salt concentration and mercuric acetate concentration. The degree of mercuration was estimated by the binding efficiency of the DNA to the SH-Sepharose column. After 20 h of reaction, over 97% of the DNA bound to SH-Sepharose and could be eluted with \(\beta\)-mercaptoethanol. Standard conditions of mercuration were subsequently defined as incubation in 10 mM-mercuric acetate, 20 mM-sodium acetate, pH 6.0, for 20 h at 50 °C. M-MuLV cDNA mercurated under the same conditions bound to SH-Sepharose with the same efficiency as SV40 DNA (data not shown).

Hybridization of Hg-cDNA to \(^{3}\text{H}\)-M-MuLV genomic RNA

The selection of pulse-labelled virus RNA by affinity chromatography on SH-Sepharose depended on the ability of mercurated (Hg) M-MuLV cDNA to hybridize \(^{3}\text{H}\)-labelled virus RNA. To test the hybridization properties of Hg-cDNA, unlabelled cDNA was synthesized in an endogenous reverse transcription reaction, purified and mercurated as described above. The Hg-cDNA was hybridized under stringent conditions to \(^{3}\text{H}\)-M-MuLV 70S virion RNA and the extent of hybridization was analysed by affinity chromatography on SH-Sepharose columns. In a parallel experiment, unmercurated cDNA was hybridized to \(^{3}\text{H}\)-virus RNA and annealing was analysed by digestion with ribonucleases A and T1. The extents of hybridization were similar as measured by the two methods and at saturation, 80% of the \(^{3}\text{H}\)-RNA was hybridized (Fig. 2a). It should be noted that 20% of the RNA
Virus RNA synthesis in MuLV-infected cells

Fig. 2. cDNA excess hybridization to M-MuLV 70S RNA: (a) 3H-70S RNA of M-MuLV (800 ct/min) was hybridized for 2 h to mercurated (●—●) and unmercurated (●—●) cDNA. The mercurated cDNA samples were chromatographed on SH-Sepharose and the percent of the 3H-RNA which bound to the columns was determined. The unmercurated cDNA samples were analysed by digestion with ribonucleases A (50 μg/ml) and T1 (500 units/ml) in 0.5 ml 0.4 M NaCl, 5 mM EDTA and 10 mM-tris-HCl (pH 7.5) for 45 min at 37°C. The percent hybridization was computed from the amount of RNA which remained trichloroacetic acid insoluble after ribonuclease digestion. (b) Competition hybridization: mercurated cDNA (0.05 μg) and 3H-M-MuLV 70S RNA (800 ct/min) were annealed for 20 h in the presence of increasing amounts of unlabelled virus RNA. The percent hybridization was determined by chromatography on SH-Sepharose columns.

could not be hybridized indicating either that 20 % of the RNA is in a non-hybridizable form or that the cDNA probe is representative of only 80 % of the virus genome.

In order to test the specificity of binding, Hg-cDNA and 3H-virus RNA were hybridized in the presence of increasing quantities of unlabelled virus RNA, and the hybrids were analysed for binding to SH-Sepharose (Fig. 2b). The unlabelled virus RNA was an effective competitor, showing a significant reduction in binding of the 3H-RNA to Hg-cDNA in the presence of excess unlabelled RNA, while the addition of heterologous RNA from rat liver had no effect on the binding of the 3H-virus RNA. The hybridization of 3H-RNA from MuLV-infected rat cells (cp-1) with cDNA was also competitively inhibited by the presence of unlabelled virus RNA, confirming the specificity of the hybridization (data not shown).

Specificity of the hybridization

In order to determine whether the hybridization was indeed specific for virus sequences, the M-MuLV Hg-cDNA was hybridized to 3H-labelled RNA extracted from uninfected rat cells (NRK) which contain little if any sequences homologous with the M-MuLV genome (Benveniste & Scolnick, 1973; Scolnick et al. 1974). These experiments indicated that 10 to 15 % of the cDNA represented cellular sequences. Virus- and cell-specific fractions of the cDNA were separated, mercurated and subsequently tested for their ability to hybridize with 3H-RNA from uninfected rat (NRK) cells (Fig. 3). Cell-specific cDNA hybridized the NRK RNA efficiently. An eight- to tenfold higher concentration of the unselected cDNA bound the same amount of NRK RNA as the cell-specific cDNA. This result was consistent with our previous determination that unselected cDNA contained 10 to 15 % cell-specific sequences. The mercurated virus-specific cDNA in contrast, hybridized much smaller quantities of the NRK RNA, with 0.1 to 0.2 % binding (Fig. 3). This background was effectively reduced by hybridization in the presence of rat liver RNA to a level of 0.05 to 0.06 % (Fig. 4).
Fig. 3. Hybridization of cDNAs to \(^3\)H-RNA from uninfected NRK cells. NRK cells were labelled with \(^3\)H-uridine for 24 h and the RNA was purified as described in the text. \(^3\)H-RNA (10,000 ct/min) was hybridized for 24 h with increasing amounts of total cDNA (○—○), cell-specific cDNA (□—□) or virus-specific eDNA (△—△) as in Methods. The extent of hybridization was determined by SH-Sepharose chromatography. Hybridization was also performed between unmercurated total cDNA and \(^3\)H-NRK RNA (10,000 ct/min; ●—●) and the hybrids were analysed by digestion with ribonucleases, as described in the legend to Fig. 2. A background of 0.2% ribonuclease resistance was subtracted from each sample.

Fig. 4. Quantification of newly synthesized virus-specific RNA in G0-arrested cells. M-MuLV-infected NRK cells were arrested at G0 phase by exposure to medium containing 0.5% serum for 24 h and cells were harvested by trypsinization. RNA was purified and hybridized to virus-specific Hg-cDNA and the hybrids were analysed by SH-Sepharose chromatography. ○—○, RNA (30,000 ct/min) from G0 arrested cells; ●—●, RNA (30,000 ct/min) from cells grown in the presence of 10% serum (unsynchronized). Also illustrated is the hybridization of \(^3\)H-RNA (30,000 ct/min) from uninfected NRK cells with virus-specific Hg-cDNA (△—△).

In order to test the specificity of this method further, mercurated cDNA was used to measure the steady state level of M-MuLV RNA in NIH Swiss cells. Whereas 1.5% of the RNA was virus-specific in infected cells, uninfected cells had a level of 0.08%. These data are consistent with those previously reported (Fan & Baltimore, 1973).

**Synthesis of M-MuLV RNA in infected rat cells**

To study the metabolism of M-MuLV RNA in the cell we have utilized a heterologous system of rat cells (NRK) infected with M-MuLV since M-MuLV has no apparent sequence homology with the endogenous rat virus or NRK cellular RNA (Benvenist & Scolnick, 1973; Scolnick et al., 1974). Cells were labelled with \(^3\)H-uridine for 4 or 24 h and the RNA was extracted and hybridized with increasing amounts of virus-specific Hg-cDNA. There was virtually no hybridization with \(^3\)H-RNA of uninfected cells (0.05 to 0.06%), Fig. 4 and this background was subtracted from the saturation values of all subsequent experiments. The infected NRK cells contained significant amounts of M-MuLV RNA. After 4 h of labelling, 0.8% of the RNA was found to be M-MuLV and at 24 h 0.5% was virus-specific. These data were obtained from saturation hybridization analysis after normalization for the hybridization potential of specific virus RNA (80%) as shown in Fig. 2.

**Virus RNA synthesis in synchronized cells**

The synchronization of cells chronically infected with M-MuLV has been found to reduce the amount of virus released into the growth medium (Paskind et al. 1975; Panet & Cedar, 1977). It was of interest to determine whether the block in virus synthesis was at the level of virus RNA transcription or at a later stage of virus formation. To this end, M-MuLV-
Virus RNA synthesis in MuLV-infected cells

Table 1. Virus RNA synthesis in synchronized and unsynchronized M-MuLV-infected NRK cells

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Serum (%)</th>
<th>Virus RNA synthesis (ct/min 10^6 cells)</th>
<th>Virus production</th>
<th>DNA synthesis (ct/min 10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
<td>0.94</td>
<td>1.167</td>
<td>62.607</td>
</tr>
<tr>
<td>1‡</td>
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<td>0.027</td>
<td>13.950</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
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<td>0.023</td>
<td>0.22</td>
</tr>
<tr>
<td>2§</td>
<td>10%</td>
<td>0.56</td>
<td>14.600</td>
<td>310,000</td>
</tr>
<tr>
<td></td>
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<td>670</td>
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<tr>
<td>Ratio</td>
<td></td>
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<td>0.046</td>
<td>0.105</td>
</tr>
</tbody>
</table>

* Virus RNA synthesis: cells were labelled with 3H-uridine for 4 h and total RNA was purified. The 3H-RNA was hybridized to saturation with virus-specific Hg-cDNA and the hybrids were analysed by SH-Sepharose chromatography as described in the legend to Fig. 4. The amount of RNA synthesized was calculated from the plateau value of hybridization.

† DNA synthesis: cells were labelled for 1 h with 3H-thymidine and the amount of radioactivity incorporated into DNA was determined as described by Panet & Cedar (1977).

‡ Expt. 1: cells were synchronized by exposure to 0.5% serum for 24 h in parallel with control dividing cells in 10% serum.

§ Expt. 2: cells were exposed to 5 ml of medium containing 2% serum for 48 h. Another 5 ml of medium without serum was added for an additional 48 h. The cells were harvested and processed as described in Methods.

Infected NRK cells were arrested in the G0 phase of the cell cycle by exposure to 0.5% serum for 24 h and the degree of synchrony attained was determined by measuring the relative amounts of cellular DNA synthesis. Virus release was determined by examination of the 24 h growth medium for the presence of reverse transcriptase associated with the virus. In the serum-depleted cells, the level of DNA synthesis and virus production were greatly reduced (Table 1, Expt. 1). Under these conditions the cells remained viable and the cell cycle could be resumed by adding back serum.

To measure RNA synthesis, G0-arrested cells were labelled with 3H-uridine for 4 h and the total cellular RNA was extracted. The RNAs were then hybridized with increasing amounts of virus-specific Hg-cDNA until plateau values were reached (Fig. 4). It was consistently found that the amount of labelled virus RNA was higher in G0-arrested (1.75%) than in unsynchronized (0.94%) cells (Table 1 and Fig. 4).

Using a different synchronization protocol, which involved leaving the cells in low serum for 4 days, DNA synthesis was even more efficiently reduced (Table 1). Virus production was measured more directly in this experiment, by determining the amount of 3H-uridine labelled virus particles released during the time of labelling with 3H-uridine. As in the previous experiment, virus production in the G0-arrested cells was greatly reduced while virus RNA synthesis continued unabated. It is possible that the higher values of newly synthesized RNA in the synchronized cells represented accumulation of RNA rather than increased synthesis, since virus release was inhibited.

To investigate further the metabolism of virus gene products in G0-arrested cells, the pool of virus-specific RNA was estimated by kinetic hybridization of 8H-cDNA with unlabelled cytoplasmic RNA from M-MuLV-infected NRK cells. No differences were found in the amounts of virus-specific RNA in unsynchronized and G0-arrested cells (data not shown). These results suggest that the restriction in virus production in G0-arrested cells was post-transcriptional.

To investigate the possible inhibition of virus protein synthesis, the level of p30 antigen in these cells was measured by competition radio-immunoassay (Fig. 5). The G0-arrested cells and the unsynchronized cells contained comparable amounts of p30 antigen (1.1 μg/mg
Fig. 5. Quantification of p30 antigen in M-MuLV-infected NRK cells arrested in G0 phase. Cytoplasmic extracts were obtained by the disruption of cells with 5 ml phosphate buffered saline (PBS) containing 10 mM-EDTA and 0.5 % Triton X-100 followed by centrifugation for 1 h at 27000 g to remove cell debris. The amount of p30 in the cytoplasmic fraction was determined by homologous competition radio-immunoassay (RIA) with M-MuLV, anti-p30 serum and 125I-p30 as described by Spira et al. (1974). ■—□, Synchronized cells (0.5 % serum); ▲—▲, dividing cells (10 % serum); ●—●, standard antigen, purified p30 of M-MuLV.

protein) which would suggest that the amount of the major structural protein, p30, was not the limiting factor in virus production in the arrested cultures.

DISCUSSION

In this communication a new technique for the isolation and quantification of newly synthesized retrovirus RNA is described. This technique is based on the solution hybridization of Hg-cDNA to virus RNA and the subsequent isolation of the hybrids by SH-Sepharose chromatography. A number of techniques have been described in the literature for measuring pulse labelled virus-specific RNA in retrovirus-infected cells (Parsons et al. 1973; Coffin et al. 1974; Jacquet et al. 1974; Bishop et al. 1976; Shih et al. 1977; Fan, 1978; Parsons et al. 1978; Bromley et al. 1979). With one exception (Parsons et al. 1978), the background of non-specific hybridization had to be reduced by ribonucleases, which precluded the isolation of intact RNA.

Several aspects of our technique may be compared with the method described by Coffin et al. (1974) and later modified by Parsons et al. (1978), in which cDNA was tagged at the 3' end with polycytidylic acid. After solution hybridization with virus RNA, the hybrids were reversibly bound to columns of polynosinic acid–agarose. This technique, however, is quite complex, involving the terminal nucleotidyl transferase catalysed addition of polycytidylic acid, in comparison with the relatively simple procedure of adding mercury to cDNA.

The major problem which we encountered in developing the assay was the presence of cell-specific sequences in cDNA preparations. The cDNA probe hybridized a significant portion of RNA from uninfected cells. Cellular sequences in virus cDNA have been described before (Garapin et al. 1973; Chattopadhyay et al. 1976; Baxt & Meinkoth, 1978), but since these sequences constituted a minor fraction of the probe, they posed no difficulty in hybridization experiments where the probe was not in excess. The nature of these cellular
sequences in cDNA preparations and whether they are covalently linked to virus sequences remain to be determined.

In order to eliminate the cell-specific sequences, the cDNA was hybridized to a large excess of mercurated rat liver RNA and passed through a SH-Sepharose column. For our purposes, this procedure to select hybrid molecules was superior to the commonly used hydroxylapatite column chromatography, since a portion of the cDNA molecules, although synthesized in the presence of actinomycin D, bound to hydroxylapatite columns probably by virtue of small stretches of double stranded DNA. Thus, the selection of mercurated hybrids by SH-Sepharose chromatography is a useful general method for the isolation of specific nucleic acid sequences since only hybridized molecules bind to the column.

Using the new technique, we have investigated the transcriptional activity of proviral genes and the influence of cell growth arrest on virus RNA synthesis. M-MuLV production has been shown previously to be inhibited after arresting chronically infected cells at the G₀ phase of the cell cycle (Panet & Cedar, 1977; Paskind et al. 1975). The addition of serum released the block and virus production resumed, even in the absence of RNA synthesis, suggesting the presence of a pool of virus RNA in the G₀-arrested cells (Paskind et al. 1975). It was also shown that the first wave of virus released after the resumption of cell growth contained RNA labelled during arrest; whether this was indeed virus RNA, or the encapsidation of cellular sequences (Levin & Seidman, 1979) was not demonstrated. We have shown directly that virus RNA synthesis was not impaired by growth arrest in G₀ phase. Moreover, the pools of intracellular virus RNA and p30 antigen were similar in actively dividing and G₀-arrested cultures. These results resembled those obtained after the treatment of M-MuLV-infected cells with interferon (Friedman, 1977). This agent, which does not seem to inhibit virus RNA (Fan & MacIsaac, 1978) or protein synthesis (Friedman et al. 1975; Pitha et al. 1976; Shapiro et al. 1977), reduces virus production. Whether the inhibition of virus production in G₀-arrested cells and interferon treated cells are at the same stage in the virus growth cycle has not yet been determined.

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