Journal of Virology, July 1979, p. 86-93
0022-538X/79/07-0086/08$02.00/0
Vol. 31, No. 1

New Procedure for Isolation of Rous Sarcoma Virus-Specific RNA from Infected Cells

P. A. BROMLEY,* P.-F. SPAHR, and J.-L. DARLIX
Département de Biologie Moléculaire, Université de Genève, CH-1211 Geneva 4, Switzerland

Received for publication 6 February 1979

The use of mercurated "strong stop" complementary DNA (complementary to the 5'-terminal 101 nucleotides of Rous sarcoma virus RNA) in the isolation of virus-specific RNA from infected chicken embryo fibroblasts is described. Strong stop Rous sarcoma virus complementary DNA was mercurated chemically, and, as a result of the low complexity of this DNA, short hybridization times (up to 15 min) and heating in the absence of formamide were found to be adequate conditions for the isolation of virus-specific RNA. The purity of the isolated RNA was demonstrated by analysis of labeled RNase T1-resistant oligonucleotides by two-dimensional polyacrylamide gel electrophoresis. The isolated RNA could be translated in the in vitro protein synthesis system derived from rabbit reticulocytes, and an analysis of polypeptides programmed by isolated RNA before and after immunoprecipitation further demonstrated both the purity of the isolated mRNA and the quantitative nature of the isolation procedure.

The analysis of virus-specific RNA in cells infected with RNA tumor viruses has until recently been based on an indirect approach, namely, the hybridization of radiolabeled DNA (complementary to the viral genome) to an excess of total or fractionated infected-cell RNA (see, for example, Fan and Baltimore [13]). Although limited to the analysis of steady-state levels of virus-specific RNA, this procedure has yielded extensive data concerning the quantity, size distribution, intracellular location, and sequence content of virus-coded RNAs (1, 12, 14, 16, 20, 28).

The analysis of newly synthesized virus-specific RNA has been performed by using pulse-labeling of infected-cell RNA and hybridization of this RNA to an excess of viral complementary DNA (cDNA) (22). A similar approach was used to analyze the in vitro mechanism of viral RNA synthesis, using the polymerase activity of isolated nuclei (26; Bromley, unpublished data).

Procedures for the isolation of virus-specific RNA (6, 7, 21, 23) have been slower to develop, due mainly to the relatively low levels of virus-specific RNA in the infected cell combined with the fact that RNA tumor virus infection does not turn off the synthesis of host cell mRNA's. We have published (2) the results of applying the use of mercurated cDNA (8) to the isolation of radioactive virus-specific RNA, and physical analysis of the RNA by finger-printing indicated a high degree of purity of isolated RNA, a result confirmed by others using different cDNA-based isolation techniques (7, 21, 23).

In this paper we describe the use of mercurated, so-called strong stop (SS)-cDNA of Rous sarcoma virus (RSV) for the isolation of virus-specific RNA; the low complexity of this SS-DNA (101 nucleotides [11]) has permitted the isolation of virus-specific RNA in high yield after short periods of hybridization in the absence of formamide. The isolated RNA is capable of efficiently directing in vitro protein synthesis.

The high degree of conservation of SS-DNA sequences and their presence on subgenomic viral RNAs (7, 18, 21) make this isolation procedure attractive as a general method of isolating avian tumor virus-specific RNAs.

MATERIALS AND METHODS

Cells and viruses. The LA23 mutant of RSV, which is temperature sensitive for cell transformation, was obtained from J. Wyke, Imperial Cancer Research Foundation, London. The virus was grown in secondary cultures of gs$^-$ Chf$^-$ chicken embryo fibroblasts (Valo eggs, SPAFAS Co., Cuxhaven, Germany) as previously described (9).

Isolation of RNA. Virion 70S RNA was isolated as previously described (9). Total RNA was extracted from chicken embryo fibroblast cells labeled with 200 μCi of $^{32}P_i$ (carrier-free, Radiochemical Centre, Amersham, England) per ml after phosphate starvation for 4 h; labeling was for 5 h at 41°C. Cells were lysed in 0.15 M NaCl, 10 mM Tris (pH 7.8), 1% sodium dodecyl sulfate, and 200 μg of proteinase K (Merck) per ml, and the lysate was incubated at 37°C for 15 min. The RNA was extracted twice with phenol saturated with 0.15 M NaCl-10 mM Tris (pH 7.8) and precipitated from the aqueous phase with 2.5 volumes of ethanol at −20°C. After a second precipitation with ethanol,

the nucleic acids were dissolved in 0.5 M NaCl-50 mM $MgCl_2$-2 mM $CaCl_2$-10 mM Tris (pH 8.5); pancreatic DNase (RNase-free, Worthington Biochemicals Corp.) was added to a concentration of 20 μg/ml, and the solution was incubated at room temperature for 15 min with constant pipetting. Proteinase K (50 μg/ml) and sodium dodecyl sulfate (1% final concentration) were then added, and the incubation was continued for 15 min, after which RNA was precipitated with ethanol. Polyadenylic acid [poly(A)]-containing RNA was selected by passing heat-denatured RNA twice over columns of polyuridylic acid-Sepharose (Pharmacia Fine Chemicals).

RSV DNA-polymerase reactions. The reconstructed reaction using purified avian myeloblastosis virus DNA polymerase (courtesy of J. Beard, Life Sciences Inc.) and 70S RSV RNA was used for the synthesis of SS-DNA in a 6-min reaction as previously described (11).

The endogenous DNA polymerase reaction was carried out with tissue culture-grown LA23 virus purified by differential centrifugation, after initial concentration in a Millipore virus concentration unit. The reaction conditions used were those of Cashion et al. (4). The SS-DNA was purified as described (11), and the alkali-treated DNA samples were labeled at their 5′ ends with γ-[^{32}P]ATP (1,200 Ci/mmol) and T4 polynucleotide kinase (Miles Laboratories). Labeled DNA samples were analyzed on 8% polyacrylamide slab gels in 50 mM Tris-borate-EDTA, pH 8.3 (15), as previously described (11).

Fingerprint analysis. Analysis of the products of T1 RNase digestion of ^{32}P-labeled RNA samples was performed by using two-dimensional polyacrylamide gel electrophoresis and direct autoradiography, as previously described (9).

In vitro protein synthesis. Messenger-dependent rabbit reticulocyte lysate was prepared as described (24). In vitro translation and immunoprecipitation using antiviral polymerase, anti-LA23 virus, and protein A-Sepharose (Pharmacia) were performed as previously described (11), except that rat liver tRNA was included in the translation mixture at a concentration of 0.1 mg/ml and the reaction products were analyzed on 10% acrylamide-0.25% bisacrylamide gels containing 0.1% sodium dodecyl sulfate and 0.375 M Tris, pH 8.7. ^{35}S-labeled polypeptides were detected by autoradiography of dried gels, using fluorography as described (19).

^{14}C-labeled protein markers were obtained from New England Nuclear Corp. and have the following molecular weights: phosphorylase B, 92,500; bovine serum albumin, 69,000; ovalbumin, 46,000; carbonic anhydrase, 30,000; cytochrome c, 12,300.

RESULTS

Synthesis of mercurated SS-DNA. SS-DNA was synthesized in an endogenous reaction, using tissue culture-grown LA23 RSV under the conditions described in Materials and Methods. An analysis of 5′-end ^{32}P-labeled product DNA is shown in Fig. 1 (lane 1). Although the labeled DNA migrates fairly heterogeneously on the gel, a major band is seen at the level of the 100-nucleotide position. This DNA comigrates with the SS-DNA of known sequence synthesized in a reconstructed reaction on two-

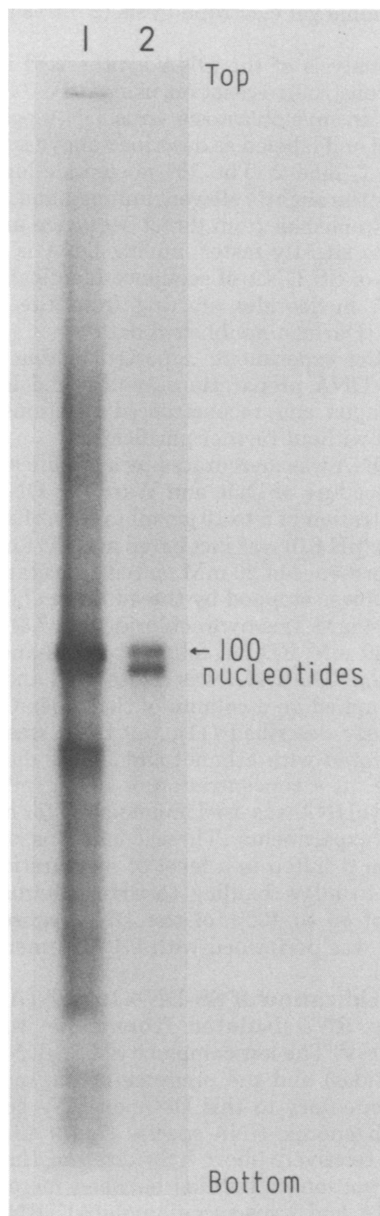

FIG. 1. *Autoradiograph of ^{32}P 5′-end-labeled DNA synthesized in (1) an endogenous DNA polymerase reaction. (2) A reconstructed reaction using LA23 virus or LA23 70S RNA (see text); the DNA was analyzed on an 8% acrylamide gel slab as described in the text, and the autoradiograph was obtained after an exposure time of 2 h. The indication of the position of a 100-nucleotide-long DNA is taken from direct sequence analysis (11).*

dimensional gel electrophoresis (25) (results not shown).

An analysis of the DNA synthesized in a 6-min reconstructed reaction, using RSV 70S RNA and avian myeloblastosis virus DNA polymerase, and end labeled as described above is shown in Fig. 1, lane 2. The 101-nucleotide-long SS-DNA is the slightly slower running band, as has been established from direct sequence analysis (11); the slightly faster moving DNA is a subspecies of SS-DNA of sequence identical to the first 85 nucleotides starting from the tRNA primer (Darlix, unpublished data).

For the experiments reported in this paper, the SS-DNA preparations synthesized in both endogenous and reconstructed reactions were utilized without further purification.

SS-DNA was mercurated by a modification of the procedure of Dale and Ward (8); DNA at a concentration of 5 to 10 μg/ml in 50 mM sodium acetate (pH 6.0) was incubated at 80°C for 7 min in the presence of 20 mM mercuric acetate. The reaction was stopped by the addition of 0.5 volume of 4 mM Tris-hydrochloride (pH 7.5)-0.4 M NaCl-40 mM EDTA, followed by ethanol precipitation. The DNA was redissolved and chromatographed on a column of Sephadex G-75 as previously described (11); the DNA was again precipitated with ethanol and finally dissolved in water at a concentration of 100 μg/ml. Mercurated cDNA was used immediately for hybridization experiments. These conditions of mercuration resulted in a level of mercuration sufficient to allow binding to SH-Sepharose columns of 80 to 100% of the DNA; subsequent elution was performed with 0.1 M β-mercaptoethanol.

Hybridization of SS-DNA to poly(A)-containing RNA isolated from cells infected with RSV. The low complexity of SS-DNA (101 nucleotides) and the presence of the sequence complementary to this DNA on RSV genomic and subgenomic RNA species (7, 18) allow the use of relatively short hybridization times for the formation of hybrids between mercurated SS-DNA and virus-specific poly(A)$^+$ RNA. We have therefore used hybridization at 67°C in the presence of 0.3 M LiCl-5 mM EDTA-10 mM Tris (pH 7.5)-2 μM β-mercaptoethanol for short time periods (0 to 15 min). These conditions were chosen to preserve both the physical integrity of the RNA and the stability of the mercurated DNA. In addition, the use of formamide can be avoided in these experiments.

Physical characterization of virus-specific RNA isolated with mercurated SS-DNA. To compare the isolated virus-specific RNA with authentic viral RNA, we have chosen to select poly(A)$^+$ RNA from the infected cell that sediments on a sucrose gradient with S values between 30 and 40; virus-specific RNA of this size class should consist mainly of full-length subunit RNA with a fingerprint essentially the same as that of virion 70S RNA.

^{32}P-labeled 30–40S poly(A)$^+$ RNA from RSV-infected chicken embryo fibroblasts was prepared as follows. Total RNA (see Materials and Methods) was fractionated by sucrose gradient sedimentation (Fig. 2), the appropriate fractions containing RNA of 30–40S were pooled as shown, and the RNA was precipitated with ethanol. The poly(A)$^+$ RNA was selected as described in Materials and Methods and concentrated by ethanol precipitation.

A total of 1.4 × 10^6 cpm of this RNA was hybridized with 1 μg of mercurated SS-DNA for 15 min at 67°C in a volume of 5 μl under the conditions described above. At the end of the reaction, 400 μl of ice-cold 0.2 M LiCl-10 mM Tris-hydrochloride (pH 8.0)-4 mM EDTA (LITE buffer) was added, and the mixture was passed over a 0.5-ml column of SH-Sepharose, which was then washed with the same buffer; the column was then washed with 2 ml of the same buffer containing 0.1 M β-mercaptoethanol to elute the mercurated SS-DNA and any ^{32}P-labeled RNA hybridized to it. Since some ^{32}P

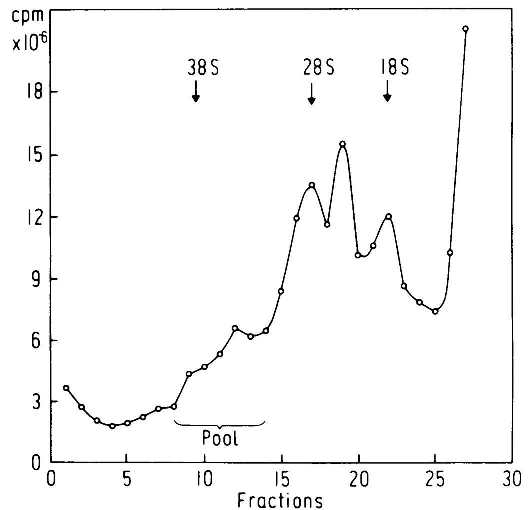

FIG. 2. *Total RNA, prepared as described in the text, was centrifuged at 37,000 rpm for 7 h at 8°C on linear 15 to 30% sucrose gradients containing 0.05 M NaCl, 0.01 M Tris-hydrochloride (pH 7.4), 0.01 M EDTA, and 0.2% sodium dodecyl sulfate. Fractions containing 30–40S RNA were selected by reference to 28S and 18S rRNA's run on a parallel gradient and pooled, and the RNA was precipitated with ethanol at −20°C.*

radioactivity was still bound to the column, a final wash with 99% formamide-10 mM Tris (pH 8) was performed. Table 1 shows the quantitative recovery of ^{32}P radioactivity in successive washes.

A sample of the RNA before hybridization and RNA from the combined flow-through and LITE buffer wash, LITE buffer-β-mercaptoethanol wash, and formamide elutions were digested with T1 RNase and fingerprinted; the results obtained, together with an authentic LA23 RSV 70S RNA fingerprint and scheme of large T1 oligonucleotides, are presented in Fig. 3. It can be seen that the RNA eluted by the LITE buffer-β-mercaptoethanol wash (Fig. 3c) gives a fingerprint very similar to that of 70S RNA (Fig. 3e). The formamide wash elutes RNA that is clearly enriched for virus specificity (see fingerprint in Fig. 3d), although some streaking and/or contaminating RNA digest products are also present. It should be noted that the virus-specific RNA fingerprint pattern is essentially invisible on the fingerprints of total (Fig. 3a) and unbound (Fig. 3b) RNA.

Thus, hybridization of infected-cell RNA to a mercurated DNA able to hybridize to the terminal 5′ 100 nucleotides of a 30-40S RNA can clearly be used to isolate intact RSV-specific RNA, as seen from the fingerprint data of Fig. 3 and as further substantiated by the translatability of isolated RNA.

Isolation of RSV-specific RNA from the infected cell and its translation in vitro. Total RNA from cells infected with the LA23 mutant of RSV was purified as described in Materials and Methods, and the poly(A)-containing RNA fraction was purified by successive chromatographies on columns of polyuridylic acid-Sepharose as described in Materials and Methods. For these experiments no size fractionation of infected-cell RNA was used other than to remove RNA and DNA fragments smaller than 8S by sucrose gradient centrifugation. Approximately 2 μg of SS-DNA (from a reconstructed reaction, see Materials and Methods) was hybridized to 200 μg of poly(A)-containing, infected-cell RNA in a volume of 100 μl under the conditions described above. At zero time (mixing at 67°C and rapid cooling on ice) and after 5 and 10 min of reaction at 67°C, samples were removed and diluted 50-fold in LITE buffer followed by chromatography on columns of SH-Sepharose as described above. The flow-through RNA and LITE wash from each time point of hybridization as well as the β-mercaptoethanol RNA eluate for each were precipitated twice with ethanol. Each RNA was further purified by G-75 Sephadex chromatography and ethanol precipitation as described (11).

A sample of each RNA was translated in the reticulocyte lysate, and the in vitro products synthesized were analyzed on sodium dodecyl sulfate-polyacrylamide gels as described in Materials and Methods. The results of the zero-time, 5-min, and 10-min flow-throughs and bound RNA translation products are shown in the autoradiogram of Fig. 4, lanes 2 to 7. Part of the in vitro translation products of each RNA sample were immunoprecipitated with antisera against total RSV and against the DNA polymerase as described in Materials and Methods, and the results obtained are shown in Fig. 4, lanes 9 through 14, respectively.

A polypeptide comigrating with Pr76 (cf. translation products from 70S LA23 RNA, Fig. 4, lane 15) is seen in the translation products of the bound and eluted RNA. Apart from the major band at about 53,000 daltons which coelectrophoresed with the major polypeptide synthesized by total infected-cell RNA (cf. Fig. 4 lane 2), one polypeptide of 60,000 molecular weight is seen in the direct isolate translations (Fig. 4, lanes 5 and 7). This polypeptide has the molecular weight expected for the product of the SRC gene (3).

Finally, the gels of the immunoprecipitates of flow-through and β-mercaptoethanol elutions at each time point (Fig. 4, lanes 9 through 14) indicate a progressive, and at 10 min essentially complete, transfer of at least the mRNA for Pr76 from flow-through to elution, indicating the quantitative nature of the isolation of active messenger by the procedure described in this paper. It should be noted that for both the physical and biological characterization of the isolated RNA, reactions were performed in the presence of the mercurated DNA after denaturing the isolated hybrids by heating at 100°C for 30 s. The presence of mercurated DNA had no

TABLE 1. *Chromatography on SH-Sepharose columns of hybridization mixtures between mercurated SS-DNA and ^{32}P-labeled 30-40S poly(A)$^+$ RNA from infected cells*

Sample	^{32}P radioactivity (cpm)	% of total
Load	1.4×10^6	100
Flow-through	6×10^5	41.4
LITE buffer wash	5.13×10^5	35.3
LITE buffer-β-mercaptoethanol	1.15×10^5	7.9
99% formamide	1.5×10^5	10.3
Total recovery		94.9
Bound fraction recovered		18.2

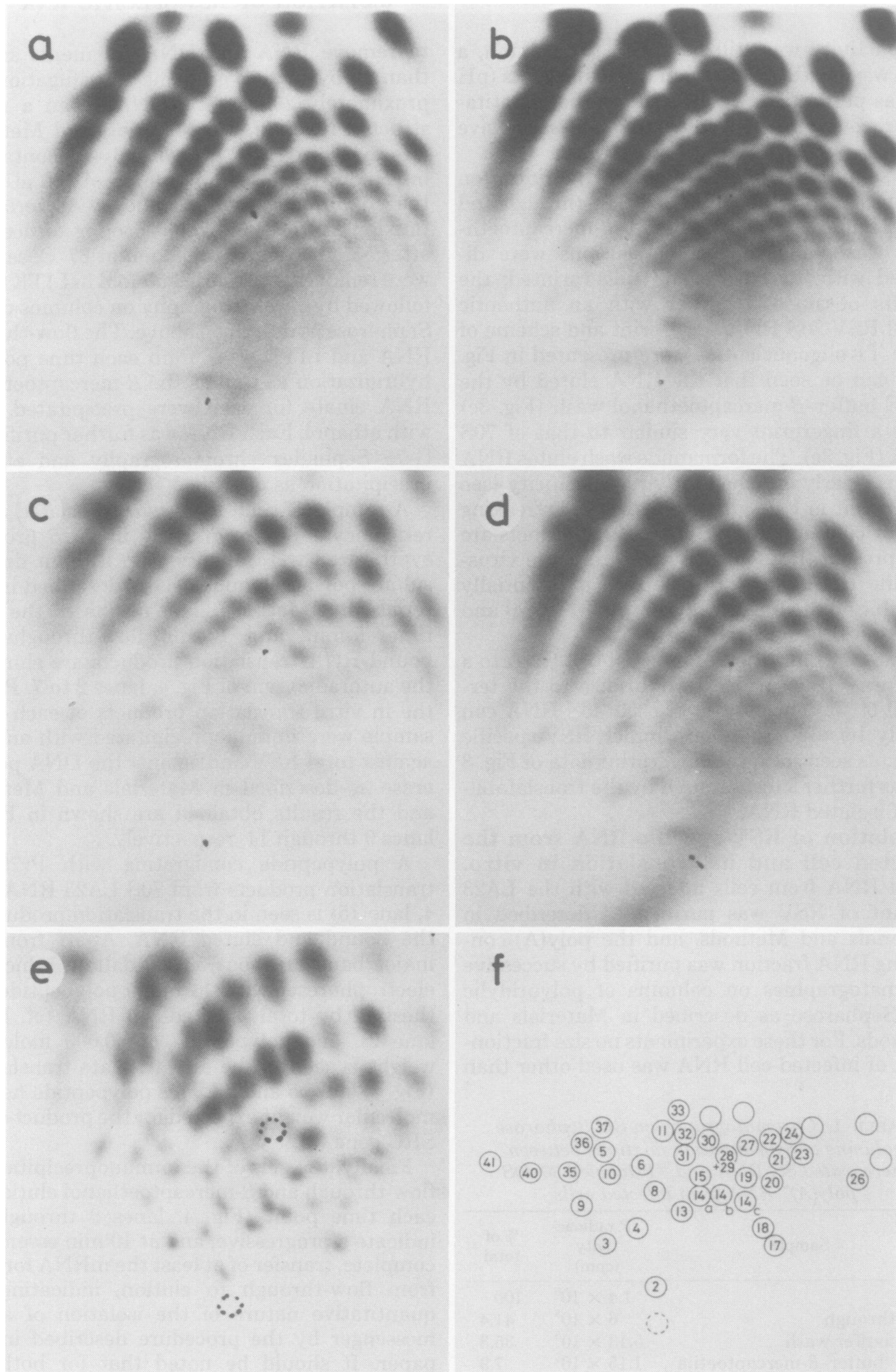

Fig. 3. *Fingerprint analysis of ^{32}P-labeled RNA samples carried out as described in the text. The nature of the samples is as follows. (a) 30–40S poly(A)$^{+}$ RNA from RSV-infected cells; (b) 30–40S RNA that flows through an SH-Sepharose column after hybridization of the RNA to mercurated SS-cDNA (endogenous reaction product); (c) 30–40S RNA bound to the SH-Sepharose column after hybridization and eluted with 0.1 M β-mercaptoethanol; (d) 30–40S RNA eluted after the β-mercaptoethanol elution, using 99% formamide at room temperature; (e) 70S LA23 viral RNA; (f) scheme of (e). The numbering system and the order with respect to the 3′ poly(A) tail and sequence analysis of large T1 oligonucleotides have been reported (10).*

inhibitory effect on either the T1 RNase digestion or the in vitro translation. If necessary, the DNA can be removed by passage of the denatured RNA-DNA hybrid over an SH-Sepharose column as described above.

DISCUSSION

The use of mercurated DNA for the isolation of complementary RNA was originally described by Dale and Ward (8). Our previous work using mercurated cDNA representative of the entire RSV genome indicated that virus-specific RNA could be isolated from infected cells (2). However, the relatively long hybridization times required to drive the reaction to completion and the use of formamide to lower the required annealing temperature did not allow the efficient isolation of biologically active mRNA. In our hands, the long exposure of mRNA to formamide has frequently reduced the biological activity of the RNA.

In this paper, the use of the mercurated SS-DNA (the sequence complexity of which represents only 1% of that of the RSV RNA genome) allowed us to carry out the hybridization reaction for short times and in the absence of formamide. Our results show that a 100-nucleotide-long DNA can hybridize to the 30-40S (10,000 nucleotides long) virus-specific RNA to form a hybrid stable enough to bind to SH-Sepharose columns. Compared with our previous work (2), this result suggests that the application of the mercurated DNA-RNA hybridization procedure to the isolation of any mRNA can be improved if a specific cDNA fragment of low complexity is available.

Until now only physical and chemical analyses have been performed on virus-specific RNA isolated by methods other than the one described here (7, 21, 23), and we are not aware of any report of template activity of such isolated RNAs. Using the method described in this pa-

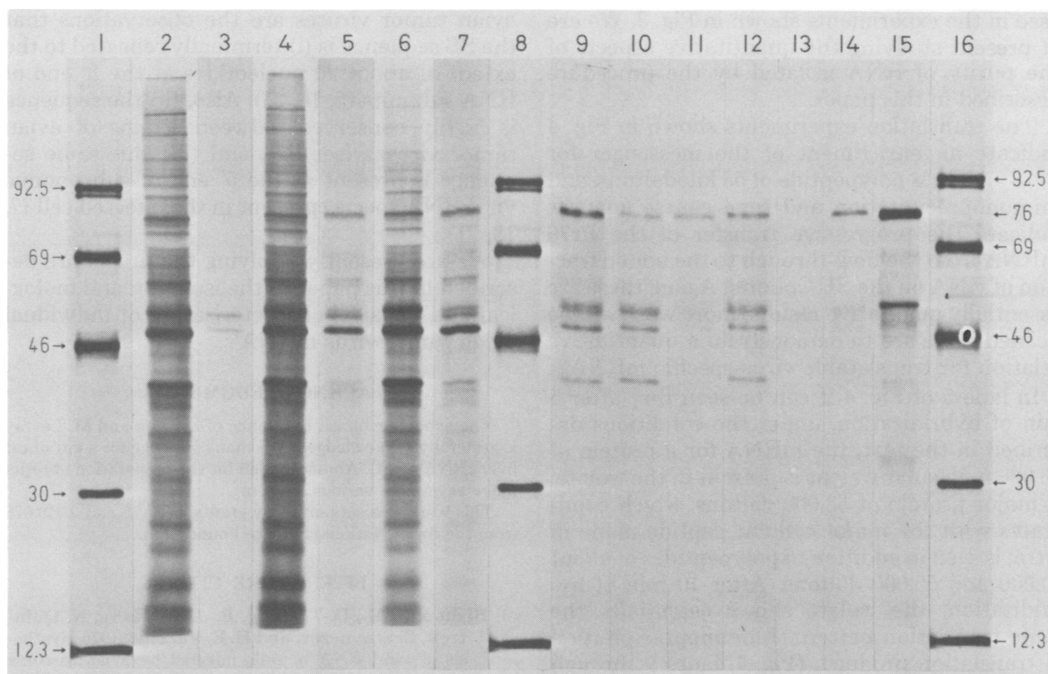

FIG. 4. Analysis on 10% polyacrylamide gels of the products of in vitro translation of total poly(A)$^+$ RNA from chicken embryo fibroblasts infected with the LA23 mutant of RSV (see text). Lanes 1, 8, and 16 show the molecular weight protein markers described in the text, and their molecular weights ($\times 10^3$) are indicated on both sides of the figure. Lanes 2, 4, and 6 show the products of translation of the RNA that flow through an SH-Sepharose column after hybridization of the RNA to mercurated SS-DNA (reconstructed reaction product) for zero time, 5 min, and 10 min, respectively. Lanes 3, 5, and 7 show the translation products of RNA initially bound and subsequently eluted with 0.1 M β-mercaptoethanol, at the same time points of hybridization. Lanes 9 through 14 show the products that are precipitated with a combination of antiviral DNA polymerase and anti-LA23 virus antisera from translation of fractions corresponding to the total translation products shown in lanes 2 through 7, respectively. Lane 15 presents a parallel immunoprecipitate of the products of in vitro translation of LA23 70S RNA. The gels were subjected to fluorography (19) and were exposed at −70°C for 3 days, using Kodak XR 5 film.

per, we show that the fingerprint analysis of RSV-specific RNA isolated from 30-40S poly(A)$^+$ RNA in infected cells indicates the high degree of enrichment achieved. Furthermore, both the purity of the isolated RNA and its biological activity are demonstrated by the relatively simple polypeptide pattern of the in vitro translation products (Fig. 4).

The results shown in Fig. 3 and 4 provide a quantitative demonstration of the purity of isolated virus-specific RNA, as is provided by visual inspection of the fingerprint and in vitro translation patterns and comparison with virus RNA and virus-specific polypeptides.

The experiments presented do not provide a quantitative analysis of the purity of isolated RNAs; such quantitation can only be provided by measuring radioactivity in each large T1 oligonucleotide and by accounting for all of the radioactivity applied to the gel. The error involved is considerable when relatively low counts are available in RNA isolates, as was the case in the experiments shown in Fig. 3. We are at present studying the quantitative aspects of the purity of RNA isolated by the procedure described in this paper.

The translation experiments shown in Fig. 4 indicate an enrichment of the messenger for Pr76 and for a polypeptide of 53 kilodaltons, and immunoprecipitation and time course analysis indicate the progressive transfer of the Pr76 mRNA from the flow-through to the bound fraction of RNA on the SH-column. Again, these are essentially qualitative results; more work will be needed if we are to demonstrate a quantitative relation for translatable virus-specific mRNA's.

In lane 5 of Fig. 4 it can be seen that after 5 min of hybridization, under the conditions described in the text, the mRNA for a protein of 76,000 molecular weight is present in the isolate. A major protein at 53,000 daltons, which comigrates with the major cellular peptide made in vitro, is seen in addition to polypeptides of about 60,000 and 55,000 daltons. After 10 min of hybridization, the isolate shows essentially the same translation pattern. Immunoprecipitation of translation products (Fig. 4, lanes 9 through 15), using antiviral and antiviral DNA polymerase antisera, demonstrates the virus-specific nature of most of the polypeptides translated from the isolated RNA (cf. lanes 12 and 5 of Fig. 4). The exceptions are that the major translation product from isolated RNA (53,000 daltons) immunoprecipitates rather poorly, whereas the 60,000-dalton product does not immunoprecipitate. These results suggest that the 53,000-dalton product may be mainly cellular in origin, whereas the 60,000-dalton product could be the gene product of the SRC gene, whose molecular weight is of this order (3).

The synthesis of p180, which has been variable in our experience, is weak or absent using either 70S RNA or total cellular mRNA (Fig. 4, lanes 15 and 9) and is not seen on translation of the isolated virus-specific RNA. A clear candidate for the *ENV* precursor is not evident but may be demonstrated if one were to use immunoprecipitation by a monospecific antibody raised against the *ENV* protein.

It should be noted that immunoprecipitation of viral 70S translation products (Fig. 4, lane 15) closely resembles that of the products of isolated RNA. In addition, the demonstration that the 76,000-dalton protein is seen by immunoprecipitation to be programmed by an mRNA that is progressively transferred from the flow-through to the eluted fraction of the SH column is indicative of the overall efficiency of the isolation procedure we describe here.

Of more general interest in the case of the avian tumor viruses are the observations that the SS sequence is (i) terminally repeated to the extent of about 20 nucleotides at the 3′ end of RNA subunits (5, 18, 21). Also, (ii) the sequence is highly conserved between strains of avian tumor viruses (see 27), and (iii) this same sequence is present at the 5′ end of subgenomic viral RNA species present in the infected cell (7, 18, 21).

We are presently applying the procedure described in this paper to the isolation and biological and physical characterization of individual avian tumor virus mRNA's.

ACKNOWLEDGMENTS

The expert technical assistance of I. Heim and M. Levray is gratefully acknowledged. We thank H. Rogg for a gift of rat liver tRNA and C. Ahmad-Zadeh for the preparation of reticulocytes and the various antisera.

This work was supported by grants 3.727.76 and 3.310.078 from the Swiss National Science Foundation.

LITERATURE CITED

1. **Bishop, J. M., D.-T. Deng, B. J. W. Mahy, N. Quintrell, E. Stavnezer, and H. E. Varmus.** 1976. Synthesis of viral RNA in cells infected by avian sarcoma viruses, p. 1-20. *In* D. Baltimore, A. S. Huang, and C. F. Fox (ed.), Animal virology. Academic Press Inc., New York.
2. **Bromley, P. A., P.-F. Spahr, J.-L. Darlix, and C. Ahmad-Zadeh.** 1977. Isolation and *in vitro* translation of Rous sarcoma virus specific RNAs, p. 369-378. *In* P. May, R. Monier, and R. Weil (ed.), Early proteins of oncogenic DNA viruses. INSERM, Paris.
3. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346-348.
4. **Cashion, L. M., R. Joho, M. A. Planitz, M. A. Billeter, and C. Weissmann.** 1976. Initiation sites of Rous sarcoma virus RNA-directed DNA synthesis *in vitro*.

Nature (London) 262:186-190.

5. Coffin, J. M. 1976. Genes responsible for transformation by avian RNA tumor viruses. Cancer Res. 36:4282-4288.
6. Coffin, J. M., J. T. Parsons, L. Rymo, R. K. Haroz, and C. Weissmann. 1974. A new approach to the isolation of RNA-DNA hybrids and its application to the quantitative determination of labeled tumor virus RNA. J. Mol. Biol. 86:373-396.
7. Cordell, B., S. R. Weiss, H. E. Varmus, and J. M. Bishop. 1978. At least 104 nucleotides are transposed from the 5' terminus of the avian sarcoma virus genome to the 5' termini of smaller viral mRNAs. Cell 15:79-91.
8. Dale, R. M. K., and D. C. Ward. 1975. Mercurated polynucleotides: new probes for hybridization and selective polymer fractionation. Biochemistry 14:2458-2469.
9. Darlix, J.-L., P. A. Bromley, and P.-F. Spahr. 1977. Extensive in vitro transcription of Rous sarcoma virus RNA by avian myeloblastosis virus DNA polymerase and concurrent activation of the associated RNase H. J. Virol. 23:659-668.
10. Darlix, J.-L., M. Levray, P. A. Bromley, and P.-F. Spahr. 1979. Characterization of the genomic RNA from a Rous sarcoma virus mutant temperature-sensitive for cell transformation. Nucleic Acids Res. 6:471-485.
11. Darlix, J.-L., P.-F. Spahr, P. A. Bromley, and J.-C. Jaton. 1979. In vitro, the major ribosome binding site on Rous sarcoma virus RNA does not contain the nucleotide sequence coding for the N-terminal amino acids of the *gag* gene product. J. Virol. 29:597-611.
12. Deng, C. T., D. Stehelin, J. M. Bishop, and H. E. Varmus. 1977. Characteristics of virus-specific RNA in avian sarcoma virus-transformed BHK-21 cells and revertants. Virology 76:313-330.
13. Fan, H., and D. Baltimore. 1973. RNA metabolism of murine leukemia virus: detection of virus-specific RNA sequences in infected and uninfected cells and identification of virus-specific messenger RNA. J. Mol. Biol. 80:93-117.
14. Haseltine, W. A., and D. Baltimore. 1976. *In vitro* replications of RNA tumour viruses, p. 175-213. *In* D. Baltimore, A. S. Huang, and C. F. Fox (eds.), Animal virology. Academic Press Inc., New York.
15. Haseltine, W. A., A. M. Maxam, and W. Gilbert. 1977. Rous sarcoma virus genome is terminally redundant: the 5' sequence. Proc. Natl. Acad. Sci. U.S.A. 74:989-993.
16. Hayward, W. S. 1977. Size and genetic content of viral RNAs in avian oncornavirus-infected cells. J. Virol. 24:47-63.
17. Junghans, R. P., S. Hu, C. A. Knight, and N. Davidson. 1977. Heteroduplex analysis of avian RNA tumor viruses. Proc. Natl. Acad. Sci. U.S.A. 74:477-481.
18. Krzygek, R. A., M. S. Collett, A. F. Lau, M. L. Perdue, J. P. Leis, and A. J. Faras. 1978. Evidence for splicing of avian sarcoma virus 5'-terminal genomic sequences onto viral-specific RNA in infected cells. Proc. Natl. Acad. Sci. U.S.A. 75:1284-1288.
19. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. 56:335-341.
20. Leong, J.-A., A.-C. Garapin, N. Jackson, L. Fanshier, W. Levinson, and J. M. Bishop. 1972. Virus-specific ribonucleic acid in cells producing Rous sarcoma virus: detection and characterization. J. Virol. 9:891-902.
21. Mellon, P., and P. H. Deusberg. 1977. Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3' half and the 5' terminus of virion RNA. Nature (London) 270:631-634.
22. Parsons, J. T., J. M. Coffin, R. K. Haroz, P. A. Bromley, and C. Weissmann. 1973. Quantitative determination and location of newly synthesized virus-specific ribonucleic acid in chicken cells infected with Rous sarcoma virus. J. Virol. 11:761-774.
23. Parsons, J. T., P. Lewis, and P. Dierks. 1978. Purification of virus-specific RNA from chicken cells infected with avian sarcoma virus: identification of genome-length and subgenomic-length virol RNAs. J. Virol. 27:227-238.
24. Pelham, H. R. B., and R. J. Jackson. 1976. An efficient mRNA dependent translation system from reticulocyte lysates. Eur. J. Biochem. 67:247-256.
25. Rensing, U. F. E., and J. G. G. Schoenmakers. 1973. A sequence of 50 nucleotides from coliphage R17 RNA. Eur. J. Biochem. 33:8-18.
26. Rymo, L., J. T. Parsons, J. M. Coffin, and C. Weissmann. 1974. *In vitro* synthesis of Rous sarcoma virus-specific RNA is catalyzed by a DNA dependent RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. 71:2782-2786.
27. Stoll, E., M. A. Billieter, A. Palmenberg, and C. Weissmann. 1977. Avian myeloblastosis virus RNA is terminally redundant: implications for the mechanism of retrovirus replication. Cell 12:57-72.
28. Weiss, S. R., H. E. Varmus, and J. M. Bishop. 1977. The size and genetic composition of virus-specific RNAs in the cytoplasm of cells producing avian sarcoma-leukosis viruses. Cell 12:983-992.