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Purification of SV-40 Messenger RNA by Hybridization to SV-40 DNA Covalently Bound to Sepharose†

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ABSTRACT: SV-40 DNA sheared form was coupled in a stable covalent bond to cyanogen bromide activated Sepharose. Under the conditions used at least 80% of the DNA was bound to Sepharose. The $T_{1/2}$ of hybridization of 0.5 μg/ml of SV-40 cRNA to SV-40 DNA-Sepharose was 1 hr. This rate of hybridization is sufficiently rapid to purify SV-40 sequences from solutions containing as little as 0.05–0.1 μg/ml. Nonspecific hybridization of RNA is in the range of 0.1–0.2% of the total input RNA. The DNA-Sepharose is fairly stable and can be reused several times to purify RNA. The SV-40 DNA-Sepharose was used to select large quantities of virus specific RNA from SV-40 infected BS-C-1 cells. The virus specific RNA when added to cell-free extracts from wheat germ was shown to direct the synthesis of the major viral structural protein VP-1.

Some DNA viruses, such as Simian Virus 40 (SV-40) (Oda and Dulbecco, 1968), do not inhibit host cell RNA synthesis after infection. In order to purify virus specific RNA, procedures utilizing hybridization of RNA from infected cells to virus DNA have been reported (Bautz and Hall, 1962; Gillespie and Spiegelman, 1965; Bautz et al., 1966; Riggsby, 1969; Weinberg et al., 1972; Prives et al., 1974a; Coffin et al., 1974; Eron and Westphal, 1974). As RNA-DNA hybridization competes unfavorably with DNA–DNA reannealing, the denatured viral DNA has been immobilized on nitrocellulose filters preventing the renaturation of the DNA. When RNA from infected cells is incubated under suitable conditions with virus DNA thus immobilized, only viral RNA forms a stable duplex with the DNA. The virus specific RNA can be subsequently eluted from the filters under hybrid denaturing conditions. This procedure has been used to isolate viral RNA from SV-40 infected cells in order to study viral RNA metabolism (Weinberg et al., 1972, 1974) and its cell-free translation into virus specific polypeptides (Prives et al., 1974a). However, there are several problems associated with this procedure. RNA eluted from the filters is translated very inefficiently most probably due to the coelution of inhibiting contaminants including possibly a small amount of viral DNA (Prives et al., 1974b). It was found that virus-specific RNA must undergo a further purification through oligo(dT)-cellulose before it could be effectively translated and this invariably results in a considerable loss of RNA. Furthermore, filters can be used only once in a selection procedure, a serious drawback when the amount of DNA is limited in

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quantity or requires complicated procedures to prepare, such as strand separation or restriction enzyme digestion.

An alternate approach is to covalently bind the DNA to a solid matrix such as cellulose or Sepharose. Several procedures are available for the covalent binding of nucleic acids to a solid supporting material (Gilham, 1968; Saxinger et al., 1972; Robberson and Davidson, 1972; Wagner et al., 1971). Shih and Martin (1974) have used this method to purify SV-40 specific RNA sequences, but the kinetics of annealing described are fairly slow which may result in RNA degradation. In this communication we report the purification and properties of SV-40 DNA covalently bound to Sepharose and its use in the purification of SV-40 specific mRNA which was shown to direct the cell free synthesis of the major SV-40 viral capsid protein VP-1.

Methods

SV-40 DNA Purification. SV-40 DNA was extracted from plaque purified SV-40 infected BS-C-1 cells 48 hr after infection. The conditions for virus preparation and infection have been previously described (Lavi and Winocour, 1972). Cells were infected with 50 PFU[1]/cell. The Hirt procedure (Hirt, 1967) was used to extract SV-40 DNA from infected cells. Generally, 5–10 μg of SV-40 DNA was extracted per culture (4×10^6 cells). Form I superhelix SV-40 DNA was obtained by subsequent gradient sedimentation (Sambrook et al., 1968). For the preparation of DNA-Sepharose, cyclic coil SV-40 DNA was used (Westphal, 1970) essentially as described by Lavi et al. (1973). This method is rapid, convenient, and yields large amounts of SV-40 DNA. ^{32}P-labeled DNA was obtained by the addition of 0.5 mCi of [^{32}P]orthophosphate (Nuclear Research Center, Beer-Sheva) to one culture 24 hr after infection in the presence of 1/100 the normal phosphate concentration.

Covalent Binding of SV-40 DNA to Sepharose. Sepharose 4B (Pharmacia) was activated by cyanogen bromide (Fluka) (Axen et al., 1967; Porath et al., 1967; Cuatrecasas, 1970). Briefly, 0.2 g of extensively ground cyanogen bromide was mixed with each milliliter of Sepharose suspension (Sepharose-H_2O, 1:1). The pH was maintained at 11 ± 0.5 with 3 *M* NaOH for 15–20 min at 0°, after which the suspension was washed with cold H_2O and equilibrated with 0.2 *M* Mes (Sigma) (Wagner et al., 1971) (pH 6.0) in 90% formamide. ^{32}P-labeled SV-40 cyclic coil DNA (50 μg/ml of settled Sepharose) which was sonicated in a Raytheon 10 KC/sec sonic vibrator at full power for 20 min, and denatured by heating to 95° for 5 min, was added to the suspension and incubated at 4° with gentle shaking. The sedimentation of the sonicated DNA was 6–7 S from which a molecular weight of 3×10^5 was calculated for the double-strand native DNA (Studier, 1965). After 24 hr the suspension was transferred to a glass column and washed extensively with 5 volumes each of: (1) 0.2 *M* Mes (pH 6.0) in 90% formamide; (2) 1 *M* NaCl; (3) 0.1 *N* NaOH, and (4) H_2O.

In the range of 0.2–50 μg of DNA/ml of Sepharose, over 80% DNA was coupled as estimated by liquid scintillation counting of an aliquot of the DNA-Sepharose suspension.

Synthesis of RNA Complementary to SV-40 DNA. RNA was translated from pure SV-40 DNA (form I) by *Escherichia coli* DNA dependent RNA polymerase and purified as described by Shih and Martin (1973). The polymerase, kindly supplied by Dr. S. Levy, was purified by the procedure of Chamberlin (Berg et al., 1971) to the glycerol gradient stage and had a final activity of 12,000 units/mg; 1.7 mg of complementary RNA (cRNA) was synthesized per 0.15 mg of SV-40 DNA. Less than 2% of the RNA reannealed after 24-hr incubation at 65° in 4 × SSC (0.15 *M* NaCl–0.015 *M* sodium citrate) as measured by resistance to pancreatic RNase A (Worthington) and T_1 RNase (Sankyo) in 2 × SSC. The molecular weight of cRNA was determined by polyacrylamide agarose gel electrophoresis (Peacock and Dingman, 1968) to be about 10^6 (20–21 S).

Extraction of Cytoplasmic RNA from SV-40 Infected BS-C-1 Cells. Fifty cultures of BS-C-1 cells were infected with SV-40 and labeled with uridine-5-^{3}*H* as described (Prives et al., 1974a). Cultures were washed three times each with sterile phosphate-buffered saline at 0° and then treated with 1 ml of NP40 buffer containing 0.1 *M* NaCl, 0.01 *M* Tris (pH 8.5), 0.005 *M* $Mg(OAc)_2$ and 0.5% Nonidet P-40 (Shell). The lysed cells were scraped from the culture plates, pooled, and centrifuged at 10,000*g* for 10 min to obtain a post-mitochondrial supernatant from which the RNA was extracted by phenol–chloroform–isoamyl alcohol (Penman, 1966), followed by ethanol precipitation. In cases where the purification of poly(A) containing RNA was required, the RNA was subjected to oligo(dT)-cellulose chromatography as described by Aviv and Leder (1972).

Hybridization of RNA to SV-40 DNA-Sepharose. SV-40 DNA-Sepharose was equilibrated with hybridization buffer (0.5 *M* Tris-HCl, 0.75 *M* NaCl, 0.5% dodecyl sulfate, 1 m*M* EDTA, and 50% deionized formamide, adjusted to pH 7.5); 1 ml of packed Sepharose beads was suspended with an equal volume of hybridization buffer. After the addition of RNA the suspension was incubated in a shaking water bath at 37° and then carefully transferred to a water-jacketed glass column maintained at 37°. Nonhybridized RNA was washed with 5 ml of hybridization buffer, resuspended in 2–3 ml of hybridization buffer, and incubated again with shaking at 37° for another hour, then washed as described above. This second wash further reduced nonspecific binding of RNA to the Sepharose beads. Hybridized RNA was eluted stepwise with 5 ml each of (1) 98% formamide in 10 m*M* Tris-HCl, 0.1 m*M* EDTA, and 0.01% dodecyl sulfate adjusted to pH 8.5, (2) 1 *M* NaCl, and (3) 0.1 *N* NaOH.

Hybridization of SV-40 DNA Immobilized on Nitrocellulose Filters. Denatured SV-40 DNA was immobilized on HAWP (Millipore) 0.45-μ filters prepared as described by Gillespie and Spiegelman (1965). Analytical hybridization was performed in 0.1 ml of hybridization buffer (0.05 *M* Tris-HCl, 0.75 *M* NaCl, 1 m*M* EDTA, 0.5% dodecyl sulfate, and deionized formamide adjusted to pH 7.5), with 0.6-cm filters containing 1.6 μg of DNA. No RNase treatment was used.

Polyacrylamide Gel Electrophoresis of RNA in Formamide. Formamide gel electrophoresis in cylindrical gels was performed as described by Staynov et al. (1972) as modified by Gould and Hamlyn (1973). The running buffer was 4 g of high resolution buffer (Gelman) (pH 8.8) dissolved in 750 ml of H_2O. Electrophoresis was performed on constant current at 1 mA/tube for 4 hr. Gels were sliced into 1-mm sections, dissolved in 0.3 ml of NCS (Amersham-Searle) tissue solubilizer, and counted in toluene.

Cell-Free Translation and Product Analysis. Messenger

[1] Abbreviations used are: cRNA complementary RNA; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PFU, plaque forming units; SSC, 0.15 *M* NaCl–0.015 *M* sodium citrate.

Table I: Specificity of RNA Hybridization to DNA-Sepharose.

Input RNA[a]	DNA-Sepharose[b]	% RNA Hybridized
SV-40 [^{3}H]cRNA	SV-40	90
SV-40 [^{3}H]cRNA	Calf thymus	<1
BS-C-1 [^{3}H]RNA	SV-40	0.2

[a] cRNA and BSC-1 cellular RNA were prepared as described in Methods. Approximately 0.5 μg of cRNA (7500 cpm) and 3 × 10^6 cpm of BS-C-1 RNA were used. [b] 20 μg of SV-40 and of calf thymus DNA-Sepharose was used.

RNA preparations were translated in the wheat germ system using wheat germ which was kindly supplied by Mr. Shildhaus of the Bar Rav Mills, Tel-Aviv. The procedure was essentially that described by Roberts and Paterson (1973) except that the wheat germ was ground in 17 ml of grinding buffer and incubation mixtures contained 50 μ*M* spermine-HCl. Reaction mixtures included 2–5 μCi of [^{35}S]methionine (175 Ci/mmol) along with the 19 other common amino acids. Generally, 0.5–1.0 μg of RNA directed the incorporation of 5–10 pmol of [^{35}S]methionine into acid-insoluble polypeptides per 50-μl reaction. Products synthesized in the wheat germ system were analyzed by dodecyl sulfate polyacrylamide slab gel electrophoresis as previously described (Prives et al., 1974a). Coomassie Brilliant Blue (Sigma) stained gels were dried and autoradiographed in order to locate the [^{35}S]methionine-labeled polypeptides synthesized in vitro.

Results

SV-40 DNA will renature rapidly at the salt concentration of the coupling reaction, resulting in the linking of double-stranded, rather than single-stranded DNA molecules to the Sepharose. RNA–DNA hybridization would then compete unfavorably with DNA–DNA reannealing causing a reduced efficiency of RNA hybridization. In order to reduce the probability of DNA–DNA reannealing the coupling reaction was carried out in the presence of 90% formamide. While as much as 80–90% of the denatured DNA binds to Sepharose under these conditions (see Methods), only 30–50% was coupled to cyanogen bromide activated cellulose (Whatman CF-11) when treated similarly. As the DNA is covalently linked to Sepharose, it is fairly stable throughout the RNA purification procedure (see Table IV). The specificity of SV-40 DNA-Sepharose was demonstrated in an experiment in which 90% of SV-40 cRNA hybridized to the immobilized SV-40 DNA while less than 1% of cRNA was bound to calf-thymus DNA-Sepharose prepared in identical fashion. Only 0.2% of [^{3}H]uridine-labeled RNA from BS-C-1 monkey cells hybridizes to SV-40 DNA-Sepharose (Table I). Furthermore, it has recently been shown that less than 0.2% of [^{125}I]globin mRNA hybridizes to SV-40 DNA-Sepharose while annealing to globin cDNA-Sepharose similarly prepared to an extent of approximately 40% (S. Levy et al., unpublished).

The kinetics of the molecular hybridization reaction in solution is dependent on the concentration of both DNA and RNA (Britten and Kohne, 1968; Wetmur and Davidson, 1968). Since DNA in this reaction is covalently bound to a solid matrix the rate of hybridization reaction is expected to be slower than in solution (Gillespie and Spiegelman, 1965; Spiegelman et al., 1973). We have measured the rate of hybridization between SV-40 cRNA and SV-40 DNA-Sepharose (Figure 1). The rate of the reaction in the range of RNA concentrations tested was dependent on the RNA concentration which differs from the expected theoretical result. However, similar findings have been observed by others with immobilized DNA (McCarthy, 1967). An identical rate was obtained when SV-40 DNA was immobilized on membrane filters. In a control experiment when calf thymus DNA-Sepharose was used practically no hybridization took place (Figure 1). The effect of DNA-Sepharose concentration on the rate of RNA hybridization was not measured.

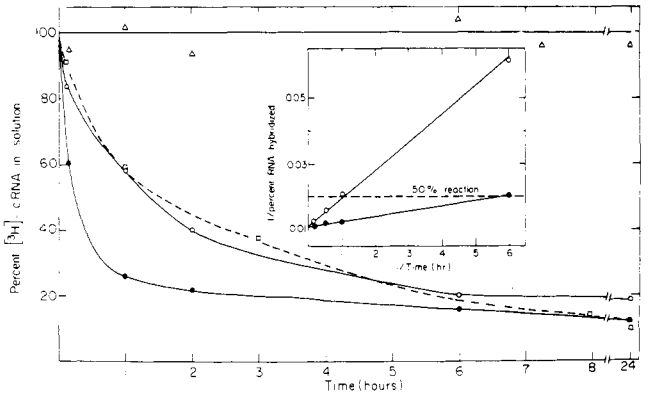

FIGURE 1: Rate of hybridization of cRNA to SV-40 DNA-Sepharose. cRNA was hybridized to 25 μg of SV-40 DNA bound to Sepharose or to nitrocellulose filters in 1-ml volume. At times indicated the suspension was centrifuged and aliquots of the supernatant were counted: (○) 0.5 μg of cRNA (7500 cpm input); (●) 3.5 μg of cRNA (52,500 cpm input) was hybridized to SV-40 DNA-Sepharose; (▲) 0.5 μg of cRNA was hybridized to 25 μg of calf thymus DNA-Sepharose; (□) 0.5 μg of cRNA was hybridized to SV-40 DNA immobilized on nitrocellulose filters.

Purification of Viral RNA from BS-C-1 Cells Infected with SV-40. RNA from SV-40 infected cells was extracted and hybridized to the immobilized DNA in order to test the feasibility of obtaining SV-40 specific mRNA in quantities sufficient for cell-free translation. [^{3}H]Uridine-labeled RNA extracted from the cytoplasm of SV-40 infected cells was prepared and hybridized to SV-40 DNA-Sepharose for 18 hr as described in Methods. The DNA-Sepharose was washed with hybridization buffer to remove the nonselected RNA which comprised 97% of the input radioactivity (Figure 2, first peak); a further 1% could be washed away by an additional 1-hr incubation in fresh hybridization buffer (Figure 2, fraction a). The hybridized RNA was eluted as follows: 50% was released with 98% buffered formamide (Figure 2, fraction b), 20% with 1 *M* NaCl (fraction c), and 30% eluted only with 0.1 *M* NaOH (fraction d). The total yield of hybridized SV-40 RNA was 2.6%, compared with 0.26% of RNA from uninfected BS-C-1 cells. It is not clear why 50% of the hybridized RNA does not elute with formamide. This treatment is required as a preliminary denaturation step in RNA elution as it must precede the NaCl elution. The amount of RNA eluted by salt (1 *M* NaCl) could not be reduced by addition of unlabeled rRNA to the elution buffer, reducing the salt concentration in the hybridization buffer, elevating the temperature during elution to 60°, siliconizing the glass column, or changing the dodecyl sulfate concentrations in the formamide elution buffer from 0 to 0.5%. This is probably a physical property of the Sepharose beads as SV-40 DNA covalently linked to cellulose does not show this peculiar elution pattern and yields over 80% of the hybrid RNA in the presence of 99% formamide.

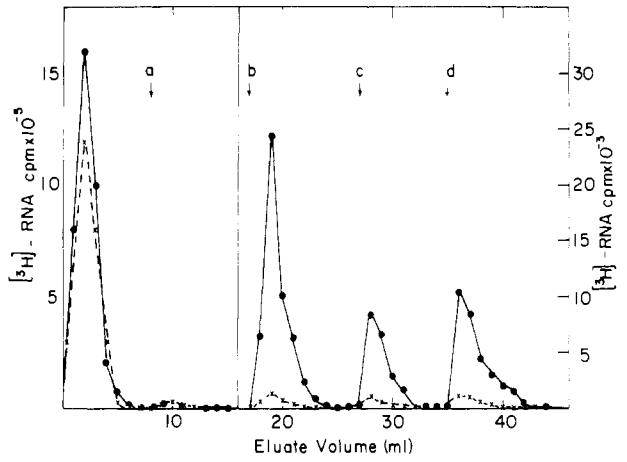

FIGURE 2: Elution pattern of RNA from SV-40 infected and mock infected BS-C-1 cells. BS-C-1 cells (4×10^6) were infected (—) or mock infected (- - -) with SV-40. At 40 hr p.i. 500 μCi of [^{3}H]uridine (40 Ci/mmol) was added per culture and 8 hr later RNA was extracted and hybridized to 25 μg of SV-40 DNA-Sepharose in 1 ml for 18 hr as described in Methods. RNA was eluted, Cl_3CCOOH precipitated, and counted as described in Methods. (a) A second incubation in hybridization buffer; (b) elution with 98% formamide; (c) elution with 1 *M* NaCl; (d) elution with 0.1 *N* NaOH.

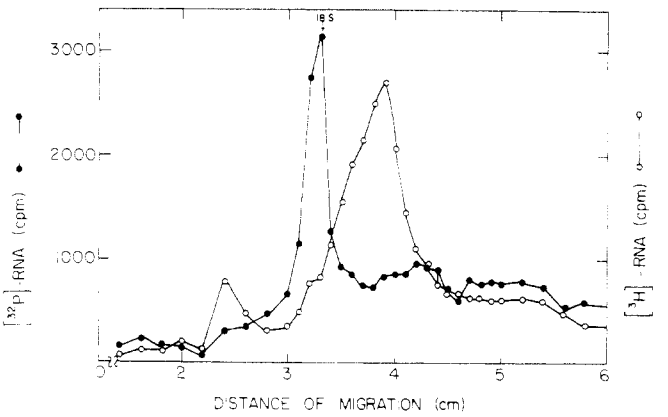

FIGURE 3: Analysis of RNA hybridized to SV-40 DNA-Sepharose by electrophoresis on polyacrylamide gels. (○) [^{3}H]RNA (24,000 cpm) eluted by formamide as described in the legend of Figure 2 was precipitated with ethanol and dissolved in small volume of sample buffer and subjected to formamide gel electrophoresis as described in Methods. (●) [^{32}P]rRNA marker (18,000 cpm).

Table II: Analytical Hybridization of RNA Fractionated by SV-40 DNA-Sepharose to SV-40 DNA Fixed to Nitrocellulose Filters.[a]

RNA	Input (cpm)	Hybridized (cpm)	Hybridized (%)
Total RNA	2.5×10^6	2.1×10^4	0.85
Nonhybridized RNA	1.4×10^5	176	0.12
RNA eluted by			
(1) Formamide (99%)	1023	720	68
(2) NaCl 1 *M*	1062	734	69
SV-40 cRNA	1925	1614	84

[a] RNA extracted from cells labeled with [^{3}H]uridine from 34 to 50 hr postinfection. RNA was prepared as described in Methods. The RNA fractions eluted from the SV-40 DNA-Sepharose were pooled, precipitated with ethanol, dissolved in water, and hybridized for 24 hr to SV-40 DNA fixed to nitrocellulose filters.

Table III: Hybridization Capacity of SV-40 DNA-Sepharose.[a]

Input		Hybridized	
μg of DNA	μg of cRNA[b]	μg of cRNA Hybridized	μg of cRNA Hybridized/μg of DNA
5.0	8.5	5.6	1.1
2.5	8.5	2.5	1.0
2.5	17.0	2.6	1.0

[a] Hybridization conditions as described in Methods. [b] [^{3}H]Uridine-labeled cRNA had a specific activity of 320 cpm/μg.

In order to compare the hybridized RNA from formamide and NaCl elution stages, each was collected, reprecipitated, and then analyzed by subsequent hybridization to SV-40 DNA immobilized on nitrocellulose filters (Table II). The formamide-eluted RNA (Figure 2b) and NaCl-eluted RNA (Figure 2c) classes hybridized to the SV-40 DNA filters to the extent of 68 and 69%, respectively, in comparison to 0.85% SV-40 specific sequences hybridized from total RNA from SV-40 infected cells. From these figures it can be estimated that SV-40 DNA-Sepharose has purified SV-40 specific sequences 80-fold in one step (68/0.85). As there still is 0.12% SV-40 RNA in the nonhybridized fraction (Table II) the yield of selection can be calculated as [1 − (0.12/0.85)] or 86%. When the RNA fractions eluted by formamide and NaCl were separately rehybridized to SV-40 DNA-Sepharose, approximately 75% of the RNA in both cases was retained on the column. Furthermore, the bound RNA in both cases was eluted from the column by formamide, NaCl, and NaOH in similar ratios, resembling the pattern depicted in Figure 1 indicating that the two populations are identical.

RNA eluted from SV-40 DNA-Sepharose has a migration pattern typical of SV-40 RNA (Weinberg et al., 1972, 1974; Prives et al., 1974b) when subjected to formamide gel electrophoresis (Figure 3). The RNA consists mainly of a "16S" component as well as minor "19S" component. The RNA in this experiment was prepared from cells which had been labeled with [^{3}H]uridine for a relatively long period (30–48 hr post infection) and the relative metabolic instability of "19S" RNA (Weinberg et al., 1974) may account for its decreased proportion under long labeling conditions. Little RNA of a size smaller than the "16S" SV-40 RNA species was observed indicating that the SV-40 RNA was generally intact after this selection procedure. The pattern of RNA eluted by NaCl is identical (not shown).

Capacity and Stability of SV-40 DNA-Sepharose. The hybridization capacity of the SV-40 DNA-Sepharose was determined by annealing quantities of SV-40 cRNA in excess of the DNA (Table III). It was found that 1 μg of RNA was hybridized per μg of DNA with a ratio of cRNA/DNA as great as 6.8. If each molecule of DNA were to bind one molecule of cRNA, the DNA capacity should be 3 μg of cRNA/μg of SV-40 DNA, as the cRNA is complementary to only one strand of DNA (Westphal, 1970) and its molecular weight was estimated as 1×10^6, while that of the sheared, denatured DNA is 1.5×10^5. Therefore, it is likely that approximately 30% of the SV-40 DNA bound to Sepharose is participating in the hybridization reaction.

An important advantage of the DNA-Sepharose is that it can be reused several times for hybridization selection. We routinely use one SV-40 DNA-Sepharose preparation at least five times to select SV-40 specific RNA from infected cells with little apparent loss of capacity. In Table IV it is

Table IV: Capacity of SV-40 DNA-Sepharose to Hybridize cRNA upon Reuse.[a]

Cycles of Hybridization	μg of cRNA Hybridized	% Capacity
1	5.6	100
2	5.5	98.2
3	5.3	94.6

[a] Hybridization was performed as described in Methods. In every cycle 5 μg of SV-40 DNA bound to Sepharose and 8.5 μg of [^{3}H]cRNA (2745 cpm) were used.

shown that the SV-40 DNA-Sepharose binds cRNA for three successive cycles of hybridization with only slight reductions in the binding capacity. When the stability of DNA-Sepharose was followed by the loss of radioactively labeled DNA from Sepharose, less than 5% of the DNA was lost in one cycle.

Cell-Free Translation of SV-40 RNA Purified by Hybridization to SV-40 DNA-Sepharose. A comparison was made between the translation product of SV-40 mRNA obtained by hybridization either to SV-40 DNA nitrocellulose filters or SV-40 DNA-Sepharose. Generally, the virus specific RNA obtained by either selection process comprised the same percent of input RNA. However, the additional oligo(dT)-cellulose purification step required by filter-hybridized RNA (Prives et al., 1974a,b) results in a minimum 50% loss of virus specific RNA.

The cell-free synthesis of polypeptides synthesized in response to various mRNA preparations was carried out in wheat germ extracts (Figure 4). The wheat germ system has extremely low levels of endogenous protein synthesis (Prives et al., 1974a) (Figure 4h). Poly(A) containing RNA from SV-40 infected BS-C-1 cells directs the synthesis of a series of polypeptides including a major product with identical electrophoretic mobility to the major SV-40 capsid protein VP-1 (Figure 4c). This polypeptide which does not appear in products directed by mRNA from uninfected BS-C-1 cells (Figure 4b) has been shown to contain a similar tryptic peptide pattern to the major viral polypeptide VP-1 (Prives et al., 1974a). SV-40 RNA obtained by hybridization to SV-40 DNA immobilized on nitrocellulose filters, followed by further purification on oligo(dT)-cellulose, has also been shown to direct the synthesis of a series of polypeptides (Figure 4d), the very predominant component which comprises at least 50% of the products made, is corresponding in its electrophoretic mobility and tryptic peptide pattern to SV-40 VP-1 (Prives et al., 1974b). Some of the smaller polypeptides are other viral, structural, and nonstructural polypeptides (Prives et al., 1974b), and some are probably early terminated peptides which are a common artificial phenomenon of cell-free translation systems. SV-40 specific RNA obtained by hybridization to SV-40 DNA-Sepharose followed by elution in formamide and NaCl directed the products seen in Figure 4e and f, respectively. The products synthesized in response to the RNA eluted by formamide and NaCl were virtually identical in this and every experiment carried out, giving further indication that these RNAs are the same. Figure 4g is the products synthesized in response to SV-40 RNA prepared by selective hybridization to and elution from SV-40 DNA-cellulose. The polypeptides directed by the various hybridization selected RNAs are all very similar indicating that these different methods do enrich for the same class of mRNA whose products differ in pattern and complexity from those directed by total mRNA from SV-40 infected or uninfected cells.

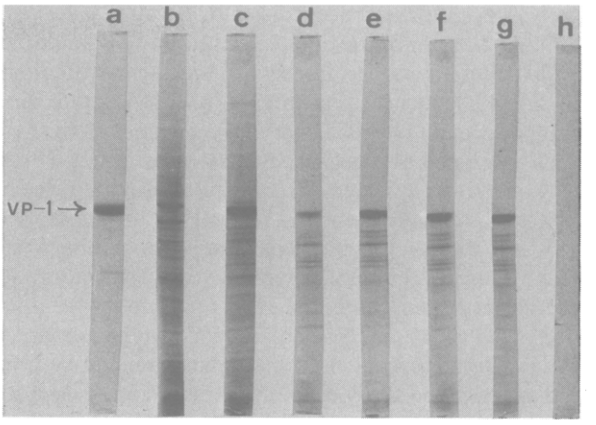

FIGURE 4: Polypeptides directed by mRNA in the wheat-germ system. Reaction mixtures (0.025 ml) contained components of the wheat germ system including approximately 0.1–1.0 μg of mRNA. Cell-free products synthesized in the wheat germ system were subjected to polyacrylamide slab gel electrophoresis. Autoradiograms of dried gels include (a) [^{35}S]methionine labeled SV-40 virion, and products synthesized in response to (b) mRNA from uninfected BS-C-1 cells; (c) mRNA from SV-40 infected BS-C-1 cells; (d) RNA from SV-40 infected cells which was hybridized to and eluted from SV-40 DNA filters, followed by oligo(dT)-cellulose chromatograph; (e) RNA from SV-40 infected cells which was hybridized to SV-40 DNA-Sepharose followed by elution with 98% formamide and subsequently with (f) 1 *M* NaCl; (g) RNA from SV-40 infected cells which was hybridized to and eluted from SV-40 DNA-cellulose; (h) no RNA added.

Discussion

We have shown that hybridization of RNA to DNA immobilized on a solid matrix proceeds with sufficiently rapid kinetics to be useful both for analytical studies of RNA and for preparation of biologically active mRNA. The introduction of a formamide denaturing step associated with the coupling reaction greatly reduced the probability of competition by DNA reannealing with RNA–DNA hybridization. The methods of coupling the denatured fragmented DNA to cellulose by cyanogen bromide activated Sepharose at pH 6.0 (Wagner et al., 1971) is relatively simple and the DNA binds with over 80% efficiency.

The kinetics of hybridization of RNA to either a suspension of DNA-Sepharose beads or to DNA immobilized on nitrocellulose filters are identical and are relatively rapid ($t_{1/2}$ = 1 hr for 0.5 μg/ml of RNA), when compared to the continuous circulation hybridization system described by Shih and Martin (1974). Furthermore, 80–90% of cRNA was bound to DNA-Sepharose or DNA-nitrocellulose in 3 hr, compared to 65% in 150 hr in this alternative method. One drawback of "solid phase" hybridization is the rather slow rate of hybridization compared to that in solution (Gillespie and Spiegelman, 1965; Kourilsky et al., 1970). Furthermore, several studies have shown that the kinetics of hybridization on nitrocellulose filters are more complex than in solution (McCarthy, 1967; Kennel and Kotovlas, 1968; Bishop, 1970; Spiegelman et al., 1973; Flavell et al., 1974a,b). For example, if RNA sequences complementary to the specific RNA required are present in the solution, the formation of double-stranded RNA may proceed more rapidly than the hybridization of RNA to immobilized DNA, thus reducing the yield of selected RNA. It seems, however, that the annealing reaction is rapid enough to purify RNA

sequences even if the concentration of RNA is as low as 0.1 μg/ml. At this concentration the expected $t_{1/2}$ would be 20 hr. This limit is rather important for the purification of early SV-40 sequences and mRNA of RNA tumor viruses which are present in cells in very low concentrations.

This method has several potential uses. Viral DNA or cDNA prepared by reverse transcription of viral or cellular RNA can be covalently bound to a solid matrix and used to purify mRNA by the methods described herein. We are currently using the DNA-Sepharose hybridization technique to purify separately the early and late SV-40 mRNA classes as well as mRNA coding for C-type tumor virus proteins. Other aspects of RNA metabolism can be investigated as well. The study of mRNA transcription or processing by nuclei or chromatin is generally hampered by the fact that solution hybridization of labeled transcribed RNA to DNA has a background of 1–5% of non-hybridized RNA which is resistant to RNase digestion. If a newly synthesized RNA species is present in quantities less than the nonspecific background values, its detection is impossible. The nonspecific binding of RNA to DNA-Sepharose can be reduced to 0.1–0.2% which can enable the detection of RNA species comprising as little as 1% of the total. During the preparation of this manuscript an alternative method for the selection of globin mRNA sequences on globin complementary DNA bound to oligo(dT)-cellulose has been described by Venetianer and Leder (1974). Their method has similar applications to that of the DNA-Sepharose described here and it is hoped that this approach of specific RNA selection can lead to a variety of experimental developments. SV-40 DNA was covalently bound to Sepharose by a similar method (Arndt-Jovin et al., 1975).

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