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# Purification By Oligo(dT)-Cellulose of Viral-Specific RNA from Sarcoma Virus-Transformed Mammalian Nonproducer Cells

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Viral-specific RNA has been purified by oligo(dT)-cellulose chromatography from sarcoma virus-transformed nonproducer cells. This RNA comprises approximately 3% of the purified RNA, as judged by RNA-DNA hybridization.

Mammalian sarcoma virus-transformed nonproducer clones of rat cells which lack replicating type-C virus have been reported (1). Although these cells are transformed, neither rat nor mouse virion structural proteins have been detectable in such cells, not even by radioimmunoassay (R. Benveniste and E. Scolnick, Virology, in press). Such cells do contain RNA which hybridizes to $^3$H-DNA products made from virus preparation of Moloney or Kirsten sarcoma virus (4, 5; R. Benveniste and E. Scolnick, Virology, in press). This hybridizable RNA is not detectable in avian sarcoma virus-transformed rat cells (R. Benveniste and E. Scolnick, Virology, in press). Thus, this RNA is virus-specific and not simply a consequence of transformation. Since the RNA present in such nonproducer cells presumably codes for proteins involved in the maintenance of transformation, we have attempted to purify and quantitate the RNA in these cells. In the case of other mammalian mRNAs, oligo(dT)-cellulose chromatography has proved useful in purifying functional mRNA (2). Such purification has been based on the observation that many mammalian mRNAs has been found to have polyadenylate [poly (A)]rich regions (3) which allow the RNA to bind to (dT)-cellulose. Thus we have attempted to purify the RNA of sarcoma virus-transformed rat cells by chromatography on (dT)-cellulose.

Polysomal and other cytoplasmic RNA was prepared from Kirsten sarcoma virus-transformed nonproducer normal rat kidney cells (1) by procedures previously described (R. Benveniste and E. Scolnick, Virology, in press). The RNA was applied to an oligo(dT)-cellulose column as shown in Fig. 1. The optical density profile at 260 nm shows that approximately 97 to 99% of the RNA was not retained on the column. Earlier studies have shown that such RNA is predominantly ribosomal (2). When the column was eluted with buffer of low ionic strength, a condition which does not favor the association between complementary polynucleotides, a small peak of RNA was eluted.

The starting material and the material which bound to and eluted from the (dT)-cellulose column was tested for its ability to hybridize to a $^3$H-DNA product made from an endogenous reverse transcriptase reaction (R. Benveniste and E. Scolnick, Virology, in press). The results are shown in Fig. 2. The starting material hybridizes to the $^3$H-DNA product and saturates the reaction at between 400 to 500 $\mu$g of RNA. The material eluted from the column gives detectable hybridization with as little as 0.5 $\mu$g of RNA, and saturates at between 8 to 15 $\mu$g of RNA. In studies not shown the synthetic polymer, polyriboadenylic acid, did not hybridize to the $^3$H-DNA product (less than 30 counts/min). Thus, approximately a 30- to 60-fold purification of the viral-specific RNA can be achieved. The total yield of hybridizable RNA in repeated studies has varied from 20 to 60% of the starting material. Presumably, any RNA lacking poly(A) or which has had its poly (A) cleaved during the extraction procedure would not bind to the column.

To estimate what proportion of the cellular RNA purified by (dT)-cellulose represented viral-specific RNA, a calibration curve was done using 70*S* viral RNA, and a comparison was made with the material eluted from oligo(dT)-cellulose. The results are shown in Table 1. Approximately 0.2 $\mu$g of 70*S* RNA is required to saturate the $^3$H-DNA product at 67% of the input counts. Approximately 6 $\mu$g of the cellular RNA is required for saturation at 31% of the input counts. The higher final levels of hybridization with 70*S* RNA are consistent with past

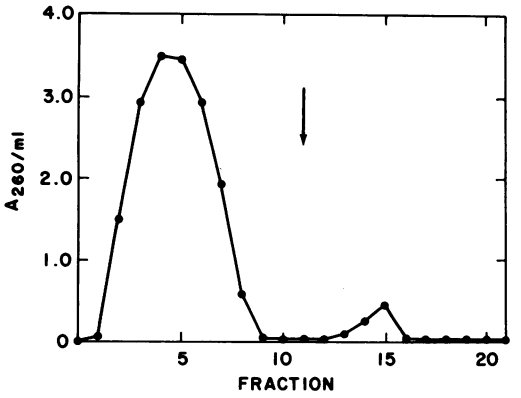


FIG. 1. *Oligo(dT)-cellulose chromatography. Forty $A_{260}$ units of crude polysomal RNA from Kirsten sarcoma virus-transformed nonproducer rat cells, dissolved in application buffer containing 0.01 M Tris-HCl, pH 7.5; 0.5 M KCl, was applied at 25 C to a 0.9- by 10-cm column of oligo(dT)-cellulose (~1.5 g dry weight). The unabsorbed material was eluted by continued washing with the application buffer at a flow rate of approximately 60 ml per h. The material retained by the column was eluted (↓) with buffer containing 0.01 M Tris-hydrochloride, pH 7.5. Each fraction is approximately 2.0 ml. The material eluted in this way was lyophilized until use, and stored at −20 C. The oligo(dT)-cellulose was purchased from Collaborative Research, Waltham, Mass.*

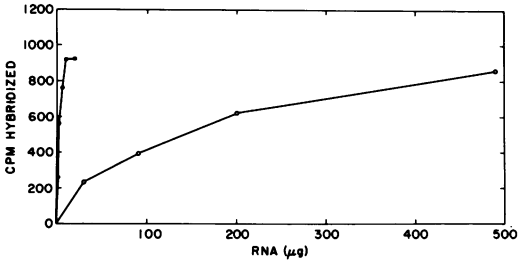


FIG. 2. *Hybridization with crude and purified nonproducer cell RNA. Approximately 3,000 trichloracetic acid-precipitable counts (0.08 pmol of $^3$H-TTP) of $^3$H-DNA product made in the presence of actinomycin D from a virus preparation containing Kirsten sarcoma and leukemia virus was hybridized to RNA under conditions previously described (R. Benveniste and E. Scolnick, Virology, in press). The counts/min hybridized were measured after digestion of the unhybridized material with nuclease S-1. The details of hybridization have been previously described (R. Benveniste and E. Scolnick, Virology, in press). Approximately 20 to 40 counts/min were resistant to nuclease S-1 with no RNA addition; RNA additions at zero time did not appreciably inhibit the enzyme (less than additional 40 counts/min over counter background). Symbols: ●, purified; ○, crude.*

TABLE 1. *Quantitation of viral-specific RNA in (dT)-cellulose-purified nonproducer cell RNA*[a]

| RNA (μg) | Percent Hybridization |
|---|---|
| 70s RNA | |
| 0.01 | 9 |
| 0.02 | 24 |
| 0.05 | 33 |
| 0.10 | 49 |
| 0.20 | 67 |
| 0.50 | 67 |
| (dT)-Cellulose RNA | |
| 1.0 | 10 |
| 3.0 | 18 |
| 6.0 | 31 |
| 10.0 | 31 |

[a] Quantitation of viral-specific RNA in (dT)-cellulose-purified RNA. 70S RNA from a virus preparation containing both Kirsten sarcoma and Kirsten leukemia virus was purified as previously described (R. Benveniste and E. Scolnick, Virology, in press). Condition of hybridization are as noted in the legend to Fig. 2. Reactions contained approximately 2,500 trichloroacetic acid-precipitable counts/min of $^3$H-DNA product; 1% of the product was resistant to nuclease S-1 with no RNA added.

results which showed that cells producing virus expressed information (presumably for proteins involved in replication), which was not expressed in nonproducer cells (6; R. Benveniste and E. Scolnick, Virology, in press). This difference makes exact comparative quantitation difficult. However, these studies would suggest that approximately 3% of the (dT)-cellulose purified RNA from nonproducer cells represents viral-specific RNA.

The present results demonstrate that viral-specific RNA can be purified by (dT)-cellulose chromatography from sarcoma virus-transformed nonproducer cells. This technique provides a method for obtaining large amounts of this RNA free of RNA concerned with the replication of type C viruses. If such RNA can be further purified, it may be useful in programming in vitro protein synthesizing systems to synthesize viral-specific protein(s) involved in transformation. In addition, the technique of oligo(dT)-cellulose chromatography may prove useful in the search for viral-specific RNA in other types of transformed mammalian cells not producing virus.

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## LITERATURE CITED

1. Aaronson, S. A., and C. Weaver. 1971. Characterization of murine sarcoma virus (Kirsten) transformation of mouse and human cells. J. Gen. Virol. **13**:245–252.
2. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligo thymidylic acid-cellulose. Proc. Nat. Acad. Sci. U.S.A. **69**:1408–1412.
3. Darnell, J. E., R. Wall, and R. J. Tushinski. 1971. An adenylic-acid rich sequence in messenger RNA of Hela cells and its possible relationship to reiterated sites in DNA. Proc. Nat. Acad. Sci. U.S.A. **68**:1325–1325.
4. Green, M., H. Rokutanda, and M. Rokutanda. 1971. Virus-specific RNA in cells transformed by RNA tumor viruses. Nature N. Biol. **230**:229–232.
5. Tsuchida, H., M. S. Robin, and M. Green. 1972. Viral RNA subunits in cells transformed by RNA tumor viruses. Science **174**:1418–1419.