JOURNAL OF VIROLOGY, Oct. 1976, p. 342-347

Vol. 20, No. 1
Printed in U.S.A.

RNA Complementary to the Genome of RNA Tumor Viruses in Virions and Virus-Producing Cells

EDWARD STAVNEZER,* GORDON RINGOLD, HAROLD E. VARMUS, AND J. MICHAEL BISHOP

Department of Microbiology, University of California, San Francisco, California 94143

Received for publication 6 April 1976

Cells producing type C (avian sarcoma virus) or type B (mouse mammary tumor virus) RNA tumor viruses contain small amounts of RNA complementary to the viral genomes. The negative strands are complementary to at least 30 to 45% of the viral genomes and are found as RNA-RNA duplexes in the nucleus and cytoplasm of infected cells and in mature virions.

RNA tumor viruses replicate by transcription of a DNA provirus integrated into the host genome (6, 27); the transcripts serve as viral genomes and mRNA's (9) and are designated "positive strands" (3). Consequently, there appears to be no requirement for RNA complementary to the viral genome (negative strands) in the replicative cycle. In a previous report (10), negative strands of murine sarcoma-leukemia virus RNA were detected in infected rat cells by hybridizing nuclear RNA to radioactive positive strands (viral genome). However, using similar techniques, attempts to detect viral negative strands in cells infected by avian sarcoma virus (ASV) have been unsuccessful (4, 6, 11; Table 1, line 1). We have noted previously (6) that the experimental approach used in these experiments may be compromised by competition between viral positive strands in the cellular RNAs and the radioactive positive strands used to detect negative strands by molecular hybridization. The extent of competition can approach 100% if, as shown below (see Fig. 3), negative strands comprise a small fraction of cellular virus-specific RNAs. To obviate this difficulty, we devised a protocol that eliminates surplus positive strands before the test for negative strands. Cellular or virion RNA was self-annealed to allow endogenous positive strands to form RNA-RNA duplexes with any negative strands. The annealed RNA was treated with RNase at high ionic strength to digest single strands, including excess viral positive strands, while preserving duplex RNA. The surviving duplex RNA was extracted to remove RNase, denatured, and assayed for viral negative strands by testing its ability to hybridize radioactive viral positive-strand RNA.

Using the protocol outlined above, we detected viral negative strands in nuclear and cytoplasmic RNAs from cells producing avian type C (B77 strain of ASV [B77 ASV]; Fig. 1a) or mammalian type B (mouse mammary tumor virus [MMTV], Fig. 1b) RNA tumor viruses. We also found negative-strand RNA in purified virions of B77 ASV (Fig. 1a). We measured the portions of the viral genomes represented in negative strands by reacting radioactive positive strands with increasing amounts of duplex RNAs to approach a plateau of hybridization (Fig. 1). Negative strands from the nuclei of infected cells hybridized with about 30% of the B77 ASV genome (Fig. 1a) and about 45% of the MMTV genome (Fig. 1b); negative strands from cytoplasm hybridized with about 20% of both the viral genomes (Fig. 1a, b). The negative strands in virions of B77 ASV represented about 15% of the genome (Fig. 1a); we have not tested virion RNA of MMTV in this manner. The labeled ASV RNA used in these studies was isolated from virus stocks composed mostly of transformation-defective mutants which bear a 15% deletion of the wild-type sarcoma virus genome (17). Therefore, the percentages given in Table 1 and Fig. 1a apply to the defective genome; we do not know whether the complement of the deleted sequence is present in negative strands.

Several low-molecular weight RNAs (tRNA's and 5S rRNA), which are present in avian cells and virions, form complexes with the ASV genome (9, 26). We have also found that nuclei of uninfected duck cells contain high-molecular-weight RNAs which anneal to the ASV genome (our unpublished data). For several reasons, these RNAs cannot account for the extensive annealing of viral genome RNA (assayed by RNase resistance) which characterizes the negative strands we have detected in infected cells and virus particles. (i) Duplexes formed by viral genome RNA and either the 4S and 5S RNAs or the high-molecular weight RNAs from uninfected cells are denatured at relatively low

TABLE 1. *Negative-strand RNA in cells producing ASV*[a]

Source of RNA	Pretreatment of RNA	% of ASV [^{32}P]RNA hybridized by:	
		Nuclear RNA	Cytoplasmic RNA
Duck cells producing ASV	1. Denatured[b]	0	0
	2. Self-annealed[c], RNase[d], denatured	30	23
	3. Alkali digested[e]	0	0
	4. RNase (low ionic strength)[f], denatured	0	0
	5. RNase, denatured	28	21
	6. Denatured, RNase, denatured	4	3
Unifected duck cells	7. Denatured	0	0
	8. Self-annealed, RNase, denatured	0	0
	9. Annealed with ASV RNA, RNase, denatured	0[g]	0[g]

[a] Nuclear and cytoplasmic RNAs were purified from uninfected duck fibroblasts or from infected duck fibroblasts producing B77 ASV as described in the legend to Fig. 1. Following the indicated pretreatments, RNA samples were assayed for viral negative strands by annealing with 0.2 ng of ASV [^{32}P]RNA as described in the legend to Fig. 1. The samples used in each annealing reaction were derived from 55 μg of nuclear RNA or 270 μg of cytoplasmic RNA from virus-producing cells. With uninfected cells, 95 μg of nuclear RNA or 200 μg of cytoplasmic RNA was used for each reaction.

[b] RNA samples dissolved in water were denatured by heating at 100°C for 4 min and then cooled rapidly in an ice bath. For the first denaturation in experiment number 6 the RNAs were dissolved in 0.01 M Tris-hydrochloride, pH 7.4, 1 mM EDTA.

[c] RNAs were incubated at 68°C to a C_rt = 100 mol of nucleotides × s/liter. At this value of C_rt, hybridization reactions driven by the endogenous (experiment 2) or exogenous (experiment 9) viral RNA are completed (E. Stavnezer, unpublished data).

[d] RNA at 0.5 mg/ml in 0.5 M NaCl, 0.01 M Tris-hydrochloride, pH 7.4, and 1 mM EDTA was treated with pancreatic RNase (100 μg/ml) and T_1 RNase (10 units/ml) at 37°C for 1 h to digest single-stranded RNA. The samples were extracted twice with phenol, and residual RNase activity was inactivated by a brief treatment with 0.05% diethylpyrocarbonate (25). The duplex RNA was ethanol precipitated after adding 50 μg of yeast RNA carrier per ml.

[e] Samples in 0.3 N NaOH were incubated at 37°C for 16 h, neutralized, and precipitated with ethanol after adding 50 μg of yeast RNA carrier per ml.

[f] Samples were treated as in *d* except the RNase digestion was performed in 0.01 M Tris-hydrochloride, pH 7.4, 1 mM EDTA.

[g] These values were obtained after subtracting the hybridization observed in a parallel experiment in which the same amount of ASV RNA (but not cellular RNA) was subjected to the same pretreatment (15% hybridization).

temperatures (melting temperature [T_m] less than 60°C in 0.02 M Na^+ for all but $tRNA^{trp}$, our unpublished data; 26). On the other hand, duplexes formed by viral genome RNA and negative strands from infected duck cells melt at high temperatures (T_m = 82°C in 0.015 M Na^+; Fig. 2). The thermal stability of the negative strand-viral genome duplex is that of a fully base-paired RNA with a base composition of about 50% guanine plus cytosine (5). (ii) Negative strands from virus-producing cells form RNase-resistant duplexes with the viral genome. Since we cannot detect negative strands in uninfected cells using an RNase assay (Table 1, lines 7-9), we assume that complexes of viral genome RNA and uninfected cell RNAs are susceptible to RNase digestion at high ionic strength; this is consistent with the low thermal stability of these complexes. (iii) Viral 70S RNA, which is a complex of low-molecular-weight RNAs and the viral genome, does not have extensive RNase resistance (ca. 4%) nor does it acquire RNase resistance when self-annealed (ca. 4 to 7%). In fact, removal of these low-molecular-weight RNAs from viral 70S RNA by agarose gel filtration subsequent to denaturation (26) did not result in the loss of virion negative strands (data not shown).

Negative strands were destroyed by treating nuclear, cytoplasmic, and virion RNAs either with RNase at low ionic strength or with alkali (Table 1, lines 3 and 4; Fig. 1, legend). Since these treatments hydrolyze duplex and single-strand RNA (29) but not DNA, we conclude that the viral negative strands are RNA and not contaminating proviral DNA.

Labeled positive-strand RNA hybridized to about the same extent with duplex RNAs pre-

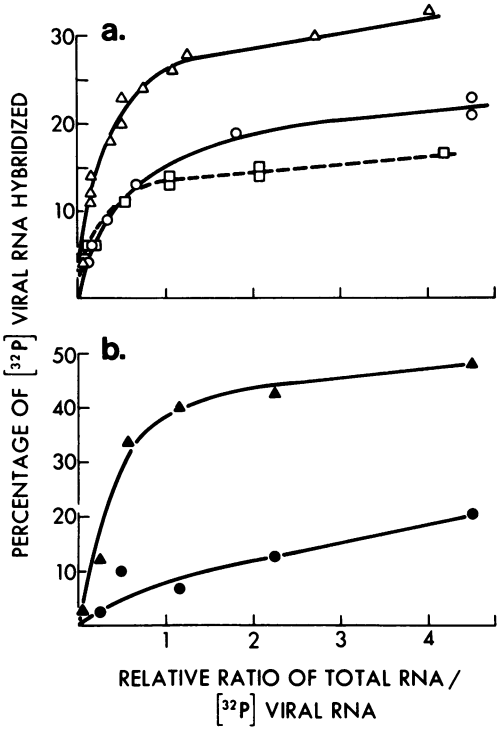

FIG. 1. *Hybridization of viral [^{32}P]RNA by complementary RNA. (a) Pekin duck embryo fibroblasts were grown in culture and infected by the B77 strain of ASV (subgroup C) as described previously (28). Fully transformed cells were fractionated into nuclei and cytoplasm (28). RNA was purified from these subcellular fractions by a combination of sodium dodecyl sulfate-Pronase treatment, DNase digestion, and phenol extraction as described previously (18). The 60-70S RNA complex was purified from Prague strain ASV (subgroup C) propagated on chicken fibroblasts (21). The RNase-resistant fractions of self-annealed nuclear (−△−), cytoplasmic (−○−), and virion (- -□- -) RNAs were prepared as in experiment 2 of Table 1. These duplex RNAs were denatured and annealed with ASV [^{32}P]RNA (10^4 to 2×10^{-4} M KCl, 1.5×10^{-5} M $MgCl_2$, and nuclei were various ratios of unlabeled to labeled RNA, the ratios having been calculated from the amount of untreated RNA which was the source of the duplex RNA used. A relative ratio of 1 equals a ratio of 4.8×10^4 for virion RNA, 2×10^5 for nuclear RNA, and 6×10^5 for cytoplasmic RNA. (b) GR mouse mammary tumor cells (22) were grown in the presence of 10^{-5} M dexamethasone. Cells were disrupted by Dounce homogenization in 10^{-4} M Tris-hydrochloride, pH 7.4, 10^{-4} M KCl, 1.5×10^{-5} M $MgCl_2$ and nuclei were separated from cytoplasm by centrifugation at 1,500 × g for 15 min. Nuclear and cytoplasmic RNAs were isolated, self-annealed, and treated with RNase at high ionic strength as described in the legend to Table 1. The RNase-resistant fractions of nuclear (−▲−) and cytoplasmic (−●−) RNA were heat denatured and annealed with MMTV [^{32}P]RNA (10^4 to 2×10^4 cpm/ng; R. Friedrich, V. L. Morris, H. M. Goodman, J. M. Bishop, and H. E. Varmus, Virology, in press) in a series of reactions containing varying ratios of cellular to labeled virion RNA. A relative ratio of 1 is equivalent to a ratio of 2.4×10^5 for nuclear RNA and 12×10^5 for cytoplasmic RNA (the ratios were calculated from the amount of untreated RNA used). Samples were annealed at 68°C for 18 h in 0.6 M NaCl, 0.04 M Tris-hydrochloride, pH 7.4, 2 mM EDTA. Annealed samples were diluted with 0.5 M NaCl, 0.01 M Tris-hydrochloride, pH 7.4, 1 mM EDTA and divided in half, and one-half was treated with pancreatic RNase (50 μg/ml) and T_1 RNase (5 units/ml) for 1 h at 37°C. The untreated (total) and RNase-resistant (hybridized) RNAs were precipitated with 10% trichloroacetic acid, collected on glass fiber filters, and counted in a liquid scintillation spectrometer. The RNase resistance of the virion [^{32}P]RNAs annealed with yeast RNA (ca. 5%) was subtracted from the other values. The negative strands in all of these preparations were completely destroyed by treatment with alkali as described in Table 1, experiment 3.*

pared without self-annealing and with duplex RNA from self-annealed samples (Table 1, lines 2 and 5). Moreover, duplexes of viral RNA were present at the same concentration in self-annealed and unannealed RNAs (data not shown). The fact that self-annealing did not appreciably affect the amount of duplex RNA isolated from nuclear and cytoplasmic fractions indicates that most or all of the viral negative strands in ASV-producing cells are contained in duplex structures.

Duplexes can be formed by single-stranded RNAs folded back on themselves in "hairpins"; these structures cannot be permanently denatured with heat because the RNA folds back again when cooled (24). We tested for negative strands in hairpins by assaying cellular RNA which had been boiled, quick cooled, and treated with RNase at high ionic strength; very little of the RNA complementary to the genome of B77 ASV survived this treatment (Table 1, line 6), and reannealing of less than 5% of the negative strands during the cooling could account for the extent of complementarity observed (ca. 4%) (see Fig. 1a). We consider it unlikely that viral negative strands are in hairpin forms, although we cannot exclude the possibility that hairpins were nicked (and rendered denaturable) either before or during extraction from the infected cells.

The duplex RNAs we isolated after treatment with RNase at high ionic strength should contain equal amounts of positive and negative strands. Consequently, the concentration of viral negative strands in a preparation of duplexes can be deduced from the concentration of viral positive strands. This is convenient because the concentration of positive strands can

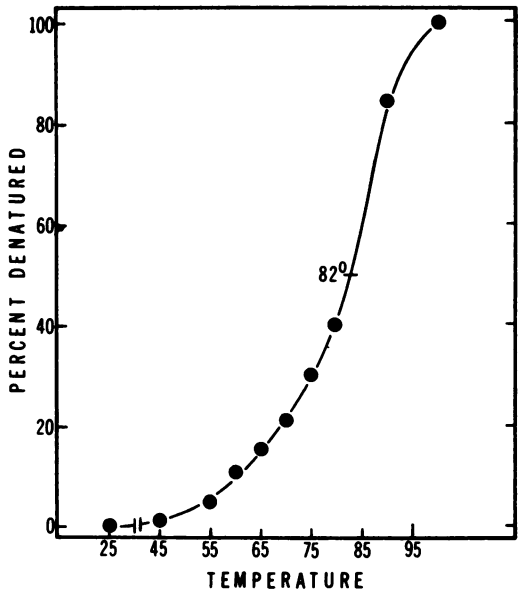

FIG. 2. *The thermal stability of ASV duplex RNA. Cellular RNA was prepared from duck fibroblasts producing B77 ASV as described for subcellular fractions in the legend to Fig. 1. Duplex RNA, prepared from 1.75 mg of cellular RNA after self-annealing, was denatured and annealed with 2 ng of ASV [^{32}P]RNA. In a control reaction, 2 ng of [^{32}P]RNA was incubated with 200 μg of yeast RNA. Equal portions of the reaction mixtures were diluted to a final NaCl concentration of 0.015 M, and a pair of samples (one from each annealing reaction) was incubated for 10 min at each temperature indicated and then cooled rapidly in an ice bath. The amount of [^{32}P]RNA that was resistant to RNase treatment after heating at the various temperatures was determined as described in the legend to Fig. 1, and the values from the control reaction (4 to 5%) were subtracted from the experimental values. The fraction of hybridized RNA that was denatured at each temperature was calculated relative to the 25°C sample, which showed that 29% of the ASV [^{32}P]RNA (909 of 3,142 cpm) had been hybridized.*

be readily measured by molecular hybridization using radioactive DNA complementary to the positive strand (cDNA) (18). By comparing the rates of hybridization with viral 70S RNA and cellular RNAs, we found that viral positive strands comprise 0.3 to 0.5% of nuclear RNA and 0.1 to 0.3% of cytoplasmic RNA in duck cells producing B77 ASV (data not shown). Similarly, by comparing the rates of cDNA hybridization with positive strands in untreated and RNase-resistant cellular RNAs (Fig. 3), we have deduced the relative concentrations of positive and negative strands. Our data indicate that the concentration of viral positive strands is about 200-fold greater than the concentration of negative strands in nuclear RNA and 500-fold greater in cytoplasmic RNA (Fig. 3). From these values we compute that negative strands comprise less than 0.005% of nuclear RNA and less than 0.0005% of cytoplasmic RNA. Although we have not made direct measure-

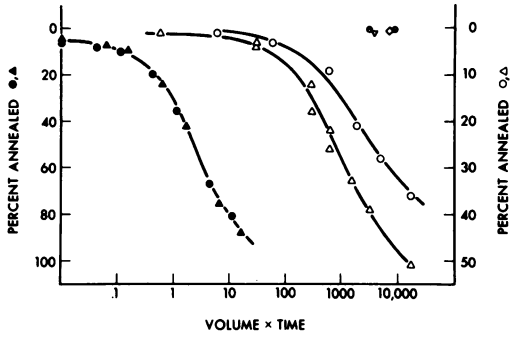

FIG. 3. *The relative concentrations of positive and negative strands of viral RNA in cells producing ASV. Labeled DNA complementary to the viral genome ([^{3}H]cDNA, 2 × 10^{7} cpm per μg) was synthesized with the endogenous DNA polymerase activity of B77 ASV (22). The [^{3}H]cDNA used in these studies was preselected as that fraction of cDNA that hybridized to viral RNA at a cDNA:RNA ratio of 100; hybrids were separated from unhybridized cDNA by fractionation on hydroxyapatite. The preselected cDNA has a more uniform representation of the nucleotide sequence of the ASV genome than unfractionated cDNA (E. Stavnezer, unpublished data; 15); it is not, however, a perfect representation of the viral genome, and the extent of its hybridization cannot be equated with a percentage of the genome. Nuclear and cytoplasmic RNAs were prepared from duck fibroblasts producing ASV, and a portion of each was used to prepare duplex RNA after self-annealing (as in Table 1). Samples containing 10 μg of RNA in either 3 μl of untreated nuclear RNA (▲) or 2 μl of untreated cytoplasmic RNA (●) were annealed with 0.2 ng of [^{3}H]cDNA for various times. Samples of duplex nuclear RNA (△) and duplex cytoplasmic RNA (○) were denatured and annealed with 0.1 ng of [^{3}H]cDNA. Control annealings were performed with undenatured duplex RNAs (nuclear, ▽; cytoplasmic, ◒) and with alkali-digested RNAs (nuclear, ◇; cytoplasmic, ◒). The samples of duplex RNA used in each annealing reaction correspond to 22.5 or 45 μl (77 or 154 μg) of the untreated nuclear RNA and 45 μl (230 μg) of untreated cytoplasmic RNA. The hybridization of [^{3}H]cDNA was assayed by fractionation on hydroxyapatite columns at 60°C and is plotted as a function of the volume of the RNA sample times the annealing time (V_0t). The parameter V_0t permits comparisons of hybridization data without knowledge of the concentration of RNA in the tested samples (22). The present data reflect the relative concentrations of total positive strands and positive strands contained only in duplexes, since in all cases V_0t is computed from the volume of untreated RNA used.*

ments, the concentration of negative strands in virion RNA must be very low because extensive self-annealing of viral RNA ($C_t t$ = 0.5 mol/s per liter) did not produce appreciable duplex structure (ca. 5% resistance to RNase at high ionic strength).

To validate our quantitative conclusions, we measured the recovery of double-stranded replicative form of poliovirus RNA (7) using our procedure for preparation of duplexes from cellular RNA (as described in legend to Table 1). Recovery of replicative form was 85 and 91% in two separate trials. We conclude that our measurements of the concentration of viral negative strands are not seriously distorted by poor recoveries of duplex RNA.

Our data demonstrate that virions of B77 ASV and the nuclei and cytoplasm of cells producing either B77 ASV or MMTV contain negative strands of viral RNA in duplex form. The negative strands are complementary to a limited portion of the viral genomes, but the significance of this observation is uncertain; we have tested only steady-state populations of RNA, and the negative strands found there may be the products of extensive processing. The viral genome is most extensively represented in the negative strands found in nuclei (30 to 40%), and negative strands in the cytoplasm represent little more of the viral genome (ca. 20%) than do negative strands in virions (ca. 15%). These observations suggest, but do not prove, that viral negative strands are synthesized in the nucleus, transferred to the cytoplasm, and incorporated into virions during their maturation. We will not be able to study the genesis and metabolism of negative-strand RNA until we have developed a sensitive assay for radiolabeled RNA complementary to the viral genome.

Viral negative strands are found as single-stranded RNA in cells infected with DNA tumor viruses (1, 2, 16, 20) and are alleged to be the product of bidirectional (or symmetrical) transcription from viral DNA. The negative strands we have found for RNA tumor viruses occur in duplexes, an unlikely product of bidirectional transcription. By contrast, duplex RNA is a hallmark of RNA synthesis by RNA replicases (8). Although RNA tumor viruses are not known to induce new replicase in infected cells, normal cells may contain a replicase (12, 19) that could transcribe tumor virus RNA. Irrespective of the mechanism by which they are synthesized, viral negative strands could represent intermediates in the genesis of viral messengers smaller than a subunit of the viral genome; messengers of this sort probably exist in infected cells (13, 23; J. M. Bishop, C-T. Deng, B. W. J. Mahy, N. Quintrell, E. Stavnezer, and H. E. Varmus, *In* D. Baltimore, A. S. Huang, and C. F. Fox, ed., *ICN-UCLA Symposia on Molecular and Cellular Biology*, vol. 4, in press).

We thank Lois Fanshier for technical assistance.

This work was supported by Public Health Service contract NO1 CP 33293 and grant CA 12705-05 from the National Cancer Institute, and American Cancer Society grant VC-70B. E.S. was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation. H.E.V. is a recipient of Public Health Service Research Career Development Award CA 70193.

LITERATURE CITED

1. **Aloni, Y.** 1972. Extensive symmetrical transcription of simian virus 40 DNA in virus-yielding cells. Proc. Natl. Acad. Sci. U.S.A. 69,2404-2409.
2. **Aloni, Y., and H. Locker.** 1973. Symmetrical *in vitro* transcription of polyoma DNA and the separation of self-complementary viral and cell RNA. Virology 54:495-505.
3. **Baltimore, D.** 1971. Expression of animal virus in genomes. Bacteriol. Rev. 35:235-241.
4. **Baluda, M.** 1974. Evidence in support of a DNA intermediate in the replication of avian RNA tumor viruses, p. 313-332. *In* R. W. Crumley (ed.), Molecular studies in viral neoplasia (25th Annual M.D. Anderson Symposium on Fundamental Cancer Research, 1972). Williams and Wilkins Co., Baltimore.
5. **Billeter, M. A., C. Weissman, and R. C. Warner.** 1966. Replication of viral ribonucleic acid. IX. Properties of double-stranded RNA from *Escherichia coli* infected with bacteriophage MS2. J. Mol. Biol. 17:145-173.
6. **Bishop, J. M., A. J. Faras, A. C. Garapin, C. Hansen, N. Jackson, W. E. Levinson, J. M. Taylor, and H. E. Varmus.** 1974. RNA-directed DNA polymerase and the replication of Rous sarcoma virus, p. 229-257. *In* R. W. Crumley (ed.), Molecular studies in viral neoplasia (25th M.D. Anderson Symposium on Fundamental Cancer Research, 1972). Williams and Wilkins Co., Baltimore.
7. **Bishop, J. M., G. Koch, B. Evans, and M. Merriman.** 1969. Poliovirus replicative intermediate: structural basis of infectivity. J. Mol. Biol. 46:235-249.
8. **Bishop, J. M., and L. Levintow.** 1971. Replicative forms of viral RNA. Structure and function, p. 1-82. *In* J. Melnick (ed.), Progress in medical virology, vol. 13. S. Karger, Basel.
9. **Bishop, J. M., and H. E. Varmus.** 1975. The molecular biology of RNA tumor viruses, p. 3-48. *In* F. F. Becker (ed.), Cancer, vol. 2. Plenum Press, New York.
10. **Biswal, N., and M. Benyesh-Melnick.** 1969. Complementary nuclear RNAs of murine sarcoma-leukemia virus complex in transformed cells. Proc. Natl. Acad. Sci. U.S.A. 64:1372-1379.
11. **Coffin, J. M., and H. M. Temin.** 1972. Hybridization of Rous sarcoma virus deoxyribonucleic acid polymerase product and ribonucleic acid from chicken and rat cells infected with Rous sarcoma virus. J. Virol. 9:766-775.
12. **Downey, K. M., J. J. Byrnes, B. S. Jurmark, and A. G. So.** 1973. Reticulocyte RNA-dependent RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. 70:3400-3404.
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. Furuichi, Y., A. J. Shatkin, E. Stavnezer, and J. M. Bishop. 1975. Blocked, methylated 5'-terminal sequence in avian sarcoma virus RNA. Nature (London) 257:618–620.
15. Garapin, A. C., H. E. Varmus, A. J. Faras, W. E. Levinson, and J. M. Bishop. 1973. RNA-directed DNA synthesis by virions of Rous sarcoma virus: further characterization of the templates and the extent of their transcription. Virology 52:264–274.
16. Kozak, M., and B. Roizman. 1975. RNA synthesis in cells infected with herpes simplex virus. IX. Evidence for accumulation of abundant symmetric transcripts in nuclei. J. Virol. 15:36–40.
17. Lai, M. M-C., P. H. Duesberg, J. Horst, and P. K. Vogt. 1973. Avian tumor virus RNA: a comparison of three sarcoma viruses and their transformation-defective derivatives by oligonucleotide finger-printing and DNA-RNA hybridization. Proc. Natl. Acad. Sci. U.S.A. 70:2266–2270.
18. Leong, J. A., A. C. Garapin, N. Jackson, L. Fanshier, W. E. Levinson, and J. M. Bishop. 1972. Virus-specific ribonucleic acid in cells producing Rous sarcoma virus: detection and characterization. J. Virol. 9:891–902.
19. Mikoshiba, K., Y. Tsukada, I. Haruna, and I. Watanabe. 1974. RNA-dependent RNA synthesis in rat brain. Nature (London) 249:445–448.
20. Pettersson, U., and L. Philipson. 1974. Synthesis of complementary RNA sequences during productive adenovirus infection. Proc. Natl. Acad. Sci. U.S.A. 71:4887–4891.
21. Quintrell, N., H. E. Varmus, J. M. Bishop, M. D. Nicholson, and R. M. McAllister. 1974. Homologies among the nucleotide sequences of the genomes of C-type viruses. Virology 58:568–575.
22. Ringold, G., E. Y. Lasfargues, J. M. Bishop, and H. E. Varmus. 1975. Production of mouse mammary tumor virus by cultured cells in the absence and presence of hormones: assay by molecular hybridization. Virology 65:135–147.
23. Schincariol, A. L., and W. K. Joklik. 1973. Early synthesis of virus-specific RNA and DNA in cells rapidly transformed with Rous sarcoma virus. Virology 56:532–548.
24. Schmid, C. W., J. E. Manning, and N. Davidson. 1975. Inverted repeat sequences in the Drosophila genome. Cell 5:159–172.
25. Solymosy, F., I. Fedorscak, A. Gulyas, G. L. Farkas, and L. Ehrenberg. 1968. A new method based on the use of diethylpyrocarbonate as a nuclease inhibitor for the extraction of undegraded nucleic acid from plant tissues. Eur. J. Biochem. 5:520–527.
26. Taylor, J. M., B. Cordell-Stewart, W. Rhode, H. M. Goodman, and J. M. Bishop. 1975. Reassociation of 4S and 5S RNAs with the genome of avian sarcoma virus. Virology 65:248–259.
27. Temin, H. M., and D. Baltimore. 1972. RNA-directed DNA synthesis and RNA tumor viruses. Adv. Virus Res. 17:129–186.
28. Varmus, H. E., R. V. Guntaka, W. J. Fan, S. Heasley, and J. M. Bishop. 1974. Synthesis of viral DNA in the cytoplasm of duck embryo fibroblasts and in enucleated cells after infection by avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. 71:3874–3878.
29. Weissmann, C., and P. Borst. 1963. Double-stranded ribonucleic acid formation *in vitro* by MS2 phage-induced RNA synthetase. Science 142:1188–1191.