Differential expression of poly(A)-adjacent sequences of mammary tumor virus RNA in murine mammary cells

(representative cDNA/3'-cDNA/ BALB/c lactating tissue/ BALB/c and C3H mammary tumor cell lines)

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ABSTRACT   Two DNA probes representative of either the entire mouse mammary tumor virus (MMTV) genome or the poly(A)-adjacent sequences at the 3' end of MMTV RNA were synthesized with calf thymus DNA or oligo(dT) primers, respectively. These probes were used to study the expression of endogenous MMTV sequences in several BALB/c mammary tumor cell lines, in normal lactating BALB/c tissue, and in a cloned C3H tumor cell line. Both probes were characterized with respect to their rates of hybridization with template RNA, their size as determined by alkaline sucrose gradient centrifugation, and the thermal stability of the cDNA·MMTV RNA hybrids. In addition, the ability of the calf thymus oligodeoxynucleotide- or oligo(dT)-primed probes to protect ^{125}I-labeled MMTV RNA or ^{125}I-labeled poly(A)-adjacent MMTV RNA sequences from S1 nuclease digestion was determined. Hybridization analysis with these two probes indicated that (i) there were approximately 20-fold more oligo(dT)-primed sequences in BALB/c lactating tissue than there were sequences representing the entire genome; (ii) in BALB/c tumor cells, the oligo(dT):random oligonucleotide-primed cDNA sequence ratio was reduced to 4:1; and (iii) in virus-producer C3H tumor cells, there was only a 2-fold excess of oligo(dT)-primed sequences over that observed with a representative cDNA. These results are consistent with the presence of subgenomic viral mRNA species, integration of partial proviral copies, or altered mRNA processing.

Previous investigations of the expression of mouse mammary tumor virus (MMTV) sequences in normal and neoplastic mouse mammary glands have been limited to the use of complementary DNA (cDNA) probes which were either uncharacterized (1) or were representative of undefined regions of the viral genome (2). These studies indicated that MMTV RNA could be detected at high levels in the mammary glands and tumors of mouse strains with high incidences of mammary cancer and at low levels in the normal nonmammary tissue of such strains. All normal or neoplastic tissue from strains of mice with low incidences of breast cancer contained low levels of MMTV RNA. However, it was not determined whether there were also qualitative differences in the expression of viral genes in the various murine cells.

In the present communication, two different cDNA probes have been used to study MMTV gene expression. cDNA primed by random calf thymus DNA oligodeoxynucleotides (calf thymus-primed cDNA) was shown to be representative of the entire MMTV genome with relative uniformity, while cDNA primed by (dT)$_{12-18}$ was shown to have a decreased genetic complexity and to hybridize predominantly with sequences adjacent to the poly(A)-terminal end of viral RNA. As described below, the oligo(dT)-primed cDNA probe detected increased levels of 3'-end MMTV RNA sequences in both lactating glands and mammary tumor tissue from BALB/c mice over that observed in virus-producer tumor cells.

MATERIALS AND METHODS

Virus and Cell Lines. Purified MMTV released by Mm5mt/c1 cells (C3H) and purified Moloney leukemia virus (MoLV) were provided by the Office of Program Resources and Logistics, Virus Cancer Program, National Cancer Institute. BALB/cCrgl mouse mammary tumor cell lines were derived from transplantable or spontaneous tumors (3). The H-1 line was generously provided by W. Parks; B/cLi cells (4) were obtained from A. J. Hackett.

Preparation of Viral and Cellular RNA. Viral RNA and cellular RNA extracts were prepared by a sodium dodecyl sulfate/phenol/chloroform extraction procedure (5, 6). Total virion RNA was heated at 80°C for 3 min, rapidly cooled in ice, and extracted three times with 3.0 M sodium acetate (pH 6.0) (7). Viral RNA prepared in this manner had a sedimentation coefficient between 10 and 30 S on neutral sucrose gradients. DNA and low molecular weight RNA were also removed from cellular RNA extracts by this method, except that the heating step was eliminated.

Synthesis of cDNA. A representative MMTV cDNA probe primed by calf thymus oligodeoxynucleotides was synthesized using modifications of Taylor et al. (8) and Young et al. (9). Reactions contained heat-denatured sodium acetate-treated MMTV RNA (35 μg/ml or greater), avian myeloblastosis virus polymerase (125 units/ml), 150 mM KCl, 1 mM unlabeled triphosphates, 200 μM [^{3}H]dCTP, 4 mM sodium pyrophosphate, 14 mM magnesium acetate, 20 mM dithiothreitol, and calf thymus DNA fragment primers at a ratio of 40:1 (primer to template) (μg/μg) in a total reaction volume of 100 μl. Reactions were incubated at 46°C for 20 min prior to purification of the cDNA. Oligo(dT)-primed cDNA was prepared in a 50-μl reaction mixture containing 100 μg of sodium acetate-treated MMTV RNA per ml, 100 μg of (dT)$_{12-18}$ per ml, 20 mM dithiothreitol, 8 mM magnesium acetate, 4 mM Na$_4$P$_2$O$_7$, 50 mM Tris·HCl (pH 8.3), 50 mM KCl, 1 mM each dGTP, dATP, and dTTP, 500 μM [^{3}H]dCTP, and 125 units of avian myeloblastosis virus polymerase per ml. Incubations were performed at 37°C for 1 hr prior to addition of sodium dodecyl sulfate, Na$_2$EDTA, and poly(A) carrier to final concentrations of 0.7%, 14 mM, and 135 μg/ml, respectively. After chromatography on Sephadex G-50, the template RNA was destroyed by alkaline hydrolysis. Average specific activities for tritium-labeled cDNA probes were 1–2 × 10^7 cpm/μg; calculations were based on an average cytosine content of 25%.

Oligo(dT)-primed MMTV cDNA exhibited low levels of hybridization (25%) to purified MoLV RNA at R$_0$t values greater than 1. (R$_0$t is the concentration of ribonucleotides in
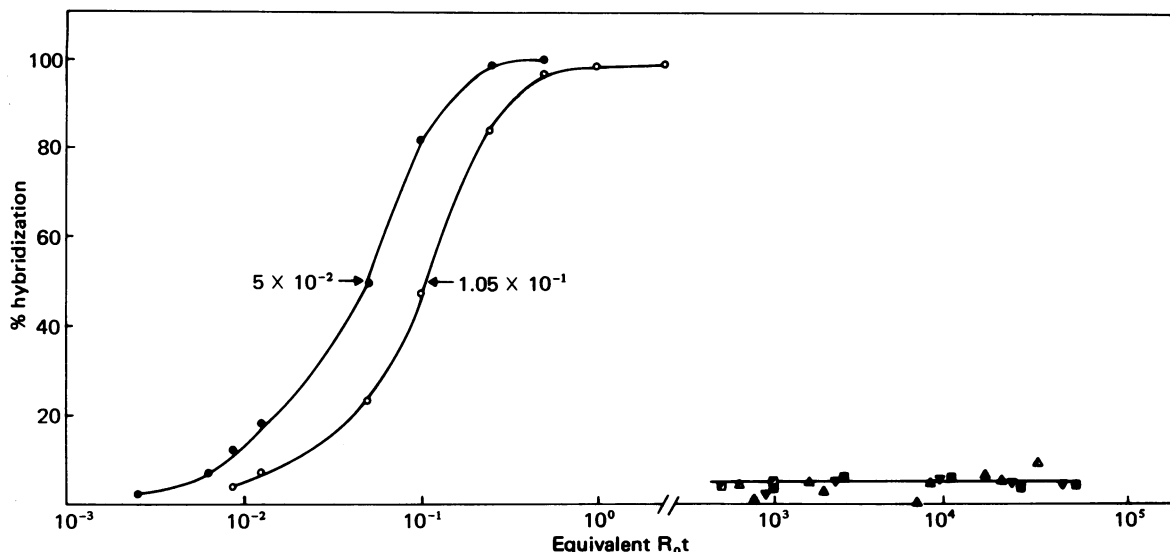
FIG. 1.   Specificity of hybridization to calf thymus-primed and oligo(dT)-primed cDNAs. Annealing of calf thymus-primed cDNA is shown with RNA from the following sources: ▲, BALB/c liver tissue; ■, simian virus 40-transformed cells (VLM); ▲, BALB/c liver cell line grown in 1 μg of dexamethasone and 10 μg of insulin per ml; ▼, BALB/c liver cell line grown in insulin alone; ■, a reconstituted mixture containing 1% MoLV RNA in yeast RNA; ●, purified 10–30S MMTV RNA. Hybridization of oligo(dT)-primed cDNA to a reconstituted mixture containing 1% MoLV RNA in yeast RNA, BALB/c liver tissue RNA, and VLM cell RNA yielded results identical to those shown for calf thymus-primed cDNA. O, Hybridization of the oligo(dT)-primed cDNA to purified 10-30S MMTV RNA.

mol/liter multiplied by time in seconds.) To remove such contaminating sequences, we hybridized oligo(dT)-primed cDNA to sodium acetate-treated MoLV RNA for 8–16 hr at 68°C (R_0t = 1.29–2.59 × 10²). The sample subsequently was adjusted to 0.14 M sodium phosphate buffer (pH 6.4–6.8) and applied to a 1-ml pre-equilibrated hydroxylapatite column. The column was washed successively prior to use with 10 vol of 1 mM, 0.14 M, and 0.5 M phosphate buffer and then equilibrated with 0.14 M phosphate buffer. Fractions were collected from the column, and those containing peak radioactivity were pooled prior to phosphate removal by Sephadex G-50 chromatography in 10 mM Tris·HCl, pH 7.6/0.1 M NaCl/2 mM Na₂EDTA. Unhybridized MoLV RNA was destroyed by alkali treatment.

RNA Excess Hybridizations and Protection Assays. Hybridizations were performed as described (10) and hybrid formation was assayed with S1 nuclease digestion (11). Viral RNA for use in nuclease protection experiments was iodinated by the technique of Commerford (12) with modifications of Woo *et al.* (13) and Prensky *et al.* (14). Fractions containing

FIG. 2.   Thermal stability of cDNA·MMTV RNA hybrids. 10–30S sodium acetate-treated MMTV RNA was hybridized to either (●) calf thymus-primed or (O) oligo(dT)-primed cDNA to a R_0t of 1.2. Samples were diluted to 0.2 M NaCl prior to thermal denaturation and assay with S1 nuclease.

RNA with sedimentation values of 10–20 S were used for the protection experiments. The melting profile of cDNA·RNA hybrids was determined by the method of Rosen and Barker (10).

RESULTS

Characterization of MMTV cDNAs. cDNA preparations used in hybridization studies were initially characterized for their specificity of hybridization, size in alkaline sucrose gradients, and representation of viral sequences. The specificity of cDNA hybridization was determined (Fig. 1). A $R_0t_{1/2}$ of 5 × 10⁻² was obtained when calf thymus-primed cDNA was hybridized to template MMTV RNA, while a value of 1.05 × 10⁻¹ was observed with oligo(dT)-primed cDNA·MMTV RNA hybrids. Although annealing occurred at a slower rate with oligo(dT)-primed cDNA than with calf thymus-primed cDNA, both reactions displayed pseudo-first-order kinetics. The slower rate of hybridization could have resulted from differences in complexity, size, or base composition of the cDNAs used (15). These possibilities will be considered below.

Calf thymus-primed cDNA exhibited only background hybridization values to all control RNAs tested, including a reconstituted mixture containing 1% MoLV RNA at R_0t values approaching 100,000 (Fig. 1). Hybridization of the oligo(dT)-primed probe to RNA extracted from simian virus 40-transformed BALB/c 3T3 cells and BALB/c liver tissue or the reconstituted MoLV preparation yielded similar results. In addition, no hybridization was detected to chicken oviduct ribosomal RNA, to poly(adenylic acid), to yeast RNA, or to RNA from two dimethylbenz[a]anthracene-induced mammary cell lines (3) with either of the cDNA preparations (data not shown). The thermal denaturation profiles of hybrids formed between MMTV 10–30S denatured RNA and cDNAs synthesized using either calf thymus DNA or oligo(dT) primers are shown in Fig. 2. The dissociation temperature (t_m) values for calf thymus- and oligo(dT)-primed cDNA·RNA hybrids were 86.4°C and 84.5°C, respectively. The slightly lower t_m (≈2°C) of the latter hybrid may be a result of its lower G+C content; however, both values are consistent with results obtained for other well base-paired RNA·cDNA duplexes (10, 11). Size analysis by alkaline
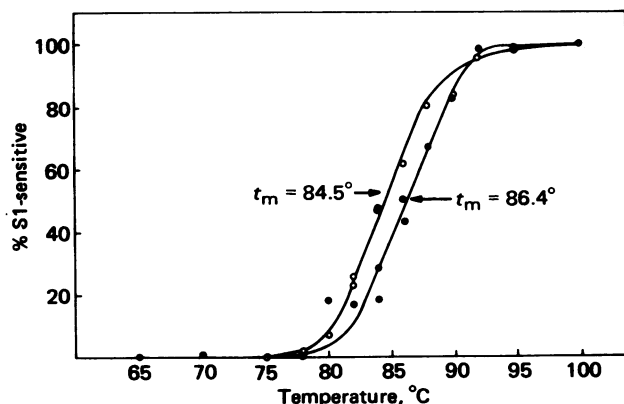
sucrose gradient sedimentation indicated that differences between the two cDNA probes were minimal; average sizes were 550 nucleotides for the calf thymus-primed cDNA and 400 nucleotides for the oligo(dT)-primed cDNA.

The complexity of the cDNA preparations was next evaluated by their ability to protect iodinated MMTV RNA from S1 nuclease digestion. Equal ratios of calf thymus-primed cDNA and 50–70S viral RNA resulted in 50% S1 nuclease-resistant material, while 90% protection was achieved at ratios of 10:1 (cDNA:RNA). Complete hybridization, indicative of 100% copying of the template MMTV RNA, was obtained at ratios of 20:1 or greater. Lower ratios of cDNA:RNA ($\approx$2:1) were needed for complete nuclease resistance with sodium acetate-treated MMTV RNA (data not shown).

In contrast, the results of a similar experiment with oligo(dT)-primed cDNA are shown in Fig. 3. cDNA-to-RNA ratios of 1:1 resulted in protection of 27% of iodinated MMTV RNA, while 8:1 ratios protected 49% of viral RNA. Comparison of the two protection experiments revealed that the complexity of calf thymus-primed cDNA was substantially different from that of oligo(dT)-primed cDNA, although considerably more of the genome RNA was rendered S1-resistant by the latter probe than would be expected from its size alone.

Since it was assumed that oligo(dT)-primed cDNA was composed primarily of sequences specific for the 3' end of viral RNA, the ^{125}I-labeled MMTV RNA was subjected to oligo(dT)-cellulose chromatography. Only the poly(A)-containing RNA fragments were isolated by washing the oligo(dT)-cellulose with intermediate KCl concentrations (as detailed in the legend to Fig. 3), thereby eliminating any oligo(A)-containing material (16). The results of a protection experiment using oligo(dT)-primed cDNA and poly(A)-containing MMTV RNA are also depicted in Fig. 3. Approximately 64% of ^{125}I-labeled
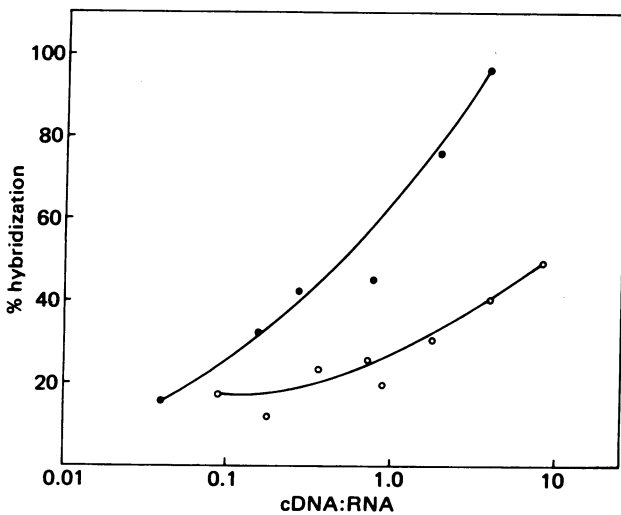

FIG. 3. Protection of ^{125}I-labeled MMTV RNA by oligo(dT)-primed cDNA before and after oligo(dT)-cellulose chromatography. Oligo(dT)-primed cDNA synthesized from 10–30S sodium acetate-treated viral RNA was incubated in increasing ratios with 0.25 ng of radiolabeled size-selected template RNA. A second portion of the ^{125}I-labeled MMTV RNA was heated at 68°C for 1 min and bound to oligo(dT)-cellulose in 0.5 M KCl/1 mM Na2EDTA/10 mM Tris·HCl, pH 7.6. The column was subsequently washed with 0.25 M KCl/1 mM Na2EDTA/10 mM Tris·HCl, pH 7.6, and then with 0.1 M KCl/1 mM Na2EDTA/10 mM Tris·HCl, pH 7.6, to elute any oligo(A)-containing viral sequences. Poly(A)-containing MMTV RNA was eluted with 10 mM Tris·HCl, pH 7.6/1 mM Na2EDTA (recovery: 1.4% of iodinated RNA). The ability of oligo(dT)-primed cDNA to protect the poly(A)-containing viral RNA (0.4 ng) was assayed with S1 nuclease. O, Before oligo(dT)-cellulose chromatography; ●, after chromatography.

RNA was S1 resistant at cDNA:RNA ratios of 1:1, and essentially complete protection was attained at ratios of 4:1.

When unlabeled viral RNA that had been chromatographed on oligo(dT)-cellulose was used, oligo(dT)-primed cDNA hybridized approximately 4-fold faster to the poly(A)-containing RNA than to the fraction not bound to oligo(dT). In contrast, hybridization of calf thymus-primed cDNA to oligo(dT)-bound MMTV RNA occurred 2.3 times more slowly than hybrid formation with non-oligo(dT)-selected viral RNA. Identical kinetics of hybridization were obtained with RNA not bound to oligo(dT) and non-oligo(dT)-selected MMTV RNA. Therefore, the majority of sequences present in oligo(dT)-primed cDNA appear to be specific for regions adjacent to the poly(A) end of MMTV RNA; the probe will subsequently be referred to as 3'-cDNA.

Expression of 3' Sequences in Mouse Mammary Cells. Since very low levels of MMTV sequences have been detected in BALB/c mammary tumor cells using a representative calf thymus-primed cDNA (i.e., less than or equal to 6 molecules per cell), it was considered possible that only a portion of the viral genome was expressed in these cells. Therefore, the 3'-cDNA probe was used to facilitate detection of poly(A)-adjacent sequences of the MMTV genome in virus nonproducer cells, in normal lactating tissue, and in virus-producer C3H tumor cells.

Hybridizations of oligo(dT)-primed cDNA and calf thymus-primed cDNA to RNA isolated from normal lactating BALB/c mammary tissue are shown in Fig. 4. Surprisingly, 3'-cDNA detected a greater number of virus-specific sequences than the representative probe. It can be calculated from the $R_0t_{1/2}$ of 15,000 for lactating gland RNA that 3'-end sequences comprised 0.0007% of the RNA, while calf thymus-primed sequences represented 0.000031% of the RNA in lactating glands based on 20% hybridization values obtained with that cDNA probe; i.e., a 23-fold difference. The hybridization of lactating RNA with oligo(dT)-primed cDNA appeared to plateau at approximately 60% hybridization, suggesting that only 60% of the sequences represented by this probe may have been present in RNA isolated from BALB/c lactating tissue. At equivalent R_0t values, no leveling off of hybridization was noted with BALB/c tumor cell lines (see Fig. 5).
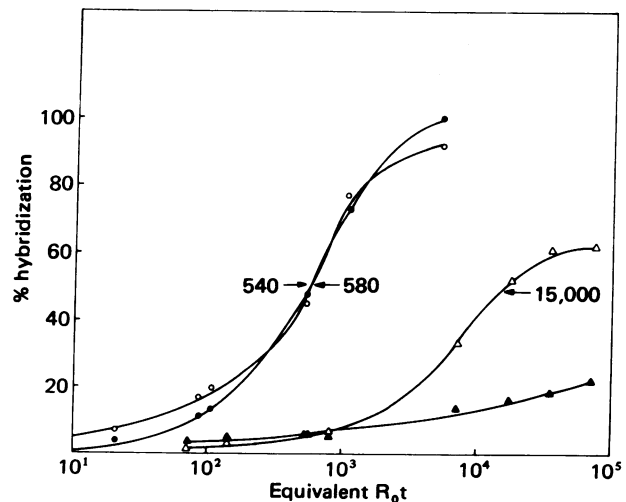

FIG. 4. Analysis of MMTV 3'-end sequences in mice with high tumor incidence (C3H) and low tumor incidence (BALB/c). Total cellular RNA was extracted from H-1 cultures and hybridized to either (●) calf thymus-primed or (O) oligo(dT)-primed cDNA. RNA from 9- to 11-day lactating BALB/c mice (pooled from two animals) was annealed to (▲) calf thymus-primed or (△) oligo(dT)-primed cDNA under the same conditions.
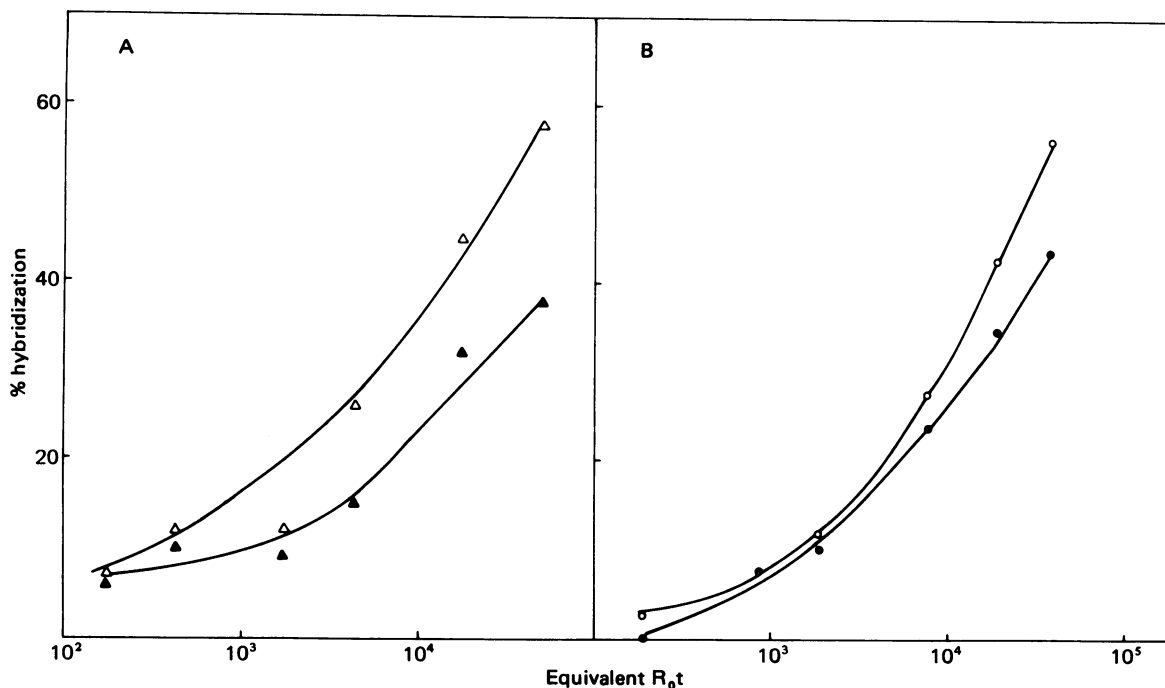
FIG. 5. Transcription of 3'-end sequences in BALB/c mammary tumor cell lines. (*A*) RNA extracted from MTV-L/BALB Cl 2 cells was annealed to either (▲) calf thymus-primed or (△) oligo(dT)-primed cDNA. (*B*) Hybridizations with ESD/BALB cellular RNA were performed using (●) calf thymus-primed or (○) oligo(dT)-primed cDNAs.

The hybridization kinetics observed with H-1 (C3H) virus producer cellular RNA are also depicted in Fig. 4. The $R_0t_{1/2}$ values obtained with oligo(dT)-primed cDNA and calf thymus-primed cDNA were nearly identical—580 and 540, respectively. Since there was approximately a 2-fold difference in the $R_0t_{1/2}$ values observed for the back-hybrids (see Fig. 1), it can be estimated that there were twice as many oligo(dT)-primed sequences as calf thymus-primed sequences in H-1 cells; i.e., 0.018% viral RNA 3'-end sequences compared to 0.0093% total genome sequences. Although both lactating tissue and virus-producer cells appeared to have more 3'-end sequences than those detected by calf thymus-primed cDNA, the ratio of these sequences was increased 10-fold in the lactating tissue relative to the C3H tumor cells. Since there was a notable difference in the levels of 3'-end sequences detected in normal BALB/c glands and in tumor cells of C3H origin, RNA isolated from several BALB/c tumor cell lines was hybridized to the oligo(dT)-primed probe (Fig. 5). As shown for ESD/BALB cells, there were approximately 4-fold more oligo(dT)-primed sequences than those detected with calf thymus-primed cDNA. This suggested that the level of 3'-end sequences in BALB/c tumor cells was intermediate between that obtained for normal, lactating BALB/c tissue and C3H tumor cells. Confirmatory results were obtained with a second BALB/c tumor cell line, MTV-L/BALB Cl 2 (i.e., there was a 7-fold enrichment of RNA representing the 3' end of the MMTV genome over that seen with a representative probe). Results identical to those shown in Figs. 4 and 5 were obtained when the experiments were repeated with oligo(dT)-primed cDNA selected by hybridization to poly(A) followed by oligo(dT)-cellulose chromatography, similar to Tal *et al.* (17).

The t_m values obtained after thermal denaturation of H-1 RNA or MTV-L/BALB Cl 2 RNA hybrids with oligo(dT)-primed or calf thymus-primed cDNAs were within 4°C of that obtained with purified MMTV RNA, indicating high-fidelity base pairing for all RNA-cDNA hybrids tested. Thus, the cDNA probes synthesized using MMTV-S are capable of detecting endogenous BALB/c RNA sequences.

DISCUSSION

Interpretation of the differential expression of MMTV RNA sequences detected by oligo(dT)-primed and calf thymus-primed cDNAs in mammary cells is dependent upon the sequence representation of such probes. The use of random oligodeoxynucleotide primers results in the production of relatively uniform copies of viral genomes (this report and refs. 8 and 9). The oligo(dT)-primed cDNA is predominantly specific for sequences at the 3' end of the RNA since lower cDNA:RNA ratios were necessary to protect poly(A)-containing RNA than unfractionated material. Oligo(dT)-primed cDNA of 400–500 nucleotides could protect 100% of an iodinated 11S poly(A)-containing fragment of MMTV RNA from nuclease digestion (Fig. 3), suggesting that the cDNA may represent a reiterated sequence in the viral genome, located presumably at the 3' end of the viral RNA. Although the 3'-cDNA probe could not represent more than 5–6% of the viral RNA [if the MMTV genome is composed of ≈9000 bases (18)], it could protect 12–17% of an unfractionated RNA preparation representing the entire genome (Fig. 3). Results reported by others are not inconsistent with a reiteration of 3'-adjacent sequences in avian sarcoma virus RNA (17) or a subgenomic mRNA species thereof (19). Although the evidence is not conclusive that oligo(dT)-primed cDNA represents a repetitive sequence in MMTV RNA, it does appear that the probe is predominantly specific for the 3' end of the viral RNA.

There were approximately 20-fold more oligo(dT)-primed sequences in BALB/c lactating glands than there were sequences representing the entire genome, while in BALB/c tumor cells the ratio of oligo(dT)-primed to calf thymus-primed cDNA sequences was reduced to approximately 4:1. In virus-producer C3H tumor cells, there was only a 2-fold excess of oligo(dT)-primed sequences over that detected with a representative cDNA. At least four different models can be devised to explain the observed results.

(*i*) An excess of oligo(dT)-primed cDNA sequences in all mammary cell RNAs tested might result from the existence of subgenomic viral mRNA species, with all partial transcripts of

the viral genome containing 3'-end sequences. The existence of subgenomic viral RNA species with sedimentation values of 28 and 21S has been demonstrated in avian sarcoma virus-infected cells (19, 20). Hayward (19) reported that there are 2-fold more 3'-end specific RNA sequences in avian sarcoma virus-infected cells than sequences detected using a 5'-proximal cDNA. In addition, Schochetman and Schlom (21) have suggested that there are independent initiation sites for different classes of MMTV proteins, implying the existence of subgenomic mRNAs in the B-type oncornavirus system as well.

There was a larger ratio of oligo(dT)-primed to calf thymus-primed cDNA sequences in BALB/c tumor cells than in virus-producer tumor cells, an effect magnified in lactating tissue in which oligo(dT)-primed sequences outnumbered calf thymus-primed cDNA sequences 20 to 1. One could speculate that the majority of viral messengers present in BALB/c tumor cells are the two subgenomic viral species (comparable to 28 and 21S RNA in the avian system), while MMTV expression in normal lactating mammary glands is limited to the smallest RNA species or a portion thereof.

(*ii*) Differential 3'-end sequence expression in producer and nonproducer tumor cells could be dependent upon the nature of the integrated MMTV provirus. Transcription in producer C3H cells might proceed off a complete provirus, while BALB/c cells might contain only partial provirus copies. Schlom *et al.* (22) found that mice of the low-cancer strain C3Hf contained only 75% of the viral sequences present in mice of high mammary cancer strains (GR and C3H). Furthermore, only a portion of the sequences of the Mason–Pfizer monkey virus are transmitted as provirus in primates (23). In this model, the difference in sequence expression between normal and neoplastic BALB/c cells could be due to the integration of an exogenous virus (or an appropriate fragment thereof).

(*iii*) The third model suggests that there are different numbers of copies of 3'-end sequences in producer tumor cells, nonproducer tumor cells, and normal nonproducer cells. Although this explanation seems unlikely, duplications of certain regions of viral genomes have been demonstrated; e.g., simian virus 40 mutants containing multiple copies of the origin of replication (for review, see ref. 24).

(*iv*) Sequences that are adjacent in mammalian mRNAs may not be juxtaposed in the cellular DNA (25–27). Therefore, it is possible that the enrichment for 3'-end sequences observed in BALB/c normal or neoplastic tissues is the result of differential mRNA processing in virus nonproducer and virus-producer cells. There is currently no precedent for such a phenomenon.

The available data do not permit discrimination between these models. However, with the availability of well-characterized MMTV DNA and derivative restriction endonuclease fragments (18), it will be possible to distinguish between these possibilities and to establish whether regulation of 3'-end sequences is controlled at the level of mRNA expression or processing or at the level of provirus integration. Identification of the genetic information localized near the 3' end of MMTV RNA is dependent upon the construction of a genetic map of the virus. One may ask whether the 3'-cDNA probe used in

these studies detects a portion of a putative *carc* gene. The biological significance of the altered expression of 3'-end viral sequences in normal and tumorigenic BALB/c mammary tissue is currently unknown.

1. Schlom, J., Michalides, R., Kufe, D., Hehlmann, R., Spiegelman, S., Bentvelzen, P. & Hageman, P. (1973) *J. Natl. Cancer Inst.* 51, 541–551.
2. Varmus, H. E., Quintrell, N., Medeiros, E., Bishop, J. M., Nowinski, R. C. & Sarkar, N. H. (1973) *J. Mol. Biol.* 79, 663–679.
3. Butel, J. S., Dudley, J. P. & Medina, D. (1977) *Cancer Res.* 37, 1892–1900.
4. Owens, R. B., Smith, H. S. & Hackett, A. J. (1974) *J. Natl. Cancer Inst.* 53, 261–269.
5. Rosen, J. M., Woo, S. L. C. & Comstock, J. P. (1975) *Biochemistry* 14, 2895–2903.
6. Rosen, J. M., Woo, S. L. C., Holder, J. W., Means, A. R. & O'Malley, B. W. (1975) *Biochemistry* 14, 69–78.
7. Palmiter, R. D. (1974) *Biochemistry* 13, 3606–3615.
8. Taylor, J. M., Illmensee, R. & Summers, J. (1976) *Biochim. Biophys. Acta* 442, 324–330.
9. Young, H. A., Shih, T. Y., Scolnick, E. M. & Parks, W. P. (1977) *J. Virol.* 21, 139–146.
10. Rosen, J. M. & Barker, S. W. (1976) *Biochemistry* 15, 5272–5280.
11. Monahan, J. J., Harris, S. E., Woo, S. L. C., Robberson, D. L. & O'Malley, B. W. (1976) *Biochemistry* 15, 223–233.
12. Commerford, S. L. (1971) *Biochemistry* 10, 1993–1999.
13. Woo, S. L. C., Rosen, J. M., Liarakos, C. D., Choi, Y. C., Busch, H., Means, A. R., O'Malley, B. W. & Robberson, D. L. (1975). *J. Biol. Chem.* 250, 7027–7039.
14. Prensky, W., Steffensen, D. M. & Hughes, W. L. (1973) *Proc. Natl. Acad. Sci. USA* 70, 1860–1864.
15. Bishop, J. M. (1972) in *Karolinska Symposia on Research Methods in Reproductive Endocrinology*, 5th Symposium, Gene Transcription in Reproductive Tissues, ed. Diczfalusy, E. (Forum Printer, Copenhagen), pp. 1–26.
16. Edmonds, M. & Caramela, M. G. (1969) *J. Biol. Chem.* 244, 1314–1324.
17. Tal, J., Kung, H.-J., Varmus, H. E. & Bishop, J. M. (1977) *Virology* 79, 183–197.
18. Shank, P. R., Cohen, J. C., Varmus, H. E., Yamamoto, K. R. & Ringold, G. M. (1978) *Proc. Natl. Acad. Sci. USA* 75, 2112–2116.
19. Hayward, W. S. (1977) *J. Virol.* 24, 47–63.
20. Weiss, S. R., Varmus, H. E. & Bishop, J. M. (1977) *Cell* 12, 983–992.
21. Schochetman, G. & Schlom, J. (1976) *Virology* 73, 431–441.
22. Schlom, J., Colcher, D., Drohan, W., Kettmann, R., Michalides, R., Vlahakis, G. & Young, J. (1977) *Cancer* 39, 2727–2733.
23. Drohan, W., Colcher, D., Schochetman, G. & Schlom, J. (1977) *J. Virol.* 23, 36–43.
24. Fareed, G. C. & Davoli, D. (1977) *Annu. Rev. Biochem.* 46, 471–522.
25. Gilbert, W. (1978) *Nature (London)* 271, 501.
26. Aloni, Y., Dhar, R., Laub, O., Horowitz, M. & Khoury, G. (1977) *Proc. Natl. Acad. Sci. USA* 74, 3686–3690.
27. Berget, S. M., Moore, C. & Sharp, P. A. (1977) *Proc. Natl. Acad. Sci. USA* 74, 3171–3175.