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Primate RNA Tumor Virus-Like DNA Synthesized Endogenously by RNA-Dependent DNA Polymerase in Virus-Like Particles from Fresh Human Acute Leukemic Blood Cells

(leukemia/molecular hybridization/type-C sarcoma virus)

R. C. GALLO*, N. R. MILLER†, W. C. SAXINGER*, AND D. GILLESPIE*

*Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20014; and †Bionetics Research Laboratory, Bethesda, Maryland

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ABSTRACT **A particle of discrete biochemical composition was purified from fresh, unfrozen peripheral blood leukocytes of human patients with acute myeloblastic leukemia. This particle endogenously synthesized DNA by use of an RNA primer and template. About half of the DNA sequences synthesized in the presence of actinomycin D hybridized to RNA isolated from type-C sarcoma viruses of primates or mice; lower annealing values were obtained with RNA isolated from other sarcoma or leukemia viruses. The results confirm and extend previous results from molecular hybridization experiments related to the existence in human leukemia of components of RNA tumor viruses.**

Recent biochemical data revealed the presence of components of certain type-C RNA tumor viruses in human acute leukemic cells. Gallo and associates isolated a protein from peripheral blood leukemic leukocytes that had biochemical properties of RNA-dependent DNA polymerase of viruses (1, 2); Todaro and Gallo have now shown that it is immunologically specifically related to RNA-dependent DNA polymerase purified from type-C sarcoma and leukemia viruses of primates (3). Work from Spiegelman's and our laboratories has demonstrated that (*1*) this DNA polymerase activity in human acute leukemia is recovered from a cytoplasmic subcellular fraction having a density characteristic of RNA tumor virus particles of animals (4, 5); (*2*) the endogenous template for the DNA polymerase reaction is likely to be a high-molecular-weight RNA molecule (refs. 5 and 6; R. E. Gallagher, unpublished); (*3*) the purified enzyme uses synthetic template–primers with a specificity like RNA-dependent DNA polymerase of viruses and different from the major DNA polymerases of normal proliferating leukocytes (2, 7); and (*4*) the purified enzyme can copy heteropolymer regions of a natural RNA template-primer complex isolated from animal tumor viruses (2, 8).

Abbreviations: Tumor viruses used as a source of RNA: SiSV (NRK), simian sarcoma virus grown in normal rat kidney cells (NRK); MuSV (Kirsten), a sarcoma–leukemia virus complex grown in NRK cells which originated by repeated infection of rats with a Gross-type murine leukemia virus; MuLV (AKR), a Gross-type murine leukemia virus grown in mouse fibroblast cells and originating spontaneously from AKR mice; AvLV (AMV), avian leukosis virus, strain avian myeloblastosis virus; MuLV (Rauscher), murine leukemia virus, strain Rauscher; FeSV (Gardner), feline sarcoma–leukemia virus, strain Gardner; FeLV (Rickard), feline leukemia virus, strain Rickard.

Powerful arguments for the presence of RNA tumor viruses or components of these viruses in human acute leukemic cells also stemmed from molecular hybridization experiments done in Spiegelman's laboratory. Leukemic, but not normal, human cells contained RNA genetically similar to mouse (Rauscher) leukemia virus RNA and not to RNA from unrelated viruses (9). Experiments by Baxt *et al.* (6), using *DNA* products synthesized endogenously by human material, leave little doubt that a small but isolatable portion of this DNA contains sequences genetically related to RNA of mouse leukemia virus but not to RNA from unrelated viruses. Unfortunately, until the present communication, these hybridization experiments were not confirmed.

We show here that the hybridization results can be reproduced with low efficiency using the mouse leukemia virus probe. We demonstrate further that the DNA synthesized endogenously by RNA-dependent DNA polymerase contained among its sequences a high proportion (50%) capable of hybridizing to RNA isolated from a primate type-C sarcoma virus and/or a murine sarcoma virus. We also find that the DNA-synthesizing activity was recovered in a particle not disaggregated by physical manipulation (unlike the vast majority of cytoplasmic particulate material), which had a density of 1.16–1.17 g/ml.

The endogenous polymerase used RNA as both template and primer for DNA synthesis. The present results stress the importance of purification of the cytoplasmic particle to obtain a suitable DNA probe. Finally, we found that virus-related RNA can be detected in the same leukemic cells by using a DNA probe synthesized by the primate type-C sarcoma virus.

MATERIAL AND METHODS

Source of Leukemic Cells. Patient G.F. was a 49-year-old woman who developed acute myeloblastic leukemia this year. The diagnosis was made from cytological examination of bone marrow and peripheral blood. The leukocyte count was elevated to 38,000 cells per mm^3. The majority (>80%) were leukemic myeloblasts. The cells used for our studies were collected by leukophoresis 4 days after the onset of prednisone treatment, which was without clinical efficacy. *Patient W.* was a 26-year-old man with chronic myelogenous leukemia since 1968. Cytogenetic studies showed the presence of the Ph1 chromosome. Commencing Feb. 1973 he was treated with

TABLE 1. *Hybridization of [^{3}H]DNA product of endogenous RNA-dependent DNA polymerase from human leukemic (G.F.) cytoplasmic particle to RNA isolated from RNA tumor viruses*

Exp.	RNA	cpm	cpm (−AMV)	% Hybridization
1	SiSV (NRK)	550	450	53
	MuSV (Kirsten)	259	159	19
	MuLV (AKR)	145	45	5
	AvLV (AMV)	100	—	—
	Input	840	—	—
2	MuLV (Rauscher, plasma virus)	261	42	5
	MuLV (Rauscher, tissue-culture virus)	264	45	5
	AvLV (AMV)	219	—	—
	Input	800	—	—

Data are presented as crude filter-bound radioactivity in counts per min (cpm), as the same data corrected for radioactivity trapped by a heterologous RNA (cpm minus AMV), or as the corrected data expressed as percent of the input DNA recovered in a hybrid structure (% hybridization). [^{3}H]DNA products were synthesized (2) in the presence of actinomycin D, with fraction 15 of Fig. 1 as a source of DNA polymerase and template-primer complex. These DNA products were purified and annealed for 8 days in 0.1 ml of 50% formamide–0.45 M NaCl–0.045 M Na-citrate (final concentrations) to 0.2 μg of immobilized 70S viral RNA or 2 μg of poly(A) (13). In Exp. 2, the unhybridized DNA from hybridizations of Exp. 1 to MuSV, MuLV, and AvLV RNA were pooled and hybridized for 2 weeks to the indicated RNA preparations.

cytosine arabinoside, and cytoxan. Commencing March 1973 he was treated only with vincristine, but he developed an acute "blast crisis." The majority of cells were immature myeloblasts similar to cells seen in acute myeloblastic leukemia. His leukocyte count was very high (576,000 leukocytes per mm^3). These were the cells used in this study.

Preparation of Subcellular Fractions and Synthesis of [^{3}H]-DNA. All operations were done at 0°. Fresh human leukemic myeloblasts (15 g) were separated from other blood components as described (11), washed twice with, and finally suspended in, 3 volumes of cold RSB buffer [10 mM NaCl–10 mM Tris·HCl (pH 7.4)–1.5 mM $MgCl_2$]. Suspended cells were allowed to swell for 10 min; then they were manually homogenized. Glycerol was immediately added (1/10 volume), and large particles including nuclei were removed by centrifugation at 2500 × *g* for 5 min. The pellet was suspended in the original volume of RSB buffer and the homogenization and centrifugation steps were repeated. The low-speed supernatants were combined and clarified by an intermediate-speed centrifugation at 12,100 × *g* for 20 min to remove mitochondria. The intermediate-speed supernatant was sedimented through 30% glycerol in TNE buffer [10 mM Tris·HCl (pH 7.4)–0.1 M NaCl–1 mM EDTA] for 2 hr at 100,000 × *g*. The sedimented material was layered over a 20–50% sucrose gradient and centrifuged at 100,000 × *g* to equilibrium (more than 12 hr). Fractions were collected and assayed for absorbance at 260 nm and for endogenous

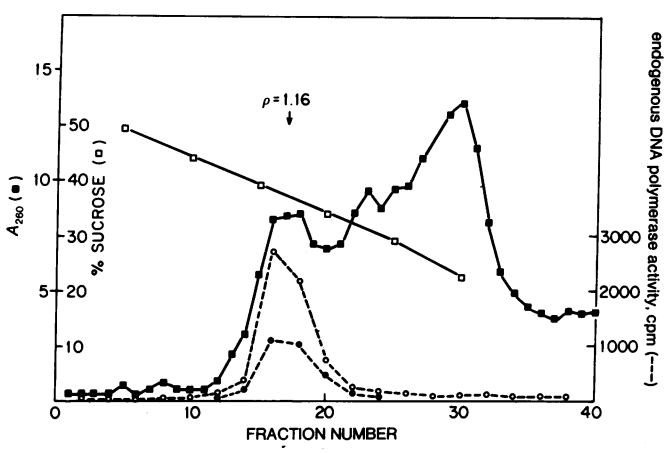

FIG. 1. Isopycnic fractionation of cytoplasmic endogenous DNA polymerase activity from patient G.F. DNA polymerase activity: (○) without RNase; (●) with RNase.

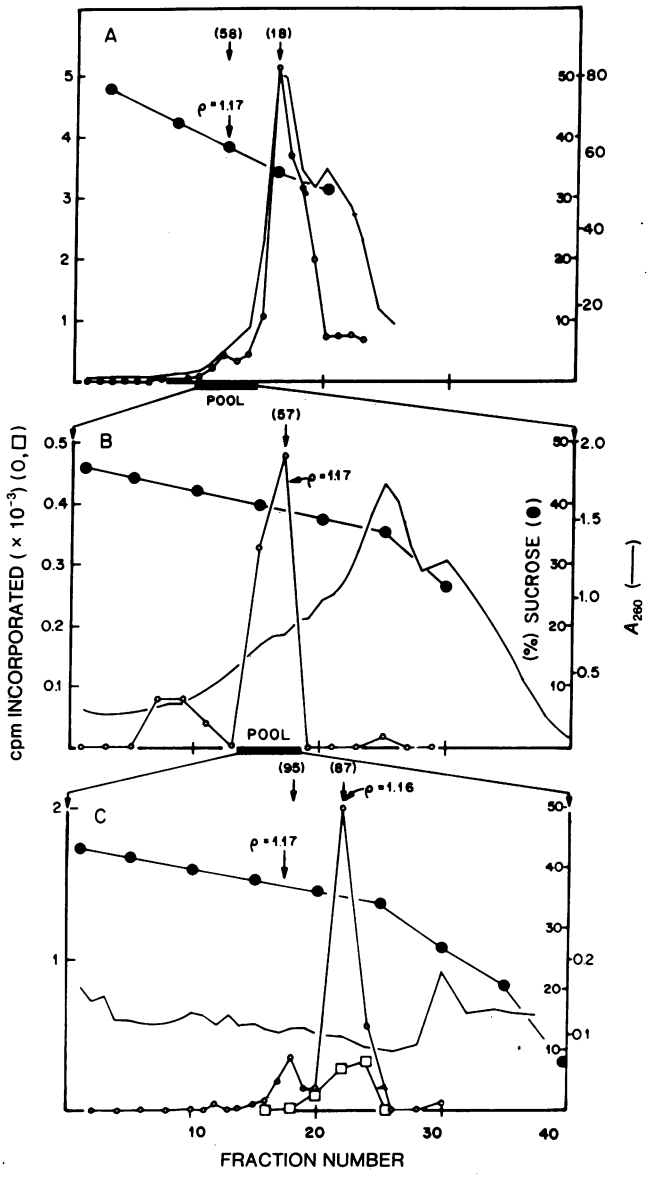

FIG. 2. Isopycnic fractionation of cytoplasm from patient W. *Numbers in parentheses* are % RNase sensitivity. DNA polymerase activity: (○) without RNase; (□) with RNase.

TABLE 2. *Hybridization of [³H]DNA product of endogenous RNA-dependent DNA polymerase reaction from human leukemic (W.) cytoplasmic particle to RNA isolated from RNA tumor viruses*

	W-1 DNA			W-2 DNA		
	cpm	cpm (−AMV)	% Hybridized	cpm	cpm (−AMV)	% Hybridized
SiSV (NRK)	580	414	23	577	357	22
MuSV (Kirsten)	965	799	41	1110	894	58
MuLV (AKR)	406	240	14			
MuLV (Rauscher, plasma virus)	507	342	19	584	365	27
MuLV (Rauscher, tissue-culture virus)	403	237	12			
FeSV (Gardner)	270	103	5	364	144	9
FeLV (Rickard)	130	0	0	155	0	0
AvLV (AMV)	166	—	0	219	—	0
Poly(A) (2 μg)	172	6	0	197	0	3
Input	1940	—	—	1380	—	—

Data are presented and [³H]DNA products were generated as described in the legend to Table 1, except that fraction 18 or 23 and 24 from Fig. 2 were used as a source of DNA polymerase and template–primer complex for synthesis of W-1 DNA or W-2 DNA, respectively. Hybridization was for 15 days and otherwise was as described in the legend to Table 1.

RNA-dependent DNA polymerase activity (2). Subcellular cytoplasmic particles from one patient (G.F.) were used at this stage to generate [³H]DNA. In samples from patient W., fractions having DNA polymerase activity in particles of a density of about 1.17 g/ml determined from the first isopycnic centrifugation were pooled, diluted to 20% sucrose in TNE buffer, and centrifuged at 100,000 × *g* for 8 hr in a 30–43% sucrose gradient in TNE buffer. The main RNA-dependent DNA polymerase activity from this first rebanding was pooled, frozen at −70°, diluted to 20% sucrose with TNE buffer, and then again centrifuged at 100,000 × *g* for 8 hr in a 30–43% sucrose gradient. Enzymatically active fractions from this final purification step were used to synthesize endogenous [³H]DNA. Sucrose gradients were monitored for polymerase activity by measuring incorporation of [³H]dTTP (18 Ci/mmol) into acid-precipitable material. DNA synthesized for hybridization purposes used [³H]dATP (17.3 Ci/mmol), [³H-]dCTP (30 Ci/mmol), and [³H]dGTP (10 Ci/mmol) and was synthesized in the presence of 40 μg/ml of actinomycin D.

Purification of Nucleic Acids. Purification of DNA and RNA followed published procedures, but was at pH 9 (10, 12, 15). DNA was treated for 20 hr at 37° with 0.3 N NaOH and then neutralized, before use in hybridization experiments. RNA from cytoplasmic fractions of leukemic cells was dissolved in TNE buffer to a final concentration of 1 mg/ml and applied to 7-mm diameter phosphocellulose filter discs as described (13). 70S viral RNA was purified from the crude viral RNA preparation by glycerol gradient centrifugation.

RESULTS

Viral-related DNA synthesized by myeloblastic human leukemic cells

Cytoplasmic Fractionation and Molecular Hybridization (Patient G.F.). Fig. 1 presents results obtained from the isopycnic banding in a 20–50% sucrose gradient of particulate cytoplasmic material from patient G.F. Most of the material at A_{260} was recovered in fractions of relative low density, although a prominent shoulder was observed at a density of 1.16–1.17 g/ml. This particular patient displayed only one peak of endogenous DNA polymerase activity, which was 61% sensitive to RNase treatment and was recovered exclusively in the 1.16–1.17 density region.

DNA product synthesized in the presence of actinomycin D (2) by the pooled active fractions was purified, digested with NaOH, and annealed for 8 days to denatured viral RNA immobilized on 7-mm phosphocellulose discs (13) (Table 1). In agreement with previously published data (6), human DNA product did not hybridize to RNA of avian leukosis virus, strain avian myeloblastosis virus [AvLV (AMV)]. A small but detectable amount of annealing was obtained with RNA isolated from murine leukemia virus (MuLV), Rauscher strain, a result that also confirms the earlier data (6). Neither the strain of MuLV (Rauscher or AKR) nor the source of the Rauscher virus (plasma or tissue culture) influenced the magnitude of the hybridization response. However, a significantly higher fraction of the input DNA (19%) was capable of hybridizing to RNA from murine sarcoma virus (MuSV).

Over half of the input human DNA annealed to RNA isolated from the primate type-C virus, simian sarcoma virus (SiSV), derived from a woolly monkey. In our minds this high percentage of hybridization of the DNA product renders untenable any interpretation resting on the assumption that the hybridization arises from the presence, by chance, of sequences complementary to the test RNA. We conclude from the data on this patient that a cytoplasmic entity having a density of 1.16–1.17 in the human cells can indeed synthesize viral-like DNA.

Repeated Isopycnic Fractionation of Cytoplasm from Patient W. Fig. 2*A* presents the initial isopycnic banding of particulate cytoplasmic material from patient W. We note the appearance of two DNA polymerase activities—a minor activity 50% sensitive to RNase, banding at a density of about 1.17 with small amounts of material at A_{260}, plus a major RNase-resistant activity, recovered at a density of 1.13–1.15 with large amounts of material at A_{260}. In our hands, this, rather than the single DNA polymerase activity observed in patient

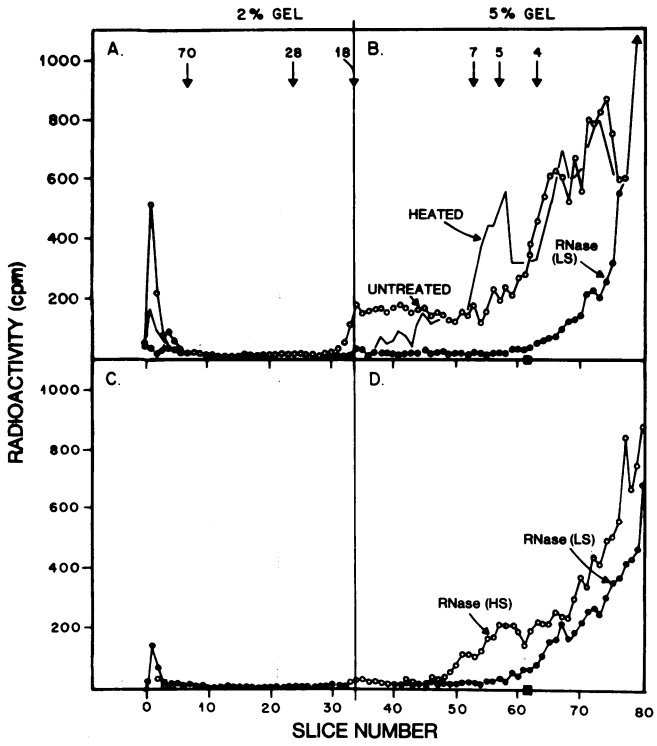

FIG. 3. Analysis on composite polyacrylamide gels of DNA synthesized by cytoplasmic particles of patient W. [^{3}H]DNA products (not treated with NaOH) were prepared. Na dodecyl sulfate–polyacrylamide gels were run according to Bishop *et al.* (18). First a 7-cm 5% gel was prepared. After it had polymerized, a 4.4-cm 2% gel was polymerized above. Electrophoresis was for about 2^1/$_2$ hr at 5 mA/gel at room temperature. Gels were subsequently frozen, extruded from their Lucite tubes, sliced with a parallel razor blade assembly, and analyzed for radioactivity. Samples were treated with boiled pancreatic RNase A for 60 min at 37° at 20 μg/ml in H_2O (*LS*) or in 10 mM Tris·HCl (pH 4.7)–10 mM $MgCl_2$–0.5 M NaCl (*HS*). Samples were heated for 5 min at 70°. The position of markers (4, 5, 7, 18, and 28S RNA) isolated from mouse fibroblast cells) was determined in parallel gels and transposed to the experimental gel by calculating their migration with respect to that of the marker dye, bromphenol blue (*solid square on abscissa*). Material migrating further than slice 75 is likely to represent trichloroacetic acid soluble material.

G.F., is representative of several cases of fractionated cytoplasm of human acute leukemic cells.

Fig. 2*B* and *C* present successive isopycnic fractionations in shallow sucrose gradients of cytoplasmic material having a density of 1.16–1.17 and containing DNA polymerase activity. In both rebandings, the material at A_{260} redistributed itself, while the DNA polymerase activity retained its characteristic density. Repeated banding of the cytoplasmic material achieved a 5000-fold purification of the material with endogenous activity in units of TMP incorporated/A_{260} unit, completely separated an active high-density material from a major active material of lower density, and resulted in purified material with endogenous activity which was 85–100% RNase sensitive. We interpret this result as evidence that the RNA-dependent DNA polymerase activity is part of a particle with a well-defined biochemical composition.

Molecular Hybridization with Viral-Like DNA Synthesized by Patient W. DNA products were prepared for molecular hybridization studies in the presence of actinomycin D using endogenous DNA polymerase activities from the satellite (ρ = 1.17, W-1 DNA) or main band (ρ = 1.16, W-2 DNA) obtained after the third isopycnic fractionation (see Fig. 2*C*). The pattern of hybridization between cytoplasmic DNA products of patient W. and RNAs of selected tumor viruses showed a remarkable similarity to the pattern described for DNA synthesized by fractionated cytoplasm of patient G.F. (Table 2). No annealing was observed with RNA from AvLV or feline leukemia virus (FeLV) or with poly(A). Low hybridization occurred with RNA of feline sarcoma–leukemia virus (FeSV). Moderate (15–25% of the input DNA) hybridization occurred with RNA from MuLV, and again neither strain of virus nor source (plasma compared with tissue culture) of the Rauscher strain significantly influenced the data. As in G.F., the best hybridization of DNA from patient W. was obtained with RNA isolated from mouse or simian sarcoma viruses and, again, the best annealing involved over half of the input DNA.

Polyacrylamide-Gel Analysis of Endogenous RNA-Dependent DNA Polymerase Products of Patient W. Fig. 3 presents a polyacrylamide-gel analysis of the reaction products of the endogenous RNase-sensitive DNA polymerase activity of the particle from patient W. (W-2 DNA). Similar analyses on DNA synthesized by animal tumor viruses showed that the purified untreated product of the RNA-dependent DNA polymerase reaction of AvLV (AMV) migrated in polyacrylamide gels as two entities—a complex of about 70 S and lower molecular weight material of heterogeneous size (4–10 S). Heating the untreated complex yielded material migrating at 5–7 S (product–primer). Treatment with alkali or with RNase in low salt concentration liberated the single-stranded DNA product, which migrated as a sharp band between 4 and 5 S. Similar analyses of intermediates synthesized by mammalian RNA tumor viruses yielded a similar pattern, except that the RNA-free DNA chains are heterogeneous in chain length and are unusually short (less than 4 S).

In order to visualize as many size classes of material as possible in the human-derived product of the RNA-dependent DNA polymerase reaction, composite gels, which consisted of a lower portion of 5% gel and an upper of 2% gel, were used.

TABLE 3. *Hybridization of SiSV DNA to RNA isolated from RNA tumor viruses*

RNA	cpm	cpm (—AMV)	% Hybridized
SiSV (NRK)	2510	2400	57
MuSV (Kirsten)	2090	1970	45
MuLV (AKR)	1830	1610	37
MuLV (Rauscher, tissue-culture virus)	202	86	2
MuLV (Rauscher, plasma virus)	236	126	3
FeSV (Gardner)	201	85	2
FeLV (Rickard)	148	32	<1
AvLV (AMV)	116	0	0
Poly (A)	163	47	1
Input	4240	—	—

[^{3}H]DNA synthesized endogenously by SiSV virions was annealed to immobilized RNA as described in the legend to Table 1.

Material 18 S and larger remain in the 2% gel while smaller material penetrates the 5% gel (Fig. 3). The untreated sample, ^{3}H-labeled in the DNA moiety, was recovered near the top of the 2% gel (Fig. 3*A*) and as heterogeneous material in the 5% gel (Fig. 3*B*). No peak at exactly 70 S was observed. The radioactivity in the untreated complex, which migrated between slices 1 and 50, was sensitive to RNase in high (Fig. 3*C* and *D*) or low (Fig. 3*A* and *B*) salt concentration. Heating the untreated complex eliminated a large fraction of the material at the top of the 2% gel (Fig. 3*A*) and the material in slices 30–45 in the 5% gel (Fig. 3*B*). Concomittantly, material assumed to be product–primer complex accumulated in slices 52–59 upon heating (Fig. 3*B*). This complex generated upon heating was sensitive to subsequent RNase digestion (Fig. 3*D*).

The high-molecular-weight complex obtained from the untreated sample (Fig. 3*A*) migrated more slowly than marker 70S RNA. However, a complex of exactly 70 S should not be demanded at this stage of experimentation, since Spiegelman's laboratory has reported variable sedimentation values when human intracellular material is analyzed (5, 6) and since there is no way of predicting the behavior of the high-molecular-weight complex on polyacrylamide gels from the existing glycerol gradient data (5, 6, 14). An analysis by the glycerol gradient method of the reaction products of the endogenous DNA polymerase reaction from patient G.F. has revealed the presence of an RNase-sensitive 35S complex (R. E. Gallagher, unpublished).

Our interpretation of the overall polyacrylamide-gel analysis is that the structure of the product-primer–template complex is supplied by RNA in both the primer and template moieties. The DNA product itself appears to be a short polynucleotide chain. In correlating the polyacrylamide-gel data with the hybridization results, it appears that only a small fraction of the product of RNA-dependent DNA polymerase migrates as a high-molecular-weight complex but that a large proportion of the DNA sequences are viral specific. It follows that if the biological material is sufficiently purified before DNA synthesis, then the hybridization assay is the more powerful tool for detecting virus-like nucleic acids.

DNA synthesized by a primate type-C RNA tumor virus

It is not clear *a priori* how much homology one would expect between viral genetic information of human origin and viral genetic information in animal model systems, e.g., from MuLV (Rauscher) (6, 9). Accordingly, we measured genetic homology among animal tumor viruses, in an effort to approach the human system in a predictive manner (15). A representative experiment germane to this communication is included here to put the data of Tables 1 and 2 in proper perspective.

Tables 3 and 4 present hybridizations between DNA synthesized by the type-C primate virus SiSV and RNA from viral or human sources. SiSV DNA contains a large proportion of sequences that anneal to RNA from the Gross-type murine viruses MuSV (Kirsten) and MuLV (AKR) (Table 3). Less homology was observed with RNA from MuLV (Rauscher) and the feline viruses, and no hybridization was detected with RNA from the avian virus. This is qualitatively the same pattern as was observed with the human DNA. The differences observed between MuLV (AKR) and MuLV (Rauscher) are reproducible. Since the SiSV virus was grown in normal rat kidney cells (NRK) it was important to assess the fraction of rat- and monkey-derived information in the SiSV genome.

TABLE 4. *Hybridization of SiSV DNA to RNA purified from various subcellular fractions from leukemic blood of patients G.F. and W.*

Source of RNA (subcellular fraction)	cpm	cpm (−AMV)
Cell-free plasma, patient G.F.	74	0
Step gradient, patient G.F.	176	80
Sucrose gradient, patient G.F.		
Density = 1.16–1.17	173	77
Density = 1.14	194	98
Density = 1.12	115	19
Sucrose gradient, density = 1.16, patient W.	789	715
AvLV (AMV)	96	—
SiSV	3240	3140

About 4600 cpm of SiSV DNA was hybridized to 5 μg of immobilized RNA for 2 weeks, as described in the legend to Table 1. Cell-free plasma was obtained by twice clarifying (at 10,100 × *g*) whole blood from which cells had been allowed to settle (11). Plasma or cytoplasmic extract was sedimented through 30% glycerol in TNE buffer onto 100% glycerol (step-gradient fraction). The step-gradient fraction was subjected to isopycnic fractionation in a sucrose gradient (sucrose gradient fractions). RNA was extracted from the subcellular fractions by the procedure at pH 9 described in *Methods*.

Appropriate hybridization experiments between radioactive SiSV RNA and cellular DNA show that the ratio of monkey:rat:mouse information in this SiSV preparation is 8:2:1 (unpublished results).

If the human cytoplasmic particle synthesizes a DNA that contains SiSV-related sequences and synthesizes this DNA from an RNA template, then it should be possible to detect an RNA species from the human subcellular fraction that will anneal to DNA synthesized by SiSV virus. Accordingly, RNA was prepared from the cytoplasmic fractions of the two patients and from the cell-free plasma of patient G.F. Table 4 demonstrates that the viral DNA probe does anneal to human leukemic RNA. For RNA from patient G.F., only the crude cytoplasmic RNA (before isopycnic banding) and high-density fractions after isopycnic banding hybridized to SiSV DNA; RNA from low-density fractions or from the cell-free plasma did not. For patient W, only RNA from the region of density 1.16 was tested, and this RNA hybridized over 20% of the viral probe.

DISCUSSION

Our experiments confirm and extend the observations that human leukemic cells can use an *endogenous* RNA template to synthesize DNA (4–6) complementary to murine leukemia virus RNA (Rauscher strain) (6). In agreement with recent experiments from this laboratory (4) and those of Kufe *et al.* (5), we find the activity in a cytoplasmic fraction having a density of 1.16–1.17. In our hands, the enzymatically active fraction sediments at high speed and retains its characteristic density upon repeated manipulation and centrifugation. The second property is unlike that displayed by the bulk of the cytoplasmic aggregates. Our experiments used fresh unfrozen cells as starting material. When this condition is adhered to, the repeated isopycnic banding method achieved 5000-fold purification of the particle containing RNA-dependent DNA

polymerase without apparent loss of total polymerase activity. After this endogenous activity was separated from other DNA polymerase activities, about 50% of the DNA synthesized in the presence of actinomycin D in the RNA-dependent DNA polymerase reaction hybridized to RNA isolated from specific type-C animal RNA tumor viruses.

Spiegelman and coworkers used MuLV (Rauscher) to search for viral-related nucleic acids in human leukemic cells (6, 9). Our data also show that although positive results can be obtained with the Rauscher probe, RNA from simian sarcoma virus or Kirsten murine sarcoma virus is much more effective. We do not know whether the efficacy of these probes resides in their leukemia genome or in their potential sarcoma-specific sequences (16). However, with the two patients examined, the murine leukemia Gross-type virus is not superior to Rauscher virus as a probe even though Kirsten sarcoma virus [which carries a Gross-type leukemia virus helper (16)] *is* superior. Additionally, FeSV appears superior to FeLV as a probe. If the particle obtained from human acute leukemic cells does in fact synthesize sarcoma-specific DNA sequences, it may be necessary to reevaluate our notions concerning the process of human leukemia, for example, in terms of selecting appropriate animal model systems or even in terms of designing the appropriate methods for obtaining biological activity from a presumptive virus from human leukemic myeloblasts.

The DNA synthesized by the human cytoplasmic particle shows a specificity pattern in hybridizations with RNA of animal tumor viruses that parallels the pattern obtained with DNA synthesized by a primate type-C RNA tumor virus, SiSV. Both hybridize well to RNA from murine viruses, both show only a limited capacity to anneal with RNA from FeSV, and neither hybridized to RNA from FeLV or AvLV. This pattern, combined with the magnitude of the hybridization responses, leaves little doubt in our minds that the human particle synthesizes a special set of sequences similar to sequences synthesized by animal RNA tumor viruses. We emphasize that both the human viral-related DNA and the human RNA-dependent DNA polymerase show taxonomic relationships that would be expected of components of primate type-C RNA tumor viruses.

The ability of the human particles to synthesize these sequences in a reaction that is RNase sensitive and in which an RNase-sensitive complex is formed, predicts that RNA complementary to the DNA product will be present in at least those subcellular fractions that show the enzymic activity. It was possible to demonstrate that this is the case of hybridizing DNA synthesized by the SiSV to small amounts (5 μg) of human RNA immobilized on phosphocellulose discs (13). This viral-specific RNA was found in the appropriate subcellular fractions, but was not observed in RNA from the cell-free plasma. The findings reported here are in keeping with our proposal (17) that if RNA tumor viruses are involved with human cancer, they are likely to be immature or replication-defective in the sense that they can only be recovered as intracellular entities.

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