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Homology exists among the transforming sequences of avian and feline sarcoma viruses

(cell transformation/*src* gene/hybridization)

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ABSTRACT **Fujinami sarcoma virus (FSV) of chickens does not contain nucleotide sequences related to the *src* gene of Rous sarcoma virus, but it carries unique sequences of at least 3000 bases, which are likely to code for the transforming protein of this virus. Using radioactive DNA complementary to FSV-unique sequences, we investigated the relatedness of FSV to other sarcoma–leukemia retroviruses in vertebrates. Under conditions of moderate stringency, no cross-hybridization was detected between FSV cDNA and RNAs of Rous sarcoma virus, Y73 avian sarcoma virus, several representative avian acute leukemia viruses, or Abelson murine leukemia virus. This cDNA, however, did hybridize with RNA of PRCII sarcoma virus of chickens to the extent of 56%. In addition, FSV cDNA was found to hybridize with RNAs of Gardner–Arnstein and Snyder–Theilen strains of feline sarcoma virus to the extent of 27% and 19%, respectively, but not with RNA of McDonough feline sarcoma virus. Studies on thermal denaturation of hybrids showed that the melting temperatures of the heteroduplexes of the FSV cDNA with RNAs of PRCII and Gardner–Arnstein feline sarcoma virus were 7°C and 12°C lower, respectively, compared with the melting temperature of the homologous hybrid of FSV, and suggested less than 10% mismatching in both heteroduplexes. These results indicate that nucleotide sequences closely related to at least a part of FSV-unique sequences are present in the genomes of other sarcoma viruses obtained in chickens and in cats.**

In the avian system, RNA tumor viruses have been classified into three main groups: (*i*) sarcoma viruses, (*ii*) acute leukemia viruses, and (*iii*) lymphoid leukosis viruses (1). Fujinami sarcoma virus (FSV) (2) is a recently characterized, potent sarcoma-forming virus in chickens (3, 4). FSV is defective in its replication and carries unique sequences of at least 3000 nucleotides in the middle of the genome flanked with helper viral sequences at both the 5′ and 3′ ends. Fujinami viral RNA was shown to be unrelated to *src* sequences of Rous sarcoma virus (RSV) by RNA fingerprinting and by hybridization experiments with DNA complementary to *src* (3, 4). $pp60^{src}$, a phosphoprotein of 60,000 daltons coded by the *src* gene of RSV (5, 6), was not detectable in FSV-transformed cells; however, a unique protein of 140,000 daltons was precipitable from these cells by antisera against viral structural proteins coded by the *gag* gene of avian retroviruses. This *gag*-related polyprotein, p140, suggested to be responsible for transforming capacity of this virus, has an associated protein kinase activity that phosphorylates tyrosine residues on immunoglobulin heavy chain, casein, and p140 itself (7).

In this paper we report the preparation of DNA complementary to FSV-specific nucleotide sequences and the distribution of these sequences among avian and mammalian retroviruses. The results indicate that RNAs of PRCII sarcoma virus (8) of chickens, and of Gardner–Arnstein (GA) (9) and Snyder–Theilen (ST) (10) sarcoma viruses of cats are cross-hybridizable with the probe specific to FSV.

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MATERIALS AND METHODS

Cells and Viruses. Chicken embryo fibroblasts negative for both chicken helper factor and viral group-specific antigen (Spafas, Norwich, CT) were used. The preparations of FSV and Fujinami-associated virus (FAV) used in this study were reported previously (3). Another stock of FSV (FSV-T) was a gift from H. Temin (University of Wisconsin). PRCII sarcoma virus in chickens, which was isolated in Scotland in 1958 (8), was supplied by P. K. Vogt (University of Southern California, School of Medicine). Y73 sarcoma virus, obtained from a spontaneous chicken tumor in Japan in 1973 (11), was provided by K. Toyoshima (Institute of Medical Science, University of Tokyo). RNAs of acute leukemia viruses in chickens were supplied by W. S. Hayward. Abelson murine leukemia virus (Ab-MuLV) and GA feline sarcoma virus (FeSV) (9) were prepared as pseudotypes of 4070-A strain of murine leukemia virus as described (12, 13). Feline leukemia virus (FeLV) pseudotypes of ST- (10) and McDonough (SM) strains (14, 15) of FeSV were prepared by superinfection of nonproductively transformed CCL64 mink cells with FeLV subgroup B.

Preparation of DNA Complementary to Viral RNA. Purification of virus particles and extraction of viral RNAs were essentially the same procedure as reported previously (16).

DNA complementary to FSV-unique sequences ($cDNA_{FSV-u}$) was prepared as follows. FSV 28S genomic RNA (3) was partially purified from heat-denatured 50–70S RNA of FSV(FAV) by a 10–25% sucrose density gradient centrifugation (16). [^{3}H]DNA complementary to 28S FSV RNA was synthesized by an exogenous reverse transcriptase (RNA-dependent DNA polymerase) reaction. The reaction mixture contained the following components in a final volume of 0.2 ml; 50 mM Tris·HCl (pH 8.0), 6 mM $MgCl_2$, 10 mM dithiothreitol, 50 mM NaCl, 18 μg of actinomycin D, 0.1 mM each dGTP, dATP, and dCTP, 21 μM [^{3}H]dTTP (47 mCi/μmol; 1 Ci = 3.7×10^{10} becquerels), 150 μg of calf thymus DNA primer (17), 200 units of avian myeloblastosis virus reverse transcriptase (provided by J. Beard, Life Sciences, St. Petersburg, FL, through the courtesy of J. Gruber, the Resource Program, National Cancer Institute), and 10 μg of 28S FSV RNA as a template.

The reaction mixture was incubated at 41°C for 60 min,

Abbreviations: FSV, Fujinami sarcoma virus; FAV, Fujinami-associated virus; RSV, Rous sarcoma virus; SR-B RSV, Schmidt–Ruppin (subgroup B) strain of RSV; RAV, Rous-associated virus; FeSV, feline sarcoma virus; FeLV, feline leukemia virus; GA-, ST-, and SM-FeSV, Gardner–Arnstein, Snyder–Theilen, and McDonough strains of FeSV; Ab-MuLV, Abelson murine leukemia virus; $cDNA_{FSV-u}$, DNA complementary to FSV-unique sequences; C_rt, product of RNA concentration in moles of nucleotide per liter and incubation time in seconds.

followed by addition of 400 μl of a stop-solution containing 30 mM NaEDTA, 0.75% NaDodSO$_4$, and 60 μg of proteinase K. After incubation for 15 min at 37°C, the sample was extracted twice with phenol/chloroform/isoamyl alcohol (1:1:0.01, vol/vol) containing 0.05% 8-hydroxyquinoline, and once with chloroform containing 1% isoamyl alcohol. The sample was treated with 0.3 M NaOH at 37°C for 3 hr to degrade RNA, neutralized, and passed through a Sephadex G-50 column. Void volume fractions were collected. DNA was cleaved by depurination to average fragment length of about 150 nucleotides (16) and precipitated with ethanol.

About 1 μg of cDNA thus obtained (2 × 10^7 cpm) was hybridized with 5 μg of total FAV virion RNA under conditions of moderate stringency (see below) in order to absorb cDNA sequences hybridizable with helper viral RNA. After the hybridization reaction reached a C_rt value (product of RNA concentration in moles of nucleotide per liter and incubation time in seconds) of 42 mol·sec/liter, the nonhybridizable portion of cDNA was separated from RNA·cDNA hybrid on a hydroxylapatite column at 60°C by eluting with 0.1 M sodium phosphate, pH 7.0, in the presence of 0.6 M NaCl. This concentration of salt is reported to stabilize the hybrid molecules between RNA and cDNA (18). The nonhybridizable fraction was then hybridized with 1.5 μg of 28S FSV RNA. At a C_rt of 5.4 mol·sec/liter, the sample was loaded onto a hydroxylapatite column at 60°C, and the column was extensively washed with a 0.10–0.16 M linear gradient of sodium phosphate buffer containing 0.6 M NaCl. RNA·cDNA hybrid fractions were eluted with 0.4 M sodium phosphate buffer/0.6 M NaCl and passed through a Sephadex G-50 column to remove sodium phosphate. The sample was treated with 0.3 M NaOH at 37°C for 2 hr, neutralized, and stored at −20°C. This probe is designated $cDNA_{FSV\text{-}u}$.

$cDNA_{FAV}$ was prepared by essentially the same procedure as above, except that 35S FAV genomic RNA (3) was used as a template RNA in place of 28S FSV RNA. 35S FAV RNA was purified from 70S FAV RNA after heat denaturation. To analyze the respresentativeness of $cDNA_{FAV}$, approximately 1.9 μg of [^{3}H]$cDNA_{FAV}$ synthesized was hybridized to 1.2 μg of 35S FAV RNA to a C_rt of 5 mol·sec/liter, and the sample was then fractionated by hydroxylapatite column chromatography; 1.1 μg of $cDNA_{FAV}$ eluted in the fraction of RNA·cDNA hybrid, indicating that $cDNA_{FAV}$ hybridized with FAV RNA at a ratio of 0.9 to 1 (wt/wt). Thus, $cDNA_{FAV}$ appears to be a relatively uniform transcript of 35S FAV RNA. This hybrid fraction between $cDNA_{FAV}$ and FAV RNA was treated with 0.3 M NaOH, neutralized, and used as the $cDNA_{FAV}$ probe in these experiments.

^{32}P-Labeled DNA ($cDNA_{SR\text{-}B}$) complementary to RNA of the Schmidt–Ruppin subgroup B strain (SR-B) of RSV was supplied by W. S. Hayward. This $cDNA_{SR\text{-}B}$ was not a representative probe for the entire SR-B genome because it was synthesized from a template that had been enriched for the 3′ end of viral RNA.

Nucleic Acid Hybridization. Hybridization was performed under conditions of moderate stringency [50°C in 30% (vol/vol) formamide/0.45 M NaCl/0.045 M sodium citrate/5 mM NaEDTA/0.1% NaDodSO$_4$] (19) and the extent of hybridization was determined by S1 nuclease digestion (16).

Thermal Denaturation of RNA·DNA Hybrids. Approximately 3000 cpm of [^{3}H]$cDNA_{FSV\text{-}u}$ was hybridized with 50–70S viral RNA or total virion RNA under the same condition as above. At a C_rt of 2 mol·sec/liter for transforming viral RNA, incubation was stopped and the sample was mixed with a hybrid between [^{32}P]$cDNA_{SR\text{-}B}$ and SR-B 70S RNA (2000 cpm) as an internal standard. The portion of RNA·cDNA hybrid in the mixture was purified by hydroxylapatite column chromatography (eluted with 0.4 M sodium phosphate, pH 6.8 at 50°C), and this hybrid fraction was diluted to 0.12 M sodium phosphate and loaded onto a hydroxylapatite column (0.6 × 3 cm) at 50°C. The column was washed four times (5 ml each) with 0.14 M sodium phosphate buffer (pH 6.8), and then the temperature of the water circulating the column jacket was raised stepwise in 4°C increments. After the water temperature was equilibrated at each step, the column was washed with 5 ml of 0.14 M sodium phosphate buffer. Acid-insoluble radioactive material in each fraction was collected on a Millipore filter and quantified in a liquid scintillation counter.

RESULTS

Preparation and Specificity of $cDNA_{FSV\text{-}u}$. FSV is defective in replication (3, 4), and most FSV(FAV) preparations contain helper virus at least several fold more abundant than transforming virus. In order to obtain a good template RNA for the preparation of FSV cDNA, we partially purified 28S FSV genomic RNA from 35S RNA of the helper virus FAV by a sucrose density gradient centrifugation. Single-stranded DNA complementary to the 28S RNA fraction was then synthesized with the reverse transcriptase reaction, using calf thymus DNA primer, and the cDNA was fractionated by sequential hybridizations with FAV and FSV RNAs (see *Materials and Methods*). About 3% of the starting cDNA was recovered as $cDNA_{FSV\text{-}u}$.

As shown in Fig. 1*A*, $cDNA_{FSV\text{-}u}$ hybridized to FSV(FAV) RNA to an extent of 90%, but failed to hybridize with FAV RNA even at a C_rt value of 10 mol·sec/liter. On the other hand, $cDNA_{FAV}$ hybridized with both RNAs of FSV(FAV) and FAV with almost identical kinetics (Fig. 1*B*). These results indicate that $cDNA_{FSV\text{-}u}$ contains very little cDNA hybridizable with the FAV genome, and thus represents cDNA specific to FSV-unique sequences. Because RNA containing only FSV-unique sequences was not available, we did not check the genomic complexity of $cDNA_{FSV\text{-}u}$. However, $cDNA_{FAV}$, which was synthesized under almost identical conditions, was shown to contain a relatively uniform representation of its template, FAV RNA (see *Materials and Methods*).

The difference in $C_rt_{1/2}$ values for the hybridization of FSV(FAV) RNA with $cDNA_{FSV\text{-}u}$ ($C_rt_{1/2}$ = 0.09 mol·sec/liter) and with $cDNA_{FAV}$ ($C_rt_{1/2}$ = 0.02 mol·sec/liter) indicates that this viral RNA preparation contains RNAs of FAV and FSV at a molar ratio of 4.5:1.

Another stock of FSV, FSV-T (supplied by H. Temin) was shown to be fully crossreactive with both probes of $cDNA_{FSV\text{-}u}$ and $cDNA_{FAV}$ described above (Table 1).

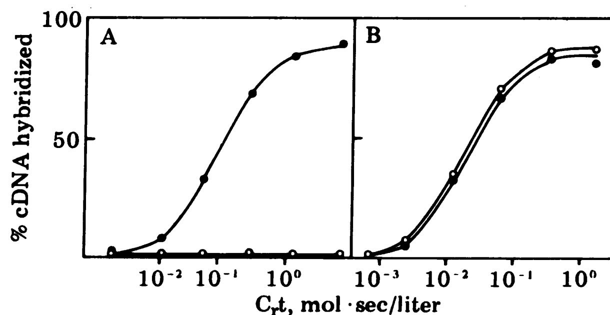

FIG. 1. Hybridization of $cDNA_{FSV\text{-}u}$ (*A*) and $cDNA_{FAV}$ (*B*) with 50–70S RNA of FSV(FAV) (●) and 70S RNA of FAV (○). About 500 cpm of [^{3}H]$cDNA_{FSV\text{-}u}$ or [^{3}H]$cDNA_{FAV}$ was mixed with 0.07 ng to 1 μg of viral RNA in a solution of 30 μl containing 30% formamide, 0.45 M NaCl, 0.045 M sodium citrate, 5 mM NaEDTA, and 0.1% NaDodSO$_4$ and incubated at 50°C for 20 hr. Extent of hybridization was measured by hydrolysis with S1 nuclease (16).

Table 1. Homology between the unique sequences of FSV and avian or mammalian retrovirus RNAs

Viral RNA*	Extent of hybridization, %	
	$cDNA_{FSV\text{-}u}$	$cDNA_{FAV}$
FSV(FAV)	90 (100)	84 (94)
FSV-T	87 (96)	87 (98)
FAV	1 (1)	89 (100)
SR-B	1 (1)	58 (65)
PR-B	1 (1)	66 (74)
RAV-2	1 (1)	62 (70)
AEV	1 (1)	65 (73)
AMV	1 (1)	66 (74)
MC29	0 (0)	67 (75)
PRCII	50 (56)	76 (85)
Y73	1 (1)	72 (81)
GA-FeSV	24 (27)	3 (3)
ST-FeSV	17 (19)	4 (4)
SM-FeSV	1 (1)	4 (4)
Ab-MuLV	1 (1)	3 (3)

Various amounts (0.05–5 μg) of viral RNAs were mixed with [^{3}H]$cDNA_{FSV\text{-}u}$ (400 cpm) or [^{3}H]$cDNA_{FAV}$ (600 cpm) and hybridized under the same conditions as in Fig. 1 at 50°C for 20 hr. Saturation level of hybridization was obtained from at least three different experiments at C_rt values of 1–100 mol·sec/liter. The figures in the parentheses are normalized to those obtained for $cDNA_{FSV\text{-}u}$ hybridization with FSV(FAV) RNA (90%) or $cDNA_{FAV}$ hybridization with FAV RNA (89%).

* PR-B, Prague (subgroup B) strain of RSV; AEV, avian erythroblastosis virus; AMV, avian myeloblastosis virus; MC29, avian myelocytomatosis virus MC29. Except for the four viruses FAV, SR-B, PR-B, and RAV-2, these viruses are associated with helper viruses.

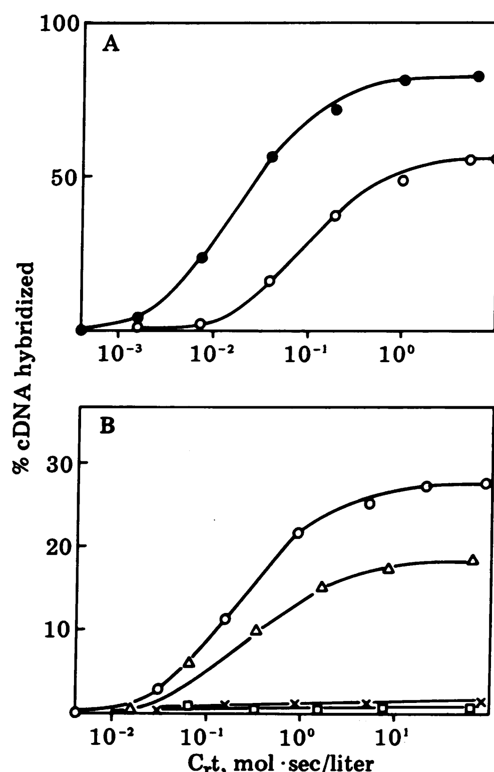

FIG. 2. (A) Hybridization of $cDNA_{FSV\text{-}u}$ (○) and $cDNA_{FAV}$ (●) with PRCII viral RNA; (B) hybridization of $cDNA_{FSV\text{-}u}$ with GA- (○), ST- (△), and SM- (□) FeSV RNAs and Ab-MuLV RNA (×). $cDNA_{FSV\text{-}u}$ or $cDNA_{FAV}$ and 4 ng to 5 μg of total viral RNA were mixed under the same conditions as in Fig. 1 and incubated for 20 hr (A) or 170 hr (B). The results were normalized to values obtained with $cDNA_{FSV\text{-}u}$ hybridization to FSV(FAV) viral RNA (90%) or $cDNA_{FAV}$ hybridization to FAV viral RNA (89%).

Nucleotide Sequences of $cDNA_{FSV\text{-}u}$ in Other Strains of RNA Tumor Viruses. Using $cDNA_{FSV\text{-}u}$ as a probe we investigated the relatedness of Fujinami unique sequences to a variety of RNA tumor viruses from both the avian and the mammalian systems. Hanafusa et al. (3) showed that FSV genomic RNA did not cross-hybridize with DNA complementary to *src* sequences in RSV. Consistent with this observation, no significant crossreaction of $cDNA_{FSV\text{-}u}$ was observed with RNAs of SR-B or Prague (subgroup B) strain of RSV (Table 1). Furthermore $cDNA_{FSV\text{-}u}$ did not cross-hybridize with RNAs of RAV-2, avian erythroblastosis virus, avian myeloblastosis virus, avaian myelocytomatosis virus MC29, Y73 avian sarcoma virus, or Ab-MuLV (Table 1).

On the other hand, the genomic RNA of PRCII avaian sarcoma virus (8), which was recently characterized to be defective and to have a nucleotide sequence coding for a *gag*-related polyprotein (20, 21), was able to hybridize with $cDNA_{FSV\text{-}u}$ to an extent of 56% (Table 1, Fig. 2A). The lower plateau value and the decrease in t_m (melting temperature) of the hybrid (see below) suggest that the PRCII genome carries a nucleotide sequence very similar, but not identical, to FSV-unique sequences. In addition, the level of cross-hybridization between $cDNA_{FAV}$ and PRCII RNA was 10–15% less than the level of homologous duplexes between $cDNA_{FAV}$ and FAV RNA (Table 1, Fig. 2A). This result indicates that helper virus(es) naturally present in PRCII stocks may be different from FAV in the FSV stock.

The most striking finding, however, was the cross-hybridization of $cDNA_{FSV\text{-}u}$ with GA- and ST- strains of FeSV (Table 1, Fig. 2B). The extent of cross-hybridization, 27% to GA-FeSV and 19% to ST-FeSV, was clearly significant compared with the level of background (less than 2%). The genome of SM-FeSV did not show any detectable cross-hybridization with $cDNA_{FSV\text{-}u}$ (Fig. 2B). The difference in plateau levels between GA- and ST-FeSV in cross-hybridization could reflect either the presence of different lengths of FSV-related nucleotide sequences or differences in homology of the unique sequences of these two FeSV genomes.

Thermal Denaturation of Duplexes Between $cDNA_{FSV\text{-}u}$ and Viral RNAs. We analyzed the degree of mismatching in duplexes between ^{3}H-labeled $cDNA_{FSV\text{-}u}$ and viral RNA by measurement of the thermal stabilities of the duplexes on hydroxylapatite column (Fig. 3). Duplexes between [^{32}P]-$cDNA_{SR\text{-}B}$ and SR-B RNA were added to each sample to standardize internally the assay for thermal stabilities. The t_m value for the hybrid of [^{32}P]$cDNA_{SR\text{-}B}$ with SR-B RNA was 79–80°C and highly constant. This t_m was slightly lower than that of homologous duplexes between $cDNA_{FSV\text{-}u}$ and FSV RNA. This may be due to the nature of this $cDNA_{SR\text{-}B}$ (see *Materials and Methods*), but because they were used just as an internal standard, we did not investigate this any further.

Duplexes between $cDNA_{FSV\text{-}u}$ and FSV(FAV) RNA and duplexes between $cDNA_{FSV\text{-}u}$ and FSV-T RNA showed almost the same t_m values of 85°C (Fig. 3 A and B). This observation and the data on cross-hybridization (Table 1) support the idea that these two FSV stocks were derived from an identical isolate.

The t_m of the duplexes between $cDNA_{FSV\text{-}u}$ and PRCII RNA (78°C) was 7°C lower than that of homologous hybrids between $cDNA_{FSV\text{-}u}$ and FSV RNA (Fig. 3C). Ullman and McCarthy (22) have shown that incorrect base pairing in a nucleic acid duplex results in a depression in the melting temperature of approximately 1.5°C per 1% mismatching. Thus, a 7°C reduction in t_m indicates about 4–5% mismatching. The duplexes

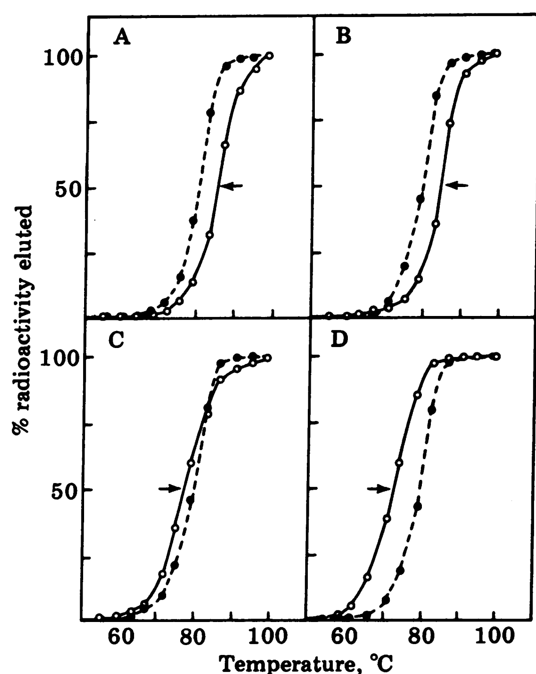

FIG. 3. Thermal denaturation of duplexes formed between cDNA$_{FSV-u}$ and viral RNAs. Viral RNAs (0.25–8 μg) were incubated with [^{3}H]cDNA$_{FSV-u}$ (3000 cpm) in 100 μl (except for 16 μl for FeSV RNA) of the same hybridization solution as in the legend of Fig. 1 at 50°C for 40 hr (except for 130 hr for FeSV RNA). After hybridization reached a C_rt value of 2 mol·sec/liter for transforming viral RNA, thermal stabilities of the duplexes were analyzed by hydroxylapatite column chromatography. Arrows indicate t_ms. Denaturation experiments were standardized internally by the addition of duplexes formed between [^{32}P]cDNA$_{SR-B}$ (2000 cpm) and SR-B RNA (●). RNAs in duplexes formed with cDNA$_{FSV-u}$ (○) are: (*A*) FSV(FAV) RNA (5 μg); (*B*) FSV-T RNA (8 μg); (*C*) PRCII RNA (3 μg); (D) GA-FeSV RNA (0.25 μg).

between cDNA$_{FSV-u}$ and GA-FeSV RNA were less stable, and the t_m (73°C) was 12°C lower than that of homologous duplexes (Fig. 3*D*), corresponding to 8% mismatching in the duplexes. Therefore a nucleotide sequence quite similar to at least a part of the FSV-unique sequences seems to be present in the genomes of GA-FeSV as well as PRCII sarcoma virus.

DISCUSSION

In the present study we prepared cDNA highly specific to the unique sequences in the FSV genome, which are unrelated to *src* sequences in RSV. Using cDNA$_{FSV-u}$, we demonstrated that PRCII sarcoma virus from chickens and GA- and ST-FeSVs from mammals contained nucleotide sequences closely related to FSV unique sequences. Several independent isolates of RNA tumor viruses obtained in the same species of animals have been reported to share homologous transforming sequences with each other. For example, RSV and Bratislava 77 avian sarcoma virus from chickens contain *src* sequences in their genomes (18), and Kirsten and Harvey murine sarcoma viruses carry homologous unique sequences (23). Therefore, the homology between FSV and PRCII is considered to be a case similar to these examples. The homology between FSV and two strains of FeSV is, however, unexpected because these viruses are of different vertebrate species.

About 60% hybridization between cDNA$_{FSV-u}$ and PRCII RNA might reflect a difference in the length of the unique sequences of PRCII and FSV, because the *gag*-related polyprotein of 105,000 daltons, p105, in PRCII-transformed cells (20, 21) is significantly smaller than the polyprotein of 140,000 daltons, p140, detectable in FSV-transformed cells (3, 4). More recently, p105 was shown to carry protein kinase activity that phosphorylates tyrosine residues similar to p140- associated protein kinase (J. C. Neil, personal communication). A similarity was also found in the tryptic fingerprints of the sarcoma-specific portion of p105 and p140 (J. C. Neil, personal communication). These observations further support the idea that these two sarcoma viruses bear quite similar unique sequences.

On the other hand, the genome of Y73, another chicken sarcoma virus, did not hybridize with the cDNA$_{FSV-u}$ (Table 1). This genome was shown to be unrelated to the *src* gene of RSV but to code for a *gag*-related polyprotein (24). Thus, Y73 belongs to another group of avian sarcoma viruses, which contains a transforming gene different from the genes of FSV and RSV.

In addition to the extensive homology between FSV and PRCII in the avian system, we found a cross-relationship between FSV and mammalian sarcoma viruses, the GA and ST strains of FeSV. The genome of ST-FeSV was shown to possess *gag*-linked unique sequences flanked with nucleotide sequences derived from FeLV at both the 5′ and 3′ ends (25). Similar genomic structures have been suggested for the GA and SM strains of FeSV (26–28). FeLV was a common helper virus for ST and SM strains of FeSV used in this study. Because cDNA$_{FSV-u}$ hybridized with the ST strain but not with the SM strain of FeSV, it is unlikely that cDNA$_{FSV-u}$ was crossreactive with the regions in the FeSV genome derived from FeLV nucleotide sequences. Instead, this observation strongly suggests that FSV and GA- and ST-FeSVs share similar nucleotide sequences at least in part of their unique sequences, which are considered to be responsible for the trasnforming activity of these viruses.

Cross-hybridization between cDNA$_{FSV-u}$ and GA- and ST-FeSV genomes was limited (approximately 20%). However, the length of unique sequences in ST-FeSV genome has been calculated to be 1500 nucleotides upon the basis of analysis of the molecularly cloned ST-FeSV genomic DNA (25). This corresponds to about 50% of the length of FSV unique sequences, which are estimated to be at least 3000 nucleotides (3). Thus, if cDNA$_{FSV-u}$ represents uniformly the region of unique sequences in FSV genome (see *Materials and Methods*), our results indicate that nearly half of the ST-FeSV unique sequences are cross-hybridizable with cDNA$_{FSV-u}$ even under the condition of moderate stringency (19).

Frankel *et al.* (28) reported that DNA compolementary to the unique sequences in ST-FeSV extensively hybridized with the genome of GA-FeSV but not with that of SM-FeSV. Furthermore, Barbacid *et al.* (26) and Van de Ven *et al.* (27) revealed that the sarcoma-specific regions of the GA- and ST-FeSV polyproteins are immunologically crossreactive and exhibit common methionine-containing peptides. Our findings on the close relationship between FSV and GA- and ST-FeSV, but not with SM-FeSV, are consistent with these observations. More recently, the GA-FeSV polyprotein was reported to carry an enzymatic activity for protein kinase (29) similar to the one found in p140 of FSV.

Recent studies on retroviruses indicate that sarcoma and acute leukemia viruses in vertebrates acquired their transforming ability as a result of recombination between retrovirus and cellular sequences (refs. 28 and 30–36; unpublished data). The original functions in normal cell metabolism of these cellular sequences remain unknown, but the wide distribution of these sequences in vertebrates and the high degree of conservation (28, 30, 33–35) suggest that each set of unique sequences codes for definite and important functions.

A hypothesis has been proposed that these cellular genes

when inserted into the viral genome would be expressed at a much higher rate as a part of the provirus, and thus the over-production of the product of this normal cellular gene could be responsible for the altered metabolism in infected cells that leads to cell transformation (32, 37, 38). However, it is not entirely clear whether or not the cellular sequences related to viral transforming genes need some modification in order to be incorporated into the viral genome and to become an active transforming gene.

Our preliminary results on hybridization between $cDNA_{FSV\text{-}u}$ and cell DNA showed that normal chicken cell DNA contains at least a part of the nucleotide sequences related to FSV-unique sequences with one or a few copies per haploid genome (data not shown). Therefore it is highly conceivable that FSV and PRCII are independent isolates from chickens in which retroviruses incorporated the same or related cellular sequences into their genomes. Likewise, the FSV-related cellular gene in cats is considered to have been incorporated into the feline leukemia virus to generate the two strains of feline sarcoma virus.

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