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Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates

(complementary DNA/RNA tumor viruses/oncogenesis)

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ABSTRACT **We have detected nucleotide sequences related to the transforming gene of avian sarcoma virus (ASV) in the DNA of uninfected vertebrates. Purified radioactive DNA ($cDNA_{sarc}$) complementary to most or all of the gene (*src*) required for transformation of fibroblasts by ASV was annealed with DNA from a variety of normal species. Under conditions that facilitate pairing of partially matched nucleotide sequences (1.5 M NaCl, 59°), $cDNA_{sarc}$ formed duplexes with chicken, human, calf, mouse, and salmon DNA but not with DNA from sea urchin, *Drosophila,* or *Escherichia coli.* The kinetics of duplex formation indicated that $cDNA_{sarc}$ was reacting with nucleotide sequences present in a single copy or at most a few copies per cell. In contrast to the preceding findings, nucleotide sequences complementary to the remainder of the ASV genome were observed only in chicken DNA. Thermal denaturation studies of the duplexes formed with $cDNA_{sarc}$ indicated a high degree of conservation of the nucleotide sequences related to *src* in vertebrate DNAs; the reductions in melting temperature suggested about 3–4% mismatching of $cDNA_{sarc}$ with chicken DNA and 8–10% mismatching of $cDNA_{sarc}$ with the other vertebrate DNAs.**

Nucleotide sequences encoding genomes of RNA tumor viruses are present in the normal cellular DNA of a wide variety of vertebrate species (see ref. 1 for review). The rate of evolution of such sequences has been estimated from the final extent of annealing and thermal stability of duplexes formed between cellular DNA and virus-specific reagents. In general, virus-specific DNA in cellular genomes appears to evolve at least as rapidly as cellular unique-sequence DNA; this pattern has been documented for viral DNA endogenous to primates (2), cats (3), rodents (4), and birds (5–10). In some instances, the apparently rapid evolution of virus-specific DNA could be explained by its relatively recent introduction into the germ line of a species by infection with a virus originating in another species (3).

In the cases cited, the hybridization reagents were not specific for defined portions of the viral genome, and the endogenous viruses under study were, in most cases, not oncogenic. Stehelin *et al.* (11) isolated radioactive DNA ($cDNA_{sarc}$) complementary to most or all of the viral gene(s) (*src*) required for transformation of fibroblasts by avian sarcoma virus (ASV) (11–13). $cDNA_{sarc}$ annealed to DNA not only from normal chickens, the presumed natural host for ASV, but also to DNA from several other birds spanning 100×10^6 years of evolution (10). This apparent conservation of nucleotide sequences related to *src* (hereafter called "sarc sequences") during avian speciation contrasted sharply with the lack of conservation of sequences encoding a nontransforming virus endogenous to chickens and closely related to ASV. Moreover, the conservation of cellular sarc sequences suggested that they might have an important, but as yet unknown, function, and that they might be present, albeit in a diverged form, in vertebrates other than birds.

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In the previous study (10), $cDNA_{sarc}$ did not anneal significantly to mammalian DNA (from calf and mouse) under relatively stringent conditions of hybridization. In this study, we have used conditions that would permit formation and detection of mismatched duplexes in order to test DNA from several vertebrate and nonvertebrate species for cellular sarc sequences. Under these conditions, we find that $cDNA_{sarc}$ anneals with the DNA of all tested vertebrates, but anneals only slightly to DNA from sea urchin, and not at all to *Drosophila* and *Escherichia coli* DNAs.

MATERIALS AND METHODS

Preparation of Cellular DNA. Unlabeled DNA was extracted from the following sources: 10- to 11-day-old chicken embryos; liver and spleen of a GR mouse; human placental tissue (provided by Y. W. Kan); sea urchin sperm and *Drosophila* cells grown in culture (obtained from Brian McCarthy); *E. coli* cells (provided by Herbert Boyer); and XC cells, derived from a tumor produced in rats with the Prague C (Pr-C) strain of ASV (14). Homogenized tissue or cells were suspended in buffer containing 0.1 M NaCl, 10 mM Tris·HCl (pH 7.4), 1 mM EDTA, 0.5% sodium dodecyl sulfate ($NaDodSO_4$), and 500 μg of Pronase per ml at 37° for 1–2 hr and extracted with phenol/chloroform/isoamyl alcohol (25:24:1 vol/vol). The high molecular weight DNA was then spooled out of solution after addition of ethanol. High molecular weight salmon sperm and calf thymus DNA (Worthington Biochemical Corp.) were treated as above.

The DNA was reduced to 6–7 S and residual RNA was digested by boiling in 0.3 M NaOH for 20 min. The samples were then neutralized with HCl, adjusted to 0.5% $NaDodSO_4$, treated with 500 μg of Pronase per ml for 45 min at 37°, extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1), and precipitated with ethanol.

^{14}C-Labeled unique-sequence DNA was prepared from BALB/c 3T3 cells or chicken embryo fibroblasts grown in the presence of [^{14}C]thymidine. The DNA was denatured and reannealed to a C_0t (concentration of DNA in moles of nucleotides × time in sec) of 500 in 0.6 M NaCl at 68°; the DNA that did not reassociate was isolated by its failure to adsorb to a hydroxylapatite column in 0.14 M phosphate buffer (pH 6.8) at 60°. Unlabeled unique-sequence calf thymus DNA was prepared in the same way except that it was selected as DNA that had not reannealed at a C_0t of 1.5×10^3.

Abbreviations: ASV, avian sarcoma virus; *src*, gene required for transformation of fibroblasts by ASV; sarc, nucleotide sequences deleted in transformation-defective deletion mutant of Prague C strain of ASV (Pr-C *td*); cellular sarc, nucleotide sequences of cellular origin related to sarc; $cDNA_{sarc}$, purified single-stranded DNA complementary to most or all of the gene (*src*); C_0t, concentration of DNA in moles of nucleotide × time in sec; $NaDodSO_4$, sodium dodecyl sulfate.

Preparation and Characterization of cDNAs. Preparation of both ^{32}P-labeled single-strand DNA complementary to the RNA genome of the B77 strain of ASV ($cDNA_{B77}$) and of [^{3}H]$cDNA_{sarc}$ have been described (11). For preparation of $cDNA_{sarc}$, the genome of Prague C strain of ASV was transcribed into complementary DNA; hybridization was then used to select for DNA specific for the region missing from the genome of a transformation-defective (*td*) deletion mutant of Pr-C of ASV. We have examined the complexity and composition of this $cDNA_{sarc}$ by two distinct procedures.

First, we hybridized $cDNA_{sarc}$ to ^{32}P-labeled ASV 70S RNA at various ratios of DNA:RNA. A maximum of 10–13% of the viral RNA formed RNase-resistant duplexes with $cDNA_{sarc}$; this value was achieved when the complementary sequences of DNA and RNA were annealed at a ratio of 1–3 (Table 1). These findings indicate that $cDNA_{sarc}$ is a reasonably uniform representation of 10–13% of the ASV genome (about 1300 nucleotides), which represents about 60% of the deletion in the strain of *td* virus used for the selection (15–18).

Second, we isolated the viral RNA that hybridized to $cDNA_{sarc}$ and analyzed the two-dimensional chromatogram of oligonucleotides released from this RNA by hydrolysis with T1 RNase (Sankyo) in low salt according to published procedures (19, 20); homochromatography was carried out with Homomix B (20). Autoradiography of the dried chromatograms was carried out with Kodak X-omat film and Dupont Cronex Lightening Plus screens. For comparison, a similar analysis was performed with the entire genome of Pr-C ASV. Our chromatogram of the viral genome was sufficiently congruent with that published by Wang *et al.* (13) to permit identification of the individual larger oligonucleotides by their position in the two-dimensional pattern (Fig. 1*A*). In particular, we could identify the two oligonucleotides (nos. 8 and 10) that Wang *et al.* have assigned to the region of the ASV genome that encodes *src* (13). The chromatogram of the RNA that hybridized to $cDNA_{sarc}$ contained two large oligonucleotides whose positions corresponded approximately to those of oligonucleotides 8 and 10 (Fig. 1*B*). However, we could not recover sufficient radioactivity in these oligonucleotides to verify their identity by chemical analysis. The remainder of the large oligonucleotides isolated from the viral genome (Fig. 1*A*) allegedly map outside the boundaries of *src* (13), and none of these oligonucleotides were present in the chromatogram of the RNA that hybridized to $cDNA_{sarc}$ (Fig. 1*B*).

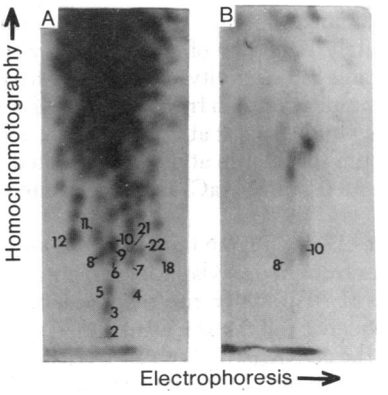

FIG. 1. Two-dimensional fractionation of RNase T1 digest of Pr-C ASV RNA and of Pr-C ASV RNA complementary to $cDNA_{sarc}$. ^{32}P-Labeled RNAs were digested with RNase T1, applied to a strip of cellulose acetate, electrophoresed at pH 3.5, and transferred to a thin-layer plate of DEAE-cellulose for homochromatography. (*A*) ^{32}P-Labeled 70S Pr-C RNA. The numbers correspond to those used by Wang *et al.* (13) for identification of RNase T1 oligonucleotides of Pr-C ASV. (*B*) ^{32}P-Labeled Pr-C RNA complementary to $cDNA_{sarc}$ was selected as follows: $cDNA_{sarc}$ (1.5 ng) was incubated with 10 ng of ^{32}P-labeled 70S RNA (6×10^4 cpm/ng) of PrC-ASV at 68° in 7.6 μl of buffer containing 0.9 M NaCl, 10 mM Tris·HCl (pH 7.4), 10 mM EDTA, and 20 μg of yeast RNA for 16 hr. After hybridization the mixture was adjusted to a final concentration of 0.3 M NaCl and 0.5 mg of RNase T1 per ml and incubated at 37° for 1 hr. The mixture was then adjusted to 0.5% $NaDodSO_4$, 1 mg of proteinase K per ml, and 0.2% diethylpyrocarbonate, incubated at 37° for 1 hr, and applied to a 30-ml Bio-Gel A 0.5 column equilibrated with 0.5 M NaCl/10 mM Tris·HCl, pH 7.4/10 mM EDTA/0.4% $NaDodSO_4$. Yeast RNA (20 μg) was added to the material in the void volume and the solution was then extracted once with phenol/chloroform/isoamyl alcohol (25:24:1). The aqueous phase was precipitated with 2 vol of isopropanol. The precipitated RNA complementary to $cDNA_{sarc}$ was resuspended in 10 mM Tris·HCl, pH 7.4/10 mM EDTA, heated at 100° for 5 min, quickly cooled, and reprecipitated with 2 vol of isopropanol before redigestion with RNase T1.

Table 1. Genetic complexity of $cDNA_{sarc}$*

Sample	Ratio (DNA/RNA)†	% RNA hybridized
Total $cDNA_{sarc}$	0.4/1	3
	1/1	10.5
	2.3/1	10
	3.3/1	11
	7.3/1	13
	16/1	11.3
$cDNA_{sarc}$ hybridized to calf thymus DNA‡	1/1	9
	3.5/1	10
$cDNA_{sarc}$ that did not hybridize to calf thymus DNA‡	2.2/1	10.2
	6.9/1	12
	22/1	12

* A constant amount of $cDNA_{sarc}$ (0.05 ng) was incubated with different amounts (0.02–1 ng) of ^{32}P-labeled 70S RNA (6×10^7 cpm/μg) of Pr-C ASV at 68° in 5 μl of 0.8 M NaCl/10 mM Tris·HCl, pH 7.4/10 mM EDTA for 72 hr. Extent of hybridization of the RNA was measured by hydrolysis with 50 μg of RNase A and 30 units of RNase T1 per ml in 0.3 M NaCl/0.03 M sodium citrate at 37° for 45 min.

† Ratio of complementary nucleotide sequences.

‡ $cDNA_{sarc}$ was fractionated on the basis of its ability to hybridize with calf thymus DNA. In this experiment, 35% of the $cDNA_{sarc}$ hybridized with calf thymus DNA and 65% of the $cDNA_{sarc}$ did not hybridize with the DNA.

To prepare cDNA representing regions of the ASV genome other than "sarc," we prepared a relatively uniform transcript of the genome of Pr-C ASV as detailed elsewhere (21) and then annealed it with RNA from *td* Pr-C virus at 4:1 ratio of cDNA:RNA. The annealed product, $cDNA_{td\text{-}rep}$, lacks sequences present in $cDNA_{sarc}$ and is representative of other portions of the ASV genome.

Annealing Conditions and Hydroxylapatite Fractionation of DNA. Denatured DNAs were annealed with [^{3}H]$cDNA_{sarc}$ or [^{3}H]$cDNA_{td\text{-}rep}$ at 59° for up to 120 hr in 1.5 M NaCl/10 mM Tris·HCl, pH 7.4/10 mM EDTA. Duplex formation was detected by fractionation on columns of hydroxylapatite (Bio-Gel-HTP, Bio-Rad) at 50°. The DNA was loaded onto columns in 0.14 M phosphate buffer and eluted with 5 column volumes of both 0.14 M phosphate buffer (single-stranded DNA) and 0.4 M phosphate buffer (double-stranded DNA). Fractions were analyzed for acid-precipitable radioactivity.

Fractionation of $cDNA_{sarc}$ on Basis of Hybridization to Calf Thymus DNA. [^{3}H]$cDNA_{sarc}$ was annealed with denatured, unique-sequence calf thymus DNA to a C_0t greater than 2×10^4 and fractionated on columns of hydroxylapatite. The 0.14 M phosphate buffer washes (containing $cDNA_{sarc}$ that did not hybridize to the calf DNA) and the 0.4 M phosphate buffer

washes (containing the calf DNA·$cDNA_{sarc}$ hybrids) were pooled separately. A portion of each fraction was analyzed for acid-precipitable radioactivity. To remove phosphate, we adjusted the remainder of each fraction to 5 mM cetyltrimethylammonium bromide, kept it at 0° for 10 min, and centrifuged it at 10,000 rpm for 20 min at 4°. The pelleted nucleic acids were resuspended in 1 M NaCl and precipitated with 2 vol of EtOH.

To separate $cDNA_{sarc}$ from the calf DNA, we denatured the pooled eluates from hydroxylapatite and annealed them with a large excess of 70S RNA from Pr-C under conditions where the unique sequence calf thymus DNA would not reanneal with itself or with the $cDNA_{sarc}$ (C_0t of calf DNA was less than 10^1). After fractionation on hydroxylapatite at 60°, the $cDNA_{sarc}$·RNA duplexes were precipitated with 5 mM cetyltrimethylammonium bromide, resuspended in 1 M NaCl, and adjusted to 0.3 M NaOH to hydrolyze the RNA (37°, 2 hr). After precipitation with ethanol, the two populations of $cDNA_{sarc}$ (one that had and one that had not hybridized with calf DNA) were again hybridized to Pr-C 70S RNA, recovered as described above, dialyzed extensively against 10 mM Tris·HCl, pH 7.2/10 mM EDTA, and precipitated with ethanol.

Thermal Denaturation of DNA·DNA Duplexes. The thermal stability of DNA·$cDNA_{sarc}$ duplexes was examined by adjusting the reaction mixture to 0.14 M PO_4^{3-} and applying it to a 6-cm column of hydroxylapatite at 50°. After three washes (8 ml each) with 0.14 M phosphate buffer, 1500 cpm of ^{32}P-labeled $cDNA_{B77}$ annealed with 17 μg of XC cell DNA (C_0t greater than 10,000) was applied to the hydroxylapatite as an internal standard. The column was washed with four additional 8-ml volumes of 0.14 M phosphate buffer at 50°, and the temperature of the water bath was raised in 4° increments; at each temperature the column was allowed to equilibrate and then washed with 8 ml of 0.14 M phosphate buffer. The fractions were analyzed for acid-precipitable radioactivity.

RESULTS

Annealing of $cDNA_{sarc}$ to Vertebrate DNAs. In preliminary studies, Stehelin *et al.* did not observe significant annealing of $cDNA_{sarc}$ to calf thymus or mouse DNA under relatively stringent conditions of hybridization (0.6 M NaCl, 68°) and of assay (resistance to S_1 nuclease and hydroxylapatite chromatography at 60°) (table 2 of ref. 10). However, when $cDNA_{sarc}$ was annealed to uninfected human, mouse, calf, or salmon sperm DNA in 1.5 M NaCl at 59°, 18–43% of the cDNA behaved like duplex DNA by hydroxylapatite chromatography at 50° (Table 2). More extensive annealing was observed with DNA from normal chicken embryos and with DNA from XC cells. Chicken DNA has been shown to contain sequences closely related to $cDNA_{sarc}$; XC cell DNA serves as a control for the formation of duplexes between homologous DNAs since XC cells (rat cells transformed by Pr-C ASV) contain approximately 20 copies of ASV DNA (22). In contrast, $cDNA_{sarc}$ annealed only slightly to the DNA from an echinoderm (sea urchin), and not at all to the DNAs from bacteria (*E. coli*) and an insect (*Drosophila*) (Table 2).

The various cellular DNAs were also tested for hybridization with single-stranded DNA ($cDNA_{td\text{-}rep}$) complementary to the RNA genome of variants of ASV that contain deletions in *src*. A major fraction of $cDNA_{td\text{-}rep}$ hybridized with DNA from uninfected chicken cells and from the ASV-transformed XC cells, but there was little or no reaction (<4%) with human, calf, mouse, salmon, sea urchin, *Drosophila*, or *E. coli* DNAs (Table 2). These results conform to the previously observed pattern (10) in that sequences related to $cDNA_{sarc}$ are conserved in DNA from divergent species, whereas sequences related to other portions of the ASV genome are found only in the DNA from normal chickens, ring-necked pheasants (9), and ASV-infected cells. There was also little or no annealing (<5%) of labeled unique-sequence chicken DNA with DNAs from calf, salmon, or *E. coli*. These results also document the specificity of duplex formation between $cDNA_{sarc}$ and the various cellular DNAs and exclude the possibility that an artifact of the hydroxylapatite assay can explain our results.

Table 2. Homology between normal DNAs and $cDNA_{sarc}$, $cDNA_{td\text{-}rep}$, and chicken single-copy DNA

DNA	% $cDNA_{sarc}$ in duplexes	% $cDNA_{td\text{-}rep}$ in duplexes	% chicken single-copy DNA in duplexes
XC	63 ± 4.6 (57–65)	67	—
Chicken	58 ± 3.6 (55–52)	66	90
Human	28 ± 4.4 (22–34)	2.7	—
Calf	30 ± 7.1 (21–43)	3.8	5
Mouse	26 ± 6.3 (18–33)	1.7	—
Salmon	24 ± 6.8 (18–34)	1.8	5
Sea urchin	6.3 ± 2.3 (2.8–9)	<1	—
Drosophila	2.6 ± 2.5 (0–5)	<1	—
E. coli	0.9 ± 1.2 (0–2.4)	2.1	<1

Reaction mixtures contained denatured cellular DNA (XC, 1–3.8 mg/ml; chicken, 2.5–4.7 mg/ml; human, 3.3–5 mg/ml; calf, 3.5–4.4 mg/ml; mouse, 1.9–4.6 mg/ml; salmon, 4.3–7.6 mg/ml; sea urchin, 3.1–6.2 mg/ml; *Drosophila*, 1.9–2.5 mg/ml; *E. coli*, 1.9–3.8 mg/ml) and [^3H]$cDNA_{sarc}$ (1.25 ng/ml, 25,000 cpm/ml) or [^3H]$cDNA_{td\text{-}rep}$ (0.94 ng/ml, 19,000 cpm/ml), or ^{14}C-labeled unique-sequence chicken DNA (550 ng/ml, 15,000 cpm/ml) in a final volume of 0.08–0.16 ml containing 1.5 M NaCl/10 mM Tris·HCl, pH 7.4/10 mM EDTA, and were incubated for 96–120 hr. Duplex formation was measured by fractionation on hydroxylapatite. Values have been corrected for backgrounds of 2% for [^3H]$cDNA_{sarc}$ hybridization, 4.2% for the [^3H]-$cDNA_{td\text{-}rep}$ hybridizations, and 5% for the unique sequence chicken [^{14}C]DNA hybridizations. Numbers in parentheses represent the range of values obtained in several independent experiments; SD is given.

The kinetics of the formation of duplexes between $cDNA_{sarc}$ and DNAs from chicken, mouse, human, and calf are shown in Fig 2. For comparison, the rates of reassociation of chicken and mouse unique-sequence DNA were also assayed (Fig. 2 *A* and *B*). Since the rate of association between $cDNA_{sarc}$ and the DNA was similar to the kinetics for the reassociation of unique nucleotide sequences, we conclude that the sequences related to $cDNA_{sarc}$ are present as single or at most a few copies in each haploid complement of the DNAs tested.

Most of the reactions between $cDNA_{sarc}$ and the DNAs from vertebrates were incomplete; 18–43% of $cDNA_{sarc}$ formed duplexes with mammalian and fish DNAs and 55–62% with chicken DNA. Either some of the nucleotide sequences represented by $cDNA_{sarc}$ were absent from the vertebrate DNAs, or the amounts of DNA complementary to $cDNA_{sarc}$ in the hybridization reactions were insufficient to drive the reactions to completion. We used two experimental approaches to distinguish between these possibilities.

(*i*) We isolated $cDNA_{sarc}$ that failed to reassociate with calf thymus DNA and tested its capacity to form duplexes with additional calf thymus DNA during a second period of incubation. The reassociations were carried out as described for Table 2 (final value of C_0t 2×10^4), with at least 2.4 mg of calf thymus DNA per ng of $cDNA_{sarc}$. After the first reaction, 65% of $cDNA_{sarc}$ eluted from hydroxylapatite as single-stranded DNA, 35% as duplex. The single-stranded $cDNA_{sarc}$ was recovered from the column eluate, incubated with fresh dena-

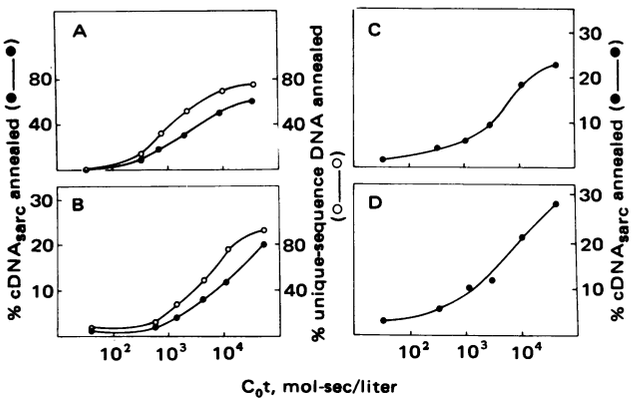

FIG. 2. Annealing of cDNA$_{sarc}$ to normal cellular DNAs. Denatured DNAs (0.25–0.365 mg) were incubated with [^{3}H]cDNA$_{sarc}$ (0.1 ng, 2000 cpm) and ^{14}C-labeled unique-sequence DNA in 0.08 ml of 1.5 M NaCl/10 mM Tris·HCl, pH 7.4/10 mM EDTA at 59° for various amounts of time (6 min–120 hr). Duplex formation was measured by fractionation on hydroxylapatite. (*A*) Chicken DNA (3.1 mg/ml) with [^{3}H]cDNA$_{sarc}$ (1.25 ng/ml, 25,000 cpm/ml) (●) and unique-sequence chicken [^{14}C]DNA (550 ng/ml, 15,000 cpm/ml) (○). (*B*) GR mouse DNA (4.6 mg/ml) with [^{3}H]cDNA$_{sarc}$ (1.25 ng/ml, 25,000 cpm/ml) (●) and unique-sequence mouse [^{14}C]DNA (500 ng/ml, 6900 cpm/ml) (○). (*C*) Calf DNA (3.6 mg/ml) with [^{3}H]cDNA$_{sarc}$ (1.25 ng/ml, 25,000 cpm/ml). (*D*) Human DNA (3.3 mg/ml) with [^{3}H]cDNA$_{sarc}$ (1.25 ng/ml, 25,000 cpm/ml).

tured calf thymus DNA, and fractionated on hydroxylapatite; 33% of the cDNA eluted as duplex (data not shown). These results indicate that stoichiometric limitations impede the reaction between cDNA$_{sarc}$ and calf thymus DNA, and that the extent of this reaction does not fully reflect the extent of homology between the cDNA and nucleotide sequences in calf thymus DNA.

(*ii*) We used molecular hybridization to determine the genetic complexity of the nucleotide sequences in cDNA$_{sarc}$ that formed duplexes with calf thymus DNA. After reassociation of cDNA$_{sarc}$ with calf thymus DNA and fractionation on hydroxylapatite, both the single-stranded cDNA$_{sarc}$ and the cDNA$_{sarc}$ in duplexes were separated from the calf thymus DNA. Both of the selected populations of cDNA$_{sarc}$ were hybridized with ^{32}P-labeled 70S RNA of Pr-C ASV at various ratios of DNA:RNA as described (Table 1). At saturation, 10–12% of the viral RNA formed RNase-resistant duplexes with either sample of cDNA$_{sarc}$; saturation occurred when complementary sequences of DNA and RNA were annealed at ratios of 1–2. These results conform to those obtained with unfractionated cDNA$_{sarc}$ (Table 1). As expected, neither fractionated nor unfractionated cDNA$_{sarc}$ hybridized to ^{32}P-labeled 70S RNA of Pr-C tdASV (data not shown).

We conclude that the cDNA$_{sarc}$ that hybridized to calf thymus DNA represents the same proportion of the ASV genome as the unselected cDNA$_{sarc}$. The failure of cDNA$_{sarc}$ to hybridize completely with the DNA from vertebrate cells is probably due to stoichiometric limitations imposed on the reactions by mismatched base pairs (see Table 3).

Thermal Stability of Duplexes Formed between cDNA$_{sarc}$ and Vertebrate DNAs. In order to analyze the degree of mismatching in duplexes formed between cDNA$_{sarc}$ and the DNA from various species, we determined the stability of the duplexes by elution from hydroxylapatite columns with a thermal gradient (Fig. 3 and Table 3). The thermal elutions were standardized internally by adding duplexes formed in a separate annealing reaction between the ASV sequences in XC cell DNA and the homologous [^{32}P]cDNA$_{B77}$ (t_m = 79° ± 1°); since the virus used to prepare cDNA$_{B77}$ consisted of at least 90% *td*

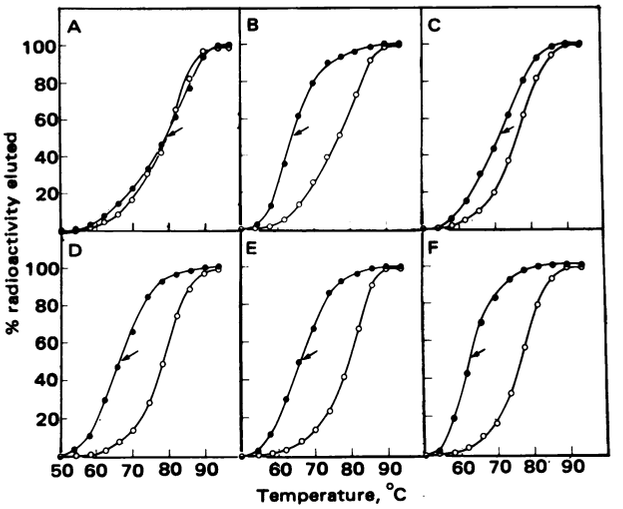

FIG. 3. Thermal denaturation of duplexes formed between cDNA$_{sarc}$ and cellular DNAs. Denatured DNAs (0.4–1 mg) were incubated with [^{3}H]cDNA$_{sarc}$ (0.2 ng, 4000 cpm) in 0.16 ml of 1.5 M NaCl/10 mM Tris·HCl, pH 7.4/10 mM EDTA at 59° for 96 hr (final C_0t > 25,000). The samples were applied to hydroxylapatite and denatured with a thermal gradient. Denaturation was standardized internally by adding duplexes formed in a separate annealing reaction between XC cell DNA and [^{32}P]cDNA$_{B77}$. ●, Duplexes with cDNA$_{sarc}$; ○, duplexes between cDNA$_{B77}$ and XC DNA (internal standard). (*A*) XC DNA (0.45 mg); (*B*) salmon DNA (1 mg); (*C*) chicken DNA (0.4 mg); (*D*) human DNA (0.53 mg); (*E*) calf DNA (0.58 mg); (*F*) mouse DNA (0.73 mg).

variants, the complementary DNA synthesized with this virus was deficient in sequences homologous to cDNA$_{sarc}$. Duplexes between cDNA$_{sarc}$ and DNA from XC cells had the highest t_m (79°; Table 3). Although rat cells presumably contain endogenous cellular sarc sequences, cDNA$_{sarc}$ should react principally with the 20 copies of ASV provirus contained in XC cells. As shown previously (10), the duplexes between cDNA$_{sarc}$ and chicken DNA were slightly less stable; the 4° depression of t_m represents about 3% mismatching of bases (23). Although the duplexes between cDNA$_{sarc}$ and mammalian or fish DNA were even less stable (t_m = 65–66.5°; Table 2), the reductions in t_m suggest only about 8–10% mismatching of bases (23). We conclude that vertebrate DNAs contain a highly conserved set of nucleotide sequences that are related to at least a portion of the transforming gene(s) of ASV.

Table 3. Thermal stabilities of duplexes between cDNA$_{sarc}$ and cellular DNAs

DNA	% DNA$_{sarc}$ in duplexes	t_m	Δt_m
XC	65	79	0
Chicken	55	74.5	−4.5
Human	29	66.5	−12.5
Calf	24	65	−14
Mouse	28	65.5	−13.5
Salmon	19	66.5	−12.5

Denatured DNAs were annealed with [^{3}H]cDNA$_{sarc}$ and adsorbed to hydroxylapatite as described in legends to Fig. 2 and Table 2. The values of t_m have been normalized to a t_m of 79° for XC DNA hybridized to [^{32}P]cDNA$_{B77}$, included as an internal standard in each analysis.

DISCUSSION

Stehelin *et al.* (10) have reported that although nucleotide sequences related to *src* of ASV were present and highly conserved in the DNA of various avian species ranging from chicken to emu, similar sequences were not detected in the DNA of two mammalian species (mouse and calf) when hybridizations were performed under relatively stringent conditions. In this report we have shown that by reducing the stringency of conditions for the formation and measurement of duplexes, at least partial homology with *src* can be detected in the DNA of all vertebrate species tested but not in the DNA from invertebrates such as sea urchins and *Drosophila* or from *E. coli*.

It is difficult, however, to determine the exact degree of homology between the cellular DNA and *src*. Thermal denaturation studies of duplexes formed between $cDNA_{sarc}$ and the various DNAs permit an estimate of base mismatching between $cDNA_{sarc}$ and cellular DNAs (3–4% for chicken DNA, 6–8% for other avian DNAs, and 8–10% for the fish and mammalian vertebrate DNAs). However, these studies were limited by the fact that only a portion of $cDNA_{sarc}$ reacted with vertebrate DNAs; 18–43% formed duplexes with mammalian and fish DNAs and 55–62% with chicken DNA. Since the reaction between mismatched complementary nucleotide sequences is slower than that between well-matched sequences, the reaction of cellular DNAs with $cDNA_{sarc}$ may not reach completion before the homologous cellular DNA strands have completely reassociated. Thus, our techniques provide minimum estimates of the extent of annealing, and maximum estimates of the fidelity of base pairing, since the most well-matched duplexes are most likely to form. However, we have demonstrated (Table 1) that the population of $cDNA_{sarc}$ that hybridized to a mammalian DNA (calf) has the same genetic complexity as the unannealed $cDNA_{sarc}$. We conclude that at least a major fraction of the nucleotide sequences in $cDNA_{sarc}$ have homologues in mammalian DNA. In quail cells, we have shown that up to 90% of $cDNA_{sarc}$ can hybridize to RNA present in uninfected cells (24); this indicates that most of the sequences in $cDNA_{sarc}$ are represented in avian DNA, although the annealing of $cDNA_{sarc}$ to cell DNA is incomplete.

The kinetics of the hybridization of $cDNA_{sarc}$ with DNAs from chicken, mouse, human, and calf are similar to the kinetics of reassociation of cellular DNA present in a single copy per haploid cellular genome (Fig. 2). Although mismatched base pairs can slow the rate of annealing between complementary nucleotide sequences (25, 26), this effect should be no greater than 5-fold for any of the reactions between $cDNA_{sarc}$ and the cell DNAs we have studied (3–10% mismatching). Thus, a maximum of 5–10 copies of DNA related to $cDNA_{sarc}$ can be present in each cell.

According to the fossil record, the separation of birds, mammals, and teleosts occurred 400 million years ago (27–29); throughout this period at least a portion of the avian cellular sarc sequences has been conserved. Moreover, in a large number of avian tissues and cells, we have recently found cellular sarc sequences to be universally transcribed into RNA, albeit at low concentrations (24). Although the conservation and ubiquity of expression of the cellular sarc sequences suggest that they might perform some critical function, the nature of that function remains obscure, and no protein product of the cellular sarc sequence has been identified.

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