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DNA and RNA from Uninfected Vertebrate Cells Contain Nucleotide Sequences Related to the Putative Transforming Gene of Avian Myelocytomatosis Virus

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The avian carcinoma virus MC29 (MC29V) contains a sequence of approximately 1,500 nucleotides which may represent a gene responsible for tumorigenesis by MC29V. We present evidence that MC29V has acquired this nucleotide sequence from the DNA of its host. The host sequence which has been incorporated by MC29V is transcribed into RNA in uninfected chicken cells and thus probably encodes a cellular gene. We have prepared radioactive DNA complementary to the putative MC29V transforming gene ($cDNA_{MC29}$) and have found that sequences homologous to $cDNA_{MC29}$ are present in the genomes of several uninfected vertebrate species. The DNA of chicken, the natural host for MC29V, contains at least 90% of the sequences represented by $cDNA_{MC29}$. DNAs from other animals show significant but decreasing amounts of complementarity to $cDNA_{MC29}$ in accordance with their evolutionary divergence from chickens; the thermal stabilities of duplexes formed between $cDNA_{MC29}$ and avian DNAs also reflect phylogenetic divergence. Sequences complementary to $cDNA_{MC29}$ are transcribed into approximately 10 copies per cell of polyadenylated RNA in uninfected chicken fibroblasts. Thus, the vertebrate homolog of $cDNA_{MC29}$ may be a gene which has been conserved throughout vertebrate evolution and which served as a progenitor for the putative transforming gene of MC29V. Recent experiments suggest that the putative transforming gene of avian erythroblastosis virus, like that of MC29V, may have arisen by incorporation of a host gene (Stehelin et al., personal communication). These findings for avian erythroblastosis virus and MC29V closely parallel previous results, suggesting a host origin for *src* (D. H. Spector, B. Baker, H. E. Varmus, and J. M. Bishop, Cell **13**:381-386, 1978; D. H. Spector, K. Smith, T. Padgett, P. McCombe, D. Roulland-Dussoix, C. Moscovici, H. E. Varmus, and J. M. Bishop, Cell **13**:371-379, 1978; D. H. Spector, H. E. Varmus, and J. M. Bishop, Proc. Natl. Acad. Sci. U.S.A. **75**: 4102-4106, 1978; D. Stehelin, H. E. Varmus, J. M. Bishop, and P. K. Vogt, Nature [London] **260**:170-173, 1976), the gene responsible for tumorigenesis by avian sarcoma virus. Avian sarcoma virus, avian erythroblastosis virus, and MC29V, however, induce distinctly different spectra of tumors within their host. The putative transforming genes of these viruses share no detectable homology, although sequences homologous to all three types of putative transforming genes occur and are highly conserved in the genomes of several vertebrate species. These data suggest that evolution of oncogenic retroviruses has frequently involved a mechanism whereby incorporation and perhaps modification of different host genes provides each virus with the ability to induce its characteristic tumors.

Avian retroviruses induce neoplastic transformation in a variety of cells, including fibroblasts, epithelial cells, and hematopoietic cells (1). The best-studied example is the avian sarcoma virus (ASV), which transforms fibroblasts via a single viral gene, *src* (32). Highly conserved nucleotide sequences homologous to *src* (denoted endogenous sarc) are present in the DNA of chickens (25, 28) and other vertebrates (25); transcripts from endogenous sarc are found in polyribosomal RNA (23) and a protein closely related to the product of *src* has been detected in uninfected vertebrate cells (6, 19a). Hence, endogenous sarc apparently encodes an essential cellular gene that may have served as progenitor for *src* of ASV. These findings raise the possibility that the capacity of avian retroviruses to transform cells other than fibroblasts was also gen-

erated from cellular genetic determinants. We have examined this possibility by analyzing the phylogenetic origins of MC29 virus (MC29V), an avian retrovirus that induces carcinomas and sarcomas in birds and transforms fibroblasts and macrophages in cell culture, yet lacks the gene *src* (7, 22, 26).

The genome of MC29V contains a sequence of approximately 1,500 nucleotides that may encode the oncogenic capacity of the virus (8, 22). We have prepared radioactive DNA ($cDNA_{MC29}$) complementary to this sequence (22) and have used this DNA to demonstrate that the putative transforming gene of MC29V is unique to MC29V and the closely related virus MH2V (D. Sheiness, L. Fanshier, and J. M. Bishop, manuscript in preparation). In the present communication, we show that the genomes of several birds and other vertebrates include a highly conserved homolog of $cDNA_{MC29}$; this homolog is transcribed into polyadenylated RNA in uninfected avian cells. Our findings, and those of Stehelin et al. (D. Stehelin, I. Saule, M. Roussel, A. Sergeant, C. Lagrou, C. Rommens, and M. B. Raes, Cold Spring Harbor Symp. Quant. Biol., in press) with avian erythroblastosis virus, indicate that each of the distinctive avian retrovirus transforming genes may have been derived from a separate genetic determinant in the avian genome. The putative progenitors of oncogenic genes appear not to be structurally related to one another, and their function in cellular growth or metabolism is not presently known. Elucidation of these alleged functions might provide useful insights into the mechanisms of oncogenesis and the origins of target cell specificity in neoplastic transformation.

MATERIALS AND METHODS

Cells and viruses. Fertile chicken eggs were obtained from H & N Farms, Redmond, Wash.; quail eggs were from Life Sciences, Gainesville, Fla.; duck eggs were from Reichard Farms, Petaluma, Calif. A clonal stock of MC29V/MCAV-A was obtained as previously described (22); MC29V/MCAV-A was propagated in C/BE chick cells which lacked the avian retrovirus group-specific antigen. XC cells were derived from a tumor produced in rats with the Prague-C strain of ASV (30) and kindly provided by J. A. Levy. The Schmidt-Ruppin subgroup D strain of ASV was obtained from Peter Vogt and propagated in random-bred chf^-/gs^- chicken embryo fibroblasts.

Preparation of RNA from uninfected and MC29V-infected chicken cells. Total cellular RNA from cultures of chicken fibroblasts was purified by a previously published method (33). Some RNA preparations were passed through a column of oligo(dT)-cellulose (T3 grade; Collaborative Research Inc., Waltham, Mass.) in buffer containing 0.5 M NaCl–0.003 M EDTA–0.02 M Tris-hydrochloride (pH 7.4)–0.5% (wt/vol) sodium dodecyl sulfate to bind the polyadenylated RNA fraction. Bound RNA was eluted with 0.01 M Tris-hydrochloride (pH 7.4)–0.003 M EDTA and then precipitated with ethanol.

Preparation of cellular DNAs. Unlabeled DNA was extracted from the following sources: 10- to 12-day-old chicken, quail, and duck embryos; MC29V-infected chicken embryo cells grown in culture; rhea lungs; BALB/c mouse mammary gland tumors; *Escherichia coli* cells (provided by Herbert Boyer); and XC cells. Rhea tissue was a kind gift from Lynn A. Griner of the San Diego Zoological Garden, San Diego, Calif. Homogenized tissues or cells were suspended in buffer containing 0.1 M NaCl, 0.02 M Tris (pH 7.4), 0.003 M EDTA, 1% sodium dodecyl sulfate, and 500 μg of Pronase per ml at 37°C for 2 to 18 h and extracted twice with phenol–chloroform (1:1). NaCl was added to a final concentration of 0.3 M, and high-molecular-weight DNA was spooled out after the addition of two volumes of ethanol. High-molecular-weight salmon sperm and calf thymus DNAs were purchased from Sigma Chemical Co., St. Louis, Mo., extracted with phenol-chloroform, and spooled out of ethanol as described above.

The DNAs were suspended in 20 mM Tris (pH 7.4)–0.01 M EDTA, reduced to an average length of 350 nucleotides by mechanical shearing in an Amicon DNA pressure cell at 40,000 to 50,000 lb/in^2, extracted with chloroform–isoamyl alcohol (24:1), and precipitated with ethanol. RNA was degraded either by digestion with 100 μg of pancreatic RNase (Worthington Biochemical Corp., Freehold, N. J.) per ml, followed by phenol–chloroform extraction and ethanol precipitation, or by treatment with 0.1 N NaOH at 37°C for 10 min followed by neutralization and ethanol precipitation. Most DNA preparations were passed through a column of Sephadex G50 before further use, or alternatively, were further purified by being precipitated three more times with ethanol.

^{14}C-labeled unique-sequence chicken DNA was purified from chicken embryo fibroblasts grown in the presence of [^{14}C]thymidine (12). After mechanical shearing, RNAse treatment, and passage through Sephadex G50, the DNA was denatured and reannealed to a C_0t of 300 in 0.6 M NaCl at 68°C; the DNA which did not reassociate was recovered by fractionation on hydroxyapatite and allowed to anneal to a C_0t of 600, and the final nonreassociated portion was isolated after another fractionation on hydroxyapatite.

Preparation of cDNA's. [^3H]$cDNA_{MC29}$ (2×10^4 cpm/ng), which was complementary to nucleotide sequences present in the genome of MC29V but not the genome of MCAV, was prepared as described previously (22).

[^{32}P]$cDNA_{B77}$ (10^5 cpm/ng) was synthesized by the endogenous reverse transcriptase of detergent-activated B77 ASV and purified as described previously (27). cDNA made by this method consists mostly of a fragment 101 nucleotides long which is complementary to the 5′ terminus of the B77 genome and also contains a small proportion of material transcribed from the entire ASV genome (11).

Nucleic acid hybridization. Stringent conditions for DNA-DNA or DNA-RNA hybridizations were 68°C in 0.6 M NaCl–0.01 M Tris (pH 7.4)–0.01 M

EDTA. Hybridization of radioactive cDNA with RNA was measured as resistance to hydrolysis by S1 nuclease (17), and hybridization between radioactive and unlabeled DNA was detected by fractionation on hydroxyapatite at 60°C. A 1- to 2-ml amount of packed hydroxyapatite was used for each 200 μg of DNA, and hybridization mixtures were loaded onto columns in 0.012 M sodium phosphate buffer (pH 6.8); single-stranded DNA was eluted with 0.12 M sodium phosphate buffer, and double-stranded DNA was eluted with 0.4 M sodium phosphate buffer. Radioactivity in eluted fractions was assayed by precipitation with trichloroacetic acid; quenching of radioactivity in samples containing more than 200 μg of nucleic acid was minimized by soaking filters overnight in a solution of 90% Omnifluor (New England Nuclear Corp., Boston, Mass.), 9% Protosol (New England Nuclear), and 1% water (Hetch-Hetchy Reservoir, Yosemite National Park, Calif.) to hydrolyze the nucleic acids.

Conditions enhancing the formation of stable duplexes between DNAs having partially mismatched sequences were 59°C in 1.5 M NaCl–0.02 M Tris (pH 7.4)–0.003 M EDTA (20). The fraction of radioactive DNA in duplex was measured by hydroxyapatite chromatography at 50°C; single-stranded DNA was eluted with 0.14 M sodium phosphate buffer, and double-stranded DNA was eluted with 0.4 M sodium phosphate buffer. Radioactivity in eluted fractions was assayed as described above.

Values for C_0t and C_rt were corrected to standard conditions (5).

Thermal denaturation of DNA-DNA duplexes. Avian DNAs were incubated with [^{3}H]cDNA$_{MC29}$ for lengths of time sufficient to allow the maximal amount of annealing, then diluted to a final volume of 5 ml of 0.14 M sodium phosphate buffer (pH 6.8). Samples were loaded onto hydroxyapatite columns in a circulating water bath at 50°C. After the columns were washed three times with 1.3 volumes of 0.14 M sodium phosphate buffer, an internal standard was applied to each column, consisting of a sample of XC cell DNA (DNA from a rat cell line transformed by ASV) annealed to [^{32}P]cDNA$_{B77}$. Columns were thoroughly washed, and then the temperature was raised in increments of 4 to 5°C. After 5 min at each temperature, thermally denatured cDNA was eluted from each column with 1.3 volumes of 0.14 M sodium phosphate buffer, and radioactivity in each wash was measured as detailed in the section describing DNA hybridization. T_m was defined as the temperature at which 50% of the originally hybridized cDNA was eluted from the column.

Thermal denaturation of RNA-DNA duplexes. RNA from uninfected or MC29V-infected chicken embryos cells was incubated with [^{3}H]cDNA$_{MC29}$ under conditions that ensured maximal hybridization. Portions were removed for determination of initial S1 nuclease resistance, and then reaction mixtures were diluted to a final concentration of 0.1 M NaCl–0.005 M Tris (pH 7.4) and a final volume of 1.0 ml. A portion of SR-D ASV RNA which had hybridized to [^{32}P]-cDNA$_{B77}$ was added to each reaction tube to serve as an internal standard, and then the mixtures were covered with a thin layer of mineral oil. Samples were placed in a circulating water bath at an initial temperature of 50°C; after 8 min, a 100-μl sample was removed to 1 ml of S1 nuclease buffer containing S1 enzyme and placed at 0°C until the completion of the thermal denaturation. The temperature was raised at 4 to 5°C intervals, and additional portions were removed after 8 min at each temperature. The fraction of cDNA remaining in hybrid at each temperature was determined by hydrolysis with S1 nuclease. T_m was defined as the temperature at which 50% of the radioactivity originally in hybrid became susceptible to S1 nuclease.

RESULTS

Genomes of birds and other vertebrates contain nucleotide sequences related to cDNA$_{MC29}$. DNA complementary to the putative transforming gene of MC29V (cDNA$_{MC29}$) annealed under stringent reaction conditions (0.6 M NaCl, 68°C) to DNA from normal chickens as well as to DNA from several other birds phylogenetically diverged from chickens (Table 1). Annealing of cDNA$_{MC29}$ to DNA from ducks and rheas, phylogenetically removed from chickens by 80×10^6 and 100×10^6 years, respectively, was somewhat less than for chicken DNA. When these annealing reactions were repeated under nonstringent reaction conditions that facilitate the formation of partially mismatched hybrids (1.5 M NaCl, 59°C), the amount of cDNA$_{MC29}$ annealing to various avian DNAs increased almost to the value seen with chicken DNA (Table 1). This increase implies that the sequences in duck and rhea DNA that are related to cDNA$_{MC29}$ have undergone divergence relative to those in chicken DNA.

DNAs from three vertebrates other than birds were tested for reactivity with cDNA$_{MC29}$. Calf thymus DNA showed very little reactivity under stringent annealing conditions (Table 1). However, approximately 20% of the cDNA behaved as duplex by hydroxyapatite chromatography after annealing to calf thymus, mouse, or salmon DNA under nonstringent reaction conditions; chicken DNA annealed to 53% of cDNA$_{MC29}$ under these same reaction conditions. Similar results have been obtained previously with cDNA corresponding to the *src* gene of ASV annealed to vertebrate DNAs (25). cDNA$_{MC29}$ annealed only slightly to *E. coli* DNA analyzed under identical conditions, thus excluding the possibility that the 20% reaction represented an artifact of our assay conditions. The specificity of the reaction of mouse DNA with cDNA$_{MC29}$ has been confirmed by the detection of a unique fragment of mouse DNA that annealed to cDNA$_{MC29}$ after cleavage with *Hin*dIII restriction enzyme (D. Sheiness, S. H. Hughes, and J. Bishop, manuscript in preparation).

Copy number of MC29V-specific sequences in chicken DNA. The kinetics of the

TABLE 1. *Homology between cDNA$_{MC29}$ and cellular DNAs*[a]

DNA source	% Homology under the following hybridization conditions:			T_m (°C)	ΔT_m (°C)	Phylogenetic distance from chickens[b]
	0.6 M Na^+; 68°C	1.5 M NaCl; 59°C				
		Expt 1	Expt 2			
MC29-infected chick cells	74	50	60	—	—	?
Chicken	62	62	53	77	0	0
Quail	—	63	—	—	—	35–40
Duck	45	54	—	73	4	80
Rhea	39	56	—	72	5	100
Cow	6	—	20	—	—	300
Mouse	—	—	23	—	—	300
Salmon	—	—	21	—	—	390
E. coli	0	2	5	—	—	~600

[a] DNAs were prepared and hybridization reactions were carried out and assayed on hydroxyapatite as described in the text. Thermal denaturations were performed as described in the text. Each reaction mixture contained approximately 1 mg of DNA in volumes of 100 to 200 μl each; final values for C_0t were $\geq 7 \times 10^4$, except in the case of DNA from MC29V-infected cells, where the values for C_0t were $\geq 10^4$.

[b] Values indicate megayears; ?, Unknown.

formation of duplexes between cDNA$_{MC29}$ and DNA from uninfected chicken embryos are shown in Fig. 1. For comparison, the rate of reassociation of unique-sequence chicken DNA was assayed in the same reaction mixture. Since ^{3}H-labeled cDNA$_{MC29}$ annealed with kinetics similar to those seen for ^{14}C-labeled unique-sequence chicken DNA, we concluded that the homolog of cDNA$_{MC29}$ is present as one or, at the most, a few copies per haploid chicken genome.

Thermal stability of duplexes formed between cDNA$_{MC29}$ and avian DNAs. We used thermal denaturation to evaluate the degree of similarity between the cDNA$_{MC29}$ homolog in chicken DNA and other avian DNAs. Hybridization reactions were performed under nonstringent conditions to allow maximal duplex formation; stability of the duplexes was determined by elution from hydroxyapatite columns with a thermal gradient (Fig. 2 and Table 1). Thermal elutions were internally standardized by the addition of duplex formed in a separate reaction mixture between the ASV sequences in XC cell DNA and [^{32}P]cDNA$_{B77}$, a cDNA homologous to the ASV genome. The duplexes between cDNA$_{MC29}$ and duck and rhea DNA were somewhat less stable than those formed with chicken DNA; the 4 to 5°C depression of T_m indicates a 3 to 4% mismatching of bases (18, 31).

Uninfected chicken embryo fibroblasts contain RNA related to cDNA$_{MC29}$. RNA extracted from chicken embryo fibroblasts was hybridized to cDNA$_{MC29}$ under stringent reaction conditions. The kinetics of this hybridization are illustrated in Fig. 3. In this and other experiments, cDNA$_{MC29}$ reproducibly reacted

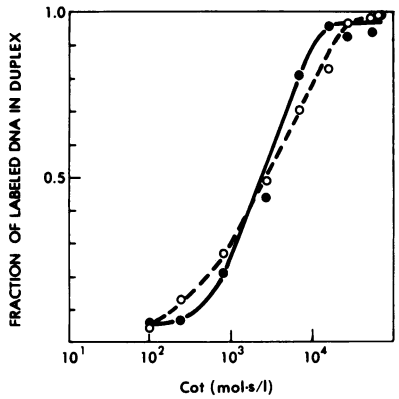

FIG. 1. *Annealing of cDNA$_{MC29}$ to chicken embryo DNA. Denatured DNA (0.6 to 1.0 mg) was incubated in 0.6 M NaCl at 68°C, as described in the text, with [^{3}H]cDNA$_{MC29}$ (0.05 ng, 1,000 cpm/reaction; ●) and ^{14}C-labeled unique-sequence chicken DNA (1,000 cpm/reaction; ○) in reaction volumes of 0.1 to 0.2 ml for time periods ranging from 3 min to 5 days. Formation of duplex was measured by fractionation on hydroyapatite as described in the text, and values for the fraction of labeled DNA in duplex were normalized to the final extent of reaction for each probe. These values were 48% for [^{3}H]cDNA$_{MC29}$ and 91% for ^{14}C-labeled unique-sequence chicken DNA.*

with chicken fibroblast RNA to a plateau value of 90%, normalized to the value to which cDNA$_{MC29}$ reacted with MC29 RNA. cDNA$_{MC29}$ hybridized equally well to polyadenylated chicken fibroblast RNA. Chicken fibroblast RNA did not hybridize appreciably with cDNA$_{B77}$ during these reactions (Fig. 3); hence, the hybridization of chicken RNA with cDNA$_{MC29}$ cannot be attributed to contamination with

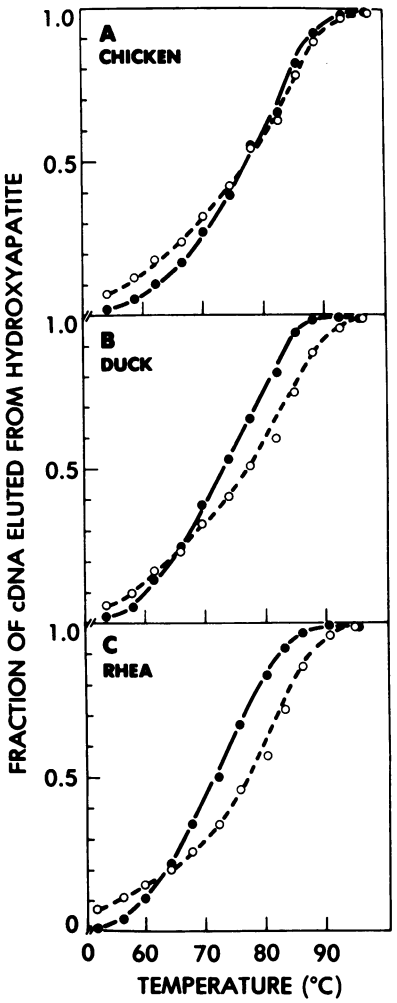

FIG. 2. *Thermal denaturation of duplexes formed between $cDNA_{MC29}$ and avian DNAs. Denatured DNAs (1.0 mg/reaction) from chicken embryos (A), duck embryos (B), and rhea lungs (C) were incubated with [^{3}H]$cDNA_{MC29}$ (4,500 cpm, 0.225 ng; ●) in 1.5 M NaCl at 59°C as described in the text for 4 to 6 days to final values of $C_0t \geq 7 \times 10^4$. Samples were applied to hydroxyapatite columns at 50°C and eluted with a thermal gradient as described in the text. Thermal elutions were internally standardized by the application to each column of duplex formed in a separate annealing reaction between XC cell DNA and [^{32}P]$cDNA_{B77}$ (○).*

MC29V RNA, since such a viral RNA contaminant would have annealed with $cDNA_{B77}$ (22).

Thermal stability of duplexes formed between $cDNA_{MC29}$ and uninfected chicken cell RNA. The extent of complementarity between chicken RNA and $cDNA_{MC29}$ was evaluated by thermal denaturation. RNA from uninfected and MC29V-infected chicken embryo fibroblasts was reacted with $cDNA_{MC29}$ to values for C_rt of 3.7×10^4 and 6×10^2, respectively; Fig. 3 indicates that RNA from uninfected cells does not react appreciably with $cDNA_{MC29}$ at a value of $C_rt = 6 \times 10^2$, whereas the hybridization of infected cell RNA with $cDNA_{MC29}$ was complete at this value for C_rt. Consequently, the duplex between RNA from MC29V-infected cells and $cDNA_{MC29}$ consisted of $cDNA_{MC29}$ hybridized to RNA transcribed from the integrated MC29V provirus, not of $cDNA_{MC29}$ hybridized to RNA transcribed from the endogenous chicken homolog of $cDNA_{MC29}$. Duplexes between $cDNA_{B77}$ and ASV RNA were added to each reaction mixture as an internal standard, and thermal denaturation was performed and analyzed with S1 nuclease as described above. Little or no difference in thermal stability was seen between hybrids formed with either uninfected or infected chicken cell RNA and $cDNA_{MC29}$ (Fig. 4). Similar results were obtained when the experiment was repeated with different preparations of cellular RNAs. Thus, by the criterion of thermal denaturation, the nucleotide sequence represented by $cDNA_{MC29}$ is indistinguishable from the homologous sequence found in chicken DNA.

DISCUSSION

Normal vertebrate cells contain a homolog of the nucleotide sequence that may encode the oncogenic capacity of MC29V. We have found nucleotide sequences homolo-

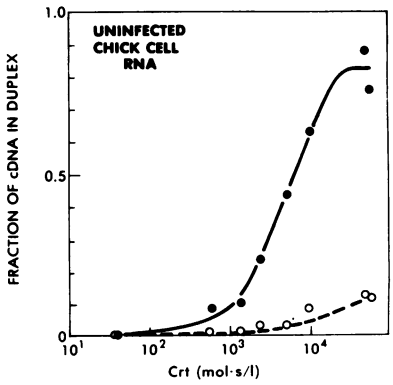

FIG. 3. *Hybridization of $cDNA_{MC29}$ with RNA from chicken embryo fibroblasts. RNA extracted from whole chicken embryo fibroblasts (150 to 500 μg/sample) was hybridized to [^{3}H]$cDNA_{MC29}$ (1,000 cpm, 0.05 ng; ●) in 0.6 M NaCl at 68°C as described in the text. The length of incubation ranged from 7 min to 56 h. [^{32}P]$cDNA_{B77}$ (1,000 cpm, 0.01 ng; ○) was included in each reaction mixture. Hybridization was measured by resistance of the cDNA to hydrolysis by S1 nuclease.*

gous to the putative transforming gene of MC29V in the genomes of several birds and other vertebrates (Table 1). Homology was detected by hybridization with $cDNA_{MC29}$ and was virtually complete in the case of the chicken genome; 90% of $cDNA_{MC29}$ annealed to RNA from uninfected chicken cells, and thermal denaturation of duplexes consisting of $cDNA_{MC29}$ and chicken RNA did not reveal the presence of any mismatched base pairs. The reaction between $cDNA_{MC29}$ and chicken DNA was less extensive (approximately 60%); we attribute the failure of this reaction to reach completion to stoichiometric limitations that can impede reactions in solution between single-stranded cDNAs and denatured cellular DNAs (4, 18).

The extent of homology with $cDNA_{MC29}$ decreases in the DNA of various vertebrates in rough accord with their phylogenetic distance from chickens (Table 1). These results are reminiscent of previous results indicating that a homolog of the ASV *src* gene is widely distributed in the DNA of vertebrate species (25). Our thermal denaturation studies, however, suggest that the avian homolog of $cDNA_{MC29}$ is slightly more conserved than is the avian homolog of *src* (25).

We observed that approximately 20% of $cDNA_{MC29}$ formed duplexes with DNAs of mammals and fish (Table 1). Comparable amounts of hybridization were seen when cDNA representing the *src* gene was annealed to DNA of vertebrates other than birds (25). Detailed investigations revealed that most of the *src* sequences were actually present in mammalian DNA, but that mismatched sequences in the duplexes between mammalian DNA and cDNA representing *src* precluded more extensive duplex formation (25). The fact that annealing of $cDNA_{MC29}$ to calf DNA occurred only under nonstringent annealing conditions (Table 1) suggests that duplexes between $cDNA_{MC29}$ and its endogenous homolog in mammalian DNA likewise contain mismatched sequences. Accordingly, we suspect that more than 20% of the sequences in $cDNA_{MC29}$ may be present in DNA of vertebrates other than birds. Fossil records indicate that the separation of birds, mammals, and fish occurred approximately 400×10^6 years ago; the detection of homology with $cDNA_{MC29}$ throughout these groups suggests that the vertebrate homolog of $cDNA_{MC29}$ must have existed as a cellular gene before the divergence of the various vertebrates and that this sequence has been highly conserved throughout vertebrate evolution.

Endogenous homolog of $cDNA_{MC29}$ may encode an essential cellular function. The

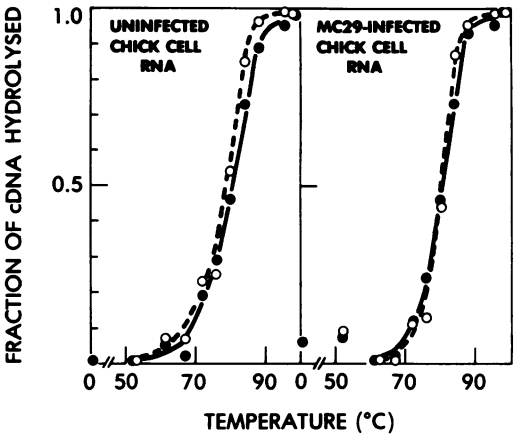

FIG. 4. *Thermal denaturation of hybrid formed between $cDNA_{MC29}$ and RNA from uninfected and MC29V-infected chicken cells. RNAs extracted from cultures of (A) uninfected (2 mg of RNA per reaction) and from (B) MC29V/MCAV-infected chicken fibroblasts (60 μg of RNA per reaction) were hybridized with [^{3}H]$cDNA_{MC29}$ (5,000 cpm, 0.25 ng; ●) in 0.6 M NaCl at 68°C as described in the text. Incubations were for 24 to 48 h to values of C_rt that allowed the maximum amounts of hybrid to form. Hybridized samples were placed in a water bath at 50°C and denatured with a thermal gradient, and the extent of denaturation was assayed with S1 nuclease as described in the text. Denaturation was standardized internally by adding hybrid formed in a separate reaction between SR-D ASV RNA and [^{32}P]$cDNA_{B77}$* (○).

widespread occurrence of sequences related to $cDNA_{MC29}$ in vertebrate genomes suggests that these sequences encode some vital cellular function which has ensured their evolutionary conservation. Our finding that sequences related to $cDNA_{MC29}$ are transcribed into polyadenylated RNA (Fig. 3) substantiates this proposal. Furthermore, the endogenous homolog of $cDNA_{MC29}$ exists in chicken DNA as a single- or low-copy sequence (Fig. 1); coding sequences for proteins are usually represented by one or a few copies in the genomic DNA (2, 9, 13, 16, 29).

The endogenous homolog of $cDNA_{MC29}$ is transcribed into only a few copies (approximately 3 to 10) of RNA per cell (Fig. 3). This low value is nevertheless similar to the intracellular concentration of transcripts from the endogenous homolog of *src* (24), which we now know to encode a protein similar to that encoded by the ASV *src* gene (6, 19a). A low level of generalized transcription is not responsible for the presence in chicken RNA of sequences hybridizing to $cDNA_{MC29}$, since globin or ovalbumin RNA could not previously be detected in chicken fibroblasts (24). Thus, despite the low

intracellular concentration of transcripts, we conclude that transcription of the endogenous homologue of $cDNA_{MC29}$ probably has functional significance.

Putative transforming gene of MC29V apparently originated from a highly conserved sequence found in vertebrate genomes. The ubiquitous distribution in vertebrate genomes of nucleotide sequences related to $cDNA_{MC29}$ indicates a high degree of evolutionary conservation for these sequences. The similarity between the chicken homolog of $cDNA_{MC29}$ and the corresponding viral sequence suggests that MC29V acquired these 1,500 nucleotides by incorporation of this conserved cellular sequence. Although the genetic element responsible for transformation by MC29V has not yet been identified, the host sequence acquired by the virus is a likely candidate for the transforming gene of MC29V. Recent investigations suggest that the sequence corresponding to $cDNA_{MC29}$ encodes a portion of a 110,000-dalton protein which is specified by the MC29V genome (16a, 19). Since no other protein encoded by the MC29V genome has been detected, the 110,000-dalton protein or some portion thereof may be responsible for the morphological changes seen in MC29V-infected cells. Thus, the genetic information acquired from a cellular sequence may encode the unique oncogenic capacity of MC29V.

The incorporation of host sequences may be a general mechanism for the acquisition of oncogenic capacities by retroviruses. A probable host origin has been previously demonstrated for the *src* gene of ASV; nucleotide sequences of avian erythroblastosis virus which may be responsible for its tumorigenicity are also found to share homology with host and other avian DNAs (Stehelin et al., Cold Spring Harbor Symp. Quant. Biol., in press). Several murine leukemia viruses simultaneously acquired sarcomagenic capacities and assimilated host nucleotide sequences into their own genomes as a result of passage through laboratory animals (10, 21). More recently, transformation-defective mutants of ASV from which the majority of the *src* gene was deleted were found to cause sarcomas when injected into chickens; ASV recovered from these tumors contained a functional *src* gene apparently acquired by assimilation of the host homolog of *src* (14, 15). These observations suggest that the evolution of an oncovirus may frequently involve the acquisition and possible modification of a normal host gene which thereafter provides each virus with its distinct oncogenic capacity.

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