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Nucleotide Sequence Relationship Between Intracisternal Type A Particles of *Mus musculus* and an Endogenous Retrovirus (M432) of *Mus cervicolor*

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Intracisternal type A particles are retrovirus-like structures found in embryonic cells and many tumors of *Mus musculus* but having no clear relationship with other retroviruses of this mouse species. We have observed a partial nucleotide sequence homology between the high-molecular-weight (32S and 35S) RNA components of intracisternal A-particles from a neuroblastoma cell line and the 70S RNA fraction from M432, a type of retrovirus endogenous to the Asian mouse *Mus cervicolor*. M432 complementary DNA (cDNA) was hybridized to the extent of 30% by the A-particle RNAs. The hybrids showed a lower thermal stability (ΔT_m, 7°C) than those formed with homologous RNA. The reaction was commensurate with that found between M432 cDNA and divergent sequences in the *M. musculus* genome. The capacity to hybridize M432 cDNA was closely correlated with the concentration of A-particle sequences in the cytoplasmic RNA of several *M. musculus* cell types. The major RNA fraction of M432 virus showed a reciprocal partial reaction with the A-particle cDNA's; the virus, which was grown in NIH/3T3 (*M. musculus*) cells, also contained a small proportion of apparently authentic A-particle nucleotide sequences. A subset of A-particle sequences seemed to be almost totally lacking in the main M432 RNA. The A-particle cDNA's hybridized extensively with divergent sequences in *M. cervicolor* cellular DNA, indicating that this mouse species may contain not only the partially homologous M432 virogene, but also a more complete genetic equivalent of the intracisternal A-particle.

Intracisternal type A particles occur regularly in early embryonic cells of *Mus musculus* (3, 5, 8) and are widespread in tumors and cultured tumor cells derived from both laboratory and feral members of this species (12, 27). The particles have an associated reverse transcriptase activity (11, 20, 26, 28, 30) and are known to contain high-molecular-weight polyadenylated [poly(A)] RNA similar in size to the RNAs of recognized RNA tumor viruses (11, 15, 20, 28, 29). The A-particle RNA is homologous to sequence elements that are reiterated in the genome of *M. musculus* (14). Recently, we have shown that A-particle-associated 35S and 28S RNA components from cultured neuroblastoma cells and a solid myeloma, respectively, can direct synthesis of the 73,000-dalton major A-particle structural protein in a cell-free system (18). Thus, the particles share many properties of known retrovirus virions.

On the other hand, it has not been possible to establish a positive connection between intracisternal type A particles and the usual type B and C extracellular viruses. The main A-particle structural protein is immunologically and structurally distinct (12), and attempts to establish sequence homologies between the A-particle RNA and the RNAs of known extracellular viruses have given conflicting, and therefore inconclusive, results (11, 15, 21, 28, 31). In a study to be reported elsewhere, we have found no homology between the 35S and 28S A-particle RNAs mentioned above and a variety of extra- and intracellular tumor virus RNAs derived from *M. musculus*; these results confirm and extend earlier observations by ourselves (15) and others (28, 31).

In the course of this survey, we tested the A-particle RNA for possible homology with the M432 viral genome. M432 is one of three retroviruses recently isolated from the Asian mouse *Mus cervicolor* (2, 6, 7). Two of these are type C viruses having distinctive patterns of homology with type C viral genomes in certain primates and other mouse species (2). The third, M432, resembled the murine type B viruses in some

biochemical and morphological respects but showed no nucleotide sequence homology with any known mouse mammary tumor or type C virus (6). There were about 25 copies of the M432-related sequences per haploid genome of *M. cervicolor* and about 3 such copies per haploid genome of NIH/3T3 cells productively infected with this virus. The DNA of uninfected *M. musculus* cells hybridized only 25% of a complementary DNA (cDNA) probe for the M432 viral nucleotide sequences, and these hybrids showed a reduced thermal stability (ΔT_m of −9.5°C) compared to hybrids formed between the cDNA and *M. cervicolor* cellular DNA. A similarly divergent set of M432-related sequences was detected in the DNA of tissues from *Mus caroli* (6) and in a retrovirus isolated from this species (7).

We have detected a comparable degree of partial homology between the purified high-molecular-weight RNA of *M. musculus* intracisternal type A particles and that of the *M. cervicolor* M432 virus.

MATERIALS AND METHODS

Cultured cell lines. The neuroblastoma line N4 (1) originated from a tumor in A/Jax mice; C127 is a line of flat cells derived from a mammary tumor in an RIII mouse; the BALB/3T3 and NIH/3T3 derived lines were as described elsewhere (10, 15). The M432 retrovirus of *M. cervicolor* (6) was propagated in NIH clone 4 cells (10); the cells were infected with a sample of the original M432 isolate (6) provided by R. Callahan, National Cancer Institute (NCI), and were maintained as a chronically productive line. Moloney murine leukemia virus (MoMuLV) was grown in NIH clone 10_{TK^-} cells (10).

Particle isolation. Intracisternal type A particles were prepared from subconfluent N4 cells as described (13). The M432 retrovirus and the MoMuLV were purified (22) from the culture media of the infected cell lines.

RNA isolation. The preparation of high-molecular-weight poly(A) RNA species (35S and 32S) from neuroblastoma A-particles has been described in detail (18); briefly, the A-particle RNA, after phenol extraction and alcohol precipitation, was heated at 65°C for 10 min in 10 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid)–1 mM EDTA, pH 7.5, and then applied to a column of oligodeoxythymidylic acid [oligo(dT)]-cellulose. Poly(A) RNA was eluted from the column and fractionated by centrifugation in isokinetic sucrose density gradients to yield the individual RNA species. The 60-70S RNA fractions (referred to as "70S RNA") of M432 and MoMuLV were purified as described elsewhere (22). The preparation of total cellular RNA has also been described (15).

Preparation of DNA. Mouse liver nuclear DNA were prepared by phenol extraction and sheared to an average size of 450 base pairs (14). DNA from *M. cervicolor* lung cells was a gift from R. Callahan, NCI.

Preparation of ^{3}H-labeled cDNA's. The cDNA's representing A-particle 32S and 35S RNAs (32S-cDNA and 35S-cDNA) were prepared in exogenous reactions, using avian myeloblastosis virus RNA-dependent DNA polymerase (15) and calf thymus DNA fragments as primer (24). This type of random priming generates cDNA's that are broadly representative of the template RNA (24). A subset of the A-particle sequences was identified in earlier studies (15) and designated the "abundant class" of sequences; the cDNA specific for this group of sequences was selected from the products of an oligo(dT)-primed exogenous reaction directed by the poly(A) RNA of mouse myeloma A-particles (15); the preparation of abundant-class cDNA was the same as that used in earlier studies (14, 15). cDNA's were prepared from gradient-purified M432 virus and MoMuLV in endogenous reverse transcriptase reactions, using calf thymus DNA fragments as primers (22). All of the cDNA's were labeled with [^{3}H]dCMP and had specific activities near 2×10^7 cpm/μg of DNA. A cDNA representing sequences common to a variety of murine (*M. musculus*) type C viruses (MuLV common-sequence cDNA) (9) was kindly provided by A. Frankel, NCI.

Hybridization reactions. ^{3}H-labeled cDNA (5×10^3 to 10×10^3 cpm) and RNA or DNA were incubated at 68°C in 25- to 50-μl reactions containing 0.75 M NaCl, 50 mM Tris-hydrochloride (pH 7.5), 0.1 mM EDTA, 0.5% sodium dodecyl sulfate, and 10 μg of *Escherichia coli* tRNA; hybrid formation was assayed with S1 endonuclease (14, 15). Fully denatured ^{3}H-labeled cDNA's were completely hydrolyzed under the S1 conditions used (background, 1.2% of input counts). The calf thymus DNA-primed cDNA's showed less than 5% S1 resistance even after long incubation times. Reaction times with the A-particle abundant-class cDNA were kept short to avoid the relatively high levels of self-annealing reported previously (15). Reaction rates have been corrected to 0.24 M sodium phosphate (PB) (4) for RNA hybridization and to 0.12 M PB for DNA hybridizations. In Fig. 1 to 3, the extent of hybridization of ^{3}H-labeled cDNA's with RNAs is expressed as the percentage of the following maximum levels of protection attained when the cDNA's were reacted with their homologous RNAs: A-particle 35S-cDNA versus 35S RNA, 74%; A-particle 32S-cDNA versus 32S RNA, 75%; M432B cDNA versus M432 70S RNA, 80%; murine (*M. musculus*) type C common-sequence cDNA versus MoMuLV 70S RNA, 65%. A-particle 35S RNA hybridized 86% of the A-particle abundant-class cDNA. Hybridization levels for reactions of cDNA's with DNA (Fig. 4) are expressed as the percentage of input.

Thermal stability curves. After hybridization, the reaction mixtures were diluted with 0.12 M PB (pH 6.8) and applied to a hydroxyapatite (HAP) column at 50°C. Elutions were carried out in this buffer at increments of 2 to 5°C.

Selection of M432 cDNA homologous to A-particle RNA. A sample of M432 ^{3}H-labeled cDNA (340,000 cpm) was hybridized to a C$_o$t of 1.8 mol·s/liter with A-particle 32S RNA (cf. Fig. 1A). The reaction mixture was applied to HAP as described above, and the bound (hybridized) fraction of cDNA (24% of total)

was eluted with 0.4 M PB at 60°C, treated with alkali to degrade the A-particle RNA, and recovered by alcohol precipitation in the presence of carrier *E. coli* tRNA.

RESULTS

Sequence homology between intracisternal A-particle and viral RNAs. Intracisternal type A particles from the N4 neuroblastoma cell line contain two main poly(A) RNA species, one of which (35S) is more active than the other (32S) in coding for the A-particle structural protein in cell-free systems (18). In spite of these apparent differences in size and functional capacity, we have not observed significant differences in sequence content upon reciprocal cross-hybridization of the two RNA fractions with their cDNA's (Lueders and Kuff, in preparation). Thus, in Fig. 1A, 32S A-particle RNA is seen to have reacted with the 35S-cDNA to the same extent and with the same kinetics as did the 35S RNA itself. Both RNAs contain the group of sequences previously shown to be specific for A-particles from a variety of mouse tumors (15). These sequences were originally defined by virtue of their high concentration in the poly(A) RNA of A-particles from myeloma cells and are referred to as the abundant class of A-particle sequences. Data showing the reaction of 35S RNA with cDNA representing this subset of A-particle sequences are included in Fig. 1A.

The complexity of the A-particle genome has not yet been precisely determined. The homologous A-particle hybridizations all fell on a curve having a $C_rt_{1/2}$ of 2×10^{-2} mol·s/liter. Electrophoresis in agarose gels containing methyl mercury hydroxide gave an estimated size of about 9,000 nucleotides for the 35S A-particle RNA (18).

Both neuroblastoma A-particle RNAs gave partial hybridization of the *M. cervicolor* M432 viral cDNA (Fig. 1A). The A-particle RNAs reacted with this cDNA over the same C_rt range as they reacted with their homologous cDNA's, showing that the heterologous reaction was not due to a minor contaminating RNA species. Maximum protection of M432 cDNA was 30% of the level achieved when this cDNA was hybridized with its own RNA. M432 cDNA was hybridized to the same extent by 28S RNA prepared from myeloma A-particles (data not shown). The hybrids formed between the A-particle RNAs and M432 cDNA had lower thermal stability (ΔT_m, 6 to 8°C) than the homologous hybrids of M432 cDNA (Table 1), indicating a significant divergence between the reacting sequences in the two nucleic acids. The reduced thermal stability of the heterologous hybrids may have contributed (23) to the slightly lower reaction rate of the A-particle RNAs with M432 cDNA as compared to the rate of the homologous reactions ($C_rt_{1/2}$'s of 4×10^{-2} and 2×10^{-2} mol·s/liter, respectively; see Fig. 1A).

The possibility was considered that A-particle RNAs were reacting primarily with the fraction of M432 cDNA which failed to hybridize with its homologous viral RNA (about 20% of the total; see Materials and Methods). To test this possibility, M432 cDNA was incubated with a saturating amount of A-particle 32S RNA (as in

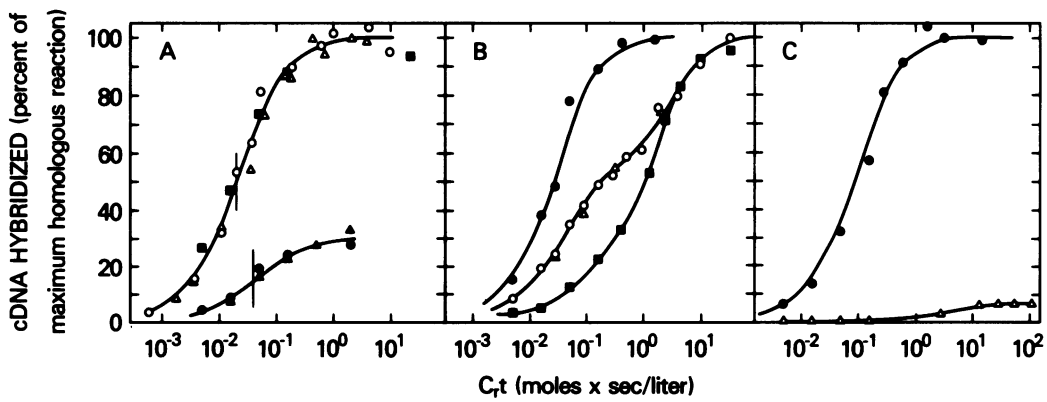

FIG. 1. *Hybridization of high-molecular-weight A-particle and M432 viral RNAs with homologous and heterologous ^3H-labeled cDNA's. (A) Neuroblastoma A-particle 35S RNA was hybridized with A-particle 35S-cDNA* (○), *A-particle abundant-class cDNA* (■), *and M432 cDNA* (●); *also, A-particle 32S RNA was hybridized with the 35S-cDNA* (△) *and M432 cDNA* (▲). *(B) M432 viral 70S RNA was hybridized with M432 cDNA* (●) *and the A-particle 35S* (○)-, *32S* (△)- *and abundant-class cDNA's* (■). *(C) MoMuLV 70S RNA was hybridized with MuLV common-sequence cDNA* (●) *and A-particle 35S-cDNA* (△). *In (A), vertical lines indicate the $C_rt_{\frac{1}{2}}$ positions for the two hybridization curves.*

TABLE 1. *Thermal stability (T_m) of hybrids between M432 (M. cervicolor) and intracisternal A-particle (M. musculus) RNAs and their respective cDNA's*[a]

RNA	T_m (°C)			
	M432 ^{3}H-labeled cDNA	A-particle ^{3}H-labeled cDNA		
		32S	35S	Abundant class
A-particle				
32S	69	**78**[b]		**75**
35S	67		**78**	**75.5**
M432				
70S	**75**	68.5[c] 74[d]		75.5

[a] The cDNA's were hybridized with the indicated RNAs to C_rt values between 1.6 and 3.1 mol·s/liter except as indicated. The samples were then applied to HAP columns at 50°C in 0.12 M sodium phosphate, and the thermal elution pattern was determined as described in the text. Values obtained for homologous hybrids are shown in boldface type.

[b] Hybrids formed at C_rt values of 0.03 and 3.1 mol·s/liter had the same T_m.

[c] Hybrids formed at C_rt of 0.11 mol·s/liter.

[d] Hybrids formed at C_rt of 20.4 mol·s/liter.

Fig. 1A), and the hybridized portion was recovered after binding to HAP (see Materials and Methods). This A-particle-homologous fraction of M432 cDNA reacted with M432 viral RNA in the same way as did the total M432 cDNA.

Callahan et al. (6) showed that M432 cDNA was fully hybridized by *M. cervicolor* cellular DNA but only partially (24%) by divergent sequences in the DNA of *M. musculus* (see also Fig. 4A). In this context, our present data suggest that intracisternal A-particles could contain the entire set of *M. musculus* sequences related to the M432 virus.

The M432 high-molecular-weight RNA reacted with its own cDNA as a single kinetic species (Fig. 1B). The $C_rt_{1/2}$ of this reaction, 2.8 $\times 10^{-2}$ mol·s/liter, was consistent with a genome size of 3×10^6 daltons (about 9,700 nucleotides) suggested by the data of Callahan et al. (6). Figure 1B also shows the reactions of M432 viral RNA with cDNA's representing the 32S and 35S A-particle RNAs and the subset of abundant-class, A-particle sequences. The abundant-class cDNA was maximally protected in a reaction having a relatively high $C_rt_{1/2}$ (1.3 mol·s/liter); that is, the M432 preparation contained all of the A-particle abundant-class sequences but at a concentration about one-fiftieth (0.028 ÷ 1.3 = 0.02) that of the RNA species responsible for the reaction with homologous (M432) cDNA. The reaction of M432 RNA with A-particle 32S- and 35S-cDNA was more complex. At higher C_rt values, the hybridization curve was congruent with that obtained for the abundant-class cDNA. However, an additional prominent kinetic component was evident in the same C_rt range as the reaction between M432 RNA and M432 cDNA.

The results in Fig. 1B show that the M432 RNA preparation contains two sets of nucleotide sequences. The major component, making up about 98% of the total, is presumably the authentic *M. cervicolor* virus RNA, responsible for full hybridization of the homologous cDNA and for the partial hybridization of A-particle 32S- and 35S-cDNA's in the lower C_rt range. A second, quantitatively minor component accounts for complete hybridization of the A-particle cDNA's at higher C_rt values; since the M432 virus is produced in *M. musculus* cells (NIH Swiss), this component could be A-particle RNA itself. In this case, one would expect the hybrids found late in a reaction between M432 RNA and 32S-cDNA to have a higher proportion of well-matched base pairs and therefore a greater average thermal stability than the partial hybrids formed at low C_rt values. The data in Table 1 confirm this expectation. In addition, it is seen that hybrids of A-particle abundant-class cDNA with M432 RNA melted at the same temperature as authentic hybrids between this cDNA and 32S A-particle RNA. Since M432 RNA gave little hybridization of the abundant-class cDNA in the low C_rt range (Fig. 1B), this class of A-particle-associated sequences is probably poorly represented in the *M. cervicolor* viral genome.

The presence of A-particle-specific sequences is not a general property of retroviruses secreted by NIH Swiss cells. 70S RNA of MoMuLV produced by a line of NIH/3T3 cells did not hybridize the A-particle cDNA significantly, even when tested at C_rt values far above those required for hybridization of a homologous type C virus cDNA (Fig. 1C). This point is discussed further below.

A-particle and viral sequences in cellular RNAs. The concentration of A-particle-related RNA sequences varies greatly among different cell types (15). We sought to determine whether the concentration of RNA sequences reacting with the M432 cDNA would vary in parallel fashion. Figure 2 presents data on three *M. musculus* cell lines that differ markedly in their content of intracisternal A-particles as judged by electron microscopy of thin-sectioned preparations. The particles are very abundant in neuroblastoma cells, appear regularly but in much reduced numbers in the C127 mammary tumor cell line, and are ordinarily not seen in BALB/3T3 cells. Figure 2 shows that all of these types of cells contain the full complement of the sequences represented in A-particle 35S-cDNA, and at concentrations that varied in the expected

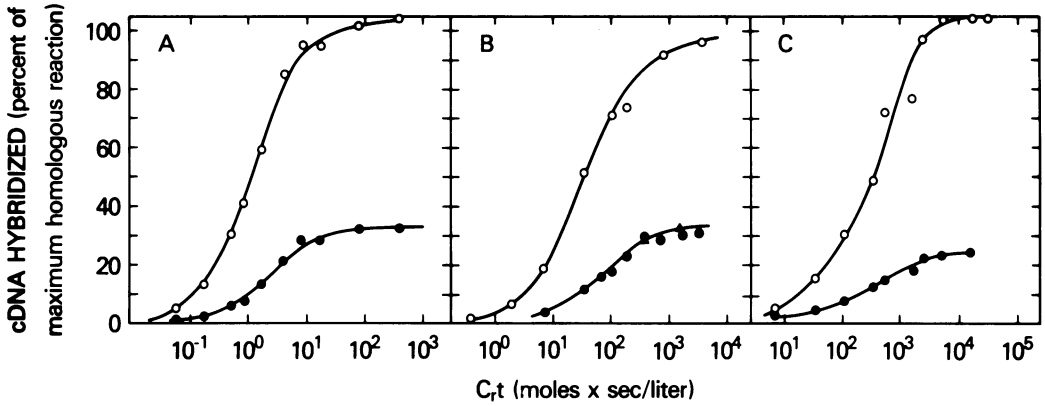

FIG. 2. *Hybridization of cellular RNAs with A-particle and M432 viral ^{3}H-labeled cDNA's. A-particle 35S-cDNA* (○) *and M432 viral cDNA* (●) *were hybridized with cellular RNAs from N4 neuroblastoma (A), C127 mammary tumor cell line (B), and BALB/3T3 fibroblasts (C). In (B), some reactions with M432 cDNA* (▲) *also contained saturating levels of 32S A-particle RNA, i.e., amounts sufficient to provide a C$_r$t of 1 mol·s/liter in the hybridization reactions shown in Fig. 1A.*

order. Thus, the C$_r$t$_{1/2}$'s for hybridization of the 35S-cDNA were 1.1, 37, and 445 mol·s/liter for the RNAs prepared from N4, C127, and BALB/3T3 cells, respectively, indicating that A-particle-specific sequences were concentrated in the neuroblastoma RNA more than 30-fold with respect to C127 and 400-fold in comparison with BALB/3T3 RNAs. All of these cellular RNAs gave a partial hybridization of the M432 cDNA (Fig. 2), and in each instance this heterologous reaction took place in the C$_r$t range corresponding to hybridization of the A-particle cDNA. Direct evidence for identity of the reacting species was obtained in a mixing experiment with C127 cellular RNA (Fig. 2B). Here, addition of saturating amounts of 32S A-particle RNA to the hybridization reaction did not increase the protection of M432 cDNA above the maximal level achieved with the cellular RNA alone. Although the C127 line originated from a mammary tumor in strain RIII mice, the cellular RNA failed to react with mouse mammary tumor virus cDNA, and we found no evidence of type B or cytoplasmic type A particle production on electron microscopy.

A-particle and viral sequences in RNA of cells productively infected with M432 and MoMuLV. RNA from M432-infected cells was assayed for virus sequences by hybridization against the M432 and A-particle cDNA's. Essentially complete hybridization of both types of cDNA was found (Fig. 3A). The curves for the A-particle cDNA's showed less displacement from the M432 reaction than was the case when hybridization was carried out with RNA from the extracellular virus (Fig. 1B); therefore, it was not possible to resolve two kinetic components

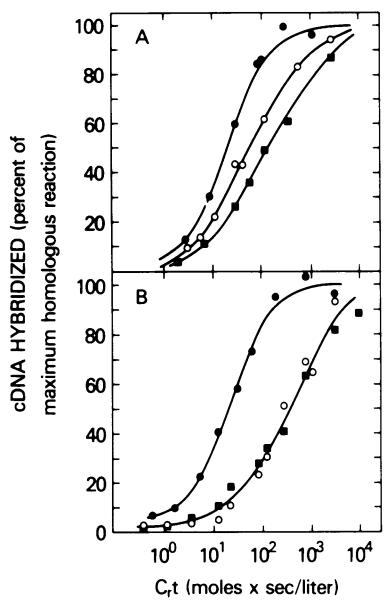

FIG. 3. *Hybridization of A-particle and viral ^{3}H-labeled cDNA's with cellular RNAs from productively infected NIH/3T3 cells. (A) RNA from cells infected with M432 virus was hybridized with M432 cDNA* (●), *A-particle 35S-cDNA* (○), *and A-particle abundant-class cDNA* (■). *(B) RNA from cells infected with MoMuLV was hybridized with MuLV common-sequence cDNA* (●) *and the same two A-particle cDNA's* (○, ■).

in the reaction with A-particle 35S-cDNA. These data almost certainly reflect a relatively higher ratio of A-particle to M432 sequences within the cell as compared to the virus and show that (as expected) encapsidation and se-

cretion of the virus select in favor of the appropriate RNA. Nevertheless, this discrimination is not complete, since A-particle sequences appear in the 70S RNA fraction of the extracellular virus. A contrasting situation was observed in the case of MoMuLV production by NIH/3T3 cells (Fig. 3B). The infected cells also contained both A-particle and leukemia virus RNA species. Yet, as seen earlier (Fig. 1C), the 70S RNA of extracellular virus was devoid of A-particle sequences. Thus, during assembly and secretion of this type C virus, there was stringent selection against the unrelated A-particle RNA.

Although A-particles can be prepared in large numbers from mouse tumor tissues, the isolated particles have been uniformly devoid of infectivity. The current study indicates that A-particle RNA can be incorporated into M432 extracellular virions more effectively than into type C virus and raises the possibility of horizontal transmission of the pseudotyped A-particle genome.

Viral sequences in the nuclear DNA of *M. musculus* and *M. cervicolor*. The reactions of A-particle and M432 cDNA's with cellular DNAs were assessed both by the development of S1 resistance over a range of C_0t values (Fig. 4) and by binding to HAP after hybridization to C_0t of 4,000 mol·s/liter (Table 2). The T_m's of HAP-bound hybrids are also shown in Table 2.

Multiple copies (≥2,000 per cell) of A-particle abundant-class sequences were known to be present in the mouse genome (14). The reaction of 35S cDNA with NIH Swiss liver nuclear DNA (Fig. 4A) had a $C_0t_{1/2}$ corresponding to about 560 copies per haploid genome, showing that the full complement of A-particle sequences was also reiterated to a remarkable degree in *M. musculus* DNA. The hybridization of M432 cDNA with its homologous (*M. cervicolor*) cellular DNA is shown in Fig. 4B. In agreement with a previous report (6), these viral sequences appear to be reiterated 25 to 30 times per haploid genome.

In the heterologous reaction between M432 cDNA and NIH Swiss DNA (Fig. 4A), 20% of the input probe was protected (29% bound to HAP [Table 2]), and the hybrids were of reduced thermal stability compared to those formed in the homologous reaction (cf. 6). The DNA sequences that reacted with M432 cDNA were multiply represented in the genome of NIH Swiss mice (Fig. 4A). Their copy number was determined more precisely using the fraction of M432 cDNA that had been selected by hybridization with A-particle 32S RNA (see Materials and Methods). Sixty percent of this selected M432 cDNA was found to hybridize with NIH

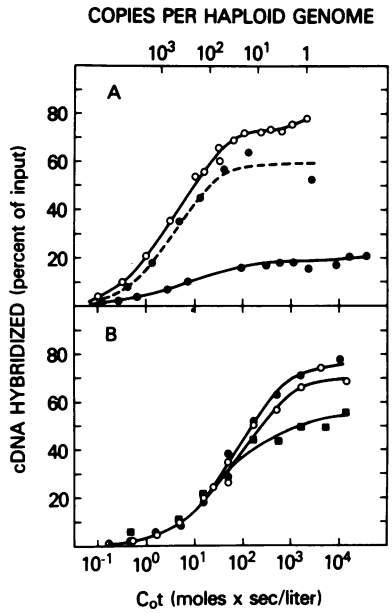

FIG. 4. *Hybridization of A-particle and M432 viral ^{3}H-labeled cDNA's with mouse liver nuclear DNAs. (A) A-particle 35S-cDNA (○) and M432 cDNA (●) were hybridized with liver DNA from NIH Swiss mice (M. musculus), and in (B), with liver DNA from M. cervicolor. In addition, (A) shows the reaction of NIH Swiss DNA with a fraction of M432 cDNA that had been selected by hybridization with A-particle 32S RNA as described in the text (●- - -●). (B) also shows the hybridization of A-particle abundant-class cDNA with M. cervicolor DNA (■).*

Swiss mouse DNA as assayed by S1 resistance in a reaction having a $C_0t_{1/2}$ corresponding to 600 copies per haploid genome (Fig. 4A). Eighty percent of the selected M432 cDNA bound to HAP after hybridization to high C_0t with Swiss mouse DNA (Table 2); these hybrids had reduced thermal stability, showing that the selected probe did not represent *M. musculus* A-particle sequences that might have been present in the original M432 cDNA preparation.

A-particle 35S-cDNA was hybridized by *M. cervicolor* liver nuclear DNA to a surprising extent (Fig. 4B, Table 2). A similarly high level of hybridization was found with DNA from cultured *M. cervicolor* lung cells (not shown). The hybrids showed a lower thermal stability than the homologous hybrids between 35S-cDNA and *M. musculus* DNA (Table 2), consistent with the evolutionary divergence between the two mouse species (19). The sequences responsible for hybridization of the A-particle cDNA and those that reacted with the M432 cDNA were reiterated to a similar extent in the *M. cervicolor* genome.

TABLE 2. *HAP binding and thermal stabilities (T_m) of hybrids between A-particle and M432 cDNA's and the cellular DNA of various Mus species*[a]

^{3}H-labeled cDNA	Cellular DNA					
	M. musculus		*M. cervicolor*		*M. caroli*	
	%	T_m (°C)	%	T_m (°C)	%	T_m (°C)
A-particle						
35S	**91**	**84**	91	77	88	78
Abundant class	**77**	**79**	67	72	65	72
M432						
70S total	28	73	**77**	**78**	39	72
70S selected[b]	80	74				

[a] The ^{3}H-labeled cDNA's were hybridized with liver nuclear DNA to C_0t values of approximately 4,000 mol·s/liter. The samples were then fractionated on HAP columns as described in the text. Binding is expressed as percentage of total cDNA which was bound at 60°C in 0.12 M sodium phosphate. Values obtained for homologous hybrids are shown in boldface type.

[b] By hybridization with A-particle 32S RNA; see text.

The hybridization of A-particle abundant-class cDNA with *M. cervicolor* liver DNA is also shown in Fig. 4B. The abundant class of A-particle sequences was reiterated in *M. cervicolor* DNA to approximately the same degree as the full complement of A-particle sequences (Fig. 4B) and showed a comparable reduction in thermal stability of the hybrids (Table 2). Previously, we reported that these sequences were reiterated in *M. cervicolor* to the same extent as they are in *M. musculus* and had minimal divergence as judged by thermal stability (14). We now know that this was an error resulting from misidentification of the mice used as the source of putative *M. cervicolor* DNA.

Reactions of A-particle cDNA with *M. caroli* nucleic acids. Callahan et al. (7) have isolated from another Asian mouse species, *M. caroli*, a retrovirus M832 whose RNA has partial homology (25%) with that of M432 and whose proteins show immunological cross-reactivity with those of the *M. cervicolor* virus. The reaction of A-particle 35S-cDNA with liver nuclear DNA of *M. caroli* was similar in extent to its reaction with *M. cervicolor* liver DNA (Table 2), and the hybrids showed a comparable reduction in T_m. RNA from an M832-producing *M. caroli* cell line (kindly provided by R. Callahan, NCI) hybridized 40% of the A-particle 35S-cDNA and 22% of the M432 cDNA in reactions having the same $C_rt_{1/2}$ of 30 mol·s/liter (reaction curves not shown). Control RNA from nonproducer *M. caroli* cells gave only a minimal reaction with the 35S-cDNA (10% hybridization at C_rt of 2,000 mol·s/liter). Thus, intracisternal A-particles may also have sequence homology with the M832 retrovirus endogenous to *M. caroli*.

DISCUSSION

Our data indicate a genetic relationship between the intracisternal type A particles of *M. musculus* and the M432 prototype retrovirus of *M. cervicolor*. The M432 cDNA was hybridized to about the same extent (25 to 30% of the homologous reaction) by both *M. musculus* cellular DNA and A-particle RNA; therefore, the entire group of M432-related *M. musculus* sequences could be represented in the A-particle RNA. On the other hand, a portion of the A-particle sequences, the so-called abundant class (15), appeared to be lacking in the M432 genomic RNA (Fig. 1B). The abundant-class cDNA was generated by an oligo(dT)-primed exogenous reaction (15), and evidence to be presented elsewhere shows that these sequences are concentrated in the 3′ regions of the A-particle RNAs. Thus, the homology between A-particles and M432 may be confined to the remaining, more 5′ portions of their genomes. A more precise analysis of this relationship would be facilitated by the use of M432 virus produced in *M. cervicolor* (rather than *M. musculus*) cells, should this type of preparation become available.

Multiple copies of both M432- and A-particle-related sequences were also present in the *M. cervicolor* DNA. However, the *M. cervicolor* genome seemed to contain a more complete representation of the intracisternal A-particle information than was found in the M432 virus, since (i) the A-particle 35S-cDNA was hybridized to a greater extent by *M. cervicolor* cellular DNA than would have been expected on the basis of its partial homology with M432 viral RNA, and (ii) sequences homologous to the A-particle abundant-class probe were present in the DNA even though they had no counterpart in the M432 viral genome. A-particle-related sequences were also detected in the DNA of *M. caroli* cells. Electron microscopy of *M. cervicolor* and *M. caroli* tumor cells and embryonic tissues should be carried out to determine

whether intracisternal A-particles are expressed in these mouse species as well as in *M. musculus*.

The cellular precursors of M432 virus are intracytoplasmic rather than intracisternal A-particles (6). Nothing is known about the proteins of the precursor particles; however, the extracellular M432 virus contains components ranging from 65,000 daltons (a glycoprotein) to 12,000 daltons, with a major internal structural protein of 24,000 molecular weight (7). The main intracisternal A-particle structural protein (73,000 daltons) is similar in size to an oncornavirus precursor polypeptide; although the particles contain small amounts of heterogeneous structurally related 45,000- and 30,000-dalton proteins (12, 16), there is little evidence for a distinct protein maturation process. Intracisternal A-particles also lack significant amounts of structural glycoprotein (12), a deficiency that could be related to the failure of the particles to associate with the plasma membrane of the producing cells. The question of protein relatedness between intracisternal particles and the M432 virus is currently under study. An interesting similarity between the two types of particles is the strong magnesium preference of their DNA polymerases (7, 26).

Intracisternal A-particles might be regarded from one viewpoint as a defective form of endogenous retrovirus that fails to achieve egress from the cells. On the other hand, it would be premature to conclude that these particles are simply the evolved *M. musculus* analog of the *M. cervicolor* and *M. caroli* retroviruses. Alternatively, a common set of 5′ sequences may have become linked to different sets of 3′ sequences to generate two related but distinct classes of particles. Precedent for such a possibility is provided by the recombinant events involved in the origin of certain other RNA tumor viruses (e.g., 22). Our data are consistent with the presence of A-particle-related information in a *Mus* progenitor common to *M. musculus*, *M. cervicolor*, and *M. caroli*. In that case, linkage between A-particle structural genes and additional information for an envelope glycoprotein could have produced a new class of replication-competent viruses in a later common ancestor (2, 19) of the two Asian mouse species.

The biological significance of intracisternal A-particles has remained obscure in spite of their widespread occurrence in embryonic and transformed cells of *M. musculus* and in tumor cells of other mammals (references in 17, 29). Our results suggest that A-particle-related sequences may be a general property of the *Mus* genus and that they could have contributed to the genome of at least one replication-competent retrovirus during the evolutionary past. The programmed expression of intracisternal A-particles in the early cleavage stages of mouse (*M. musculus*) embryogenesis (3, 5, 8) shows that this type of particle can be regulated by normal developmental mechanisms. Thus, the particles may represent an intermediate stage of virus evolution (25) with potential for further exchange of information with endogenous viral genomes or other cellular genetic elements.

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