

Nucleic Acid Homology of Murine Type-C Viral Genes

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The nucleic acid sequence homology between various murine, endogenous type-C viruses (three host range classes of BALB/c virus, the AT-124 virus, and the CCL 52 virus) and two laboratory strains of murine leukemia virus (Rauscher and Kirsten) was determined by DNA:RNA hybridization. The viral sequences exhibit varying degrees of partial homology. DNA:DNA hybridizations were performed between [³H]DNA probes prepared from N- and X-tropic BALB/c endogenous viruses and cellular DNAs from BALB/c, NIH Swiss, and AKR inbred mouse strains as well as from California feral mice and the Asian mouse subspecies *Mus musculus molossinus* and *M. musculus castaneus*. All of these strains of mice are shown to possess multiple (six to seven per haploid genome), partially related copies of type-C virogenes in their DNAs. Thermal melting profiles of the DNA:RNA and DNA:DNA hybrids suggest that the partial homology of the viral nucleic acid sequences is the result of base alterations throughout the viral genomes, rather than the loss of discrete segments of viral sequences.

Three separate host range classes (N-tropic, B-tropic, and X- or xenotropic) of murine type-C viruses have been isolated, either spontaneously or after treatment with inducing agents, from clonal (previously virus-free) cell lines derived from BALB/c mice (1, 2, 6, 33). Similar host range classes of type-C virus have been recovered from BALB/c animals *in vivo* (21, 26). These viral isolates are endogenous, encoded by type-C virogenes vertically transmitted from parent to progeny cell with the chromosomal DNA.

In the present report, we examine the extent of nucleic acid homology among the three host range classes of BALB/c endogenous type-C viruses, and their relationship to two leukemogenic murine type-C viruses (Rauscher [R-MuLV] and Kirsten [Ki-MuLV] strains) and to certain other murine type-C viruses. The results demonstrate that, whereas all of these murine type-C viruses are somewhat related to one another, there are significant differences among them as shown by nucleic acid hybridization.

When normal mouse cell DNA is examined, type-C viral-related sequences are found both in inbred laboratory strains (BALB/c, NIH Swiss, and AKR mice) and in feral mice from California and from Asia (*Mus musculus molossinus* and *Mus musculus castaneus*). It is shown that normal animals from each of these different mouse strains contain multiple type-C virogenes (per haploid genome) which are partially

related to BALB/c N- and X-tropic endogenous type-C viral sequences.

MATERIALS AND METHODS

Viruses. Table 1 briefly describes the viruses used in this study and lists the cell lines on which they were propagated. All cells were grown in Dulbecco modified Eagle minimum essential medium containing 10% calf serum (Colorado Serum Co., Denver, Colo.) and were transferred using 0.1% trypsin in phosphate-buffered saline. R-MuLV and Ki-MuLV were obtained from ElectroNucleonics Laboratory (Silver Spring, Md.). All viruses were banded twice in sucrose density gradients prior to use. Viral RNA was labeled with ³²P (carrier free) as described previously (5).

Animals and tissues. BALB/c and NIH Swiss mice were obtained from the Animal Production Unit of the National Institutes of Health (Bethesda, Md.); AKR/J mice were obtained from Jackson Laboratories (Bar Harbor, Maine). Livers from these animals were used as the source of DNA and were extracted as previously described (5). One hundred pooled livers from wild mice freshly trapped in Bouquet Canyon, Calif. (generously supplied by M. B. Gardner, University of Southern California, Los Angeles) and the pooled brain, kidney, liver, spleen, and lung tissues from several animals of the Asian subspecies *M. musculus molossinus* and *M. musculus castaneus* (a gift of M. Potter, National Cancer Institute, Bethesda, Md.) were used as DNA sources for these strains.

Preparation of [³H]DNA. [³H]thymidine-labeled DNA was synthesized by the endogenous reverse transcriptase reaction with detergent-disrupted type-C virus in the presence of actinomycin D (30 μg/ml) as previously described (7). After 16 h of

TABLE 1. *Type-C viruses studied*

Viruses	Characterization	Source of virus for studies
S2CL3 ^a	N-tropic virus spontaneously released from a BALB/3T3-derived cell line (33)	Released from S2CL3 cell line
R4/B ^a	B-tropic virus spontaneously released from a BALB/3T3-derived cell line after chronic treatment with BrdU (4, 6)	Released from R4/B cell line
S16CL10(I) ^a	X- or xenotropic virus induced from a BALB/3T3-derived cell line after brief treatment with BrdU (6)	Propagated in FCf2Th dog thymus cells or SIRC rabbit cornea cells
AT-124 ^a	X- or xenotropic virus recovered from human RD tumor cells passaged in immunosuppressed NIH Swiss mice (34)	Propagated in RD cells
CCL 52 ^a	Virus spontaneously released from NCTC 3959 (CCL 52) amelanotic melanoma cell line (from C × DBA mouse).	Released from NCTC 3959 cell line
R-MuLV ^a	NB-tropic leukemogenic virus; mouse strain of origin uncertain (27)	Propagated in BALB/c derived cells (JLSV9)
Ki-MuLV ^a	NB-tropic leukemogenic virus; from C3H/G strain mice (18)	Propagated in NIH/3T3 cells
RT21C ^b	Spontaneously released from RT21C rat cell line (32)	Released from RT21C cell line
VNRK ^b	Spontaneously released from NRK rat kidney cell line (20)	Released from NRK cell line

^a Murine viruses.^b Rat viruses.

incubation at 37 C, the [³H]DNA product was deproteinized and further purified by fractionation on hydroxylapatite and chromatography on G-50 Sephadex as described previously (7). The specific activity of the [³H]DNA was 1.5×10^7 counts per min per μg . The proportion of 70S viral RNA that was represented in the [³H]DNA probes was determined. This was done by hybridizing the [³H]DNA probes to the homologous [³²P]-labeled viral RNA (specific activity of 0.5×10^6 to 1×10^6 counts per min per μg) and by assaying for the protection of the RNA from pancreatic RNase digestion (5). The [³H]DNA probes used in these experiments contained 60 to 80% of the respective 70S viral RNA at a [³H]DNA to [³²P]RNA molar ratio of 1.5.

Hybridization. Cellular or viral RNA (extracted as previously described [5]) and [³H]DNA were incubated for 72 h at 65 C in reaction mixtures containing 0.01 M Tris (pH 7.4), 0.40 M NaCl, 2×10^{-3} M EDTA, 0.05% sodium dodecyl sulfate, 15,000 to 20,000 counts/min of [³H]DNA per ml (1.3 ng), and either 2 to 4 mg of cytoplasmic RNA or 5.0 μg of viral RNA per ml. Hybridizations were initiated by heating the reaction mixtures to 98 C for 10 min, cooling on ice, and incubating at 65 C. DNA:DNA hybridizations were performed at 65 C with 0.75 M NaCl as previously described (5). C_0t values (C_0 is the concentration of

cellular DNA in moles of nucleotide per liter and t is the time in seconds) were calculated (as suggested by Britten and Kohne [10]) as the product of \ln and time (h) divided by 2 absorbancy at 260 nm (A_{260}) per ml and corrected to a monovalent cation concentration of 0.18 M (11). Hybridization reactions were analyzed with the single-strand nuclease S_1 (5).

Annealing of unique sequence BALB/c DNA. The BALB/c-derived cell line S2CL3 (33) was labeled for 48 h with 50 μCi of [³H]thymidine per ml of culture medium. The DNA was extracted as described previously (5). [³H]thymidine-labeled S2CL3 DNA (40,000 acid-precipitable counts/min [3.2 μg]) was added to 3 mg of cold, S2CL3 nuclear DNA. The mixture was heated to 98 C for 10 min in 1.0 ml of solution containing 0.01 M Tris (pH 7.4), 0.75 M NaCl, 2×10^{-3} M EDTA, and 0.05% sodium dodecyl sulfate. The solution was cooled in ice and incubated at 65 C. Samples of 0.05 ml were removed at various times, and the extent of hybridization was determined with nuclease S_1 .

RESULTS

Viruses studied. Table 1 summarizes the origin and propagation of the murine and rat type-C viruses used in these studies. Data from

an experiment demonstrating the host range of murine virus stocks used are presented in Table 2. S2CL3 is a strictly N-tropic virus; R4/B is a B-tropic virus; and S16CL10(I) is an X- (xenotropic) virus, unable to replicate in mouse cells (17, 19). All three viruses are endogenous type-C viruses derived from transformed subclones of a clone (A31) of a BALB/c embryo cell line, BALB/3T3. AT-124 is an X-tropic virus recovered from NIH Swiss mice (34), whereas CCL 52 is a morphologically and biochemically typical type-C virus which is released from the NCTC 3959 melanoma cell line for which no permissive host cell for replication has yet been found, despite extensive testing (Lieber, unpublished data). Kirsten and Rauscher murine leukemia viruses, standard, laboratory strain leukemogenic viruses, score as NB-tropic viruses in this assay.

Homology between type-C viruses as determined by RNA:DNA hybridization. We compared the relatedness of the viruses described in Table 1 by measuring the extent of hybridization between [³H]DNA prepared in vitro by the "endogenous reaction" of the viral reverse transcriptase and RNA either extracted from purified virus or from the cytoplasm of virus-producing cells. These data (Table 3) represent the normalized percent hybridization values at saturation as determined with S₁ nuclease. By this technique, all the murine type-C viruses studied are significantly related by nucleic acid sequence homology. Although both rats and mice are members of the same taxonomic family (*Muridae*), they have evolved to the point

where there are considerable differences between the unique sequences of their cellular DNAs (24, 25). The data in Table 3 show that none of the murine viruses, whether endogenously or exogenously infectious leukemia viruses, share significant homology with the rat type-C viruses studied.

The representative N-tropic endogenous BALB/c virus, S2CL3, and the representative B-tropic BALB/c virus, R4/B, are highly related to each other by nucleic acid homology. By this technique, the X-tropic, endogenous BALB/c virus is considerably less related to either the N- or the B-tropic viruses. Thus, three different endogenous viruses encoded in the same cellular genome and coming from the progeny of the same clonal cell line can differ significantly in sequence homology as well as in viral host range (6).

The AT-124 virus, an X-tropic virus derived from NIH Swiss mice (34), and R-MuLV (an NB-tropic virus) differ significantly in their nucleic acid sequences from the N-, B-, and X-tropic endogenous BALB/c viruses. In contrast, Ki-MuLV, an NB-tropic virus derived from C3Hf/G mice, and the noninfectious CCL 52 virus share a majority of sequences in common (~90%) with the N-tropic BALB/c virus, although these viruses are all markedly different in derivation and biological activity. Furthermore, both Ki-MuLV and R-MuLV are more closely related to the mouse-tropic endogenous viruses by these criteria than to the X-tropic endogenous viruses.

Thermal stability of viral [³H]DNA:RNA hybrids. The data presented above do not show whether the partial homologies observed between the different murine viruses are due to long conserved segments of identical sequences combined with segments which are radically different, or whether they are due to a general accumulation of base-pair substitutions throughout the viral genomes. To resolve this point, the thermal stability of the nucleic acid hybrids was determined. Thermal melting curves for N-tropic endogenous BALB/c viral [³H]DNA and X-tropic endogenous BALB/c viral [³H]DNA, which were hybridized to various viral RNAs, are depicted in Fig. 1A and 1B, respectively. The temperature at which 50% of the hybrid has disassociated is the *T_m*. The highest *T_m* (88 C) is obtained for the homologous hybrids. Lower *T_m* values occur with viral nucleic acids shown to be less related by the S₁ nuclease technique (Table 3). Although the *T_m* of hybrids formed between the BALB/c N-tropic [³H]DNA probe and the Ki-MuLV or BALB/c B-tropic viral RNA are relatively high

TABLE 2. Host range of murine type-C viruses

Infecting virus	Supernatant reverse transcriptase assay ^a			
	BALB/3T3 (BALB/c mouse embryo)	NIH/3T3 (NIH Swiss mouse embryo)	SIRC (Rabbit cornea)	FCf2Th (Canine thymus)
S2CL3	2.0	181.2	2.0	1.8
R4/B	85.0	1.1	1.1	2.2
S16CL10(I) ^b	1.9	1.6	195.6	958.4
AT-124	1.3	1.2	333.6	250.8
CCL 52	1.5	1.4	2.4	1.8
Ki-MuLV	289.9	1,610.2	0.9	2.7
R-MuLV	165.5	450.6	1.8	1.3

^a Host cell lines were infected with viruses, and the supernatant reverse transcriptase assay (counts per min $\times 10^{-3}$ [³H]TMP incorporated) was performed exactly as described by Lieber et al. (21). Host cell lines were assayed at 28 days postinfection.

^b Growing in SIRC cells.

TABLE 3. Nucleic acid homology between mouse and rat type-C viruses

Viruses	RNA ^a	Homology (%) ^b of [³ H]DNA probe ^c					
		BALB/c N-tropic	BALB/c B-tropic	BALB/c X-tropic	Ki-MuLV	R-MuLV	RT21C
Endogenous mouse	BALB/c N-tropic (S2CL3)	100	95	58	95	43	ND
	BALB/c B-tropic (R4/B)	98	100	60	98	49	6
	BALB/c X-tropic (S16CL10(I))	57	61	100	62	26	5
	NIH Swiss X-tropic (AT-124)	39	54	44	47	19	4
	CCL 52	100	90	77	80	54	ND
Laboratory strain murine leukemia	Ki-MuLV	100	95	61	100	51	ND
	R-MuLV	40	45	49	49	100	6
Rat	RT21C	3	5	0.4	ND	3	100
	VNRK	3	ND	ND	2	ND	45

^a RNA was extracted from purified virus preparations or from cells that produce virus. Both sources of RNA yielded similar saturating values.

^b The percent hybridization values have been normalized to 100% with respect to the saturating percent hybridization obtained with the homologous viral or cytoplasmic RNA extracted from a virus-producing culture. The actual values for the respective [³H]DNA probes were: BALB/c N-tropic, 80%; BALB/c B-tropic, 90%; BALB/c X-tropic, 70%; Ki-MuLV, 70%; Rauscher MuLV, 70%; and RT21C, 80%; and ND, not determined. The average saturating percentage values were determined by either the addition of increasing amounts of RNA or increasing periods of incubation.

^c The [³H]DNA probes used in this study have been shown to contain most of the sequences in proportions similar to their content in the homologous viral 70S RNA.

(87 C and 86 C, respectively), they can be distinguished from the homologous hybrid during the initial part of the melting curve. Hybrids formed with BALB/c X-tropic, R-MuLV, and NIH Swiss X-tropic viral RNAs melt at considerably lower temperatures (81 C, 79 C, and 79 C, respectively). Moreover, the thermal stability of hybrids formed with heterologous viral RNA and the BALB/c X-tropic, [³H]DNA probe (Fig. 1B) are all considerably lower than the homologous hybrid (Ki-MuLV and BALB/c B-tropic, 82.5 C; BALB/c N-tropic, 80.5 C; R-MuLV, 79.5 C; and NIH Swiss X-tropic, 78.5 C).

There is an inverse relationship between the thermal stability of a nucleic acid hybrid and the degree of base-pair mismatch between the two component sequences (9, 24). We have compared the differences in thermal stability of heterologous and homologous hybrids (ΔT_m) to the final extent of hybridization achieved with either the BALB/c N- or X-tropic viral [³H]DNA probe. Figure 2 shows a plot of ΔT_m versus percent hybridization. Comparisons between the various endogenous viruses and between endogenous and laboratory strains of murine leukemia virus show a direct correlation between percent hybridization and the thermal stability of the hybrid. This suggests that the differences in sequence homology between the

various murine type-C viruses result from random base-pair alterations in their nucleotide sequences.

Reassociation kinetics of type-C viral sequences in the nuclear DNA of various strains of mice. The reassociation kinetics of N-tropic BALB/c [³H]DNA with nuclear DNA from BALB/c, AKR, NIH Swiss, feral mouse, and *M. musculus molossinus* from Asia are shown in Fig. 3A. The final extent of hybridization detected with the S₁ nuclease technique is greatest for BALB/c and AKR cellular DNAs, with the wild mice and NIH Swiss DNAs yielding significantly lower values. A similar observation was made by Chattopadhyay et al. (12) when [³H]DNA prepared from AKR N-tropic virus was hybridized to cellular DNA from NIH Swiss mice. The C₀t_{0.4} for the reassociation of sequences related to N-tropic BALB/c [³H]DNA is 2.2 × 10², compared with a C₀t_{0.4} of 1.4 × 10³ for the self-annealing of BALB/c unique-sequence DNA. These data indicate that there are approximately six to seven copies of sequences related to the N-tropic viral probe per haploid genome in BALB/c mice. The reassociation curves also suggest the presence of multiple virogene copies in each of the other mouse strains studied.

As shown in Fig. 3B, similar results are detected with a [³H]DNA probe prepared from

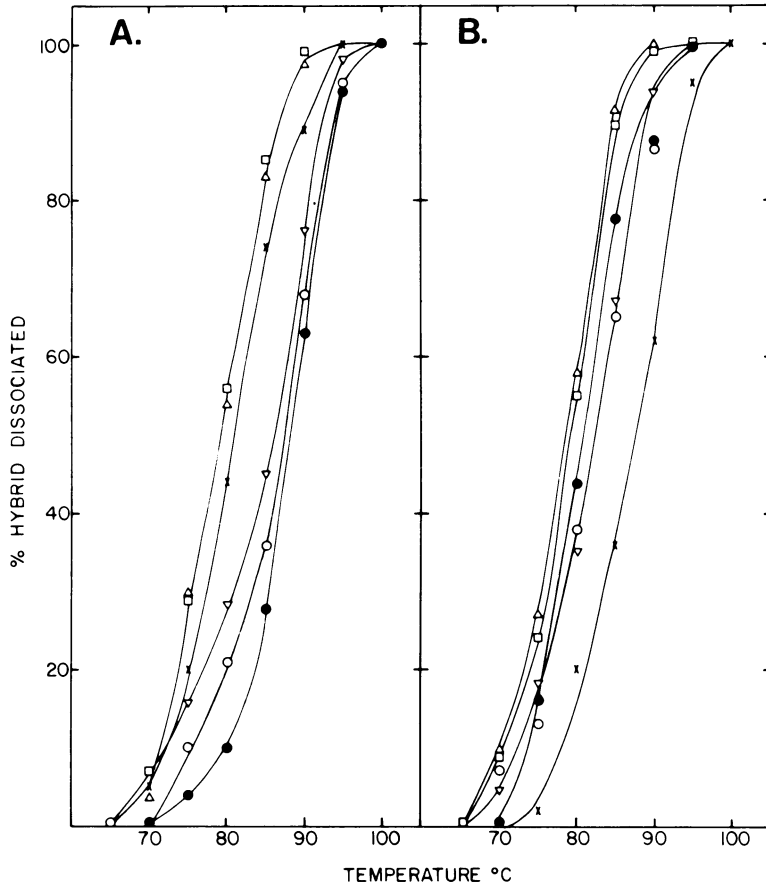


FIG. 1. Thermal stability of hybrids formed between BALB/c endogenous N- and X-tropic viral [^3H]DNA probe and various mouse viral RNAs. Hybridizations were carried out at 65 C. Samples of the hybridization reaction mixtures were diluted 10-fold in 0.4 M NaCl, heated at the listed temperature for 5 min, and then digested with S_1 nuclease. (A) The hybrids tested are between [^3H]DNA from BALB/c endogenous N-tropic virus (S2CL3) and RNA from S2CL3 (●); R4/B (○); Ki-MuLV (▽); S16CL10(I) (×); R-MuLV (□); AT-124 (Δ). (B) The [^3H]DNA probe was prepared from BALB/c endogenous X-tropic virus (S16CL10[I]). Symbols are the same as in (A).

the X-tropic endogenous BALB/c virus. The highest final extent of hybridization to the probe is found in BALB/c DNA, whereas DNAs from all the other strains (including AKR) show a much lower final extent of hybridization. In a previous report (R. E. Benveniste and G. T. Todaro, *Nature* [London], in press), it was shown that the $C_{0t_{1/2}}$ for the hybridization of the BALB/c X-tropic viral [^3H]DNA probe to a dog cell line (FCf2Th, Table 1) which was infected with this virus is 1.2×10^3 , which is identical to the $C_{0t_{1/2}}$ for the reassociation of the unique-sequence, cellular DNA of this cell line. As depicted in Fig. 3B, we compared the reassociation kinetics of the BALB/c X-tropic viral-related sequences in BALB/c DNA to those in the DNA from the infected dog cell line. The

$C_{0t_{1/2}}$ of 1.8×10^2 observed for the former reassociation reaction suggests again approximately six to seven sets of viral-related sequences per haploid genome in BALB/c mice.

Thermal stability of viral [^3H]DNA:DNA hybrids. The failure to detect full sequence homology between the BALB/c endogenous viral probes and the genomes of the other mouse strains could be the result of the gradual evolutionary divergence of the virogene sequences with the general accumulation throughout the virogenes of base-pair differences, or the result of the discrete loss of a long segment of virogene information. To study this question, we measured the thermal stability of hybrids formed between the BALB/c endogenous viral [^3H]DNA probes and nuclear DNAs from the

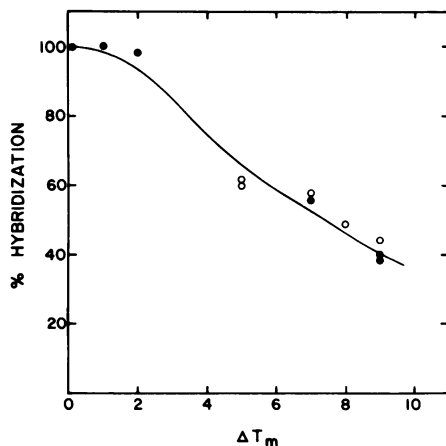


FIG. 2. Relationship between the final percent of DNA:RNA hybridization and ΔT_m . The final percent hybridization values are given in Table 2. The ΔT_m values were computed by deducting the T_m of the heterologous hybrid from that of the homologous hybrid as determined from Fig. 1. Symbols: hybrids formed between the [^3H]DNA probe prepared from the BALB/c endogenous N- (●) and X- (○) tropic viruses and RNAs tested in Fig. 1.

various mouse strains (Fig. 4A and 4B). We compared the melting profile of these hybrids to those formed with DNA from a non-mouse cell line exogenously infected with a mouse virus. In Fig. 4A and 4B, this is DNA from Ki-MuLV-infected rat cells and X-tropic BALB/c virus-infected dog cells, respectively.

A higher T_m is observed for the hybrids between BALB/c N-tropic DNA probe and Ki-MuLV-infected rat cell DNA (91.5 C), and between BALB/c X-tropic probe and S16CL10(I)-infected (see BALB/c X-tropic virus, Table 1) dog cell line DNA (89 C), than between these probes and BALB/c nuclear DNA (86.5 C and 85 C, respectively). Since the exogenously infected cells contain one (or at most two) viral gene copies per haploid genome whereas the BALB/c cells contain six to seven copies per haploid genome, we are comparing the average thermal stability of multiple viral copies with that of a single viral sequence. The results obtained (i.e., a lower T_m) are consistent with the conclusion that the BALB/c genome contains multiple, nonidentical but related, viral sequences.

The hybrids formed between the BALB/c N-tropic and X-tropic viral probes and nuclear DNAs from the other strains of *M. musculus* are even less thermally stable. An exception is the hybrid formed between the N-tropic viral DNA probe and AKR DNA, where no significant

difference in thermal stability could be detected when the hybrid was compared with BALB/c cellular DNA. The data obtained using the thermal stability of the hybrids are consistent with the final extent of hybridization data measured with S_1 nuclease. Thus, the thermal melting profiles suggest that the differences among the viruses are the result of generalized sequence divergence of the virogene information, rather than the result of loss (or gain) of discrete virogene segments.

DISCUSSION

Three different host range classes of endogenous type-C viruses (N-, B- and X-tropic) have been obtained from subclones of the BALB/3T3 cell line (6). Despite their varying host ranges, these viruses share immunologically related reverse transcriptases and p30 antigenic determinants (6). The [^3H]DNA:RNA hybridization studies reported here demonstrate that the viral genomes of these three BALB/c endogenous viruses share extensive homology but are nonidentical. The thermal melting profiles of the DNA:RNA hybrids suggest that the partial homology between the different BALB/c endogenous viruses can be accounted for best by the accumulation of small segments of base substitutions throughout the viral genomes rather than by the retention of a common, identical, long segment of base sequences coupled to sequences completely unique to each virus class.

The data presented indicate that BALB/c, AKR, and NIH Swiss laboratory strains of mice, and feral mice from California and Asia, contain in their cellular genomes type-C virogene sequences in multiple copies that are related by varying degrees to both the endogenous N-tropic and X-tropic viruses of BALB/c mice. These results are consistent with those from previous studies with probes made from exogenously infecting murine leukemia viruses (13, 31), and from the N-tropic virus of AKR mice (12) in demonstrating that the DNAs from normal mice of various strains contain multiple copies per genome of sequences with extensive homology to the infectious disease-producing, murine type-C viruses. The data with wild mice from two geographic locales indicate that type-C viral genes are a general feature of the genome of the species *Mus musculus* and are not restricted to inbred laboratory mouse strains. Further, the mouse is not unusual in possessing multiple copies of related but nonidentical endogenous type-C virogene sequences; multiple, related virogene copies are also present in the

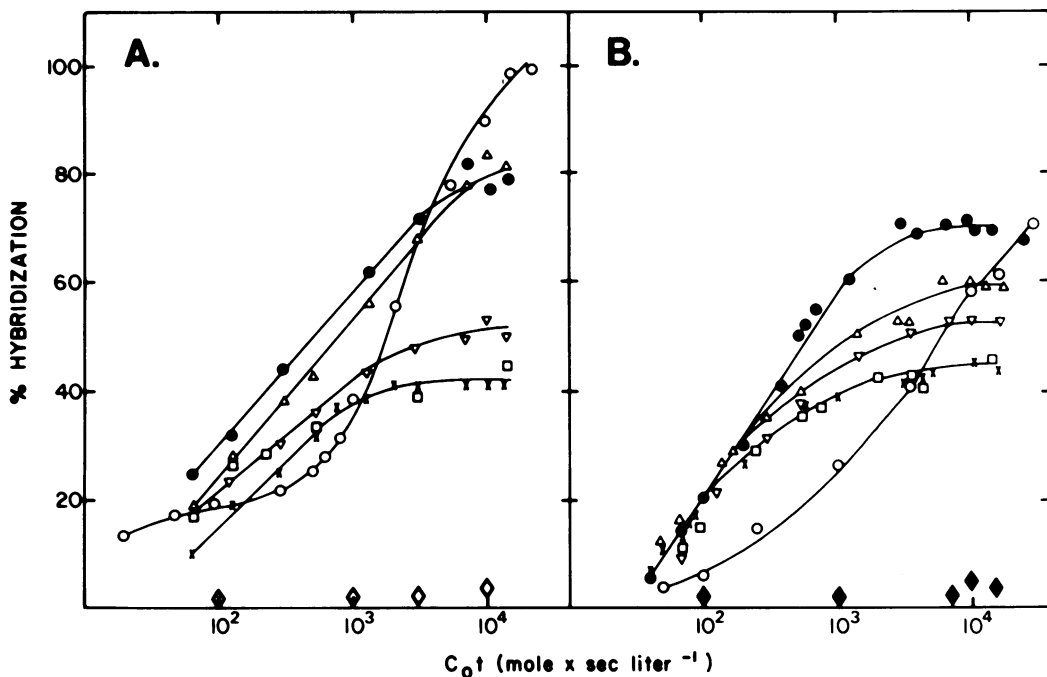


FIG. 3. Hybridization of BALB/c endogenous N- and X-tropic viral [^3H]DNA probe to DNA extracted from various cell lines and mouse tissues. Hybridization reactions contained 1,000 counts per min per 0.05 ml of either [^3H]DNA product added; the ratio of cold, nuclear DNA to [^3H]DNA probe was 1.5×10^6 to 4×10^6 . Further details of hybridization have been described previously (5). (A) [^3H]DNA probe was prepared from BALB/c endogenous N-tropic virus (S2CL3) and was hybridized to nuclear DNA extracted from: BALB/c liver (\bullet); AKR liver (Δ); California feral mouse liver (∇); *Mus musculus molossinus* pooled organs (\square); NIH Swiss liver (\times); and NRK (\diamond). The self-annealing of BALB/3T3 is shown (\circ). (B) [^3H]DNA probe was prepared from the BALB/c endogenous X-tropic virus (S16CL10[1]). The symbols are the same as in (A) except for: FCf2Th (dog thymus cell line) (\circ); and FCf2Th infected with S16CL10(1) virus (\circ).

cell DNA of the rat, pig, domestic cat, and baboon species (R. E. Benveniste and G. J. Todaro, *Nature* (London), in press).

The results obtained with the two BALB/c viral probes indicate that there are marked differences among the endogenous type-C virogens of the inbred strains and feral mice studied. Chattopadhyay et al. (12) detected a major difference between the endogenous virogens of AKR and NIH Swiss mice with an N-tropic AKR viral probe. We have found similar differences between BALB/c, AKR, and NIH Swiss laboratory strains of mice as well as three strains of feral mouse with both N-tropic and X-tropic BALB/c viral probes. Lack of homology between the BALB/c X-tropic probe and RNA from the AT-124 virus, an X-tropic virus isolated from NIH Swiss mice (34), also supports a major difference between the virogens of these two strains. Such readily detectable differences in type-C viro-gene sequences are in marked contrast to the reported inability of overall unique-sequence DNA:DNA hybridiza-

tion to detect differences among various inbred strains of *M. musculus*, and between the laboratory strains and *M. musculus molossinus* (29). As shown previously for the group of Old World monkeys, viro-gene probes are much more sensitive detectors of evolutionary divergence than overall, unique-sequence DNA (5).

Thus, at two different biologic levels, i.e., (i) the individual mouse cellular genome, and (ii) comparison of one mouse (or inbred strain) genome with other mouse genomes, there exist groups of related but nonidentical type-C virogens. Within the individual genome, the differences between virogens seem to be the probable result of gene duplication and subsequent divergent evolution from a common, primitive, type-C viro-gene. In contrast, the major source of viro-gene diversity between the inbred mouse strains can probably be attributed to what Mayr has called the founder principle (23). This designates the evolutionary consequences of "the establishment of a new population by a few original founders . . .

which carry only a small fraction of the total genetic variation of the parental population. The descendant population contains only the relatively few genes that the founders had brought with them until replenished by subsequent mutation or by immigration" (23). The inbred mouse strains represent the ultimate application of the founder principle, since they were established from the progeny of a single fertilized female and have been maintained by brother-sister matings for hundreds of generations. Thus, the particular type-C virogene sequence of the founders has been perpetuated, excluded from the gene flow from other members of the species. Clearly, however, the random processes of mutation and recombination will have also contributed to virogene divergence between the various mouse strains in the hundreds of generations separating their common origin. Presumably, within natural populations of feral mice in which there is substantial gene flow, there are complex degrees of poly-

morphism involving a large variety of endogenous type-C virogenes. Nevertheless, the data presented here suggest that at least some of the feral mouse virogenes in both California and Asia are substantially related to the highly inbred BALB/c strain virogenes. Further, one supposes that the existence of partial homology of type-C virogenes within the genome and among strains must favor the possibility of extensive recombination among type-C virogenes during meiosis.

There is ample evidence that endogenous type-C viruses from various inbred mouse strains activated spontaneously (14), or after radiation (15, 22) or carcinogen treatment (3, 28, 35, 36), are leukemogenic for other mice of the same strain and also for other strains of mice. Such viruses usually require a long latent period and newborn hosts to clearly demonstrate their pathogenicity. It seems reasonable that most laboratory strains of highly potent, leukemogenic type-C virus (e.g., Moloney,

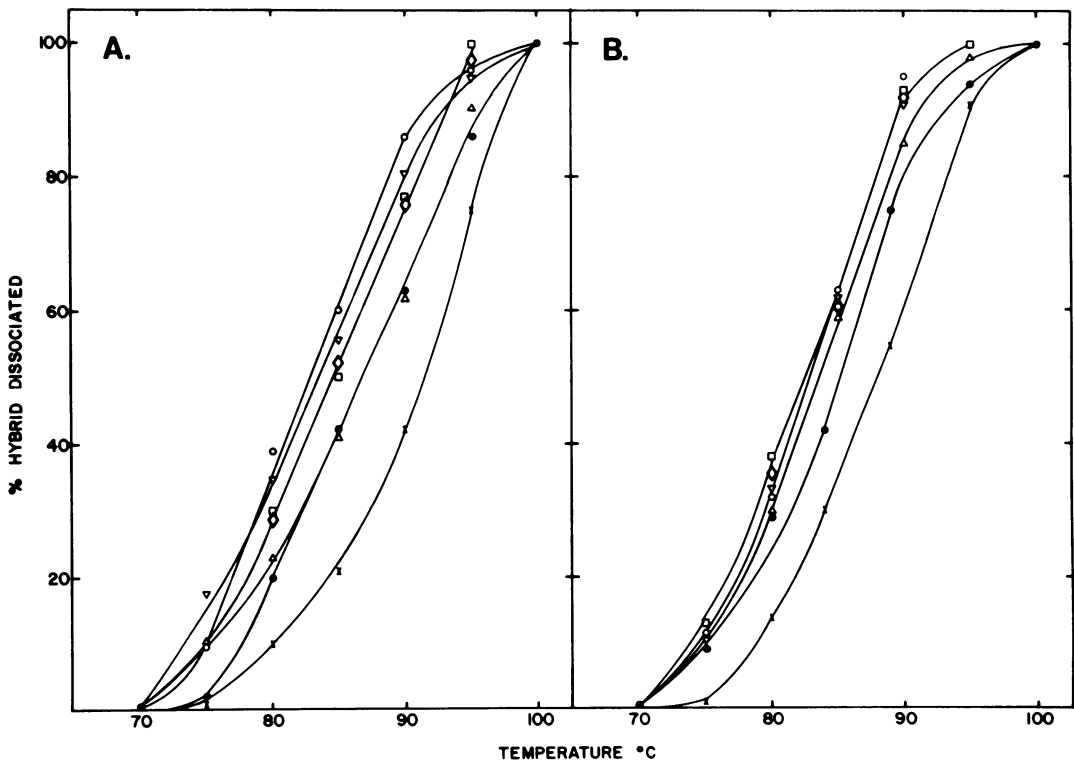


FIG. 4. Thermal stability of hybrids formed between BALB/c endogenous *N*- and *X*-tropic viral [^3H]DNA probes and nuclear DNA from various strains of mice. Hybridization was carried out to a C_{50} of 1×10^4 at 65°C. Samples of the hybridization reaction were diluted 10-fold in 0.7 M NaCl, and treated as described in the Fig. 1 legend. (A) The hybrids tested are between [^3H]DNA prepared from BALB/c endogenous *N*-tropic virus (S2CL3) and nuclear DNA extracted from: Ki-MuLV-infected NRK rat cells (\times); BALB/c liver (\bullet); AKR liver (Δ); *Mus musculus molossinus* pooled organs (\square); *Mus musculus castaneus* pooled organs (\diamond); feral mouse liver (∇); NIH Swiss liver (\circ). (B) The [^3H]DNA probe was prepared from BALB/c endogenous *X*-tropic virus (S16CL10[I]). The symbols are the same as in (A) except: (S16CL10[I])-infected dog thymus cells (\times).

Rauscher, Friend, Kirsten, etc. murine leukemia viruses) must have arisen originally from the endogenous murine type-C viruses. The close homology between Ki-MuLV, a recent leukemogenic isolate with a relatively short passage history, and the N-tropic endogenous BALB/c virus shown here and previously (8) directly supports this possibility. Moreover, it indicates that murine type-C viruses with very small differences in nucleic acid sequence can have major differences in biological activity, including tumorigenicity. In contrast, despite the pathologic similarity of R-MuLV- and Ki-MuLV-induced diseases, R-MuLV (an extensively passaged virus stock) is much more distantly related to the endogenous viruses studied here. Previous studies by Sweet et al. (31) have shown only a moderate degree of homology between the Rauscher probe and the DNAs of either BALB/c or NIH Swiss mice, the strains from which the virus was originally derived (27). This suggests to us that the strong selective pressures brought to bear on a virus stock during serial passaging to select for the most potent leukemogenic virus can rapidly generate nucleic-acid sequences significantly different from those of the original, endogenous type-C viruses. When an endogenous virus becomes activated and begins to act like a typical, exogenously infecting type-C virus rather than like a cellular gene, the frequency of replication of the viral genes is markedly accelerated by several orders of magnitude, as are concomitant possibilities for mutation and recombination. An additional possible mechanism responsible for this change is suggested by the high degree of infidelity with which avian myeloblastosis virus reverse transcriptase transcribes ribonucleotide homopolymers *in vitro* (30). Viral reverse transcriptase is required for type-C virus replication but is probably not used in the normal replication of endogenous type-C virogenes as cellular genes. Such transcription with relative nonfidelity would greatly increase the rate of divergence of infectious type-C viruses from endogenous virogenes.

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