Proc. Nat. Acad. Sci. USA
Vol. 71, No. 1, pp. 167-171, January 1974

Evidence That the AKR Murine-Leukemia-Virus Genome Is Complete in DNA of the High-Virus AKR Mouse and Incomplete in the DNA of the "Virus-Negative" NIH Mouse

(mouse leukemia virus/AKR and NIH mouse DNA and RNA/IdU activation/reassociation kinetics)

SISIR K. CHATTOPADHYAY, DOUGLAS R. LOWY*, NATALIE M. TEICH, ARTHUR S. LEVINE†, AND WALLACE P. ROWE

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, and † Section on Infectious Diseases, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Communicated by Robert M. Chanock, September 7, 1973

ABSTRACT **The AKR mouse has a high titer of murine leukemia virus early in life, and virus-negative cells derived from embryos of this mouse strain can be activated to yield murine leukemia virus by treatment with 5-iododeoxyuridine. In contrast to this high-virus strain, the NIH Swiss mouse has a low incidence of leukemia and no murine leukemia virus has been isolated from it (virus-negative). We have investigated this difference between AKR and NIH mice by examining the sequences specific for murine leukemia virus in nucleic acids of these mice. A single-stranded viral-DNA probe synthesized *in vitro* using murine-leukemia-virus from the AKR mouse contains at least 87% of the sequences present in the 70S viral RNA; most of these sequences are in proportions similar to their content in the 70S RNA. Using this probe in nucleic acid hybridization experiments, we have shown that NIH-mouse-cell DNA and AKR-mouse-cell DNA differ with respect to sequences specific for AKR murine-leukemia-virus: NIH-mouse-cell DNA lacks some of the virus-specific sequences present in AKR-mouse-cell DNA, and there are two distinct sets of virus-specific sequences in AKR-mouse-cell DNA, whereas there is only one set in NIH-mouse-cell DNA.**

RNA from virus-negative AKR-mouse cells grown in tissue culture contains some, but not all, virus-specific RNA sequences; however, within 48 hr after initiating treatment of these cells with 5-iododeoxyuridine, the complete viral genome is represented in cellular RNA.

The AKR mouse strain has high titers of murine leukemia virus (MLV) early in life (high virus), preceding the development of leukemia (1). In contrast, the NIH Swiss mouse does not produce infectious MLV ("virus-negative") nor does it develop leukemia, yet virus-specific functions may be expressed (2).

Genetic mating and 5-iododeoxyuridine (IdU) activation studies have established that the potentially infectious genome of MLV is present in all cells of AKR mice (AKR cells) (3–5) and in certain other virus-positive mouse strains (6) as a heritable component. Using DNA·DNA or DNA·RNA hybridization techniques, several laboratories have demonstrated virus-specific sequences in high-molecular-weight cellular DNA in both murine (7) and avian systems (8, 9). Surprisingly, in none of these experiments was it possible to distinguish between the DNA of virus-positive and virus-negative strains of mice or of group specific antigen (gs)$^+$ and gs$^-$ chickens. Either a double-stranded [^{3}H]DNA synthesized endogenously using viral 70S RNA as the template (8) or an isotope-labeled viral 70S RNA (9) was used to detect the virus-specific sequences. With the double-stranded [^{3}H] DNA, the number of virus-specific sequences was estimated by comparing the rate of viral probe hybridization in the presence or absence of cellular DNA. Recently, it has been shown that a single-stranded DNA probe made endogenously in the presence of actinomycin D is more representative of viral 70S RNA (10). In the experiments reported here, a single-stranded probe prepared from MLV from AKR mouse cells (AKR-MLV) was used to detect virus-specific sequences in DNA from AKR and NIH mice (AKR-DNA and NIH-DNA, respectively). Our results indicate that the complete MLV genome is present in AKR cells as DNA, and that distinct differences exist between high-virus and virus-negative strains of mice with regard to the presence of viral-specific sequences.

Abbreviations: gs, group-specific antigen; TCA, trichloroacetic acid; IdU, 5-iododeoxyuridine; MLV, murine leukemia virus; AKR cells, cells from the AKR mouse strain; AKR-MLV, MLV from AKR cells; AKR-DNA, DNA from AKR cells; NIH-DNA, DNA from cells of NIH-Swiss-mice.

* Present address: Department of Dermatology, Yale University School of Medicine, New Haven, Conn. 06511.

MATERIALS AND METHODS

Cells. Primary cells were prepared from 14- to 16-day NIH-Swiss-mouse embryos (11) and maintained in Eagle's minimal essential medium (MEM) containing 10% fetal-bovine serum (FBS). The establishment and propagation of the clonal cell line 32-C from virus-negative, AKR-mouse embryos has been reported (4). Vero (monkey) cells were maintained as described (12).

Virus. AKR-MLV has been serially passaged in secondary NIH-mouse-embryo (NIH-ME) cells.

Unlabeled Virus. Primary NIH-ME cells were treated for 45 min with 25 μg/ml of DEAE dextran 1 day after seeding, and then infected with AKR-MLV at a multiplicity of 0.1. Supernatant fluids from confluent secondary cultures of these cells were harvested every 12–18 hr for several days. The fluids were centrifuged at 1500 rpm for 10 min at 4°, and then at 9150 $\times$ g for 15 min. The virus was banded (by Drs. J. Olpin and R. Gilden, whose generosity is gratefully acknowledged) by continuous-flow zonal centrifugation in a 25–50% sucrose gradient in a CF-32 rotor (L350 Spinco centrifuge). The aliquots containing virus (measured by $A_{280 \text{ nm}}$ and complement fixation) were pooled and pelleted at 99,000 $\times$ g. The pellet was resuspended in NTE buffer [0.1 M sodium chloride, 0.01 M Tris·HCl (pH 7.4), 0.001 M EDTA] and stored at $-70°$.

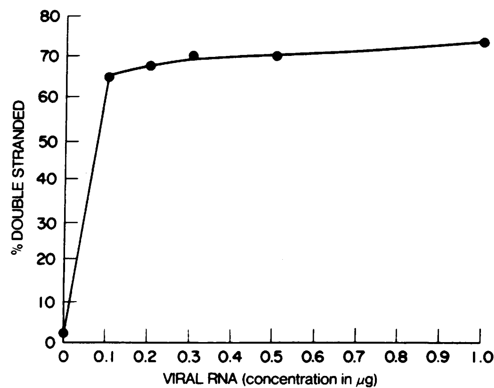

FIG. 1. Hybridization of nonradioactive 70S viral RNA with the *in vitro* synthesized [^{3}H]DNA probe (specific activity = 2×10^7 cpm/μg). Hybridizations were carried out in 1-ml sealed ampules with a 200-μl incubation mixture containing 6×10^{-5} μg of [^{3}H]DNA probe (1200 cpm), 0.1% SDS, 0.48 M phosphate buffer, 5×10^{-4} M EDTA, and RNA. Each incubation mixture was boiled in a water bath for 3 min in the presence of 0.1 M phosphate buffer; additional salt was then added. The mixtures were quickly cooled, sealed, and incubated for 22 hr at 66°. Each incubation mixture was then diluted to a final concentration of 0.14 M phosphate buffer and 0.4% SDS. Hybridization was assayed by hydroxyapatite chromatography. Unhybridized molecules were removed from the column with 0.14 M phosphate buffer plus 0.4% SDS at 60° while the hybridized molecules were removed with the same buffer at 100°. Samples were counted either by precipitation with TCA or by adding 12 ml of "Insta-gel" (Packard Instrument Co.) to 8 ml of aqueous solution.

Unlabeled Viral RNA. Viral RNA (60–70S) was prepared by the method of Neiman (9), dialyzed against TE buffer, and concentrated by vacuum dialysis.

Viral [^{32}P]RNA. Confluent secondary NIH-mouse-embryo cells, infected with AKR-MLV, were prelabeled for 14 hr in phosphate-free MEM containing 10% FBS and 100 μCi/ml of ^{32}P (carrier-free orthophosphoric acid) from New England Nuclear Corp. ([^{32}P]medium). The fluids from three consecutive 5-hr incubations with [^{32}P]medium were pooled and clarified by centrifugation as described for unlabeled virus. The virus was banded in a discontinuous 15 and 60% sucrose gradient for 70 min at 80,000 × *g*. The visible virus band was collected and repurified in a linear 20–60% sucrose gradient at 94,000 × *g* for 12 hr. Fractions containing virus were pooled, the virus was treated with pronase (100 μg/ml) for 10 min at 30°, and RNA was extracted by the method of Robinson *et al.* (13). The 60–70S viral RNA was purified as described (9), concentrated by vacuum dialysis against 0.2 × saline sodium citrate [0.15 M NaCl, 0.015 M trisodium citrate, (pH 6.9)] with 5×10^{-4} M EDTA, and stored at 4°.

Single-Stranded Virus-Specific [^{3}H]DNA. The single-stranded virus-specific [^{3}H]DNA probe was synthesized in an endogenous reverse transcriptase reaction in the presence of actinomycin D (14) using detergent-lysed, purified AKR virus. The final product was stored at 4° in 0.01 M phosphate buffer [equimolar concentrations of monobasic and dibasic sodium phosphate (pH 6.8)] with 5×10^{-4} M EDTA; no appreciable degradation of the DNA, as measured by trichloroacetic acid (TCA) precipitability and by hybridizability, was noticed after storage for 4 months. The synthesized probe was 98% single-stranded as determined by hydroxyapatite chromatography, and all of the radioactivity was TCA precipitable.

Cellular DNA for Hybridization. DNA from tissue culture cells and from embryos was isolated by the method of Varmus *et al.* (15). All DNAs were sheared to a length of 150–250 nucleotide pairs by passing them through an orifice with a pressure drop of 40,000 lb/in.^{2} using a French pressure cell (American Instrument Co.). The sheared DNA solution was then forced through a Metricel GA-6 filter (16). DNAs extracted by this method had an $A_{260/280\,nm}$ ratio of 1.85–1.95. DNA concentrations were determined from absorbance at 260 nm.

Cellular RNA. The sodium dodecyl sulfate(SDS)-hot phenol method for cellular RNA extraction has been reported (17). RNA concentrations were determined by the orcinol reaction (18).

Nucleic-Acid Hybridizations. Hydroxyapatite (BioGel HT for RNA·DNA, BioGel HTP for DNA·DNA; Biorad) was used to separate unhybridized material from nucleic-acid hybrids (19). In one RNA·DNA hybridization experiment, the extent of hybridization was measured by *Aspergillus oryzae* S-1 nuclease (20). At the end of the hybridization, 4.9 ml of 0.033 M sodium acetate (pH 4.5), 0.0002 M zinc sulfate, and 0.13 M NaCl were added to each reaction mixture (14). Each sample was then divided into two equal aliquots, purified S-1 nuclease (gift of Dr. E. Scolnick) was added to one, and both were incubated for 90 min at 45°. The samples were precipitated with cold TCA, the precipitate was collected on nitrocellulose filters, and the radioactivity was determined.

RESULTS

Characterization of the Single-Stranded [^{3}H]DNA Probe. To characterize the extent to which the [^{3}H]DNA probe was complementary to its 70S viral-RNA template, we deter-

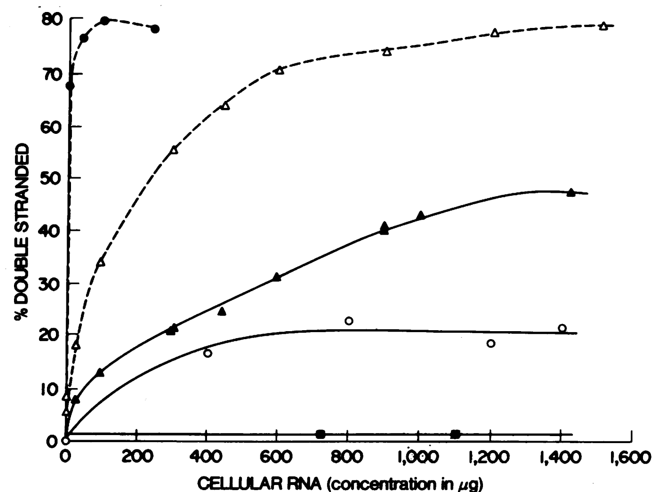

FIG. 2. Hybridization of unlabeled cellular RNAs with the *in vitro* synthesized [^{3}H]DNA probe. Conditions and assay method for hybridization are described in the legend of Fig. 1, except that 1×10^{-4} μg (2000 cpm) of [^{3}H]DNA was used in each incubation mixture. Origin of cellular RNA: (●) productively infected NIH-mouse cells; (△) IdU-treated 32-C AKR-mouse cells; (▲) normal 32-C AKR-mouse cells; (○) Uninfected NIH-mouse cells; (■) Vero (monkey) cells.

mined the proportion of the probe which is hybridizable to the 70S RNA. The results indicate that 75% of the probe was hybridized in the presence of saturating amounts of purified viral RNA (Fig. 1).

The proportion of the 70S viral RNA that is represented in the [^{3}H]DNA probe was also examined. 70S [^{32}P]RNA from AKR-MLV was incubated in the presence of excess probe (DNA:RNA = 15:1; higher ratios were not tested). As shown in Table 1, 87% of the ^{32}P-counts were resistant to S-1 nuclease, indicating that at least 87% of the 70S viral RNA sequences are represented in the DNA probe. When hybridization was carried out at a DNA:RNA ratio of 1.5:1, 69% of the ^{32}P-counts were resistant to S-1 nuclease. These results indicate that some of the viral-specific sequences in the probe DNA are not present in proportions equal to their abundance in 70S RNA, but that about two-thirds of the 70S viral-RNA sequences are well represented in the probe.

Hybridization of Probe with Cellular RNAs. We determined the extent to which the probe hydridized with saturating amounts of RNA from NIH-ME cells chronically infected with AKR-MLV (Fig. 2). A maximum of 80% of the probe hybridized to the infected cellular RNA, while no more than 3% of the probe hybridized to the control Vero-cell RNA. RNA from uninfected NIH-mouse cells hybridized to 20% of the probe; this value probably reflects the expression of MLV-specific antigens often observed in NIH mice (2).

To determine the extent of transcription of virus-specific sequences in AKR cells, the DNA-probe was hybridized to RNA from the virus-negative AKR clonal cell line. The results (Fig. 2) show that at saturation only 40% of the DNA probe is hybridized, indicating that some, but not all, virus-specific sequences are present in the cellular RNA. It cannot be determined from these experiments whether all or only some cells in the population contain this viral-specific RNA. However, when these virus-negative cells were treated with IdU (20 μg/ml for 24 hr), which activates infectious virus within 72 hr (3), the complete viral genome appears to have been transcribed into RNA within 48 hr after initiating treatment, as cellular RNA extracted at this time hybridized to 80% of the DNA probe (Fig. 2). Similar results have been obtained with the RNA from BALB/c mouse cells (21).

Hybridization of the Probe with Cellular DNAs. The presence of virus-specific sequences in cellular DNA was determined by incubating the DNA-probe with bulk cellular DNA from 15 day AKR-mouse embryos, which is completely free of virus (4), and from NIH-ME cells; the reassociation kinetics (19) are shown in Fig. 3*A*. A maximum of 50% of the probe hybridized to NIH-DNA whereas 80% hybridized to AKR-DNA. Identical kinetics were obtained when the ratio of cellular DNA to probe was increased or decreased by a factor of 4, indicating that the cellular DNA is in vast excess. Also, when virus-negative AKR cells grown in tissue culture were the source of cellular DNA, the kinetics were the same as noted with AKR-mouse embryo DNA. The $C_0t_{1/2}$ value for AKR-DNA:probe reassociation is 250 mol $\times$ sec/liter and 130 mol $\times$ sec/liter for NIH-DNA:probe reassociation; this would correspond to approximately 6 and 12 copies, respectively, of virus-specific DNA sequences per haploid cellular genome. In both cases, the thermal elution profiles of probe:cellular DNA hybrids were identical to those of cell–cell DNA hydrids, indicating that there was no mismatching of hybridized sequences.

Since the proportion of probe which hybridized to AKR-DNA (80%) was the same as that obtained with 70S RNA and with RNA from NIH-mouse cells infected with AKR-MLV, it is very likely that the complete viral genome is present in normal AKR-DNA. To further document this point, the probe was first hybridized with saturating amounts of RNA from productively infected NIH-ME cells, and the DNA·RNA hybrids were isolated by elution from hydroxyapatite. After alkaline hydrolysis of the cell RNA, the DNA probe was reconcentrated. When this probe was reincubated with AKR-DNA or RNA from productively infected NIH-mouse cells, again only 80% of the probe was hydridized (data not shown). These results strongly suggest that 80%, for technical reasons, is the maximum hybridization level which can be obtained and that the probe is thus fully hybridizing with the same sequences in infected cell RNA and normal AKR-DNA.

Another feature of the reassociation kinetics (Fig. 3*A*) is that virtually none of the probe appears to hybridize with the highly reiterated fraction of cellular DNA (> 1000 copies per haploid genome, $C_0t < 1$); this fraction forms about 25% of the total cellular DNA. To demonstrate this point, highly repetitious DNA was removed from total DNA by allowing the denatured DNA to reassociate to a C_0t of 10 (Fig. 3*B*); this C_0t value was chosen to prevent removal of slowly reassociating viral sequences from the cellular DNA. Moreover, if some of the virus-specific sequences reassociate more rapidly than others, they might also be removed at this C_0t. As can be seen, the $C_0t_{1/2}$ of the unique DNA is about 1400 mol $\times$ sec/-liter while the $C_0t_{1/2}$ for the AKR-DNA:probe reassociation is about 250 mol $\times$ sec/liter. Thus it appears, as in Fig. 3*A*, that there are about five to six copies of viral-DNA sequences per haploid AKR-cell genome.

In order to examine more closely the number of copies of the viral genome in cellular DNA, it is useful to plot the reassociation kinetics as the reciprocal of the proportion of unhybridized probe DNA *versus* the C_0t (Wetmur–Davidson plot, ref. 22). If all virus-specific sequences are present in the cellular DNA in equal numbers, the results would describe a single straight line with a slope proportional to the number of copies of that set of sequences. On the other hand, if several sets of virus-specific sequences were present, each in different proportions, several lines would be described. It can be seen in Fig. 4 that the viral probe reassociates with AKR-DNA as though there are two distinct sets of virus-specific sequences; one set has a slope about 10 times as steep as that of unique cellular DNA, whereas the second set has a slope four times as steep, suggesting 10 and 4 copies, respectively, per haploid cell genome. In contrast, the NIH-DNA:probe reassociation describes a single straight line, with a slope about 15 times as steep as that of unique cellular DNA. From these data we conclude that NIH-DNA is lacking one set of virus-specific sequences which can be detected in AKR-DNA.

DISCUSSION

This study indicates that the complete AKR-MLV genome (as measured by the [^{3}H]DNA probe) is represented in the cellular DNA of AKR-mouse embryos and tissue culture cells, whereas NIH-DNA is lacking about $^1/_3$ of the AKR-MLV sequences; that different portions of the AKR-MLV genome are present in unequal numbers in the AKR-DNA; that some, but not all, of this genome is expressed as RNA in virus-negative AKR cells; and that by 48 hr after treatment of virus-

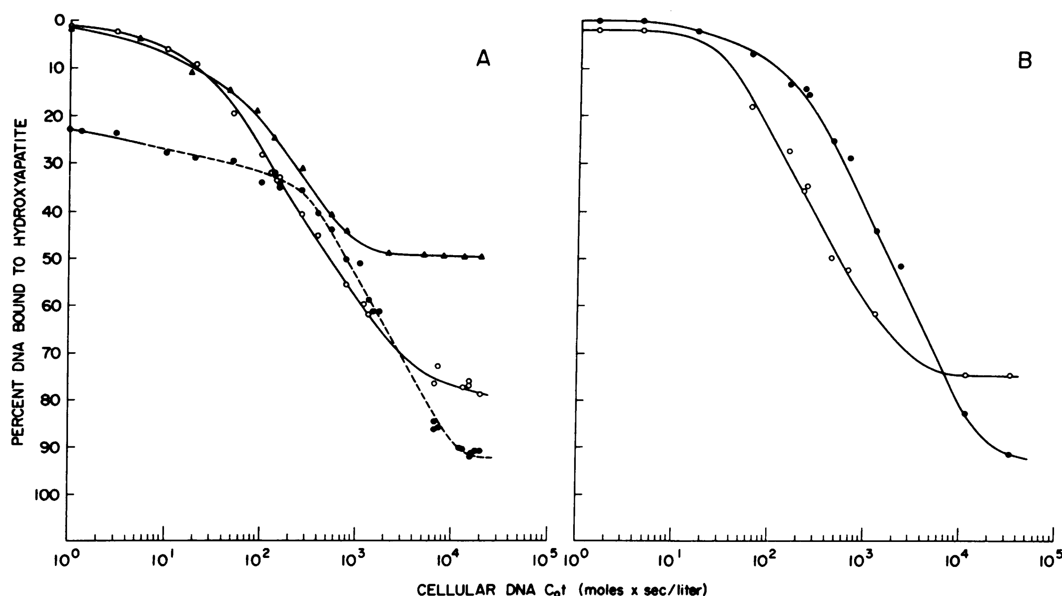

FIG. 3. Reassociation kinetics of the [^{3}H]viral DNA probe with bulk or unique mouse cellular DNAs. (*A*) Sheared cellular DNAs (4.25 mg/ml) were mixed with 3.3 × 10^{-4} μg/ml of [^{3}H]viral DNA probe (specific activity = 2 × 10^7 cpm/μg). The mixtures were then denatured in 0.12 M phosphate buffer (0.18 M Na^+) and brought to 0.48 M phosphate buffer (0.72 M Na^+) where needed. Each 75-μl reaction mixture was sealed in a 1-ml ampule and incubated at 65° for the desired time period. Each incubation mixture was then diluted to a final concentration of 0.14 M phosphate buffer + 0.4% SDS. The extent of reassociation was measured as described in the legend of Fig. 1. Each fraction obtained from hydroxyapatite chromatography was measured for absorbance at 260 nm before measurement of radioactivity. C_0t is equal to (nucleic-acid absorbancy at 260 nm) times (hours of incubation divided by 2). When mixtures were incubated at 0.72 M Na^+ concentration to achieve higher C_0ts, the equivalent C_0t at 0.18 M Na^+ was calculated according to Britten (23). (○——○) sheared AKR cellular DNA; (▲——▲) sheared NIH cellular DNA; (●——●) sheared mouse DNA-DNA self-hybridization. (*B*) Reassociation kinetics of the viral [^{3}H]DNA probe with sheared, predominantly unique AKR-DNA (i.e., cellular DNA from which rapidly reassociating fractions have been removed after allowing the denatured DNA to reassociate up to a C_0t of 10.0). Conditions for hybridization, assay procedures, and symbols employed on the graph are the same as described above.

negative AKR cells with IdU, the complete viral genome is transcribed into RNA.

There are several advantages to the system used here compared to those used in previous studies. (*i*) A single-stranded viral-DNA probe is much more representative of the viral 70S RNA than a double-stranded probe (10). (*ii*) To resolve the question of whether the whole endogenous virus genome is present in cellular DNA, it is necessary to use the homologous endogenous virus, rather than a laboratory strain, to synthesize the probe. (*iii*) The comparison of hybridization of probe with saturating amounts of RNA from infected cells or DNA from virus-negative cells (which contain the endogenous virus genome), permits the critical determination that the complete viral genome is present in cellular DNA. (*iv*) By allowing the hybridization reaction to occur in the presence of a vast excess of DNA, the reassociation can be followed until the maximum hybridization level is reached. (*v*) Analysis of the reassociation kinetics by the Wetmur–Davidson plot allows one to distinguish between components of the probe which reassociate at different rates according to their abundance in cellular DNA. (*vi*) With a single-stranded probe, the cell–cell reassociation kinetics provide an indicator that the hybridization reaction is proceeding as expected and allow a quantitative interpretation of the kinetics.

We have determined that 75–80% of the [^{3}H]DNA probe hybridizes with viral 70S RNA (when RNA is in excess), 80% with infected NIH-mouse-cell RNA, 80% with RNA from IdU-treated AKR cells, and 80% with normal AKR-

TABLE 1. *Hybridization of 70S AKR-MLV RNA with the in vitro synthesized [^{3}H]DNA probe**

DNA added (μg)		70S [^{32}P]RNA from AKR-MLV (μg)	DNA:RNA ratio	[^{32}P]TCA precipitable counts		Percent S-1 nuclease resistant
[^{3}H]-Probe	Calf thymus			Without S-1 nuclease	S-1 nuclease resistant	
—	7 × 10^{-3}	0.46× 10^{-3}	—	241	7	3
6.9 × 10^{-3}	—	0.46 × 10^{-3}	15:1	257	223	87
5.0 × 10^{-3}	—	3.3 × 10^{-3}	1.5:1	717†	494†	69

* Reaction mixtures contained the DNA and RNA in 75 μl of 5.3 × SSC and 0.05% SDS. After 72 hr at 65°, these mixtures were diluted with buffer and treated with S-1 nuclease [which degrades single-stranded but not double-stranded nucleic acids (18)], as described in *Materials and Methods*.

† Only 40% of the total sample was used.

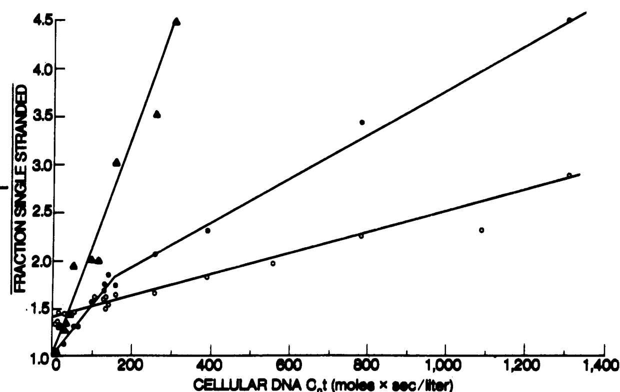

FIG. 4. Analysis of reassociation kinetics of the viral [^{3}H]-DNA probe with sheared cellular DNAs. The cell-cell and cell-virus probe hybridizations were carried out as described in Fig. 3. The maximum observed probe:cell hybridization was normalized to 100%. (○——○) mouse cell DNA:DNA self-hybridization; (●——●) AKR-DNA:probe DNA hybridization; (▲——▲) NIH-DNA:probe DNA hybridization.

cell DNA. Furthermore, neither the 80% hybridization level nor the reassociation kinetics change when the probe is hybridized with DNA from AKR cells exogenously infected with AKR-MLV (data not shown). Therefore, we conclude that all of the genetic information for the production of AKR-MLV is present in normal AKR cells as DNA. The conclusion that the stable inheritance of the MLV genome is due to the presence of a complete set of virus-specific sequences in the cellular DNA is further supported by the fact that one cannot detect the complete virus genome in the RNA of AKR cells. The finding that the virus-specific DNA content of the noninfectious, clonal, AKR-cell line 32-C is the same as that of AKR-mouse embryos implies that the clonal cell lines are indeed representative of the totality of AKR cells (4).

An analysis of the reassociation kinetics with the linear transformation of the Wetmur-Davidson plot strongly suggests that the AKR viral information in AKR cells consists of two sets of DNA sequences; one of these sets is represented about 10 times, the other about four times per haploid AKR-cell genome. Thus, the data suggest that there are about four complete copies of the viral genome per haploid cell genome in normal AKR cells. It is possible that the set which is present in greater abundance may reflect the presence of incomplete AKR-MLV genomes or the presence of other endogenous viruses which do not share complete homology with the prototype AKR-MLV. There is evidence to support the concept that two widely divergent types of endogenous C-type virus may be present in a single mouse (24).

We found that DNA from the virus-negative NIH-Swiss-mouse cells differs from AKR-DNA in two respects. First, the maximum hybridization of NIH-DNA with the probe is 50%, which means that NIH-DNA lacks some of the virus sequences represented by the probe. In addition, as seen from the reciprocal plot, only one set of DNA sequences, present in about 15 copies, is represented in NIH-DNA, compared to the two sets seen in AKR-DNA. Whether this set of virus-specific sequences in NIH-DNA is identical to that found as 10 copies in AKR-DNA, has yet to be determined. The fact that NIH-DNA is lacking a significant portion of the AKR-MLV genome may explain the inability to detect biologically or to activate an endogenous virus of the AKR type from this mouse. These results do not, of course, exclude the possibility that NIH cells contain a complete endogenous MLV genome which shares only partial homology with AKR-MLV. Further study on the recently described ATS-124 virus, which may be of endogenous NIH origin (25), should clarify this point.

Despite the marked biological differences between virus-positive and virus-negative mice, and gs$^+$ and gs$^-$ chickens, previous workers have been unable to distinguish between the virus-specific nucleotide sequences of various strains (7–9). Since the techniques employed in this study make it possible to differentiate between DNAs of high-virus or virus-negative strains of mice, it is now possible to explore the molecular basis of endogenous C-type virus expression in a wider variety of mouse strains.

This project was supported in part by The Virus Cancer Program of the National Cancer Institute.

1. Rowe, W. P. & Pincus, T. (1972) *J. Exp. Med.* **135**, 429–436.
2. Huebner, R. J. (1970) in *Comparative Leukemia Research*, ed. R. M. Dutcher (S. Karger, Basel), pp. 22–44.
3. Lowy, D. R., Rowe, W. P., Teich, N. & Hartley, J. W. (1971) *Science* **174**, 155–156.
4. Rowe, W. P., Hartley, J. W., Lander, M. R., Pugh, W. E. & Teich, N. (1971) *Virology* **46**, 866–876.
5. Rowe, W. P. (1972) *J. Exp. Med.* **136**, 1272–1285; Rowe, W. P. & Hartley, J. W. (1972) *J. Exp. Med.* **136**, 1286–1301.
6. Aaronson, S. A., Todaro, G. J. & Scolnick, E. M. (1971) *Science* **174**, 157–159; Stephenson, J. R. & Aaronson, S. A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2798–2801.
7. Gelb, L. D., Aaronson, S. A. & Martin, M. A. (1971) *Science* **172**, 1353–1355; Gelb, L. D., Milstien, J. B., Martin, M. A. & Aaronson, S. A. (1973) *Nature New Biol.* **244**, 76–79.
8. Varmus, H. E., Weiss, R. A., Friis, R. R., Levinson, W. & Bishop, J. M. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 20–24.
9. Baluda, M. A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 576–580; Neiman, P. (1973) *Virology* **53**, 196–204.
10. Garapin, A. C., Varmus, H. E., Faras, A. J., Levinson, W. E. & Bishop, J. M. (1973) *Virology* **52**, 264–274.
11. Hartley, J. W., Rowe, W. P., Capps, W. I. & Huebner, R. J. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 931–938.
12. Yasumura, P. & Kawakita, Y. (1963) *Nippon Rinsho* **21**, 1201–1219.
13. Robinson, W. S., Pitkanen, A. & Rubin, H. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 137–143.
14. Benveniste, R. E. & Scolnick, E. M. (1973) *Virology* **51**, 370–382.
15. Varmus, H. E., Bishop, J. M. & Vogt, P. K. (1973) *J. Mol. Biol.* **74**, 613–626.
16. Kohne, D. E. (1968) *Biophys. J.* **8**, 1104–1118.
17. Levin, M. J., Oxman, M. N., Diamandopoulos, G. T., Levine, A. S., Henry, P. H. & Enders, J. F. (1969) *Proc. Nat. Acad. Sci. USA* **62**, 589–596.
18. Brown, A. H. (1946) *Arch. Biochem.* **11**, 269–278.
19. Britten, R. J. & Kohne, D. E. (1968) *Science* **161**, 529–540.
20. Sutton, W. D. (1971) *Biochim. Biophys. Acta* **240**, 522–531.
21. Benveniste, R. E., Todaro, G. J., Scolnick, E. M. & Parks, W. P. (1973) *J. Virol.* **12**, 711–720.
22. Wetmur, J. G. & Davidson, N. (1968) *J. Mol. Biol.* **31**, 349–370.
23. Britten, R. J. (1969) in *Problems in Biology: RNA in Development*, ed. E. W. Hanly (Univ. of Utah Press, Salt Lake City), pp. 187-216.
24. Aaronson, S. A. & Stephenson, J. R. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2055–2058.
25. Todaro, G. J., Arnstein, P., Parks, W. P., Lennette, E. H. & Huebner, R. J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 859–862.