Proc. Natl. Acad. Sci. USA
Vol. 73, No. 7, pp. 2448–2452, July 1976
Cell Biology

Increase of AKR-specific sequences in tumor tissues of leukemic AKR mice

(leukemia/DNA annealing kinetics/gene amplification)

ANTON BERNS* AND RUDOLF JAENISCH

Tumor Virology Laboratory, The Salk Institute, Post Office Box 1809, San Diego, California 92112

Communicated by Paul Berg, May 6, 1976

ABSTRACT **AKR mice produce, from shortly after birth, high titers of their endogenous Gross type murine leukemia virus, and develop a thymus-derived leukemia at 7–9 months of age. We show that this oncogenesis is accompanied by an increase in the number of AKR-specific DNA sequences in the tumor tissues, whereas the "non-target" organs are not affected. Sequence increase was determined by kinetic analysis of DNA reassociation using an AKR-murine leukemia virus (MuLV)-specific cDNA and also by hybridization with excess AKR cDNA. The AKR cDNA was selected to recognize AKR sequences without significant crossreaction with DNA sequences of other endogenous viruses. The results show that during the development of the leukemia, the number of AKR-MuLV-specific genes increases in tumor tissues by a factor of 1½ to 2.**

Recently it has been established that several mouse strains carry one or more copies of the Gross-type N-tropic endogenous murine leukemia virus (AKR-MuLV) integrated in their genome and that this information is transmitted to their offspring in a Mendelian fashion (1, 2). In contrast, exogenous viruses such as Moloney leukemia virus (M-MuLV) are not transmitted genetically but infect only specific target cells after infection of an animal (3, 4). Production of endogenous AKR virus in AKR mice is controlled by two dominant loci, *AKV*-1 and *AKV*-2. *AKV*-1 has been identified as a structural gene for AKR-MuLV and has been mapped on chromosome number 7 (5). When newborn AKR mice are infected with AKR-MuLV, the development of leukemia occurs with a decreased latency (6), although in both cases the animals are viremic from birth. This observation and the strong association found between high titers of virus and the onset of leukemia (7) suggested the possibility that exogenous reinfection of lymphatic cells with AKR-MuLV might be crucial for the development of lymphomas. Therefore, we investigated whether the development of leukemia in AKR mice is accompanied by an increase in the number of AKR-MuLV-specific genes in the tumor tissues. An increase in M-MuLV-specific copies has been observed recently during Moloney-induced leukemia (3). Molecular hybridization experiments were performed to quantitate the AKR virus-specific sequences in normal and tumor tissues of AKR mice.

MATERIALS AND METHODS

Virus. AKR virus (Lot no. 325-68-4) for the preparation of 70S RNA and cDNA was obtained from Electronucleonics Laboratory. This virus has been passed for a long time on NIH mouse cells. M-MuLV clone number 1 was supplied by P. MacIsaac and Dr. H. Fan of the Salk Institute.

Abbreviations: M-MuLV, Moloney murine leukemia virus; AKR-MuLV, AKR murine leukemia virus; HA, hydroxyapatite; C_0t, initial concentration of total DNA (mol of nucleotide per liter) × time (sec); $C_0t_{1/2}$, ½ value of initial concentration of total DNA (mol of nucleotide per liter) × time (sec).

* Present address: Laboratory of Biochemistry, University of Nÿmegen, Geert Grooteplein N21, Nÿmegen, The Netherlands.

Mice. AKR, C3H, BALB/c, and strain 129 mice were obtained from the Jackson Laboratories, Bar Harbor, Me. The AKR mice developed spontaneous leukemias at 7–9 months of age, characterized by extreme enlargement of the thymus and spleen, and often associated with pronounced enlargement of lymph nodes and liver.

Complementary DNA (cDNA). Virus-specific DNA probes were prepared from MuLV stocks in the presence of 100 μg/ml of actinomycin D (a gift of Merck) and 2 mg/ml of calf thymus primer (8), with the endogenous polymerase reaction (9). Calf thymus primer was prepared by digesting calf thymus DNA with 20 μg/ml of DNase for 2 hr at 37° in 0.01 M Tris·HCl, at pH 7.4, 0.005 M Mg acetate, 0.1 M NaCl; calf thymus DNA concentration was 20 mg/ml. After autoclaving, the precipitate was spun down and the supernatant was used directly in the polymerase reaction. Either d[^{32}P]CTP with a specific activity of 200 Ci/mmol was used or a combination of d[^{3}H]CTP and d[^{3}H]TTP (New England Nuclear), with specific activities of 22 and 43 Ci/mmol, respectively. Nonradioactive precursors were at a concentration of 2 mM each, whereas radioactive deoxyribonucleotides were at 50 μM. Under these conditions, 20–50% of the input viral RNA (RNA was estimated to be 1% of total viral protein) was transcribed into complementary DNA.

Selection of AKR-MuLV cDNA. To remove sequences from the AKR cDNA which crossreact with other endogenous viruses, the AKR-MuLV cDNA (100 ng) was hybridized with 10 mg of strain 129 mouse DNA at 20 mg/ml for 10 hr at 68° in 1 M NaCl. The DNA was then applied on a hydroxyapatite (HA) (Bio-Rad) column (20 ml packed column vol) at 60° in 0.14 M phosphate and 0.1% sodium dodecyl sulfate. The single-stranded DNA was eluted with 0.14 M phosphate, dialyzed overnight against 0.01 M Tris·HCl, at pH 7.0, 0.001 M EDTA, and rehybridized to strain 129 mouse DNA. The fraction of the cDNA that did not hybridize to strain 129 mouse DNA was subsequently hybridized to a 2-fold excess (based on weight) of 70S AKR RNA under standard annealing conditions (1 M NaCl, 68°). The hybrid was bound to HA at 55° in 0.05 M phosphate, 0.25 M NaCl, and 0.1% sodium dodecyl sulfate. After washing with 0.14 M phosphate, the hybrid was eluted with 0.4 M phosphate and dialyzed overnight. RNA was hydrolyzed with 0.3 M NaOH for 10 hr at 45°, and the DNA was precipitated in the presence of 20 μg/ml of calf thymus DNA. Total recovery during this procedure was 15–20%. Average size of the cDNA was 4–4.5 S on an alkaline sucrose gradient.

Selection of Moloney cDNA. In order to monitor with the M-MuLV cDNA sequences different from AKR, the Moloney cDNA was hybridized with excess 70S AKR RNA. The mixture was applied on an HA column at 0.05 M phosphate as described for the AKR probe, and the 0.14 M fraction was taken. This fraction hybridized to less than 10% with AKR 70S RNA as opposed to 40% hybridization before HA chromatography.

Isolation of 70S Viral RNA. 70S AKR RNA was prepared as described (9).

Extraction of Mouse DNA. Mouse tissues were removed and extracted by the Kirby method (10), banded in ethidium Br-CsCl and boiled in 0.2 M NaOH (3). This isolation procedure excludes contamination with RNA. The size of the DNAs was determined on 1.5% agarose slab gels in the presence of ethidium Br (11). Samples were diluted to 200 μg/ml, and heat denatured with 5 μl aliquots applied to the gel. As reference, *Hpa* II fragments of polyoma virus were used (kindly provided by Dr. M. Vogt). The average size of the mouse DNA was 300 to 400 nucleotides.

Molecular hybridization

(*A*) Kinetic Analysis. DNA·DNA hybridizations were carried out as described previously (3, 4, 12). Hybrid formation was monitored by resistance to S_1-nuclease digestion (12). The mouse tissue DNA was in 2×10^7-fold excess over the AKR-MuLV [^{32}P]cDNA and in 2×10^6-fold excess over the ^{3}H-labeled M-MuLV (minus AKR sequence) cDNA. The results are corrected to standard annealing conditions (13).

(*B*) Excess cDNA Hybridization. Excess cDNA hybridizations were performed under conditions which would allow a final level of hybridization between 10 and 50%. Hybridizations were performed at 68° in 1 M NaCl with about 12 ng/ml of cDNA and 0.5–2.5 mg/ml of mouse DNA. This represents a 2- to 10-fold molar excess of cDNA over the AKR-MuLV specific endogenous sequences. After reaching the endpoint (60 hr) S_1-nuclease resistance of the cDNA was determined. With these conditions, after subtraction of background hybridization measured with strain 129 mouse DNA, the relative amount of cellular sequences complementary to the cDNA can be computed from the equation $X/(1 + X) = \%/100$ in which X is the fraction of AKR sequences in the cell DNA as compared with the input cDNA (1 in this equation). The calculation of the number of AKR-MuLV sequences per haploid mouse genome was based on the molar ratios of cDNA to cell DNA (see Table 3). For this, the complexity of the cDNA has been assumed to be 35% of the AKR-MuLV genome (see *Results* section).

RESULTS

Characterization of AKR and Moloney cDNAs

Two different cDNA probes were used in these experiments to detect different sets of viral related sequences. AKR-MuLV-specific sequences were monitored with a specific ^{32}P-labeled probe from AKR virus and, as an internal control, non-AKR related sequences were monitored with a M-MuLV ^{3}H-labeled cDNA. Unfractionated AKR-MuLV cDNA hybridizes to 90% to AKR DNA and up to 60% to DNAs from mouse strains which do not contain the AKR endogenous virus (Table 1 and ref. 1). The 60% hybridization is likely to be due to partial homology between AKR and other endogenous viruses which are present in multiple copies in the mouse genome. To increase the sensitivity of detection of AKR specific sequences, these "common" sequences were removed by hybridizing AKR cDNA with strain 129 mouse DNA, which lacks the AKR endogenous virus (1). A similar technique has been used to select a cDNA probe which recognized specifically DNA sequences in leukemic human cells (14). The cDNA was hybridized twice with a relatively low excess of strain 129 mouse DNA and the fraction which did not anneal was isolated on HA. In a final purification, the cDNA was hybridized to a 2-fold excess (on mass ratio) of 70S RNA and the annealed cDNA (70%) was isolated on an HA column. This indicates that this cDNA represents at least 35% of the genome, in accordance with a hybridization level of 60% of the unselected AKR-MuLV cDNA to 129 DNA which was used to select the AKR-specific cDNA (Table 1). The actual representation might be somewhat higher as contamination of 70S RNA by ribosomal RNA aggregates was not excluded by the isolation method of 70S RNA which was purified on a single sucrose gradient. This would result in an overestimation of the quantity of 70S RNA added in the final annealing step. The selection method used insures that the cDNA was not enriched for a minor fraction of the genome. The characteristics of the unselected and selected AKR probes are given in Table 1. From this table it can be seen that the selection procedure selects a probe which recognizes AKR sequences with high sensitivity but hybridizes to only 13% to DNA from 129 mice which do not carry AKR-MuLV copies. M-MuLV cDNA was selected by removing the sequences which this cDNA has complementary to AKR-MuLV 70S RNA. M-MuLV cDNA selected in this way hybridized to only 10% to AKR 70S RNA but still to 55% to AKR mouse DNA (see Table 1).

Table 1. Endpoint hybridization of selected and unselected AKR-MuLV and Moloney-MuLV cDNA with mouse DNA

	AKR cDNA		Moloney cDNA	
	% Unselected	% Selected	% Unselected	% Selected
Calf thymus DNA	4	6	4	5
129 DNA	60	13	60	55
BALB/c DNA	77	66	54	52
AKR DNA	88	85	52	54
AKR 70S RNA	95	95	42	10

Final levels of hybridization were determined by hybridizing to a C_0t value of 35,000 and determining the S_1 nuclease resistance. Hybridization was in 1 M NaCl at 68°. Mouse DNA was present at 10 mg/ml and AKR and Moloney probes were in 2×10^7- and 2×10^6-fold excess over cDNA respectively. Selection of probes was performed as described in the *Materials and Methods* section.

Quantitation of AKR-MuLV sequences in normal and tumor tissues of AKR mice by reassociation kinetics

AKR-specific cDNA has been used to compare the number of AKR virus copies from normal tissues and AKR embryos with the number of copies in DNA extracted from tumor tissues. In all kinetic analyses, a ^{32}P-labeled cDNA to AKR-MuLV was used, together with a ^{3}H-labeled cDNA to M-MuLV (from which the sequences complementary to AKR 70S RNA were removed) as an internal reference in the same reaction mixture. The results of such an experiment are shown in Figs. 1 and 2. In Fig. 1, the percent of probe hybridized is plotted against the C_0t [initial concentration of total DNA (mol of nucleotide/liter) × time (sec)] for the reaction with AKR and Moloney cDNAs, respectively (15). Fig. 2 shows the reciprocal of the fraction of DNA remaining single-stranded as a function of C_0t (16). The annealing kinetics of AKR embryo DNA and of DNA extracted from brains of leukemic animals are similar, whereas AKR tumor DNA annealed with a faster rate. The slope of the curves in Fig. 2 indicate that AKR tumor tissue contains 1.5–2 times the number of AKR-MuLV sequences of AKR embryos or non-tumor tissue DNA. The $C_0t_{1/2}$ [½ value of initial concentration of total DNA (mol of nucleotide per liter) × time (sec)] values were also calculated from these data. The $C_0t_{1/2}$ values for BALB DNA was about 3500, for AKR embryos 1800 and

Table 2. Number of AKR-MuLV specific sequences in normal and tumor tissue of AKR mice

	Total	Brain	Liver	Spleen	Thymus	Tumor
BALB/c embryo	1 (2)	—	—	—	—	—
C3H embryo	1 (2)	—	—	—	—	—
AKR embryo	2 (2)	—	—	—	—	—
Non-leukemic AKR	—	—	2.0	—	2.0	—
Leukemic						
AKR 1	—	—	3.4	4.0	—	—
AKR 2	—	2.0	2.2	3.2	3.6	—
AKR 3	—	2.0	3.2	3.8	3.6	3.6
AKR 4	—	—	2.0	2.8	—	—
AKR 5	—	2.0	—	—	3.4	3.8
Four pooled leukemic AKR	—	2.0	3.2	—	—	3.6
Four pooled leukemic AKR	—	—	2.8	—	—	3.2

Number of AKR-MuLV specific sequences in normal and tumor tissue of AKR mice per haploid mouse genome equivalent. Hybridization conditions were as described in the *Materials and Methods* section. Calculations were based on C_0t for unique mouse DNA of 3,200. Figures in parentheses show the number of different DNA preparations tested. All values are obtained from at least two independent kinetic analyses.

for AKR tumor tissues between 900 and 1500. Unique cell DNA annealed by our conditions with a $C_0t_{1/2}$ of 3200–3500. The M-MuLV cDNA annealed with a $C_0t_{1/2}$ of about 200, with all the DNA preparations tested. These values suggest one copy of AKR-MuLV-related sequence per haploid genome equivalent in BALB/c mice and about two copies in AKR embryos and non-tumor DNA in agreement with earlier observations (1). The AKR tumor tissues contain three to four copies per haploid genome equivalent. In contrast, the number of sequences detected with the M-MuLV cDNA was rather constant and was not increased in tumor tissues.

DNA preparations from different organs of a large number of individual AKR mice were annealed with AKR-specific cDNA as described above. The results are summarized in Table 2. Increase of AKR-MuLV sequences was observed in all tumors of leukemic animals tested. Some animals showed also an increase in AKR sequences in livers. This probably reflects infiltration of the liver with lymphoma cells in the advanced stage of the disease. As compared with AKR embryos or brain DNA, most tumor tissues contained 1.6–1.8 as many AKR sequences.

In addition, the thermal stability of hybrids between AKR cDNA and DNA from the different tissues were determined. No detectable difference in t_{m50} was observed between AKR DNA derived from embryos or tumor tissue (Fig. 3). BALB DNA showed a slightly lower t_{m50}.

cDNA excess hybridization

In order to measure the increase of AKR-specific DNA sequences with a different method, we performed low excess cDNA hybridizations and determined the final level of hybridization. All hybridizations were carried out to a C_0t value twice as high as necessary to reach saturation. The number of AKR-MuLV copies per haploid genome equivalent was cal-

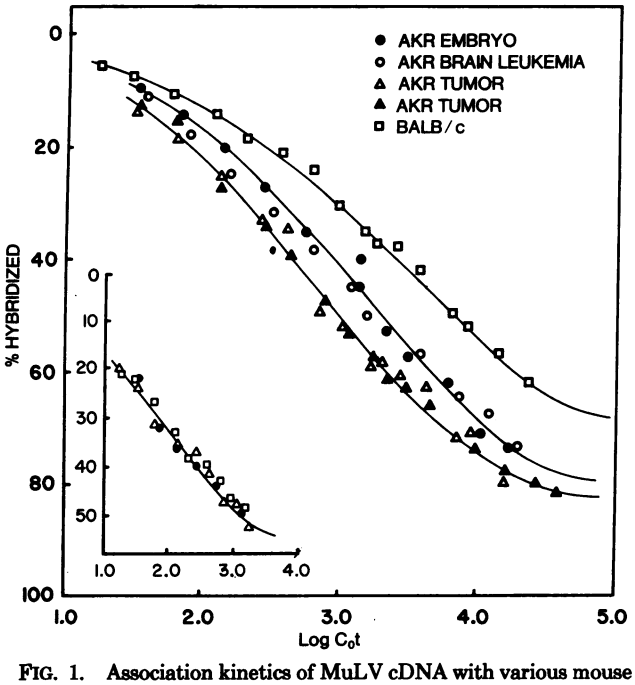

FIG. 1. Association kinetics of MuLV cDNA with various mouse DNAs. DNA was extracted and purified as described in *Materials and Methods*. DNA concentration was measured at 260 nm in 0.3 M NaOH, 0.1 M NaCl, with 26.0 A_{260} = 1 mg of DNA. Annealing conditions were as described in *Materials and Methods*. In the insert, the annealing with M-MuLV ^{3}H-labeled cDNA is shown. AKR-MuLV ^{32}P-labeled cDNA and M-MuLV ^{3}H-labeled cDNA were used in the same reaction mixture.

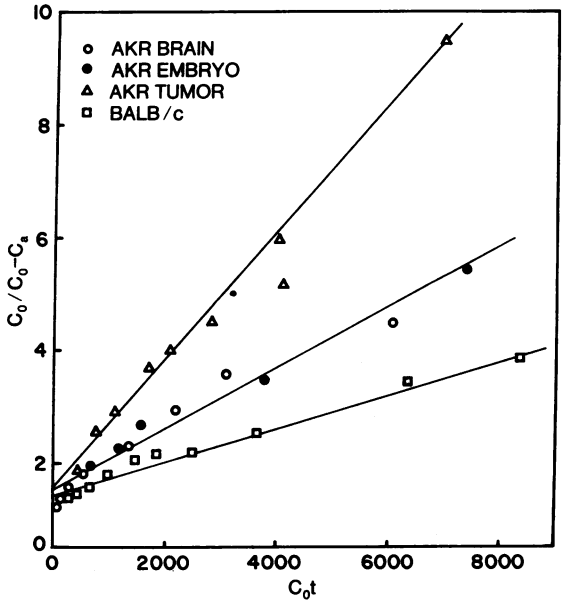

FIG. 2. Wetmur and Davidson plots of association of AKR-MuLV specific ^{32}P-labeled cDNA with various mouse DNAs. Data from Fig. 1 are plotted according to Wetmur and Davidson (16). Maximal level of hybridization was normalized to 100%.

Table 3. Hybridization of AKR and Moloney MuLV cDNA in excess with mouse DNAs

DNA	AKR cDNA			Moloney cDNA		
	% Hybridized	X	No. of copies	% Hybridized	X	No. of copies
Strain 129 liver	—	—	—	41.3	0.70	11
BALB/c liver	6.6	0.071	1.3	42.5	0.74	12
C3H liver	7.4	0.079	1.5	39.7	0.66	11
AKR embryo 1	15.5	0.184	3.5	43.9	0.78	12
AKR embryo 2	15.8	0.187	3.5	42.7	0.75	12
Thymus						
1 month AKR	14.9	0.175	3.3	N.T.	—	—
2 month AKR	16.6	0.199	3.8	N.T.	—	—
Brain leukemic AKR 5	15.5	0.183	3.5	44.9	0.81	13
Thymus leukemic AKR 5	26.7	0.363	6.9	43.8	0.78	12
Tumor leukemic AKR 5	27.3	0.376	7.1	43.6	0.78	12
Spleen leukemic AKR 5	25.0	0.333	6.3	45.9	0.85	14
Tumors of four pooled AKR	27.0	0.369	7.0	41.9	0.72	11

AKR—Mouse DNA (15 μg) was hybridized with 208 pg of selected AKR cDNA (7500 cpm) in 20 μl for 60 hr. The hybridization level with strain 129 DNA (14%) which was added to all reactions (50 μg) was subtracted from all values. Calculations were based on the assumption that the probe represents 1.25×10^6 of the genome (35%). For the haploid mouse genome, size was taken to be 2×10^{12}.

Moloney—Mouse DNA (15 μg) was hybridized with 378 pg of Moloney cDNA probe. The hybridization level with calf thymus DNA was subtracted from all values. Maximum level of hybridization (55%) was used to calculate the input hybridizable cDNA. Representation of Moloney probe was assumed to be 50% of genome size. For definition of *X* and calculations, see *Materials and Methods*.

culated from these data. The values obtained in this way are all higher than observed with the kinetic analyses. This might be due to an underestimation of the representation of the AKR-MuLV genome in the selected cDNA. If we assume a 50% representation, then the copy numbers are almost identical to the values obtained with the reassociation kinetics. In Table 3, the actual hybridization levels of the same DNA preparations described in Table 2 are shown, and the number of copies is calculated. This analysis essentially confirms the results obtained with kinetic analyses (Table 2). All tumor tissues tested contain 1.7–2 times the number of AKR-MuLV-specific sequences as compared with AKR embryo DNA, brain DNA, or DNA from a thymus of a 1–2 month old AKR mouse, although the absolute number of copies differs from the number obtained by kinetic analyses (see *Discussion*). In contrast, no increase of M-MuLV-related "common" sequences was observed.

DISCUSSION

Unselected cDNA, synthesized in an endogenous reaction from AKR-MuLV shows a high degree of cross-homology with other endogenous MuLVs. Cross-hybridization of the AKR probe with these common sequences reduces the sensitivity of detection of AKR-specific sequences considerably. Prehybridization against DNA lacking the specific virus information has been used successfully to increase the sensitivity of detection of specific viral sequences (14). We used this approach by prehybridizing AKR cDNA to cell DNA of mouse strain 129, which does not contain the endogenous AKR-MuLV, but hybridizes to 60% with AKR cDNA. In order to make this probe representative for the unique part of the AKR genome, the cDNA was hybridized to a 2-fold excess of AKR 70S RNA and the hybrid isolated on HA. The 70% hybridization level obtained in this final step indicated that the cDNA represented at least 35% of the AKR genome. This probe was highly specific as it hybridized 10–15% to DNA from strain 129 mice but 85% to AKR DNA. The single component curves obtained with this AKR-MuLV cDNA in the Wetmur Davidson plotting (Fig. 2) (16) indicate that most of the sequences which the unselected AKR cDNA has in common with other endogenous sequences in AKR mice are removed by this method. The use of this probe in reassociation kinetics and cDNA excess hybridization showed that in the tumor tissue of AKR mice the number of AKR-specific sequences was increased by a factor of 1½ to 2. A lower increase was also found in some tissues in the advanced disease. The highest increase was predominantly found in lymph node tumors and thymus. When newborn mice were injected with M-MuLV, Moloney-specific sequences were found in tumor tissue. In non-tumor tissues the presence of Moloney-specific sequences depended on the extent of infiltration of these organs with lymphoma cells, which suggests that only the target cells of an animal are susceptible to virus infection (3, 4). In analogy to these observations, the increase of AKR-specific sequences in some of the livers of leukemic mice might therefore reflect the degree of infiltration of these organs with lymphoma cells which carry the maximum number of AKR copies. This would suggest that the increase of AKR-specific sequences might be restricted to tumor cells of AKR mice and does not occur in cells

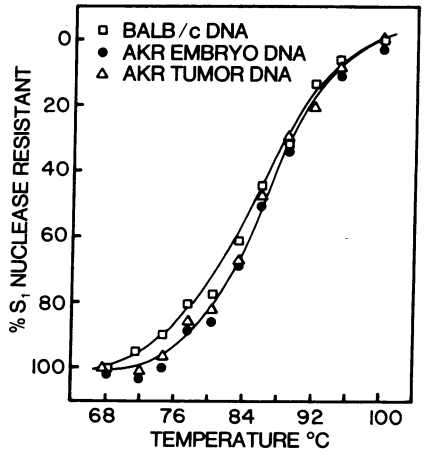

FIG. 3. Thermal stability profile of AKR ^{32}P-labeled cDNA with various mouse DNAs. Thermal stability was determined by melting hybrids between AKR-MuLV ^{32}P-labeled cDNA and mouse DNA. Hybrids were formed under standard annealing conditions. Temperature was increased stepwise and at each point aliquots were withdrawn to assay for S_1-nuclease resistance.

of "non-target" organs. Although both the kinetic analysis and the cDNA excess hybridization show this increase, the actual number of copies determined with the two methods differs. The kinetic analysis could give an underestimation as the cDNA is smaller in size than the mouse DNA. The excess cDNA hybridization could easily overestimate the number as only a minimum representation (35% of the 70S viral RNA) for this probe is known. Most probably the number of AKR-MuLV-specific sequences is between 1 and 2 copies per haploid genome equivalent for BALB/c and C3H, 2–4 for AKR embryos and non-tumor tissues, and 3–7 for tumor tissues. Whereas the number of AKR-MuLV-specific sequences varied in animals and tissues, the M-MuLV probe from which AKR-MuLV sequences had been removed detected the same number of copies in all DNA preparations from strain 129, BALB/c, or AKR origin. This served as an internal control in our annealing reactions and substantiates the difference observed with the AKR-MuLV cDNA. The C_0t values and the excess cDNA hybridization indicated that these sequences were present in 10–20 copies per haploid genome in agreement with earlier observations. We cannot exclude, however, that a minor increase of 1–2 copies occurs in the "common" sequences, as this would not be detectable with our technique.

Amplification of virus-specific sequences has also been observed in mice carrying the M-MuLV sequences integrated in their germ line (3). Amplification of Moloney sequences was found in the target organs and tissues infiltrated by the tumor comparable with the results reported in this paper. It is not known if the observed increase of AKR-MuLV sequences is due to further integration of the viral gene into the mouse genome, if it represents unintegrated proviral DNA, or if it is the result of selective chromosome duplication in transformed cells. The latter possibility deserves serious consideration as trisomy of specific chromosomes has been observed in AKR lymphoma cells (17). However, it is tempting to speculate that the increase of AKR-MuLV sequences is related to the onset of the leukemia in AKR mice. Reinfection and integration in tandem or in a new site on the mouse genome might be essential for this transformation. The observation that injection of AKR mice with AKR virus shortens the latency of the disease is in favor of this explanation.

We thank all members of the Tumor Virology Laboratory for help and criticism. The assistance of Mrs. Virginia Cox and Jessica Dausman is gratefully acknowledged. This work was supported by Grant no. CA-11561-02 and Core Grant no. CA-14195 from the National Institutes of Health. This investigation was supported in part by a Public Health Service International Research Fellowship no. 1 FO5 TW 02210-01 and by a stipend to A.B. from the Niels Stensen Foundation, The Netherlands.

1. Chattopadhyay, S. K., Lowy, D. R., Teich, N. M., Levine, A. S. & Rowe, W. P. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 1025–1101.
2. Rowe, W. P., Hartley, J. W. & Bremner, T. (1972) *Science* **178**, 860.
3. Jaenisch, R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1260–1264.
4. Jaenisch, R., Fan, H. & Croker, B. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4008–4012.
5. Chattopadhyay, S. K., Rowe, W. P., Teich, N. M. & Lowy, D. R. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 906–910.
6. Rudali, G., Duplan, J. F. & Laterjet, R. (1957) *Bull. Cancer* **44**, 440–443.
7. Lilly, F., Duran-Reynals, M. & Rowe, W. (1975) *J. Ex. Med.* **141**, 882–889.
8. Taylor, J., Illmensee, R. & Summers, J. (1976) *Biochim. Biophys. Acta*, in press.
9. Fan, H. & Baltimore, D. (1973) *J. Mol. Biol.* **80**, 93–117.
10. Kirby, K. (1966) in *Methods in Enzymology*, ed. Jacoby, W. (Academic Press, New York), Vol. 22, pp. 87–95.
11. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **12**, 3055–3063.
12. Jaenisch, R. & Mintz, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1250–1254.
13. Britton, R., Graham, D. & Neufeld, B. (1974) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 29, pp. 363–418.
14. Baxt, W. G. & Spiegelman, S. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3737–3741.
15. Britten, R. J. & Kohne, D. E. (1968) *Science* **161**, 529–540.
16. Wetmur, J. & Davidson, N. (1968) *J. Mol. Biol.* **80**, 93–117.
17. Dofuku, R., Biedler, J., Spengler, B. & Old, L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1515–1517.