Molecular Mechanisms Involved in the Differential Expression of *gag* Gene Products by Clonal Isolates of a Primate Sarcoma Virus

KEITH C. ROBBINS,¹ HIROMI OKABE,² STEVEN R. TRONICK,³ RAYMOND V. GILDEN,² AND STUART A. AARONSON^{3*}

Hazleton Laboratories, Vienna, Virginia 22180¹; Frederick Cancer Research Center, Frederick, Maryland 21701²; and Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Maryland 20014³

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Clonal isolates of an early passage stock of woolly monkey sarcoma virus (WSV) have been shown to code for different numbers of woolly monkey helper leukemia virus *gag* gene products. In the present report, the molecular mechanisms responsible for their differential expression of *gag* gene products have been analyzed. Three WSV RNA genomes were shown to possess sedimentation coefficients consistent with the differences demonstrated in their allotments of helper viral sequences. The WSV variant (WSV clone 9) that expressed no detectable proteins was shown to contain the largest amount of helper viral information. Moreover, there was no additive hybridization of the WLV complementary DNA probe by RNA of this WSV clone and that of a WSV clone coding for several *gag* gene products. These results suggest that the lack of expression of *gag* gene products by WSV clone 9 is not due to a major deletion of helper viral *gag* gene sequences. Similar levels of WLV-specific RNA were demonstrated in cells nonproductively transformed by each WSV clone, arguing that the ability to express *gag* gene proteins was not related to the magnitude of viral RNA transcription. Taken together, the results are most consistent with a mechanism by which small deletions or point mutations in the genomes of some WSV variants result in premature termination of translation or synthesis of immunologically nonreactive *gag* gene proteins. The present findings have implications concerning the effects of evolutionary selective pressures on helper viral genetic information in mammalian transforming viruses.

Known RNA-containing mammalian sarcoma viruses are defective in functions necessary for their replication (1, 3, 5, 9, 18, 19, 21). A type C RNA helper virus provides its structural proteins (3, 19) and reverse transcriptase (37) to the defective sarcoma viral genome. Evidence has accumulated that the sarcoma viral genome contains some sequences of the helper virus initially isolated in association with it (2, 4, 6, 8, 10, 13, 22, 24, 30, 31, 38). Cells transformed by certain sarcoma virus strains in the absence of a replicating helper virus do not express any detectable helper viral proteins (3, 5, 19, 21), whereas transformants of other strains code for helper viral proteins (2, 4, 9, 24, 25, 29) ranging up to all of those coded within the *gag* (7) region of the helper viral genome (8).

The mechanisms responsible for differential expression of helper viral proteins by a particular sarcoma virus are not yet well understood. Recent studies have indicated that the number of

helper virus proteins expressed by cells nonproductively transformed by different murine sarcoma viruses correlates with the presence of specific helper viral nucleotide sequences in the genome of the transforming virus (24, 28). These findings have led to the conclusion that the lack of expression of particular helper virus gene products results from deletion of those helper viral sequences (24, 28). Recently, clonal variants of an early passage stock of the woolly monkey sarcoma virus (WSV) have been shown to induce expression of different numbers of *gag* gene products (4, 8). The present studies were undertaken in an attempt to elucidate the mechanisms responsible for the differential expression of specific woolly monkey helper virus (WLV) *gag* gene proteins by these WSV variants.

MATERIALS AND METHODS

Medium. Cells were grown at 37°C in the Dulbecco modification of Eagle medium supplemented with 10%

calf serum (Colorado Serum Co., Denver, Colo.) in 50-mm plastic petri dishes (Falcon Plastics, Los Angeles, Calif.) or 490-cm² plastic roller bottles (Corning Glass Works, Corning, N.Y.).

Viruses and cells. WLV was grown in NRK (14) or human A204 cells (17). Clonal WSV variants of an early passage stock of WSV have been described previously (4). Nonproducer cell lines transformed by prototype WSV variants have been characterized immunologically with respect to their expression of the WLV *gag* gene proteins p12, p30, and p10 (4, 8). WSV clone 11 codes for all three proteins, whereas WSV clones 2 and 9, respectively, cause expression of p12 or none of these three *gag* gene proteins. The transforming viruses do not code for the expression of the *env* gene-coded gp70 (4). A clonal isolate of Kirsten murine leukemia virus (Ki-MuLV) was also used (5).

Virus assays. Sarcoma viruses were assayed for focus-forming activity as previously described (5). The quantitation of virion-associated reverse transcriptase in tissue culture fluids by using polyriboadenylic acid-oligodeoxythymidylic acid was performed by published procedures (35).

Viral RNA extraction. Sucrose gradient-purified virus was disrupted by incubation at 37°C for 30 min in 0.05 M sodium acetate (pH 5.1)–2 mM EDTA–0.1 M NaCl–1% sodium dodecyl sulfate–50 µg of self-digested Pronase per ml and then deproteinized twice with a solution containing 0.9 volume of redistilled phenol, 0.1 volume of chloroform-isoamyl alcohol (24:1), and 0.1 volume of 4 M NaCl. After ether extraction, RNA was precipitated by the addition of 2 volumes of cold ethanol. Precipitates were collected by centrifugation and suspended in TNE (0.01 M Tris-hydrochloride [pH 7.4]–0.1 M NaCl–0.001 M EDTA). For preparation of ³²P-labeled 70S viral RNA, A204 cells, chronically infected with WLV, were incubated for 12 h in phosphate-free medium containing 10% dialyzed calf serum and 100 µCi of H₃³²PO₄ (carrier-free; New England Nuclear Corp., Boston, Mass.) per ml. The medium was collected at three successive 6-h intervals. After clarification of tissue culture fluids at 12,000 × *g* for 10 min, the labeled virus was pelleted through 0.05 M Tris-hydrochloride (pH 7.4)–0.1 M KCl–20% glycerol at 100,000 × *g* for 90 min at 4°C. The virus pellet was resuspended in 0.4 ml of TNE, layered on a 15 to 60% (wt/wt) sucrose gradient, and centrifuged at 40,000 rpm overnight at 4°C in an SW41 rotor. Fractions containing ³²P-labeled virus were diluted fivefold and repelleted. RNA was extracted as described above and layered on a 5 to 20% (wt/wt) sucrose gradient. After centrifugation at 40,000 rpm for 140 min in an SW41 rotor, RNA sedimenting in the 60 to 70S region was ethanol precipitated and resuspended in TNE. The specific activity of viral RNA was 3 × 10⁵ cpm/µg.

Preparation of cellular RNA and DNA. Cellular RNA was extracted by the hot-phenol method of Scherrer (27). Cellular DNA was purified by a modified Marmur procedure as described by Britten et al. (11) and sheared to a mean size of 6 to 8S at 50,000 rpm (–20°C) in a VirTis homogenizer (VirTis, Gardiner, N.Y.). After precipitation with 2 volumes of ethanol, the DNA was resuspended at 2 mg/ml in 0.3 N NaOH, incubated at 45°C for 16 h, neutralized with

acetic acid, and dialyzed against distilled water. DNA fragments were concentrated by lyophilization, resuspended in TNE at 10 to 20 mg/ml, quick-frozen, and stored at –20°C.

Radioactive, unique-sequence cellular DNA was prepared from NRK cells, grown for 24 h in the presence of 10 µCi of [³H]thymidine (Schwartz/Mann, Orangeburg, N.Y.) per ml. Cellular DNA was extracted, sheared, and denatured as described above. Unique-sequence DNA was isolated after self-association to a C₀t of 50 by fractionation on hydroxyapatite (11). Its specific activity was 3 × 10⁶ cpm/µg.

Preparation of labeled viral cDNA. Viral complementary DNA (cDNA) was prepared in reaction mixtures containing 20 mM Tris-hydrochloride (pH 7.8), 20 mM KCl, 1 mM dithiothreitol, 6 mM MgCl₂, 0.1 mM each dATP, dCTP, and dGTP, 10^{–5} M [³H]-TTP (50 Ci/mmol; Schwartz/Mann), 20 µg of actinomycin D (Calbiochem, La Jolla, Calif.) per ml, 1.6 mg of DNase I-digested calf thymus DNA per ml (36), 0.014% (vol/vol) Triton X-100, and 100 µg of virus per ml. After incubation at 37°C for 6 h, the reaction was terminated by the addition of an equal volume of 0.1 M Tris-hydrochloride (pH 7.5)–0.2 M NaCl–0.05 M EDTA–0.8% sodium dodecyl sulfate–5 µg of calf thymus DNA per ml–1 mg of self-digested Pronase per ml. After incubation for 1 h at 37°C, viral cDNA was phenol extracted, ethanol precipitated, and passed over a Sephadex G-50 column. The molecular weights of labeled and unlabeled sheared cellular DNAs, as well as that of cDNA, were shown to be similar (6 to 8S), as determined by alkaline sucrose gradient sedimentation. The specific activity of the cDNA was 2 × 10⁷ cpm/µg. To estimate its genetic complexity, WLV cDNA was annealed to ³²P-labeled 70S WLV RNA. At a ratio of cDNA to RNA of 1:1, 40% of the [³²P]-RNA became RNase resistant (15). Greater than 70% of the [³²P]RNA was hybridized at a ratio of cDNA to RNA of 4:1.

Hybridization. RNA-DNA hybridization was performed by the addition of [³H]cDNA (1,000 to 1,200 cpm) to reaction mixtures containing 0.01 M Tris-hydrochloride (pH 7.4), 0.6 M NaCl, 1 mM EDTA, and varying amounts of viral RNA or cellular RNA in a volume of 0.05 ml. Incubation was for 18 h at 68°C. DNA-DNA hybridization reaction mixtures consisted of 2 to 4 mg of DNA, 12,000 cpm of [³H]cDNA probe, 0.01 M Tris-hydrochloride (pH 7.4), 0.6 M NaCl, and 1 mM EDTA in a final volume of 0.5 ml. After boiling for 3 min, reaction mixtures were incubated at 62°C. At varying times, 0.025-ml portions were removed and frozen at –70°C. All hybridization was assayed by the S1 nuclease method (10, 20). The results are expressed as the fraction of [³H]cDNA resistant to S1 nuclease at a given C₀t (or C₀t) value. C₀t and C₀t are, respectively, the product of the initial concentration of DNA or RNA in moles of nucleotide per liter and incubation time in seconds (12). No correction was made for Na⁺ ion concentration.

Sedimentation analysis of cellular and viral RNA. Cellular RNA, purified as described previously (27), was layered on 15 to 30% glycerol gradients in TNE and centrifuged at 25,000 rpm for 16 h at 4°C in an SW41 rotor. Fractions were collected from the bottom and analyzed for WLV-specific sequences by

hybridization to WLV cDNA as previously described (23). The sedimentation coefficient of virus-specific RNA in cellular RNA was estimated by comparison with ^{32}P -labeled Rauscher MuLV 35S RNA, unlabeled 28S and 18S rRNA, and 4S tRNA. Viral RNA, purified as described above, was layered on 5 to 20% sucrose gradients in TNE and centrifuged at 40,000 rpm for 140 min at 4°C in an SW41 rotor. Fractions were collected from the bottom, and each fraction was used to hybridize Ki-MuLV and WLV cDNA's.

RESULTS

Detection of WLV nucleotide sequences in DNAs of WSV nonproducer cells. Previous studies have demonstrated that clonal isolates of WSV code for different numbers of *gag* gene proteins in the absence of detectable *env* gene expression (4). Moreover, these properties were stable upon virus passage to new cells, indicating that each variant represents a distinct genotype (4). Studies aimed at determining the mechanisms responsible for differential viral antigen expression induced by these sarcoma viruses were initiated by the examination of DNAs of cells nonproductively transformed by each WSV variant for nucleotide sequence homology with the helper virus (WLV).

Figure 1 shows that DNA prepared from WSV-transformed NRK cells productively in-

fectured with WLV hybridized the WLV cDNA probe to an extent of over 70%. The kinetics of reassociation indicated two to three copies of WLV DNA per haploid genome when compared with unique-sequence NRK cellular DNA reassociation kinetics. DNAs of clonal nonproducer NRK lines transformed by each of the three WSV variants annealed about 30% of the cDNA probe at a C_{ot} of 10^4 , whereas only 10% hybridization was observed with NRK cellular DNA. Thus, no significant differences in extents of WLV cDNA annealing were observed among the WSV variant-transformed cellular DNAs.

Reconstruction experiments were used to determine whether the low extent of hybridization of WLV cDNA by the WSV nonproducer cellular DNAs was due to a low copy number of helper viral sequences or to the absence of a portion of the WLV genome in these clones. When WLV-infected cell DNA was diluted six-fold with NRK cellular DNA to approximate one-half copy of WLV information per haploid genome, the mixture annealed the probe to a greater extent (45%) than that achieved with WSV nonproducer cell DNAs. Thus, the lower levels of hybridization of WLV cDNA achieved with the WSV nonproducer cell DNAs must be due to the absence of a portion of the WLV genome in these clonal lines.

WLV sequences in WSV clones rescued by a heterologous helper virus. In an effort to compare more quantitatively the representation of helper virus sequences within the different WSV variants, each virus was rescued with a heterologous helper virus, Ki-MuLV, and grown to high titer for biochemical analysis. Figure 2 shows that WLV RNA annealed more than 95% of the WLV cDNA, whereas neither Ki-MuLV RNA nor NRK cellular RNA appreciably hybridized the same probe. The extents of hybridization by RNAs isolated from Ki-MuLV pseudotypes of the WSV variants were 43, 50, and 78% for WSV clones 11, 2, and 9, respectively. Findings that WSV clone 9 RNA demonstrated the highest level of hybridization were unexpected, in view of the fact that this variant caused the expression of no detectable helper virus proteins (4).

If the viral cDNA were reasonably representative of the WLV genome, the above results suggested that the size of the WSV clone 9 genome might be larger than that of the other variants. To test this possibility, RNAs extracted from Ki-MuLV pseudotypes of each WSV variant were subjected to sucrose gradient sedimentation analysis. Hybridization of gradient fractions was performed by utilizing WLV and Ki-MuLV cDNAs, the latter providing an internal standard for comparison with each

FIG. 1. Detection of WLV nucleotide sequences in DNAs of clonal NRK lines nonproductively transformed by WSV variants. Hybridization of WLV cDNA was as described in the text. Cellular DNAs were prepared from NRK cells ($\square$) and from NRK clonal lines nonproductively transformed by WSV clone 2 ($\blacksquare$), WSV clone 9 ($\blacktriangle$), and WSV clone 11 ($\circ$). Cellular DNA from WSV clone 11-transformed cells superinfected with WLV was also used ($\bullet$). Broken line represents annealing of NRK unique-sequence ^3H -labeled cellular DNA by NRK total cellular DNA. Results represent the mean values of two separate experiments.

FIG. 2. Hybridization of WLW cDNA by RNAs of different WSV variants. Viral RNA extraction and annealing conditions were as described in the text. Viruses were propagated in NRK cells and included WLW (●), Ki-MuLV (▼), WSV clone 2 (Ki-MuLV) (■), WSV clone 9 (Ki-MuLV) (▲), and WSV clone 11 (Ki-MuLV) (○). NRK cellular RNA (□) was also used.

WSV variant RNA. Figure 3 shows that WLW and Ki-MuLV RNAs demonstrated approximately the same sedimentation coefficients (around 70S), whereas WSV clone 9 was slightly smaller than Ki-MuLV RNA. RNAs of the other WSV clones showed lower sedimentation coefficients, consistent with their lesser extents of hybridization of the WLW cDNA probe.

The possibility was next considered that WSV clones 2 and 9, although they appeared to contain quantitatively more of the WLW genome than did WSV clone 11, lacked the *gag* gene sequences present in WSV clone 11 RNA. If this were the case, the extent of hybridization of the WLW cDNA by the sum of RNAs of WSV clone 11 and either of the other WSV clones should be greater than that achieved with either RNA alone. Table 1 shows that no increase in hybridization was achieved with saturating amounts of either WSV clone 2 or 9 RNA by the addition of a saturating amount of WSV clone 11 RNA. In contrast, the addition of WLW RNA resulted in over 95% annealing of the WLW cDNA. These results indicate that the lack of expression of *gag* gene products by WSV clone 2- and 9-transformed nonproducer cells was not the result of a major deletion of WLW *gag* gene sequences associated with the genomes of these WSV clones.

Viral RNA transcription in cells non-productively transformed by WSV clones. In a further effort to explain the differences in the expression of gene products by the three WSV variants, the levels of transcription of WLW-specific information by nonproducer cells transformed by each were compared. Figure 4 shows that at a C_{t} of 10^3 , cellular RNA of the

WSV clone 9 transformant hybridized 60% of the cDNA probe, whereas WSV clone 2- and 11-transformed cellular RNAs annealed 45 and 35%, respectively. These results were in agreement with those obtained utilizing the viral RNAs. The concentrations of WLW-specific RNA in each nonproducer cell line, estimated

FIG. 3. Sedimentation analysis of WSV variant RNAs. Viral RNA extraction, annealing conditions, and sedimentation were as described in the text. Virus-specific RNA in each fraction was determined by hybridization of both WLW (●) and Ki-MuLV (○) [^3H]cDNA's. Hybridization of WLW cDNA was normalized to the maximum level achievable with each WSV clone RNA (see Table 1). WLW and Ki-MuLV RNA hybridized no more than 5% of the heterologous viral cDNA. Markers included WLW ^{32}P -labeled 70S RNA and 28 and 18S rRNA. Viruses included WSV clone 2 (Ki-MuLV) (A), WSV clone 9 (Ki-MuLV) (B), WSV clone 11 (Ki-MuLV) (C), and a mixture of Ki-MuLV and WLW (D).

TABLE 1. Lack of additive hybridization of WLW cDNA by WSV variant RNAs^a

RNA source	Final extent of hybridization (%)
WSV clone 11	41
WSV clone 11 + WLW	95
WSV clone 2	50
WSV clone 2 + WSV clone 11	50
WSV clone 2 + WLW	96
WSV clone 9	78
WSV clone 9 + WSV clone 11	72
WSV clone 9 + WLW	96

^a Saturating amounts of viral RNAs, alone or in the indicated combinations, were used to hybridize WLW cDNA. Each WSV variant was propagated with a Ki-MuLV helper as previously described (4). Ki-MuLV annealed no more than 5% of the WLW cDNA. The results are mean values from two separate experiments.

FIG. 4. Expression of WLV-specific RNA in WSV-transformed nonproducer clones. The conditions of hybridization were as described in the text. Cellular RNAs were prepared from NRK cells ($\square$) and from NRK clonal lines nonproductively transformed by WSV clone 2 ($\blacksquare$), WSV clone 9 ($\blacktriangle$), and WSV clone 11 ($\circ$). Cellular RNA from WLV-infected NRK cells was also used ($\bullet$). Results represent the mean values of two separate experiments.

by $C_{T1/2}$ analysis, were very similar, around 30-fold lower than those of WLV-infected NRK cells. Thus, the concentration of viral RNA in WSV clone 9-transformed nonproducer cells could not explain the lack of *gag* gene expression by these cells.

Cellular RNAs were prepared from each WSV nonproducer transformant and subjected to sedimentation analysis to compare the size distribution of WLV-specific RNAs. Figure 5 shows that RNA of WLV-producing cells demonstrated a major peak with a sedimentation coefficient of 35S detected by hybridization. The major peaks of WLV-specific RNA associated with each of the WSV transformants were smaller and more heterogeneous, with sedimentation coefficients ranging from 25S to 33S. Each transformant also demonstrated a virus-specific peak at around 18S. Whether the 18S peak represents a breakdown product of the major subunit or a discrete mRNA remains to be resolved. Consistent with its ability to hybridize the WLV cDNA to the greatest extent, virus-specific RNA in cells transformed by WSV clone 9 possessed the highest molecular weight among the three transformants analyzed.

DISCUSSION

It is well documented that mammalian transforming viruses contain genetic information in addition to that shared with their helper viruses (6, 16, 28, 31–33). These findings have led to the hypothesis that the origin of the transforming virus involves genetic recombination between a portion of the helper leukemia virus and transformation-specific sequences of the host cell. The present studies were undertaken

to characterize the molecular basis for the differential expression of helper viral *gag* gene products in nonproducer cells transformed by clonal variants of the primate sarcoma virus, WSV. It has been shown previously that cells nonproductively transformed by WSV clone 11 express the *gag* gene proteins p12, p30, and p10, whereas those transformed by WSV clones 2 and 9 contain, respectively, only p12 or none of these three viral proteins (4, 8). The hybridization results presented here demonstrate that RNAs of these WSV variants contained different allotments of the nucleotide sequences represented in the WLV cDNA transcript. Moreover, the sizes of the viral genomes roughly correlated

FIG. 5. Sedimentation analysis of cellular RNA from WSV-transformed NRK cells. About 100 μ g of RNA from nonproducer cells and 30 μ g from producing cells were sedimented through glycerol gradients and analyzed for virus-specific RNA by hybridization with WLV [3 H]cDNA as described in the text. Hybridization was carried out with 800 cpm of [3 H]cDNA in 100 μ l containing 0.3 M NaCl at 67°C for 18 h and assayed by S1 nuclease. Cellular RNAs analyzed were from NRK cells nonproductively transformed by WSV clone 2 (A), WSV clone 9 (B), and WSV clone 11 (C). WLV-producing NRK (D) was also used. Uninfected NRK cell RNA did not show any hybridization with this [3 H]cDNA. Profiles of absorbance at 260 nm (A_{260}) (broken line) served as internal markers of 28S and 18S rRNA. Sedimentation is from right to left.

with their amounts of helper viral information. Molecular size analysis of RNAs of clonal isolates of the Moloney strain of murine sarcoma virus similarly have revealed variations in subunit sizes (22). In fact, major deletions of helper virus sequences have been shown to provide one mechanism for differences in the abilities of murine transforming viruses to express specific helper viral proteins (24, 28).

In the present report, it is noteworthy that the WSV clone that induced the largest number of *gag* gene products (WSV clone 11) contained the smallest allotment of helper viral sequences. If major deletions in the *gag* gene region were invoked to account for the lower number of *gag* gene-coded products expressed by WSV clones 2 and 9, then WSV clone 9 RNA might be expected to contain the smallest contribution of helper viral sequences. Instead, WSV clone 9 RNA possessed the largest portion of the helper viral genome. If *gag* gene sequences were missing from WSV clone 9 viral RNA, then mixtures of WSV clones 9 and 11 should have demonstrated additive hybridization of the WLV cDNA probe. As this was not observed, we presume that the majority of *gag* gene sequences are present in WSV clone 9 RNA.

These conclusions are dependent upon the representation of the *gag* gene within the cDNA transcripts used. Since WLV cDNA was able to hybridize a large majority of labeled WLV 70S RNA, it seems likely that the *gag* gene sequences were represented within the viral cDNA probe. Furthermore, hybridization results generated with a high-molecular-weight WLV cDNA with a sedimentation coefficient of up to 23S in alkaline sucrose (26; H. Okabe, unpublished data) substantiated the data presented here. Recent studies utilizing cDNA's specific to the 5'-terminal region of the WLV genome indicate that each WSV variant contains this region of the helper viral genome (S. R. Tronick, W. Haseltine, and S. A. Aaronson, unpublished data). Finally, the fact that the differences in allotments of helper virus sequences in each WSV clone correlated with estimates of the sedimentation coefficients of the respective viral RNA genomes provides further evidence that the viral cDNA transcripts gave a reasonably reliable reflection of the total viral genome.

RNAs of cells nonproductively transformed by each of the WSV variants were shown to contain similar concentrations of WLV-specific RNA, indicating that the lack of detectable *gag* gene products in the WSV clone 9 transformant was not due to a lower magnitude of viral RNA transcription. In other studies, the rates of degradation of WLV proteins, when incubated with extracts of each transformant, did not differ (J.

Krakower, unpublished data). These findings indicate that the absence of proteins in cells transformed by WSV clone 9 could also not be accounted for by their more rapid degradation. Thus, the present results are most consistent with a mechanism by which small deletions or point mutations in the WSV clone 9 RNA result in either premature termination of translation or the synthesis of immunologically nonreactive proteins. In the same fashion, the limited expression of *gag* gene products by WSV clone 2 could also be explained by small deletions or point mutations after the region coding for p12.

Several models can be considered to account for the different allotments of helper viral sequences within each of the WSV variants studied here. The variants may have arisen from independent recombinational events involving the helper virus when the transforming viral genome was initially formed. Alternatively, a single-progenitor transforming virus may have given rise to new variants by secondary cycles of recombination with the helper virus or may have progressively lost helper viral sequences in subsequent replication cycles. The very rare occurrence of sarcoma viruses in nature would tend to argue against the possibility that three distinct variants arose independently. Evidence against high-frequency recombination with the helper virus derives from studies indicating that the genetic properties of a clonal transforming virus remain stable through one or two cycles of virus transmission to new cells (2, 4; S. A. Aaronson, unpublished data). We, thus, favor the hypothesis that helper virus sequences may be lost in the course of replication of the initial transforming virus. However, the present findings indicate that small genetic alterations can also lead to the loss of the ability of the sarcoma virus to express helper viral gene products.

It is likely that the major selective pressures acting upon transforming viruses would be for the preservation of transformation-specific sequences and of helper viral sequences that allow the sarcoma virus to efficiently replicate in association with the helper leukemia virus. It is well documented that the expression of helper viral proteins by a sarcoma virus may be selected against *in vivo*, since nonproductively transformed cells that express no detectable helper virus proteins are far less immunogenic than are productively infected cells (34). The present results indicate that functions required for sarcoma virus transformation and replication can be maintained with a WSV genome containing as little as 40% of its helper viral sequences, whether or not the virus induces the expression of any known helper viral gene products. These findings lead to a prediction that, given a suffi-

cient time for selection, the transforming virus may eventually contain only the minimum amount of viral helper information needed for efficient replication in association with a helper virus. The analysis of sarcoma virus variants after prolonged virus propagation *in vitro* may be useful in elucidating the nature of those essential helper viral sequences.

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