Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 3001–3005, June 1979
Microbiology

Recombination between the defective component of an acute leukemia virus and Rous associated virus 0, an endogenous virus of chickens

(defective RNA tumor virus/oligonucleotide mapping/genetic recombination)

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Communicated by Wallace P. Rowe, April 11, 1979

ABSTRACT **The ability of the defective acute leukemia virus of chickens, MC-29, to participate in recombination was investigated by testing the ability of the MC-29 genome to donate sequences to its helper virus. The endogenous virus Rous associated virus 0 (RAV-0) was used as a helper for MC-29, and its genome was compared by fingerprinting to that of the original RAV-0. In three separate isolates, it was found that the RAV-0 used as helper for MC-29 had acquired new sequences near the 3′ and 5′ ends of its genome. The new 3′ proximal sequences resembled the C region found in exogenous but not endogenous avian oncoviruses, and it probably imparted a higher growth rate to the recombinant as compared to RAV-0. One isolate also showed recombination within the *env* gene. Because we could exclude the possibility that the recombination was with host cell information or with the original helper of MC-29, we conclude that the acquired sequences were derived from the MC-29 genome, and therefore this replication defective virus is not defective in recombination.**

MC-29 is an avian retrovirus, myelocytomatosis virus, strain 29, that was isolated in 1960 in Bulgaria (1). It was later found that this virus could transform both macrophages and fibroblasts in tissue culture (2) and could cause myelocytomatosis, carcinomas, and occasionally erythroblastosis upon injection into susceptible chickens (1, 3, 4). This and other viruses of the acute leukemia/carcinoma group (5)—e.g., avian myeloblastosis virus (AMV), avian erythroblastosis virus (AEV), and Mill Hill virus-2 (MH-2) of chickens and Friend leukemia virus and Abelson leukemia virus of mice—are mixtures of two components. One component has a genome with a complexity of about 5700 nucleotides (6) and is highly defective in that it lacks all genes for virion proteins in a functional form (4) although it does contain some information related to that of viral structural genes (6, 7).

The genome of the nondefective transforming avian retroviruses has a complexity of about 10,000 nucleotides with four defined genetic regions (8): *gag*, coding for viral structural proteins; *pol*, coding for reverse transcriptase; *env*, coding for envelope glycoproteins; and *src* (or *onc*), responsible for transformation. In addition, all avian retroviruses [with the exception of spontaneously produced endogenous viruses (9) and a number of BrdUrd-induced endogenous chicken viruses (unpublished results)] that have been tested contain a common region at the 3′ end that is about 800 nucleotides long (10) and is known as the C region (11); its function is unknown. The only function known to be coded for by the viral RNA of the defective component of MC-29 is transformation (4, 7), but the putative transforming gene has no relationship to the transforming gene of Rous sarcoma virus (6, 12) and has not yet been adequately defined. The other component is a competent virus (helper virus) that provides the virion structural proteins to the defective component. Although both the helper and the defective component replicate in the same cells, a helper-independent recombinant virus from the two has never been described.

The failure of viruses of this type to give rise to nondefective transforming virus may result from an inability of these viruses to act as partners in recombination or the part of the genome responsible for transformation may not be transferrable in a way that leaves an intact genome (13). To study these possibilities, the original helper of MC-29 was replaced with Rous associated virus 0 (RAV-0), an endogenous virus of chickens (14). The genome of RAV-0 is closely related to that of exogenous avian oncoviruses in the regions coding for virion proteins but is distinctive near the 3′ end and unrelated to the C region in the same location in exogenous virus genomes (9). RAV-0 was selected for these experiments because it grows to very low titers and, when used as a partner in recombination with exogenous viruses, the recombinants invariably grow to significantly higher titers (unpublished data). In this communication, we report that RAV-0 used as a helper for MC-29 is altered by recombination with the defective virus component.

MATERIALS AND METHODS

Cells and Viruses. Turkey embryo fibroblasts (T/BD cells) were prepared from fertile turkey eggs purchased from Wilmar Poultry Farm (Wilmar, MN). MC-29-transformed nonproducer quail cells line Q5 (4, 7) and methylcholanthrene-transformed quail (QT 6) cells (15) were kindly provided by Peter Vogt. Before use, these cells were tested and found to be negative for virion production as assayed by sedimentable DNA polymerase activity.

RAV-0 was isolated from cultures of line 100 chicken embryo fibroblasts originally provided by L. B. Crittenden. MC-29 with RAV-0 helper [MC-29 (RAV-0)1] was kindly provided by H. Robinson. The exchange of the original helper with RAV-0 was achieved by superinfecting RAV-0-producing cells with MC-29 at a low multiplicity of infection and selecting for a pseudotype transforming virus with subgroup E (RAV-0) envelope. MC-29 (RAV-0)2 and MC-29 (RAV-0)3 were derived by superinfection of MC-29-transformed nonproducer quail cells with RAV-0. The infected cells were transferred three times and the supernatant was used to infect T/BD cells. Virus produced by these cells was diluted 1:100 or 1:10,000 (to select the most abundant species) and again used to infect T/BD cells which were subsequently labeled with ^{32}P for preparation of virus RNA. The

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Abbreviations: MC-29, myelocytomatosis virus, strain 29; RAV, Rous associated virus; Pr-RSV, Prague strain of Rous sarcoma virus.

helper of MC-29 (RAV-0)1, designated RAV-0-MC-1, was isolated by end-point dilution of the MC-29 (RAV-0)1 complex.

Methods. Large T1 oligonucleotide fingerprints of the viral RNA were prepared by two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled 70S genome RNA after complete digestion with RNase T1 as described (16). The 3′ ends of the viral genome (average length, about 300 nucleotides) were prepared by mild alkaline hydrolysis and poly(U)-Sephadex chromatography (9). Oligonucleotides were characterized by digestion with RNase A and separation of the digestion products by ionophoresis on DEAE-paper (17).

RESULTS

Comparison of Genome of RAV-0 with Genome of Helper of MC-29 (RAV-0). In order to test for alterations of the RAV-0 genome as a result of recombination with MC-29, fingerprints of ^{32}P-labeled 70S RNA from RAV-0 and MC-29 (RAV-0)1 were prepared (Fig. 1 *A* and *B*). The fingerprint of the RAV-0 genome was identical to that previously described (9) and contained no detectable minor species. Its oligonucleotide map is shown at the bottom of Fig. 1. As expected, the MC-29 (RAV-0)1 fingerprint was consistent with a mixture of two viruses, with a major and a minor pattern of oligonucleotides. We attribute the minor pattern to the defective MC-29 genome. The major pattern of oligonucleotides was that of a virus closely related, but not identical, to RAV-0. Specifically, oligonucleotide 08, which maps near the 3′ end of the RAV-0 genome, was missing from MC-29 (RAV-0)1 and a new oligonucleotide, M8, was present in equimolar yield. Furthermore, oligonucleotides 13 and 8, which map near the 5′ end of the RAV-0 genome, were present in lower than molar yield and two closely related oligonucleotides, 13A and 8A, appeared. Partial sequence analysis of the relevant T1 oligonucleotides is shown in Table 1.

Table 1. Large T1 oligonucleotides from MC-29 (RAV-0) isolates

Oligonucleotide	Composition*
M1	AU,AC,4C,3-U,G
M2	AU,2AC,4C,3U,G
M3	A_3C,A_2C,2AU,AC,5-6C,3-4U,G
M4	A_4X,A_3X,AU,AC,5C,3U,G
M5	A_2C,AU,AC,4C,2-3U,G
M6	A_3C,2AC,3C,U,G
M7	A_3C,AC,C,4U,A_4G
M8	A_2U,AC,C,2-3U,G
M9†	A_2U,AU,3-4C,5-6U,G
(111)	(A_2U,AU,4C,6U,G)
8A†	A_5C,6C,6U,A_2G
(8)	(A_4C,6C,6U,A_2G)
13A†	m^7GpppG_mC,2-3AU,2-3AC,4-6C,4-6U,G
(13)	(m^7GpppG_mC,3AU,3AC,5C,5U,G)

* As determined by complete digestion with RNase A.

† These oligonucleotides are related to the RAV-0 oligonucleotides shown in parentheses. The composition of the RAV-0 oligonucleotides has been published (9).

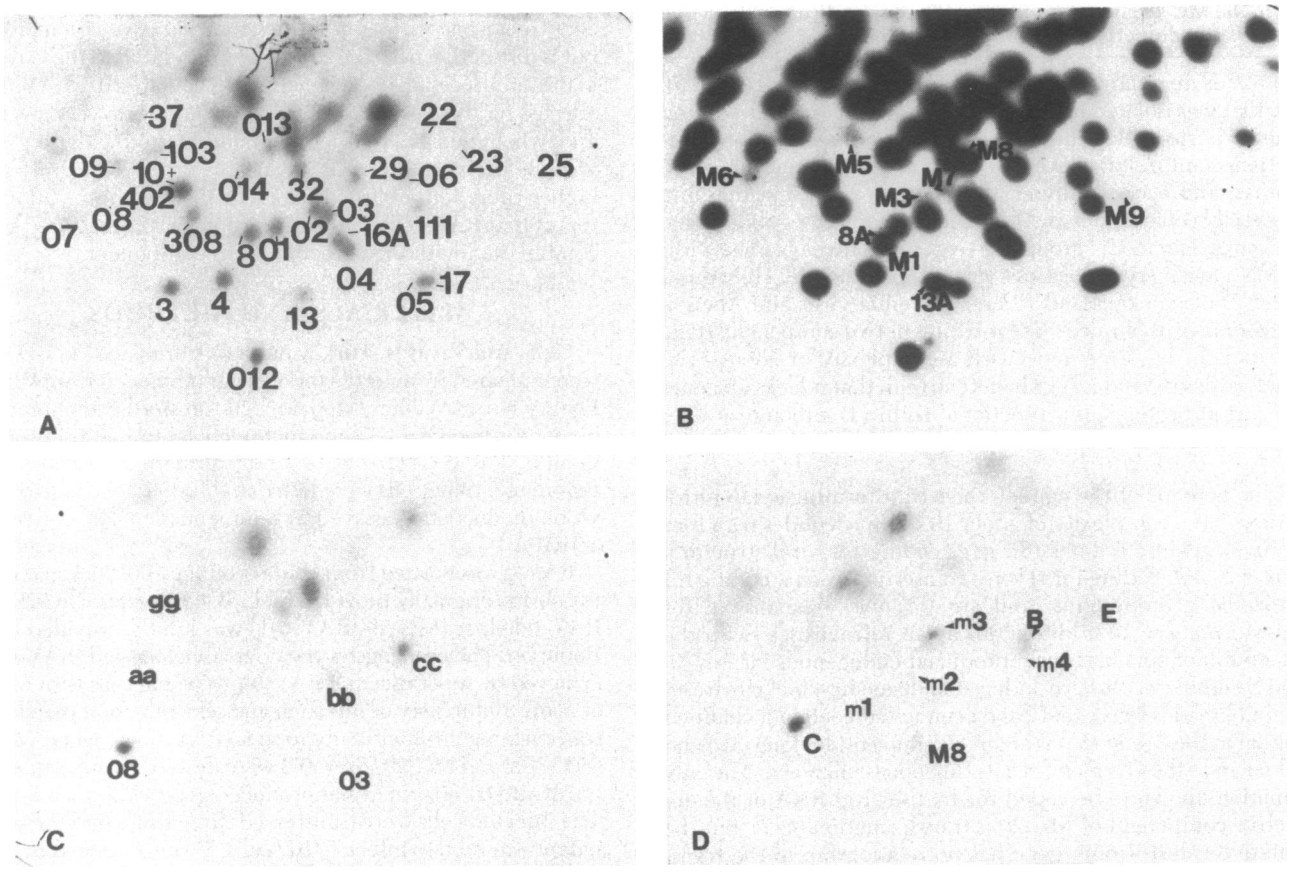

FIG. 1. Genomes of RAV-0 and MC-29 (RAV-0)1. T1 oligonucleotide fingerprints were prepared from RAV-0 70S RNA (*A*), MC-29 (RAV-0)1 70S RNA(*B*), the 3′ end of the RAV-0 genome (*C*), and the 3′ end of the MC-29 (RAV-0)1 genome (*D*). The fingerprints of the 3′-terminal fragments were run only two-thirds as far as those of total genomes in the second dimension to retain smaller oligonucleotides. Only those oligonucleotides not found in RAV-0 are numbered in the fingerprint of MC-29 (RAV-01)1 (*B*). The composition of the non-RAV-0 oligonucleotides is shown in Tables 1 and 2. The numbers at the bottom show the order of the large oligonucleotides in the RAV-0 genome (9).

Table 2. T1 Oligonucleotides derived from 3′ end of MC-29 (RAV-0)1 and RAV-0

Oligonucleotide	Composition
	MC-29 (RAV-0)1
M8	A_2U,AC,C,2-3U,G
C*	A_3C,A_2U,AU,AC,G
m1	A_4X,AU,2U,G
m2	AU,2C,3U,A_2G
m3 (mixture)	2AU,2AC,3C,4U,A_2G,AG,G
m4	AU,2C,3U,G
B*	AU,2C,3U,AG
E*	AU,3U,A_2G
	RAV-0
03	$3A_2U$,2AU,AC,5C,6U,AG
08	A_3U,A_3C,AU,AC,6C,G
aa	A_3C,AC,U,A_2G
bb	2AU,AC,2C,U,G
cc	2AU,2U,A_2G
gg	A_2C,2AC,C,U,G

* These oligonucleotides are also found in the genome of Prague strain of Rous sarcoma virus B (9).

These results suggest that more than 95% of the helper RAV-0 genomes contained new information near the 3′ ends and about 60% had acquired related, but distinct, information near the 5′ end, based on visual estimates of the relative amounts of oligonucleotides 08 and 8A. Such information could have been derived by recombination with the genome of the defective component of MC-29, the genome of the original MC-29 helper, or virus-related cellular genetic material.

To verify that the 3′ end of RAV-0 had been altered or replaced and to determine to what extent this had occurred, 3′-terminal fragments of both RAV-0 and MC-29 (RAV-0)1 were prepared by partial alkaline hydrolysis (to about 300-nucleotide-long pieces) and poly(U)-Sephadex chromatography. Fingerprints of these fragments are shown in Fig. 1 *C* and *D* and partial sequence analysis of the unique oligonucleotides from these fingerprints is shown in Table 2. The results show that oligonucleotide M8 maps near the 3′ end of the MC-29 (RAV-0)1 genome and that the 3′-terminal region of MC-29 (RAV-0)1 shares three oligonucleotides (B, C, and E) with that of other exogenous avian leukosis/sarcoma viruses. The 3′-terminal region of the RAV-0 genome was identical to that described (9) and shared no unique (i.e., large) oligonucleotides with MC-29 (RAV-0)1. From these results, we conclude that the RAV-0 genome used as helper for MC-29 was altered by the replacement of the portion of the genome nearest the 3′ end with new information. This new information is related to the C region of exogenous avian oncoviruses, which is not detectably related to sequences found in the RAV-0 genome (9).

These experiments, performed with the mixture of MC-29 and its helper, suggested that the helper virus genome had been altered by recombination with the defective MC-29 component. To show formally that the RAV-0 helper of MC-29 was altered, a clone of this virus (RAV-0-MC-1) free of the defective MC-29 component was isolated by limiting dilution on turkey cells. The fingerprint of this virus together with the fingerprint of its 3′ end is shown in Fig. 2. As expected, the minor pattern of oligonucleotides attributed to MC-29 was not present. Again, 3′-end RAV-0 oligonucleotide 08 and 5′-end RAV-0 oligonucleotide 13 were missing and oligonucleotides M8 and 13A were acquired. Oligonucleotide 8, but not 8A, was present, suggesting a crossover near the 5′ end. The 3′-terminal region of RAV-0-MC-1 was identical by this analysis to the 3′ end of MC-29 (RAV-0)1. These results show that the genome of RAV-0 used as helper for MC-29 had been altered, at both its 3′ and 5′ ends.

Rescue of MC-29 Defective Component from MC-29-Transformed Nonproducer Quail Cells by Using RAV-0 as Helper. Because of the method by which MC-29 (RAV-0)1 was isolated, it was possible that the observed recombination had taken place between RAV-0 and the original helper (MC-associated virus) rather than the defective MC-29 genome. To exclude this possibility we used RAV-0 to rescue MC-29 from line Q5. This line of MC-29-transformed nonproducer quail cells has been extensively studied and has been shown not to express any helper virus gene products (4, 7). In addition, this line does not contain any rescuable helper virus information (4), suggesting lack of the helper virus genome. The nonproducer cells were superinfected with RAV-0 at a multiplicity of infection of about 0.1 infectious unit per cell and passaged three times. The virus produced by the third passage culture was passaged once through T/BD cells and then serially diluted and used to infect T/BD cells. Two isolates of MC-29 (RAV-0) [i.e., MC-29 (RAV-0)2 and MC-29 (RAV-0)3] obtained from different dilutions were fingerprinted (Fig. 3 *A* and *B*). The pattern of the minor species in these fingerprints was almost identical to the pattern of the minor species in the fingerprint of the MC-29 (RAV-0)1 that had been isolated in a different laboratory by a different method. This result indicates that the minor pattern in these fingerprints was due to the defective MC-29 component.

The major (helper) component of these two isolates differed from RAV-0 in the same way as did MC-29 (RAV-0)1. Both MC-29 (RAV-0)2 and MC-29 (RAV-0)3 contained the same set of 3′-terminal oligonucleotides as did MC-29 (RAV-0)1. 5′-Oligonucleotides 13 and 8 were again partially replaced by 13A and 8A in MC-29 (RAV-0)2 and oligonucleotide 13 was totally replaced by 13A in MC-29 (RAV-0)3. It is of interest that two

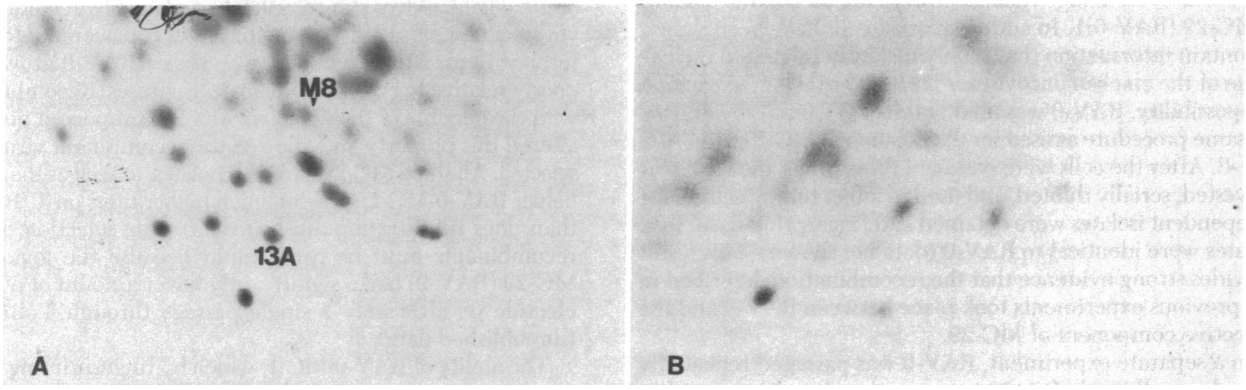

FIG. 2. Genome of the helper component of MC-29 (RAV-0)1. T1 oligonucleotide fingerprints were prepared from RAV-0-MC-1 70S RNA (*A*) and its 3′-terminal region (*B*).

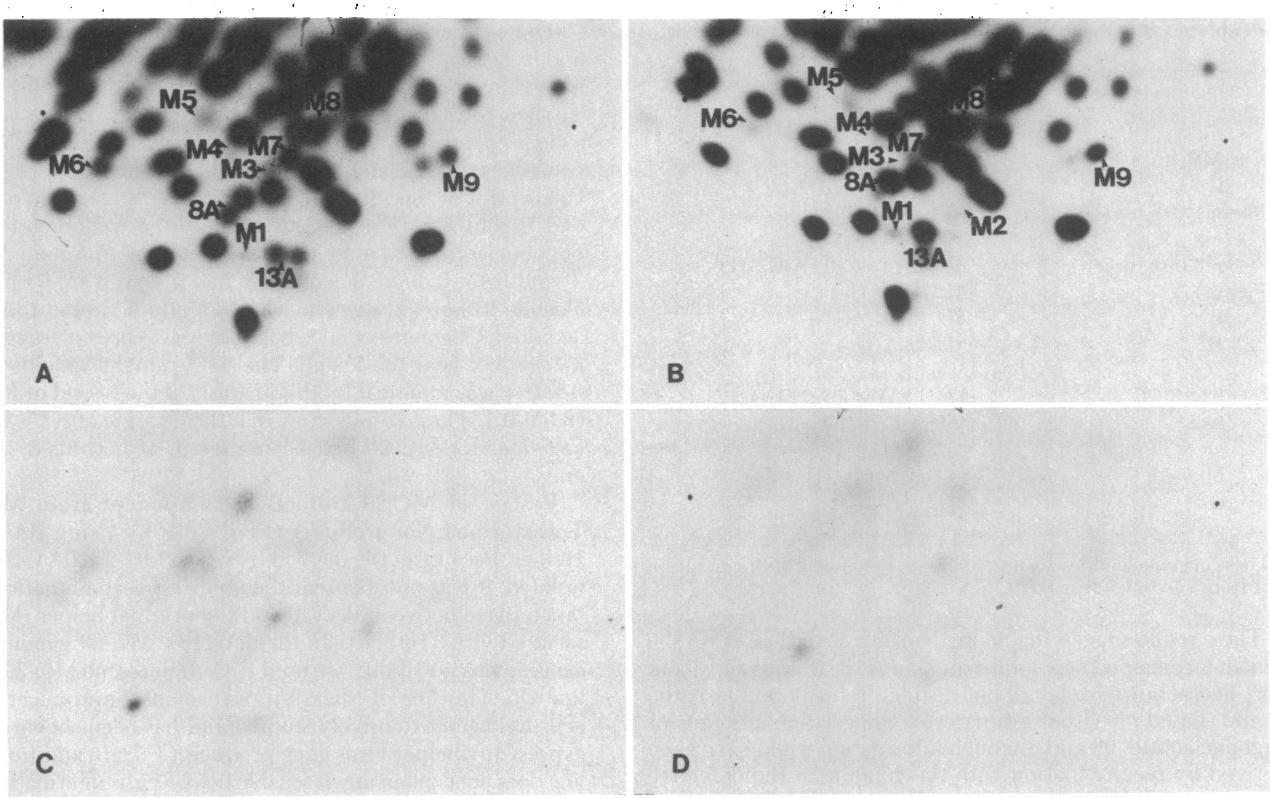

FIG. 3. Genomes of two other MC-29 (RAV-0) isolates. T1 oligonucleotide fingerprints were prepared from 70S RNA of MC-29 (RAV-0)2 (*A*) and MC-29 (RAV-0)3 and (*B*) and their 3′-terminal regions (*C* and *D*). The fingerprints shown in *C* and *D* are identical to those in Figs. 1*D* and 2*B*.

oligonucleotides, M7 and M9, that were seen in low yield in the MC-29 (RAV-0)1 and had been identified as specific MC-29 oligonucleotides now appeared in equimolar yield with the rest of the helper virus oligonucleotides and RAV-0 oligonucleotide 111, which is closely related to M9 (Table 2) and which maps in the envelope gene (9), appeared in much less than molar yield in both isolates.

Because the cells used in this experiment were nonproducers, the recombination observed must have occurred in the absence of the original nondefective helper component of MC-29. In addition the recombination involved parts of the genome other than the 3′ and 5′ ends.

The possibility that the recombination took place between RAV-0 and cellular genetic material of the transformed quail cells was unlikely in view of the fact that the helper of MC-29 (RAV-0)1 isolated by H. Robinson presents a similar pattern of recombination as the one seen in MC-29 (RAV-0)2 and MC-29 (RAV-0)3, even though quail cells were not used in the cloning of MC-29 (RAV-0)1. In addition, quail cells have been shown to contain information that is only distantly related to the genome of the chicken oncoviruses (18). Nevertheless, to exclude this possibility, RAV-0 was used to infect QT 6 cells by exactly the same procedure as used for the isolation of MC-29 (RAV-0)2 and -3. After the cells were passaged three times, the virus was harvested, serially diluted, and used to infect turkey cells. Four independent isolates were obtained and fingerprinted. All four isolates were identical to RAV-0 (data not shown). This result provides strong evidence that the recombination described in the previous experiments took place between RAV-0 and the defective component of MC-29.

In a separate experiment, RAV-0 was passaged repeatedly in turkey cells and after 20 passages the virus RNA was fingerprinted and was found to be unaltered (data not shown).

DISCUSSION

The experiments reported in this paper give evidence that, when RAV-0 is used as helper for MC-29, it becomes altered by recombination with the defective MC-29 component. Such recombination would not be observed by biological testing of the virus, because the bulk of the coding region of the RAV-0 genome is unaffected, and phenotypic characters such as subgroup specificity are identical in a helper virus before and after interaction with the MC-29 genome (4). A nondefective transforming recombinant has not been isolated from MC-29 stocks probably because the transforming gene replaces a large portion of the genome (including parts of the defective *gag*, *pol*, and *env* regions) (19) and legitimate recombination would always result in a defective transforming virus (13). Nondefective recombinants have also not been found with defective sarcoma viruses, although recombination similar to that seen here has been reported (20). In our experiments we found that the original helper virus was completely replaced by recombinants within a few passages. We attribute this replacement to a difference in growth rate of the two viruses. RAV-0 grows very poorly relative to exogenous avian retroviruses. Recombination with the genome of the defective MC-29 component probably altered this property and allowed the recombinant virus to be selected. All three MC-29 (RAV-0) isolates, as well as the cloned helper RAV-0-MC-1, grow to much higher titers on T/BD cells than does RAV-0 (unpublished data). The selection of such recombinants must be quite rapid because the genome of MC-29 (RAV-0) had a significantly lower amount of oligonucleotide 08 after only a single passage through T/BD cells (unpublished data).

The ability of RAV-0-MC-1, which by fingerprinting differs from RAV-0 only at the extreme 3′ and 5′ ends, to grow to relatively high titers supports the contention that this viral prop-

erty is provided by one of these genomic segments. Because more of the RAV-0 genomes contained new 3′ terminal than 5′ terminal sequences, the C region is implicated in this property. This interpretation agrees with the evidence obtained from experiments in which recombinants were made between RAV-0 and Prague strain Rous sarcoma virus B or its transformation-defective derivative. Fifty-four cloned recombinants were tested and all inherited the exogenous Rous sarcoma virus 3′ end. Our findings regarding the selective advantage of the exogenous virus 3′ end together with the finding by Hsu *et al.* (21) and Shank *et al.* (22) that the extreme 3′-terminal sequences of the viral genome are repeated at both ends of the proviral DNA, indicate that this portion of the viral genome may play a role in the control of viral RNA synthesis.

In addition to the recombination observed at the 3′ and 5′ ends of the helper virus genome, analysis of MC-29 (RAV-0)2 and MC-29 (RAV-0)3 RNA showed that recombination occurred in at least two more sites. One of these, represented by oligonucleotide M9, is in the envelope region as shown by the fact that M9 replaced the closely related RAV-0 *env* oligonucleotide 111 (9). Although MC-29 does not code for functional virion proteins, clearly it must contain at least fragments of functional genes. Such fragments can replace the corresponding portions of the helper virus genome, probably as a result of chance additional crossovers during recombination.

Our results showed changes in the helper virus genome after interaction with the defective component of MC-29. It is likely that during this process the defective component of MC-29 acquired new information by recombination with the helper. Such recombination is also suggested by the variability of different MC-29 laboratory strains (P. Duesberg, personal communication). Acquisition of helper sequences by the MC-29 genome could explain some observations from other laboratories regarding the role of the helper in transformation and induction of malignancy by similar viruses. Moscovici *et al.* (3) have reported that the pathogenicity of MH-2 can be altered by changing the helper. One explanation of this result could be the host range of the helper, but the possibility exists that the MH-2 genome altered by recombination with the new helper possesses altered transforming potential. Rosenberg and Baltimore (23) have shown that Abelson leukemia virus acquired or lost the ability to transform bone marrow cells *in vitro*, depending on the helper component of the virus preparation although transformation of embryonic fibroblasts was unaltered. Furthermore, the transformation ability of the virus on bone marrow cells seemed to correlate with the ability of the virus preparation to cause leukemia in susceptible mice (23). Similar phenomena have been found by Scher (24) using Abelson leukemia and Kirsten sarcoma virus. The system presented here may provide a means to elucidate the mechanisms responsible for these phenomena.

If it is correct that the defective component of MC-29 or of other avian and murine defective retroviruses can be altered every time a new helper is used to rescue it, experiments in which this kind of virus manipulation is being used should be interpreted with great caution. In any case, our results show that the genome of MC-29, and probably other similar defective viruses, is not defective in recombination.

We thank H. Robinson for her gift of MC-29 (RAV-0)1, P. Vogt for his gift of QT6 and MC-29-transformed nonproducer quail cells, and M. Champion and C. Barker for expert technical assistance. This project was supported by Grants CA17659 from the National Cancer Institute and NP246B from the American Cancer Society. P.N.T. has a Postdoctoral Fellowship from the National Cancer Institute. J.M.C. has a Faculty Research Award from the American Cancer Society.

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