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# Evidence for Crossing-Over Between Avian Tumor Viruses Based on Analysis of Viral RNAs

**(gel electrophoresis/oligonucleotide fingerprinting/analytical complexity)**

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**ABSTRACT** **The RNAs of several avian tumor virus recombinants that had inherited their focus-forming ability from a sarcoma virus and their host range marker from a leukosis virus were investigated. Electrophoretic analyses showed that the cloned sarcoma virus recombinants contained only size class *a* RNA, although they had acquired a marker that resided on class *b* RNA in the leukosis virus parent. Class *a* RNA of different recombinant clones, derived from the same pair of parental viruses and selected for the same biological markers, differed slightly in electrophoretic mobility from each other and from the parental sarcoma virus. They were also found to have different fingerprints of RNase T1-resistant oligonucleotides.**

**The average complexity of the 60–70S RNA prepared from Prague Rous sarcoma virus of subgroup B was estimated to be $3.5 \times 10^6$ daltons from the size of 20 RNase T1-resistant oligonucleotides, which represented 3.9% of the RNA and that of a recombinant to be $3.3 \times 10^6$ daltons from 23 oligonucleotides, which represented 4.7% of the RNA. This result suggests that the genome of wild-type and of recombinant RNA tumor viruses is polyploid.**

**The sum of these observations led us to propose that recombination among avian tumor viruses occurred by crossing-over between homologous pieces of nucleic acid.**

Nondefective avian sarcoma viruses can undergo high frequency genetic recombination with avian leukosis viruses (1–3). Since the 60–70S RNA of avian tumor viruses consists of several pieces (4), it appeared likely that this recombination represented reassortment of markers situated on different genome subunits. However, preliminary analyses of the RNAs of sarcoma virus recombinants, carrying a host range marker from a leukosis virus, led us to propose that recombination between avian tumor viruses involved crossing-over: (*i*) It was found that such cloned recombinant sarcoma viruses contained only 30-40S RNA species of size class *a* (5–8). Class *a* RNA is typical of nondefective avian sarcoma viruses and is larger than class *b* RNA found in all leukosis or transformation-defective viruses (6, 9–11). (*ii*) Class *a* RNAs of some but not all recombinants selected for the same two markers were found to differ slightly in electrophoretic mobility from parental class *a* RNA (7, 8, 12), suggesting that the primary structure of the viral RNA was affected during recombination. (*iii*) The number of different fingerprint patterns observed in comparing different recombinants, which were selected for the same host range and transforming markers, could be accounted for more readily by crossing-over than by reassortment of 30-40S RNA subunits (7, 8, 12).

Abbreviations: PR-A, PR-B, and PR-C, Prague Rous sarcoma virus, subgroups, A, B, and C, respectively; RAV-1, Rous associated virus, type 1, subgroup A; RAV-2, Rous associated virus, type 2, subgroup B; RAV-3, Rous associated virus, type 3, subgroup A.

The present report confirms and extends our earlier observations and includes comparative analyses of the analytical complexity of wild-type and recombinant sarcoma viruses based on RNase T1-resistant oligonucleotides. The results favor crossing-over and suggest that the genome of RNA tumor viruses is polyploid.

## MATERIALS AND METHODS

*Viruses and RNA.* Cloned wild-type and recombinant virus strains were propagated as described (1, 3, 6, 9, 13). Isolation, electrophoresis, and fingerprinting of viral RNA (4, 6, 13, 14) are detailed in figure legends.

## RESULTS

*Recombinants Selected for a Host Range Marker from a Leukosis Virus and a Transforming Marker from a Sarcoma Virus Contain Only 30–40S RNA of Size Class a.* Fig. 1 shows electropherograms of heat-dissociated 60–70S RNAs of five cloned recombinants derived from crosses between sarcoma virus PR-B and leukosis virus RAV-3. It can be seen that all five recombinants contained only or almost only 30–40S RNA of size class *a*, which electrophoresed with a class *a* RNA standard of PR-B (Fig. 1A, C–F) or PR-C (Fig. 1B). A class *b* RNA standard is included in Fig. 1B and is thought to represent the RNA of a transformation-defective PR-C segregant that had formed during nonclonal passage of this virus stock (6, 10, 13). The RNA of five different cloned

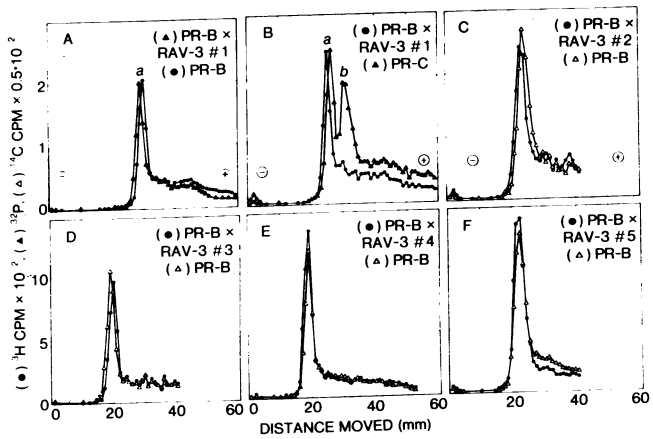


FIG. 1. (A–F) The RNAs of five different recombinants, PR-B × RAV-3 (nos. 1–5), after heat-dissociation and electrophoresis with a standard of PR-B or PR-C RNA. Appropriate amounts of radioactively labeled 60–70S RNAs, extracted from virus harvested at 3- to 5-hr intervals from infected cells, were mixed, heated in electrophoresis sample buffer, and subjected to electrophoresis in 2% polyacrylamide gels as described (6).

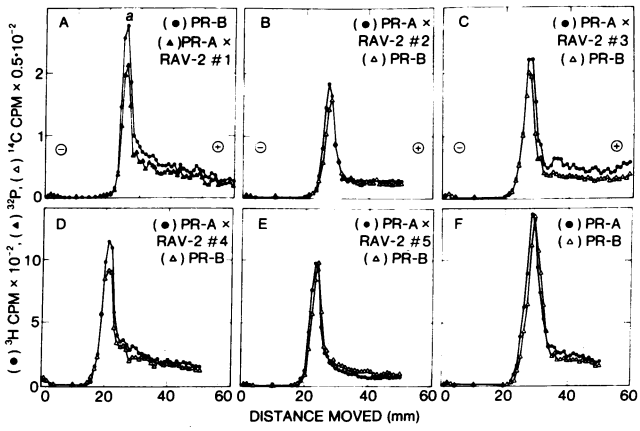


FIG. 2. (A–E) The 60–70S RNAs of five different recombinants, PR-A × RAV-2 (nos. 1–5), after heat dissociation and electrophoresis with a standard of PR-B RNA. (F) Simultaneous electrophoresis of PR-B and PR-A RNA. Conditions were as described for Fig. 1.

recombinants between PR-A and RAV-2 also contained only 30–40S RNA of size class *a* (Fig. 2). The RNA standard used in the analyses of Fig. 2 was the same preparation of PR-B as in Fig. 1. It is shown in Fig. 2F that class *a* RNA of PR-B has a slightly higher electrophoretic mobility than that of PR-A, the parental sarcoma virus of the crosses analyzed in Fig. 2.

We conclude that possibly all recombinants carrying the focus-forming marker of a sarcoma virus and the host range marker of a leukosis virus contain only 30–40S RNA of size class *a*.

It may be argued that part of the recombinant RNA that migrated faster than class *a* (Figs. 1 and 2) might include class *b* RNA, perhaps acquired by reassortment from the leukosis virus parent. This possibility has been virtually excluded because the fingerprint pattern of undegraded class *a* RNA was found to be indistinguishable from that of smaller RNA from the same recombinant, PR-A × RAV-2 no. 2 (7, 8, 12).

*Different Recombinants Derived from the Same Pair of Parental Viruses and Selected for the Same Markers Have RNAs of Different Size.* Of several genes that may presumably be exchanged between leukosis and sarcoma viruses, only two have been selected for in the recombinants studied here. If crossing-over takes place, it may theoretically occur at any point on the genetic map between the focus-forming and the host range loci. In this case, the RNAs of recombinants selected for the same markers, but derived from different crossover events, could differ in their sequences. This was suggested by small electrophoretic differences observed between the class *a* RNA of some recombinants and that of the parental sarcoma virus. For example, several independent preparations of RNA of PR-B × RAV-3 no. 1 were found to migrate a little slower than PR-B RNA (Fig. 1A and B) (7, 8, 12). The RNAs of other PR-B × RAV-3 recombinant clones fell into three electrophoretic classes. (*i*) PR-B × RAV-3 no. 2 had a lower mobility than class *a* RNA of PR-B (Fig. 1C). (*ii*) PR-B × RAV-3 no. 3 had a higher mobility than parental RNA (Fig. 1D). This recombinant was produced by transformed cells in 10- to 20-fold lower titers than other sarcoma viruses, perhaps indicating a defective replicating function. (*iii*) PR-B × RAV-3 no. 4 and no. 5 had virtually the same mobility as parental PR-B RNA (Fig. 1E and F).

The RNAs of five recombinants between PR-A and RAV-2 had a similar size distribution. Three of them, RNAs of PR-A × RAV-2 no. 1, no. 3, and no. 4 (Fig. 2A, C, and D), had the same electrophoretic mobility as a PR-B RNA standard, while those of nos. 2 and 5 (Fig. 2B and E) had a slightly lower electrophoretic mobility. None of the PR-A × RAV-2 RNAs tested had a higher electrophoretic mobility than that of PR-B. These experiments indicate that the primary structure of at least some recombinant RNAs differs from that of the parental RNA.

The apparent molecular weight by which certain recombinant RNAs differ from parental, wild-type RNA (± one fraction) is estimated to be around 70,000 on the basis that class *a* and class *b* RNAs differ by about five fractions or about 350,000 daltons under the same conditions (see Fig. 1) (6, 9, 11). The size differences observed among the RNAs of distinct recombinants were stable after several successive clonings. This observation suggests that the size variations are not likely to be host modifications similar to those observed earlier in two specific cases, which were not stable on passage of the virus in different cells (6).

*RNAs of Sarcoma Virus Recombinants Derived from the Same Pair of Parental Viruses and Selected for the Same Markers Have Different Fingerprint Patterns.* If crossing-over is responsible for the small electrophoretic differences observed among the RNAs of recombinants, it would be expected that these RNAs also differ in their sequences. This possibility was tested by fingerprinting the RNase T1-digested RNAs (15, 16). While the oligonucleotide patterns of the RNAs of five PR-B × RAV-3 recombinants are very similar, they differ in at least 2–3 out of about 20 major RNase T1-resistent oligonucleotide spots (Fig. 3A–E). Some spots that are found in one but not in all recombinants are indicated by arrows. The pattern of RNA of wild-type PR-B is shown in Fig. 3F and that of RAV-3 in Fig. 3G. Their patterns differ from those of the recombinants more extensively than the recombinant patterns differ from one another. The oligonucleotide patterns of the RNAs of five recombinants between PR-A and RAV-2 also differed from each other, however, in fewer spots (see arrows in Fig. 4A–E) than the PR-B × RAV-3 recombinants. The fingerprint patterns of parental PR-A and RAV-2 RNAs are shown in Fig. 2F and G.

We have not determined which of the large oligonucleotides of the recombinants are derived unchanged from the parental virus strains and which of these oligonucleotides contain new sequences representing sites at which crossing-over may have taken place. However, at least one spot of recombinant PR-B × RAV-3 no. 3 and one of PR-B × RAV-3 no. 5 (circled in Fig. 3C and E) appeared to be new and not to have a counterpart in either parental virus (Fig. 3F and G)

From the differences observed by fingerprinting, it may be concluded that the recombinants analyzed differ in RNA sequences. This observation supports the possibility that crossing-over points between focus-forming and host range markers are not at a fixed site.

*The 60–70S RNA of Wild-Type and Recombinant Tumor Viruses Appears to be Largely Polyploid.* If the 30–40S subunits of a given 60–70S RNA of a tumor virus were identical, the complexity of the RNA should be equal to that of each of the 30–40S pieces. However, if 60–70S RNA were haploid, its complexity would be higher than that of an individual 30–40S subunit. The complexity of an RNA species uniformly labeled with $^{32}P$ can be estimated if the sizes of

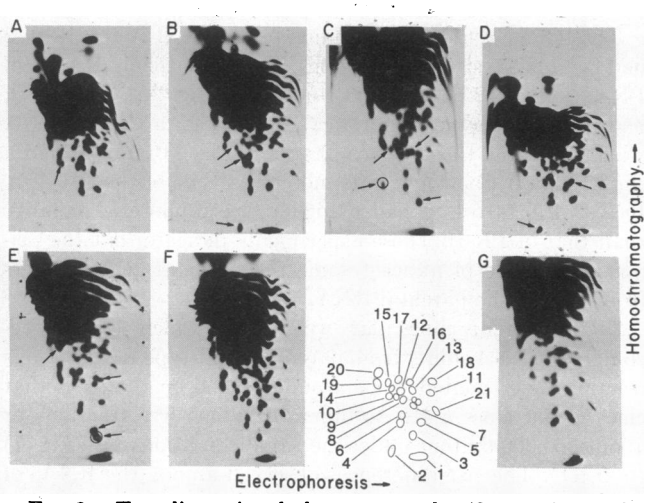


FIG. 3. Two-dimensional chromatography (fingerprint analyses) of the RNase T1-digested, 60–70S [$^{32}$P]RNAs of the five recombinants, PR-B × RAV-3 no. 1 (A), no. 2 (B), no. 3 (C), no. 4 (D), and no. 5 (E), as well as of PR-B (F) and RAV-3 (G). The 60–70S [$^{32}$P]RNA of virus harvested at 12-hr intervals was digested and analyzed as described (13, 15) except that a 3% homomixture *b* (16), prepared with yeast RNA (P. L. Biochemical Co.) containing 3 mM EDTA (pH 7–7.5), was used. Further, to ensure complete transfer of all RNase T1-digested RNA fragments from the cellulose acetate strip used for electrophoresis to the DEAE–cellulose thin-layer plate used for chromatography, the following modification was used to prepare fingerprints E, F, and G. The area of the DEAE–cellulose thin layer to be covered by the cellulose acetate strip was sprayed with water until it was shiny. Subsequently, the strip was stretched tightly over the wet region of the thin-layer plate and taped to its back. The strip was sprayed repeatedly with water until it was completely adsorbed to the thin-layer plate, and air bubbles trapped in between were removed by hand using a disposable rubber glove. Finally, the thin-layer plate, still carrying the cellulose acetate strip, was developed by homochromatography as described (16). After transfer by this method, 90–100% of the label originally applied to the cellulose acetate strip could be recovered from the DEAE–cellulose layer after complete hydrolysis in 400 ml of 0.4 M KOH for 48 hr at room temperature. The arrows in A–E indicate spots not found in all of the five recombinants analyzed. The circled spots in C and E have no homologous counterpart in the patterns of either parental virus (F and G). A schematic tracing of the large oligonucleotides of PR-B (F) identifies spots that were analyzed as described in Table 1.

unique oligonucleotides derived from it are determined, and the radioactivity of these oligonucleotides is compared with the total radioactivity of the intact RNA molecule (17). The average complexity of PR-B RNA as determined from about 20 RNase T1-resistant oligonucleotides, representing 3.9% of the RNA and resolved as described in Fig. 3F, amounted to 3.5 × $10^6$ daltons (Table 1). This is slightly higher than the molecular weight estimates for viral 30–40S subunits obtained by other methods (11).

A polyploid RNA for wild-type tumor viruses raises two possibilities for the RNA of recombinants. (*i*) If crossing-over is involved in recombination, the complexity of the recombinant RNA should be approximately the same as that of wild-type virus. Further, the 30-40S RNA species of a given recombinant should be identical, as observed in the electrophoretic analyses described above. (*ii*) If stable reassortment were involved in recombination, the complexity of the recombinant RNA should be higher than that of the parental virus. In addition, the 30–40S RNA species of such a recombinant may contain both *a* and *b* subunits. (This was not observed in any of the recombinants analyzed above.) The complexity of the RNA of PR-A × RAV-2 no. 5 (Fig. 4) was found to be about 3.3 × $10^6$ on the basis of 23 RNase T1-resistant oligonucleotides, which represented 4.7% of the 60–70S RNA (Table 2).

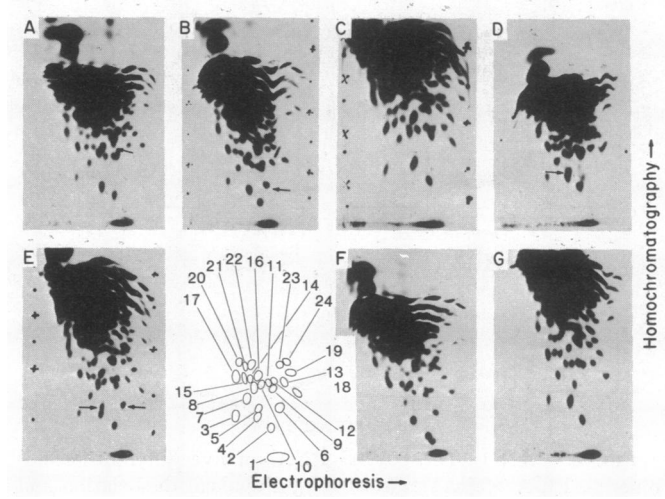


FIG. 4. Fingerprint analyses of the RNase T1-digested, 60–70S [$^{32}$P]RNAs of the five recombinants, PR-A × RAV-2 no. 1 (A), no. 2 (B), no. 3 (C), no. 4 (D), and no. 5 (E), as well as of PR-A (F) and RAV-2 (G). Conditions were as described for Fig. 3 (E, F, and G). The arrows in A–E indicate spots not found in all five recombinants analyzed. A schematic tracing of the large oligonucleotides of PR-A × RAV-2 no. 5 (E) identifies spots that were analyzed as described in Table 2.

The fingerprint pattern of 60–70S RNA, which was used in these analyses, has been shown to be identical to that of 30–40S RNA (13). Therefore, none of the large oligonucleotides studied were derived from small RNA molecules associated with the 60–70S complex. However, further work will be required to explain the fluctuations observed among complexity estimates based on different oligonucleotides. These are thought to be due to two complications. (*i*) The 60–70S [$^{32}$P]RNA prepared from virus harvested at 12-hr intervals is known to be inhomogeneous and partially degraded (18–20). Thus, some oligonucleotides may be present in greater than equimolar amounts, leading to a low complexity estimate; and others may be found in less than equimolar amounts, leading to an overly high complexity. These errors, however, should be small when many oligonucleotides are used to calculate an average complexity. (*ii*) In addition, analysis of some oligonucleotides is complicated because some radioactive RNA from neighboring spots may elute with a particular oligonucleotide and interfere with its subsequent analysis. Our complexity estimates are probably maximal since losses of 10–20% were encountered in carrying distinct oligonucleotides through this procedure (not shown), perhaps due to partial degradation during homochromatography.

We may conclude that RNAs of both wild-type and recombinant tumor viruses have an approximate genomic complexity of 3.4 × $10^6$ daltons, corresponding to 10,500 nucleotides. Thus, the 60–70S viral RNA appears largely polyploid and, consequently, recombination is likely to involve crossing-over.

## DISCUSSION

*Recombination Involves Crossing-over.* The sum of all the experiments described here favors the conclusion that re-

TABLE 1. *The complexity of PR-B RNA**

| Oligonucleotide spot no.† | cpm | RNase A digestion products | Complexity in daltons ($\times 10^{-6}$)‡ |
|---|---|---|---|
| 1 | 15,560 | Poly(A) | — |
| 2 | 3,300 | $UC_2G(AC)_3(AAU)(A_4C)_3$ | 3.20 |
| 3 | 3,100 | $U_5C_7G(AC)(AU)_2(AAC)(AAU)_2$ | 3.42 |
| 4+6§ | 5,440 | $U_8C_{15}G(AC)_3(AU)_2(AAC)$-$(AAG)(A_3C)$ | 3.05 |
| 5 | 2,840 | $U_4C_4G(AC)_3(AU)_3(A_3C)$ | 3.32 |
| 7 | 1,990 | $U_5C_3G(AC)(AU)(AAC)(AAU)$ | 3.62 |
| 8 | 1,920 | $U_5C_6(AAG)(A_5C)$ | 3.94 |
| 9¶ | 1,470 | $U_4C_6G(AC)_2(AU)(A_3C)$ | 5.40 |
| 10 | 1,960 | $U_2C_6G(AC)_2(AU)(AAC)$ | 3.48 |
| 11 | 2,240 | $U_8C_6G(AU)$ | 2.87 |
| 12‖ | 2,900 | $U_5C_8G(AC)_4(AU)_2(AG)$ | 3.65 |
| 13 | 2,220 | $U_6C_3(AC)(A_3C)(A_3G)$ | 3.24 |
| 14 | 1,790 | $U_2C_4G(AC)_2(AU)(AAC)$ | 3.38 |
| 15 | 1,550 | $UC_2(AC)_2(AU)(AG)(AAC)$ | 3.42 |
| 16 | 1,990 | $U_6C_8G(AU)$ | 3.23 |
| 17 | 1,730 | $U_4C_4(AC)(A_4G)$ | 3.27 |
| 18 | 1,860 | $U_6C_3G(AU)(AAU)$ | 3.05 |
| 19‖ | 3,750 | $U_2C_{13}G(AC)_5(AU)(AG)_2(AAC)$-$(AAU)(A_4C)$ | 4.33 |
| 20§ | 1,630 | $UC_5(AC)_3(AAG)$ | 3.48 |
| 21 | 1,860 | $U_6C_2G(AU)_4(AAU)$ | 4.06 |
| | | Average | 3.45 ± 0.37 |

* PR-B 60–70S [$^{32}$P]RNA (up to 20 μg, including in some experiments carrier tobacco mosaic virus RNA), derived from virus harvested at 12-hr intervals, was exhaustively digested for 4 hr at 38° with 3 units of RNase T1 (Cal Biochem) in 100 μl of 50 mM Tris·HCl (pH 7.4) and 0.5 mM EDTA. The digest was lyophilized and redissolved in water. A 3-μl aliquot was subjected to two-dimensional chromatography (as described for Fig. 3F). Two identical patterns were prepared. One, consisting of $1.17 \times 10^6$ cpm, was used to determine the total radioactivity in a given spot and the total recovery of the $^{32}$P digest as follows: A desired spot was circled with pencil and moistened with 50% glycerol. It was then transferred to a scintillation vial and incubated for 4 hr at 60° in a toluene-based scintillation fluid containing 10% NCS (Nuclear Chicago) before determination of its radioactivity in a scintillation counter. Subsequently the remaining [$^{32}$P]-digest on the thin layer was quantitated as described for Fig. 3. The other pattern, with $10 \times 10^6$ cpm, was used to analyze the RNase A-resistant fragments of large oligonucleotides. Elution of oligonucleotides followed published procedures (16). Subsequent digestion was for 2 hr at 38° in 10 μl of 0.01 M Tris (pH 7.4), 1 mM EDTA, and 0.2 mg/ml of RNase A (Worthington). Resistant fragments were analyzed by electrophoresis on DEAE-cellulose paper (16), and after autoradiographic location, fragments were cut out and quantitated in a scintillation counter. The composition of most fragments was determined from their position on the pherogram. Some large fragments were eluted from the DEAE-cellulose, and their base compositions were determined after complete digestion with RNases A, T1, and T2 (16).

† Numbers refer to diagram in Fig. 3E and refs. 7, 8, and 12.

‡ The complexity was calculated from an average nucleotide molecular weight of 323, determined from the base composition of PR-B RNA (24.4% cytidine, 23.8% adenosine, 28.8% guanosine, 23.0% uridine) and the known molecular weights of the nucleotides.

§ Spots 4 and 6 were not well separated in the fingerprint patterns used here and were combined for analysis; by contrast, spot 20 was resolved into two distinct spots of which the most slowly chromatographing one was analyzed.

combination among avian tumor viruses involves crossing-over. However, the suggestion that the number of different fingerprint patterns observed in each set of recombinants can be accounted for more readily by crossing-over than by reassortment is only compelling if the following is considered. If recombinants arose by reassortment of segments in a haploid genome, these recombinants should show a limited number of fingerprint patterns. In recombinants selected for two markers, the two segments containing these markers must be the same. Sequence diversity could be caused only by genome segments not carrying the selected markers. Therefore, if there are at most four RNA segments per genome (4), a maximum of two segments could be variable. The number of possible genome variations would then be four. If there are three segments per genome, the same recombinants could occur in only two fingerprint variations. Furthermore, only one fingerprint pattern would be expected if 60–70S RNA consisted of two segments, as suggested by recent electron microscopy of the RNA (22). Since we have already observed five distinct fingerprint patterns in the PR-B × RAV-3 cross and in the PR-A × RAV-2 cross, our data would be compatible with reassortment only if the genome has more than four genetically unique segments.

*The Complexity of 60–70S Tumor Virus RNA Appears to be Around 3 × 10⁶ Daltons.* We estimated that the complexities of the 60–70S RNA of PR-B and of a recombinant between PR-A and RAV-2 were around $3.4 \times 10^6$ daltons. By RNA·DNA reassociation kinetics, the complexity of 60–70S RNA of tumor virus was estimated to be $8–9 \times 10^6$ daltons by some (23, 24) and about $3 \times 10^6$ daltons by others (25). Although hybridization kinetics have been shown to be approximately a linear function of the complexity of DNA or RNA (26–28), up to 8-fold deviations from a linear relationship between complexities and reassociation rates were observed when different species of nucleic acids with different base compositions were compared (26, 27, 29). Since the base compositions of the RNA standards used to determine the complexity of tumor virus RNA differ (30, 31), these complexity estimates may be subject to such variations.

*What is the Mechanism of Tumor Virus Recombination?* Since there is no precedent and no plausible molecular mechanism for high frequency crossing-over between viruses containing single-stranded RNA, it appears likely that recombination between avian RNA tumor viruses involves the synthesis of the DNA provirus (32, 33). The high frequency recombination among RNA tumor viruses could then be a direct consequence of polyploidy. The progeny of a doubly infected cell would be largely heterozygous, containing different genomes in a 60–70S complex. Transcription of such a heterozygous RNA into DNA would bring homologous DNAs together

¶ This spot is probably derived from sequences present only in class *a* RNA of PR-B (Duesberg and Vogt, unpublished). Since the virus used for this experiment was not cloned for two passages, it presumably contained a significant proportion of class *b* RNA due to the presence of *td* PR-B segregating spontaneously from PR-B (10, 11, 13). Therefore, this spot is not expected to be present at equimolar concentration with the others and was not used to calculate the complexity.

‖ Spots 12 and 19 contained 2 or 3 guanosine residues. This is due to either 2 or 3 unresolved spots or to incompletely digested RNA. Heterogeneity of some spots is also suggested by their autoradiographic appearance; see for example spots nos. 19 and 20 in Fig. 3F.

TABLE 2. *The complexity of RNA of PR-A × RAV-2 no. 5*

| Oligonucleotide spot no.* | cpm† | RNase A digestion products†,‡ | Complexity in daltons (× $10^{-6}$)§ |
|---|---|---|---|
| 1 | 130,083 | Poly(A) | — |
| 2 | 19,770 | $U_5C_7G(AC)(AU)_2(AAC)(AAU)_2$ | 3.66 |
| 3 | 11,300 | $CG(AC)_2(A_4C)_2$ | 3.65 |
| 4 | 19,800 | $U_4C_8(AC)_2(AU)(AAC)(AAG)$ | 3.12 |
| 5 | 14,800 | $U_3C_6G(AC)(AU)(A_3C)$ | 3.14 |
| 6 | 20,739 | $U_5C_4G(AC)_3(AU)_3(A_3C)$ | 3.24 |
| 7 | 13,056 | $U_3C_6(AC)_2(AU)(AG)(AAC)$ | 3.96 |
| 8 | 25,864 | $U_6C_8G(AC)_3(AU)(A_3C)_2(A_5G)$ | 3.68 |
| 9 | 15,313 | $U_3C_4G(AC)(AU)_2(AAC)(AAU)$ | 3.38 |
| 10 | 13,045 | $U_4C_5(AAG)(A_5C)$ | 3.56 |
| 11 | 14,084 | $U_4C_8G(AU)_3$ | 3.49 |
| 12 | 14,980 | $U_4C_3(AC)_2(AU)_3(AG)$ | 3.28 |
| 13 | 14,005 | $U_4C_4G(AU)_2(A_5U)$ | 3.51 |
| 14 | 11,959 | $U_2C_5G(AC)_2(AU)(AAC)$ | 3.68 |
| 15 | 13,769 | $C_5G(AC)_2(AU)(AAC)$ | 2.82 |
| 16 | 25,525 | $U_7C_{10}G(AC)_5(AU)_2(AG)$ | 2.94 |
| 17 | 21,164 | $C_6G(AC)_3(AU)(AG)(AAC)(A_4C)$ | 3.06 |
| 18 | 13,088 | $U_5C_2G(AU)_4(AAU)$ | 3.75 |
| 19 | 14,161 | $U_3C_{10}G(AU)$ | 2.92 |
| 20 | 10,648 | $C_3(AC)(AU)(AG)(A_5C)$ | 3.63 |
| 21 | 11,222 | $C_5(AC)_2(AU)(AAG)$ | 3.22 |
| 22 | 26,693 | $U_4C_4G_2(AC)_4(AU)_2(AAU)(A_3C)(A_5C)$ | 3.37 |
| 23 | 15,206 | $U_2C_6G(AAC)(AAU)$ | 2.55 |
| 24 | 12,435 | $U_4C_4G(AU)(AAU)$ | 2.92 |
| | | Average | 3.33 ± 0.35 |

* Numbers refer to diagram in Fig. 4E.

† An aliquot of 8 × $10^6$ cpm from the same preparation of 60–70S [$^{32}$P]RNA digested with RNase T1 was used for each experiment and analyzed as described for Fig. 4E and Table 1.

‡ More than one guanosine residue was found in the RNase A fragments of spots 8, 16, 17, and 22 (see Table 1).

§ Complexity was calculated as described for Table 1, using an average molecular weight per nucleotide of 323, derived from the base composition of RNA of PR-A × RAV-2 no. 5 (25.5% cytidine, 24.3% adenosine, 28.2% guanosine, 22.0% uridine) and the known molecular weights of the nucleotides.

and could increase the chances of crossing-over (5, 32, 33). This model is compatible with genetic experiments (34) in which recombination was shown to involve a heterozygous intermediate. Polyploidy provides a rationale for an obligate heterozygous intermediate in tumor virus recombination.

**Note Added in Proof.** A complexity of 3.44 ± 0.15 × $10^6$ daltons was determined from three RNase A-resistant, unique oligonucleotides of PR RSV-B RNA, indicating that polyploidy is not limited to those sequences resistant to RNase T1.

Most of the data described here are also included in a report presented at the XXXIX Cold Spring Harbor Symposium on Tumor Viruses (12). We thank Sun Yung Kim, Marie Stanley, and Philip Harris for excellent help with these experiments and Jerry Keith (Univ. California, Berkeley) and Rich Roberts (Cold Spring Harbor Lab.) for help and advice with the fingerprinting technique. We gratefully acknowledge personal communication at the XXXIX CSH Symposium on Tumor Viruses, June 1974, of complexity estimates of tumor virus RNA on the order of 3 × $10^6$ daltons by Drs. Quade, Smith, and Nichols (Durham, N.C.), Drs. Billeter, Parsons, Coffin, and Weissmann (Zurich, Switzerland), and Dr. Shyam Dube (Göttingen, Germany), based on RNase T1-resistant oligonucleotides, and by Drs. Baluda, Shoyab, Markham, Evans, and Drohan, based on RNA·DNA hybridization kinetics. The work was supported by Public Health Service Research Grants CA 11426 and CA 13213 from the National Cancer Institute and by the Cancer Program-National Cancer Institute, under Contract no. N01 CP 43212 and Contract no. N01 CP 43242.

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