JOURNAL OF VIROLOGY, May 1979, p. 453-461
0022-538X/79/05-0453/09$02.00/0

Vol. 30, No. 2

Transformation-Defective Mutants of Rous Sarcoma Virus with Longer Sizes of Genome RNA and Their Highly Frequent Occurrences

MITSUAKI YOSHIDA,[1]* MAKOTO YAMASHITA,[1] AND AKIO NOMOTO[2]

Department of Viral Oncology, Cancer Institute, Kami-Ikebukuro, Toshima-ku, Tokyo 170,[1] and Department of Public Health, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108,[2] Japan

Received for publication 22 November 1978

Transformation-defective (td) mutants with different sizes of genomic RNA were isolated from the Prague strain of Rous sarcoma virus, subgroup C (PR-C). All six td viruses (tdTYPR-C) isolated from a single UV-irradiated stock of PR-C (clone 2 of TYPR-C) had slightly longer RNA than did the ordinary class *b* RNA of tdB77 and Rous-associated virus-7. td viruses spontaneously segregated in uncloned TYPR-C also contained genomic RNA of a size similar to tdTYPR-C RNA. On the other hand, two td mutants isolated from another stock of PR-C (LAPR-C) had the class *b* RNA. Fingerprint analysis confirmed that tdTYPR-C and tdLAPR-C were derived by deletion from clone 2 of TYPR-C and LAPR-C, respectively, and also showed that clone 2 of TYPR-C had sequences in its genome RNA different from those of LAPR-C, although it gave a fingerprinting pattern similar to the latter. These results strongly suggest that differences between the nucleotide sequences in TYPR-C and LAPR-C RNA may result in different extents of deletion.

The nondefective strain of avian sarcoma viruses (ASV) shows high spontaneous conversion to transformation-defective (td) mutants (14), in which the genomic RNA (class *b*) is about 16% smaller than the parental nondefective ASV RNA (class *a*) (3, 4, 7, 10, 12). The deletions occur in a specific portion of the ASV genome, including the *src* gene located near the 3′ terminus (5, 15). Since most independent isolates of td mutants contained class *b* RNA (4), the deletion is thought to be of rather uniform size. However, a few recently isolated td mutants had a slightly longer RNA genome than class *b* RNA (6, 8, 13, 16), and some of them were shown to retain a part of the *src* gene (6).

This paper reports that all six td mutants isolated from a viral stock of the Prague strain of Rous sarcoma virus, subgroup C (TYPR-C), had an RNA genome slightly longer than class *b* RNA. No ordinary td mutants with class *b* RNA were isolated from this stock, although they were isolated from another viral stock of the Prague strain (LAPR-C). We found that these two PR-C stocks gave slightly different RNA fingerprints, suggesting that their different extents of deletion were due to differences in the RNA sequences of the genomic RNA.

MATERIALS AND METHODS

Cells and viruses. Chicken fibroblast cultures were prepared from 10-day-old embryos of the C/E or C/BE phenotype as described previously (17). The subgroup C variant of PR-C, donated by K. Toyoshima and maintained in our laboratory, was designated as TYPR-C, and the same strain donated by P. K. Vogt was named LAPR-C. ASV strain B77 and the td derivative tdB77 were supplied by K. Toyoshima.

Radioactive labeling and purification of virus. Radioactive labeling and purification of virus were carried out as described by Duesberg and Vogt (4). The infected culture was mixed with 0.1 mCi of [^{3}H]uridine (specific activity, 46 Ci/mmol) per ml and incubated for 8 to 10 h. The medium was collected, and fresh medium without [^{3}H]uridine was added twice more for 4-h periods. The media were then pooled, and the virus was purified by the method of Duesberg et al. (2). The virus band between the 20 and 60% sucrose layer was collected for extraction of viral RNA.

Preparation of viral RNA. Viral RNA was extracted with sodium dodecyl sulfate (SDS)-phenol from semipurified virus. Radioactive virus from the sucrose gradient was mixed with 200 μg of yeast tRNA and disrupted by treatment with 0.5% SDS and 0.1% diethylpyrocarbonate. The mixture was kept for 5 min at room temperature and then extracted twice with an equal volume of phenol. The RNA was precipitated from the aqueous phase by adding 3 volumes of ethanol, leaving the mixture overnight at −20°C, and then centrifuging it at 8,000 rpm for 10 min. The precipitate was dissolved in buffer consisting of 0.01 M Tris-hydrochloride (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 0.1% SDS, and ethanol precipitation was

repeated once more. The final pellet of RNA was dissolved in 0.1% SDS–0.01 M Tris-hydrochloride (pH 7.4) and stored at −70°C.

Electrophoresis. Polyacrylamide gel electrophoresis of viral RNA was carried out as described by Duesberg and Vogt (4). ^{3}H-labeled RNA was mixed with ^{14}C-labeled viral RNA and then with 1/10 volume of 10-times-concentrated sample buffer (40 mM Tris-hydrochloride, pH 7.4, 20 mM sodium acetate, 1 mM EDTA, and 0.1% SDS). The mixture was heated at 95°C for 1 min, cooled rapidly in ice water, and layered on a 2.2% polyacrylamide gel in the same buffer containing 2 M urea. Electrophoresis was carried out at 6 V/cm for 7 to 8 h. After electrophoresis, gels were wrapped in Parafilm and frozen at −70°C. They were then cut into 1-mm sections, and the sections were extracted overnight with 0.5 ml of 0.1 N NaOH in scintillation vials. The extracts were neutralized with HCl and mixed with 10 ml of toluene-based scintillator containing 30% Triton X-100, and radioactivity was counted in a Beckman scintillation spectrometer.

Assay of reverse-transcriptase activity. The procedure for assay of reverse-transcriptase activity was as described by Wang et al. (15). The culture fluid was centrifuged to remove cells and cell debris and then incubated for 30 min at 37°C in a reaction mixture containing 50 mM Tris-hydrochloride (pH 8.0), 6 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol, 1 μM [^{3}H]TTP (specific activity, 42 Ci/mmol), 2 μg of polyadenylic acid, 0.4 μg of oligo(dT_{12-18}), and 0.1% Triton X-100. The reaction was stopped by adding 10% trichloroacetic acid, and the precipitate was collected on a nitrocellulose membrane filter and washed three times with 3 ml of 5% trichloroacetic acid. The membrane was then dried, and its radioactivity was counted in a toluene-based scintillator.

Oligonucleotide fingerprinting. ^{32}P-labeled viruses were prepared by the method of Wang et al. (15), and RNA was extracted as described above. 35S viral RNA was heat dissociated as described by Duesberg (1) and purified on a sucrose gradient. The purified ^{32}P-labeled 35S RNA was digested with RNase T_1 and separated by two-dimensional gel electrophoresis according to a modification (Y. F. Lee, unpublished data) of the methods described by Lee and Wimmer (9).

RESULTS

Isolation of td mutants from PR-C. Initially, UV irradiation was used to increase the frequency of isolation of td mutants (11). A viral stock (clone 2) of the Prague strain of RSV (TYPR-C) was irradiated with UV light for 6 to 9 min, resulting in more than 95% inactivation of the virus. The UV-irradiated virus was then inoculated into a secondary culture of chicken embryo fibroblasts and covered with a layer of agar. The smaller foci that developed were transferred to normal chicken embryo fibroblasts. After two cell passages, some cultures lost transformed cells, but most cultures became completely transformed.

Measurement of the reverse-transcriptase activity in the culture fluid showed that the six cultures that lost transformed cells all produced viruses (Table 1). These viruses seemed to be defective in transforming ability, because they did not form foci under an agar layer and the cells had an apparently normal morphology. Cells that were fully infected with these viruses were challenged with the parental TYPR-C and also with the Schmidt-Ruppin strain of Rous sarcoma virus, subgroup D (SR-D), or PR-A to determine the subgroup of the virus. Complete interference with transformation by the parental PR-C but not with transformation by SR-D or PR-A was observed. These results indicate that these nontransforming viruses belong to subgroup C, like the parental strain of TYPR-C. Thus, these viruses were designated as tdTYPR-C, tdTY4–9 (Table 1).

Genome size of tdTYPR-C. To determine whether the tdTYPR-C viruses were deletion mutants, we examined their heat-dissociated virion RNA by polyacrylamide gel electrophoresis. As shown in Fig. 1, the RNA subunits of one of them, tdTY9, migrated more rapidly than did those of the parental PR-C, which had been cloned recently. This clearly indicates that tdTY9 was a deletion mutant. However, tdTY9 RNA was found to migrate slightly slower than the subunits of tdB77, used as a marker of ordinary class *b* RNA (Fig. 1B). This slightly larger size of tdTY9 RNA was also confirmed by coelectrophoresis of tdTY9 RNA with Rous-associated virus-7 and tdLAPR-C, which were previously shown to have class *b* RNA (Fig. 1C and D) (4, 16). From the electrophoretic mobilities, tdTY9 RNA was estimated to be smaller than

TABLE 1. *Characterization of nontransforming viruses isolated from various stocks of PR-C*

Parental stock	td viruses	Focus formation	Reverse-transcriptase activity in medium[a]	Interference with focus formation by:	
				PR-C	SR-D
None	None	−	2.6	−	−
TYPR-C clone 2	None	+	42.3		
	tdTY4	−	18.2	+	−
	tdTY5	−	12.1	+	−
	tdTY6	−	21.3	+	−
	tdTY7	−	15.4	+	−
	tdTY8	−	27.3	+	−
	tdTY9	−	47.8	+	−
TYPR-C clone 3	tdTY105	−	108.0		
	tdTY107	−	18.8		
LAPR-C	tdLA1004	−	20.8		
	tdLA1045	−	15.3		

[a] Incorporation (counts × 10^{3} per minute) of [^{3}H]TTP into the trichloroacetic acid-insoluble fraction per 30 μl of culture fluid.

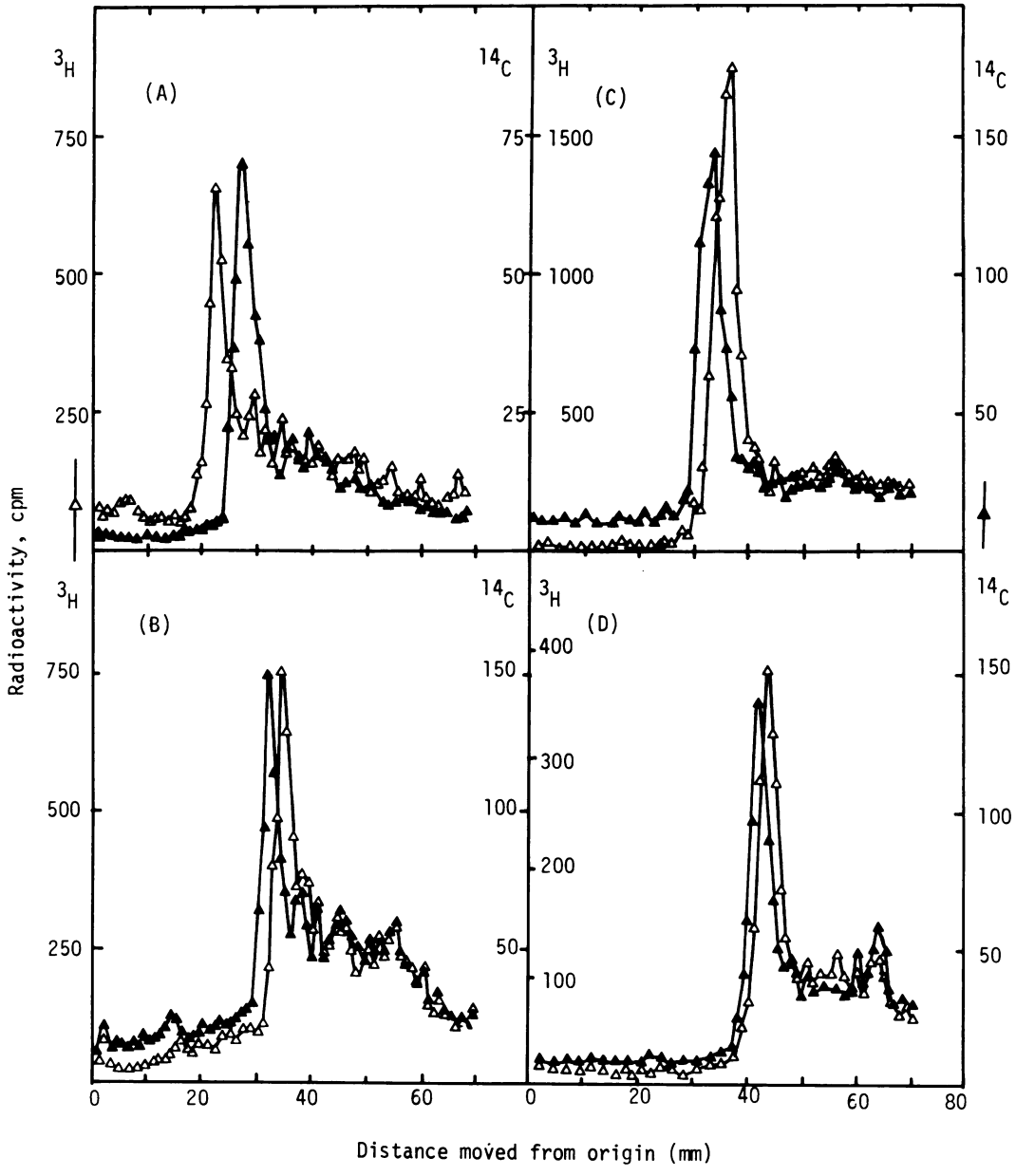


FIG. 1. *Polyacrylamide gel electrophoresis of 35S RNA of tdTY9 isolated from TYPR-C, clone 2. (A) ^3H-labeled TYPR-C, clone 2 (△) and ^{14}C-labeled tdTY9 (▲); (B) ^3H-labeled tdB77 (△) and ^{14}C-labeled tdTY9 (▲); (C) ^3H-labeled tdLAPR-C (△) and ^{14}C-labeled tdTY9 (▲); (D) ^3H-labeled Rous-associated virus-7 (△) and ^{14}C-labeled tdTY9 (▲).*

class *a* RNA by about 12% of the total genome RNA, assuming that normally 16% deletion occurs (7, 12). The other five isolates were all found to have the same-sized RNA as tdTY9, which was slightly larger than the ordinary class *b* RNA, indicating that the unusual intermediate size of tdTY9 RNA was not a unique feature of the one isolate (data not shown).

Genome size of spontaneously formed td mutants. The unexpected finding that all of the isolates of tdTYPR-C had the same intermediate size of genomic RNA could be a result of the isolation procedures, i.e., UV irradiation of the virus stock and selection of transformed cells that regressed later. To exclude this possibility, original TYPR-C viral stock that had not been

cloned recently was labeled with [^{3}H]uridine, and its RNA size was analyzed. Figure 2 clearly shows that the td mutants that spontaneously accumulated in the TYPR-C viral stock had the same intermediate size of genome RNA as that of tdTY9. On the other hand, spontaneously segregated td mutants in uncloned B77 virus stock had class *b* subunits, which are shorter than those in tdTY9. These findings clearly indicate that the isolation procedures did not affect the size of the deletion and also that the sizes of the deletions in the two viral stocks, TYPR-C and B77, were different, but that they were fairly uniform within either of the stocks. Attempts to isolate ordinary td mutants from TYPR-C have so far failed, indicating that the frequency of formation of ordinary td mutants, if they are formed, is very low.

td mutants from another stock of PR-C. As described above, all td mutants from TYPR-C had RNA slightly larger than that of tdB77. These results were not consistent with the report by Duesberg and Vogt (4) that td mutants isolated from PR-C and B77 had the same size of RNA genome, i.e., class *b* subunits. To exclude the possibility that this difference is due to the type of cells used for propagation of viruses, the isolation procedure, and/or the technique for analysis of viral RNA, we tried to isolate td mutants from another stock of PR-C, LAPR-C. Analysis of two isolates, tdLAPR-C 1004 and 1045, showed that both contained class *b* RNAs, which are shorter than those of tdPR-C (Fig. 3A). One td mutant isolated and supplied by P. K. Vogt was also confirmed to have the ordinary class *b* RNA as reported by Duesberg and Vogt (4) (data not shown).

We also isolated the td mutants tdTY105 and tdTY107 from clone 3, cloned from the original stock of TYPR-C, by the same procedure as that used for the other td viruses and found that the RNAs of these td viruses were also longer than those of ordinary td viruses and similar to that of tdTY9 (Fig. 3B).

Oligonucleotide fingerprints of 35S RNA. The reason for the highly frequent occurrence of different sizes of deletion in TYPR-C and LAPR-C viral stocks was examined by comparing the oligonucleotide fingerprints of their RNAs. RNase T_1-resistant oligonucleotides of the purified ^{32}P-labeled 35S RNA samples were separated by two-dimensional gel electrophoresis (Fig. 4). Comparison of the fingerprints of tdTY9 and the original TYPR-C showed that the dense spots 8, 15 + 15′, and 17, which are indicated in Fig. 4A, were found to be composed of two or three oligonucleotides, as judged from the yield of ^{32}P counts (Table 2). The yields of these three spots from tdTY9 were lower than those from TYPR-C, indicating that tdTY9

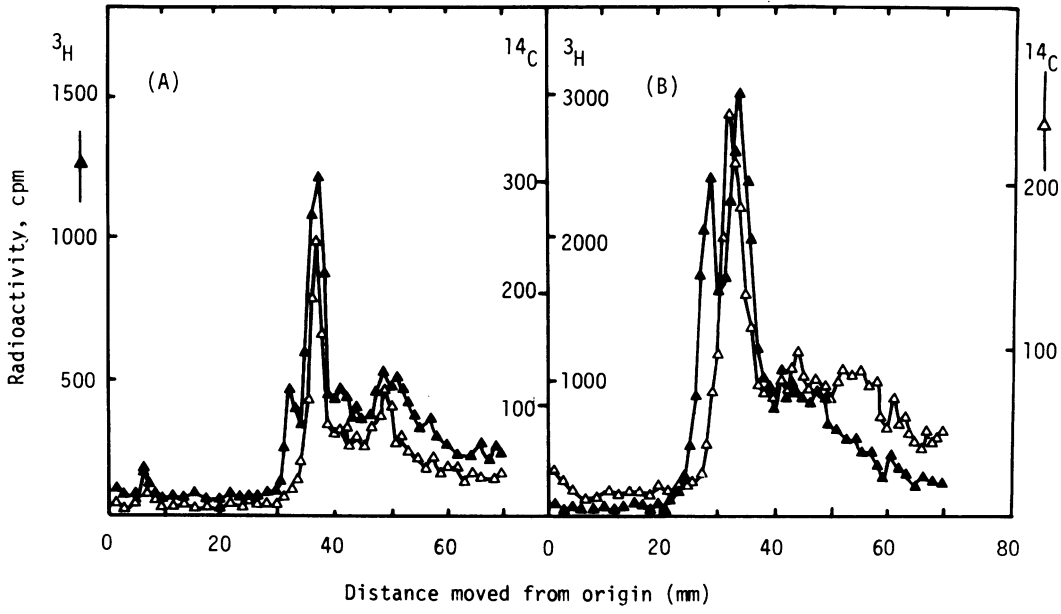


FIG. 2. *RNA size of spontaneously segregated td viruses from TYPR-C (A) and B77 (B). Uncloned viral stock of TYPR-C and B77 was infected, the viral progeny was labeled, and heat-denatured viral RNA was analyzed on a polyacrylamide gel. (A) Uncloned ^{3}H-labeled TYPR-C (▲) and ^{14}C-labeled tdTY9 (△); (B) uncloned ^{3}H-labeled B77 (▲) and ^{14}C-labeled tdTY9 (△).*

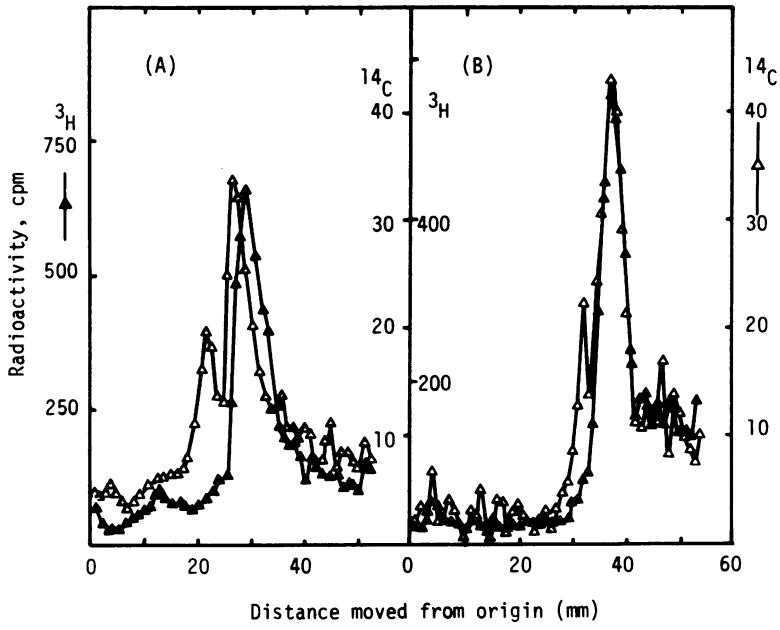


FIG. 3. *RNA size of td viruses isolated from LAPR-C and another clone of TYPR-C, clone 3. (A) ^{3}H-labeled tdLA1045 isolated from LAPR-C (▲) and uncloned ^{14}C-labeled TYPR-C (△); (B) ^{3}H-labeled tdTY105 isolated from TYPR-C, clone 3 (▲), and uncloned ^{14}C-labeled TYPR-C (△).*

lacked the three large oligonucleotides by the deletion (Table 2). The comparison also showed that two spots, 11 and 22, which were produced in lower yields and marked by closed symbols in Fig. 4A, are missing from the fingerprint of tdTY9 (Fig. 4D). The lower yield of these spots might be explained by the presence of a minor population of modified or variant viruses in the viral stock of clone 2. Isolation of tdTYPR-C clone 9 would eliminate these variant viruses. The spots in the fingerprint of tdLAPR-C were identical to those of the original LAPR-C, except that three dense spots of large oligonucleotides, 8, 15 + 15', and 17, were missing (Fig. 4B and E and Table 2). These results clearly indicated that tdTY9 and tdLAPR-C are deletion mutants derived from the respective parental strains.

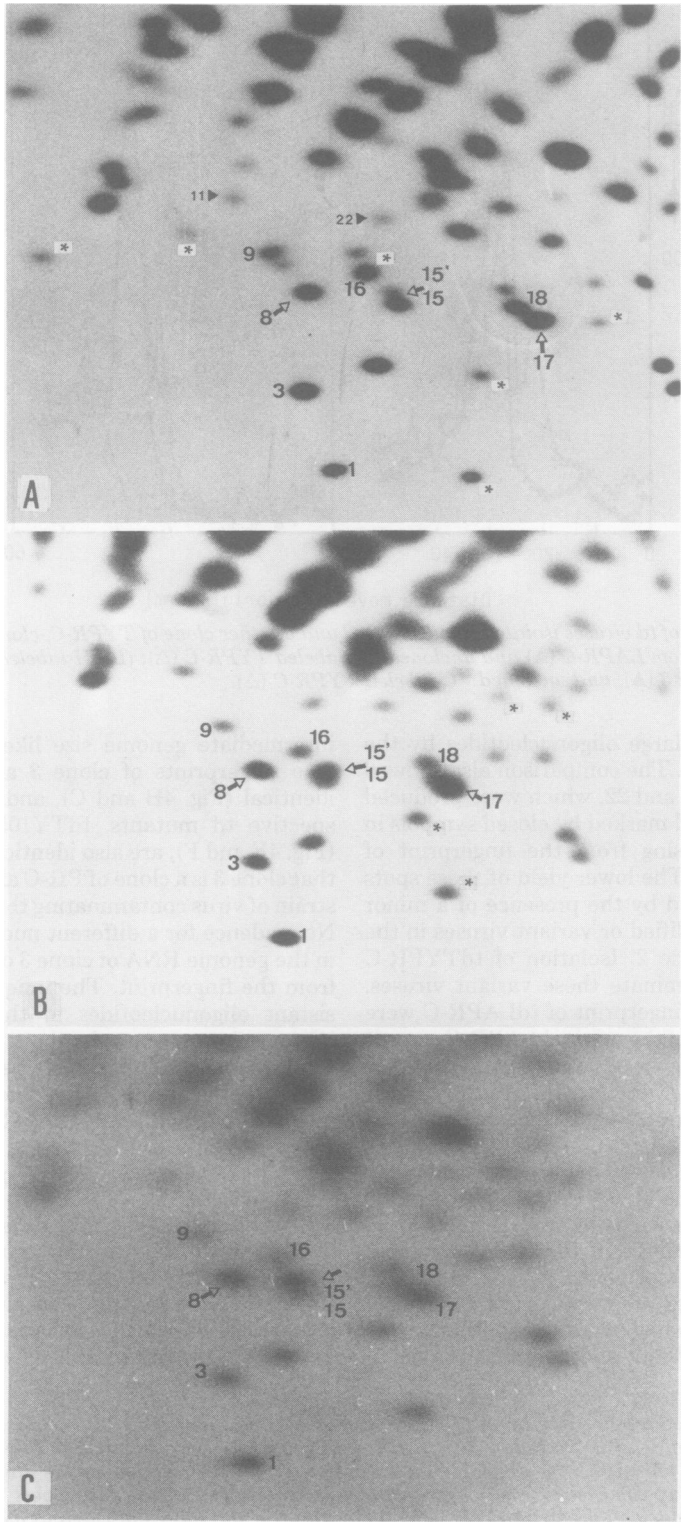
The fingerprints of TYPR-C and LAPR-C showed clear differences in several spots (Fig. 4A and B), indicating that the RNA sequences of these two viral stocks of PR-C are different.

In addition, td mutants (tdTY105 and tdTY107) were isolated from another clone (clone 3) of TYPR-C and found to have an intermediate genome size like that of tdTY9. The fingerprints of clone 3 and LAPR-C are identical (Fig. 4B and C), and those of the respective td mutants, tdTY105 and tdLA1045 (Fig. 4E and F), are also identical. This confirms that clone 3 is a clone of PR-C and not of another strain of virus contaminating the TYPR-C stock. No evidence for a different nucleotide sequence in the genome RNA of clone 3 could be obtained from the fingerprint. The unique RNase T_1-resistant oligonucleotides in these fingerprints, however, represent only about 10 to 20% of the total genome; therefore the results do not exclude the possibility that the nucleotide sequences in clone 3 and LAPR-C RNAs differ.

DISCUSSION

This study showed that different viral stocks of PR-C can produce td mutants with different sizes of genomic RNA. Our viral stock (TYPR-C) produced tdTYPR-C containing 12% smaller genomic RNA, which is longer than the ordinary td mutants formed by 16% deletion (12). On the

FIG. 4. *Oligonucleotide fingerprinting of TYPR-C and LAPR-C and their td mutants. The purified ^{32}P-labeled 35S RNAs of TYPR-C, clone 2 (A), LAPR-C (B), TYPR-C, clone 3 (C), tdTY9 from clone 2 (D), tdLAPR-C (E), and tdTY105 from clone 3 (F) were digested with RNase T_1, and the oligonucleotides were then separated by two-dimensional gel electrophoresis, as described in the text. Spots indicated by open arrows are the oligonucleotides present only in wild-type viruses, and those indicated by asterisks are specific to the viral stock as judged by comparison with TYPR-C and LAPR-C.*


11
22
9
16
15'
15
8
18
17
3
1
A
9
16
15'
15
18
8
17
3
1
B
9
16
18
15'
15
8
17
3
1
C

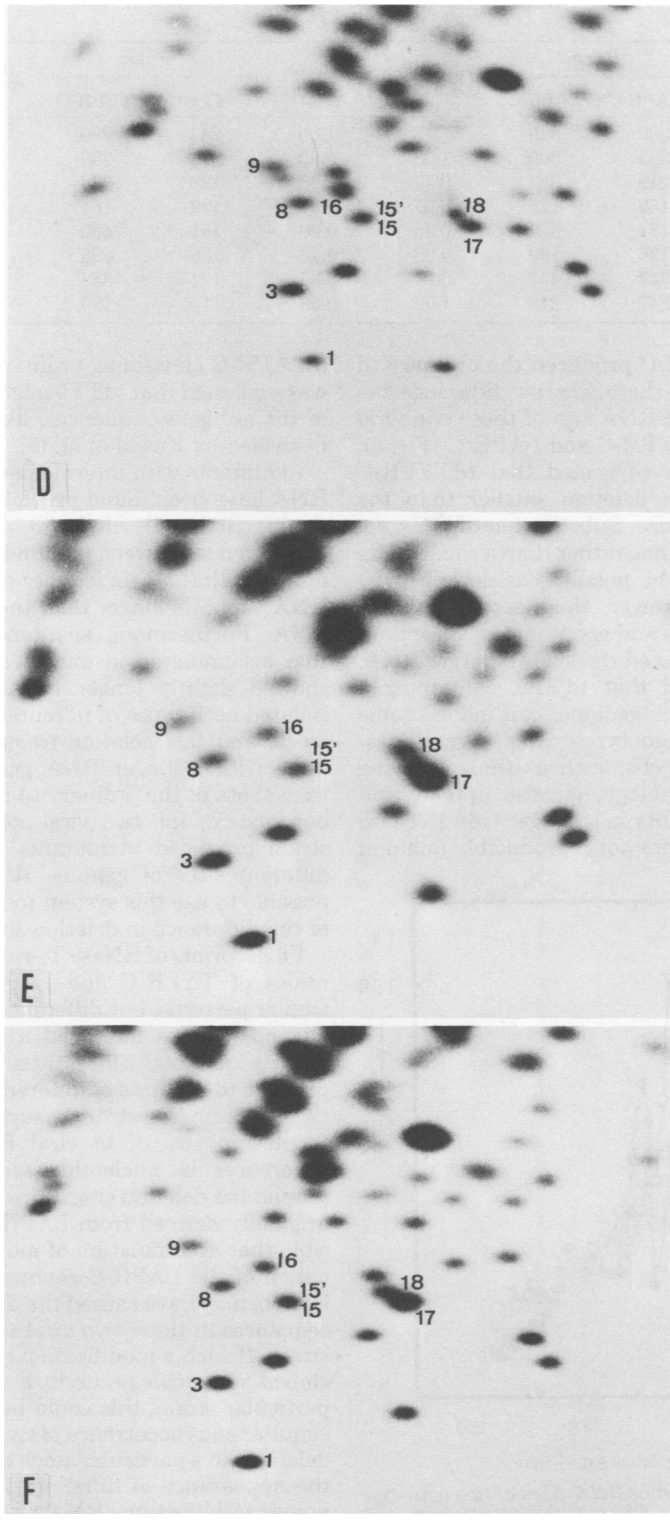

9
8
16
15'
15
18
17
3
1
D
9
16
15'
15
8
18
17
3
1
E
9
16
15'
15
8
18
17
3
1
F

TABLE 2. *Recovery of some oligonucleotides from 35S RNA of two stocks of transforming and td viruses of PR-C*

Spot	^{32}P cpm		tdLAPR-C/LAPR-C	Relative ratio	^{32}P cpm		tdTY9/TYPR-C	Relative ratio
	tdLAPR-C	LAPR-C			tdTY9	TYPR-C		
1	389	329	1.18	1.00	243	296	0.82	1.00
3	335	309	1.08	0.92	205	260	0.79	0.96
8	219	387	0.57	0.48	128	338	0.38	0.46
9	169	175	0.97	0.81	122	165	0.74	0.90
15 + 15′	181	520	0.35	0.30	131	452	0.29	0.35
16	195	199	0.98	0.73	180	295	0.61	0.74
17	343	443	0.77	0.65	171	343	0.50	0.61
18	247	243	1.02	0.86	114	190	0.60	0.73

other hand, LAPR-C produced the ordinary td mutants. Because there was no difference between the genomic RNA size of these two viral stocks of PR-C, TYPR-C and LAPR-C (Fig. 5), these observations suggested that tdTYPR-C were formed by a deletion smaller than the ordinary deletion size. Substitution of RNA sequences in td mutants, rather than a smaller size of deletion, could be possible, as described by Lai et al. (8); however, this seemed unlikely because all isolates and spontaneously accumulated TYPR-C showed the slightly larger RNA. We have reported that tdTY9, containing a slightly larger RNA genome, can induce some transformation phenotypes after several passages of infected cells, such as those showing altered cell morphology, glucose uptake, and colony-forming ability in soft agar (16). Because these properties were not reproducibly found in tdLAPR-C containing ordinary class *b* RNA, it was suggested that tdTY9 might still retain some of the *src* gene sequences, like the td mutants described by Kawai et al. (6).

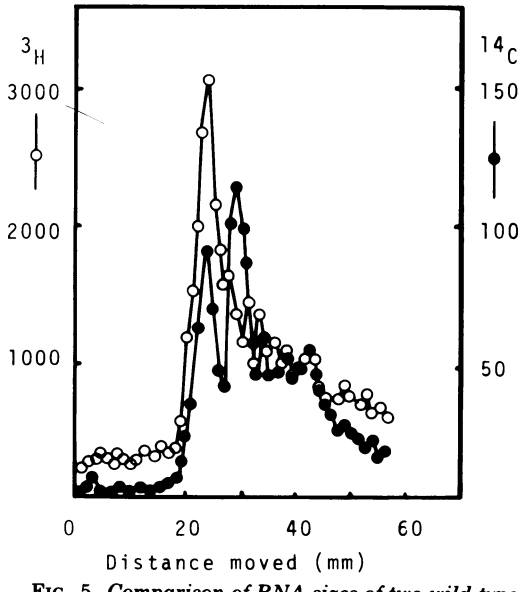


FIG. 5. *Comparison of RNA sizes of two wild-type viral stocks of uncloned ^{14}C-labeled TYPR-C (●) and ^{3}H-labeled LAPR-C (○) RNAs.*

td mutants with intermediate sizes of genome RNA have been found previously in other ASV strains, SR-A (6), SR-D (8), and also B77 (13). A difference between our findings and those of others is that all six isolates of tdTYPR-C had RNA subunits longer than the ordinary class *b* RNA. Furthermore, spontaneous td mutants that accumulated in uncloned viral stock also showed slightly longer RNAs. Other workers isolated both types of td mutants from one viral stock, and the isolation frequencies of td mutants with a longer RNA genome were lower than those of the ordinary td mutants. Since, in our system, the two viral stocks of the same strain produced td mutants with respectively different sizes of genome RNA, it should be possible to use this system to analyze the cause of the difference in deletion size.

Fingerprints of RNase T_1-resistant oligonucleotides of TYPR-C and LAPR-C gave rather similar patterns, but differences in several large oligonucleotides indicated a difference in the RNA sequences. Since these two viral stocks produce td mutants of different respective RNA sizes, this finding strongly suggests that the difference in size of td viral RNA was due to differences in nucleotide sequences, possibly around the deletion site. Since our TYPR-C was originally derived from LAPR-C, it seems possible that accumulation of mutations or modification of the LAPR-C genome, followed by selection, may have caused the differences in RNA sequences in these two viral stocks of the same strain. If such a modification or mutation of the cloned virus can occur in a short period in a particular strain, this could be the cause of the simultaneous occurrence of two different sizes of deletions in a particular stock of virus. Of course, the appearance of different deletions from genomes with the same RNA sequences is possible, as discussed by Lai et al. (8).

The oligonucleotide fingerprint of tdTY9 was similar to that of TYPR-C, except that three large oligonucleotides were missing. This finding clearly indicates that tdTY9 was derived from TYPR-C by deletion.

ACKNOWLEDGMENTS

We are indebted to K. Toyoshima and P. K. Vogt for gifts of virus strains and for invaluable advice and to Y. F. Lee for his advice on two-dimensional gel electrophoresis techniques. We also thank Y. Ikawa for helpful discussions and Y. Hirayama and H. Arakawa for skillful technical assistance.

This work was partly supported by a grant-in-aid for cancer research from the Ministry of Education, Sciences and Culture, Japan, and by a grant from the Cancer Research Fund of Princess of Takamatsu.

LITERATURE CITED

1. **Duesberg, P. H.** 1968. Physical properties of Rous sarcoma virus RNA. Proc. Natl. Acad. Sci. U.S.A. **60**:1511–1518.
2. **Duesberg, P. H., H. L. Robinson, W. S. Robinson, R. J. Huebner, and H. C. Turner.** 1968. Proteins of Rous sarcoma virus. Virology **36**:73–86.
3. **Duesberg, P. H., and P. K. Vogt.** 1973. Gel electrophoresis of avian leukosis and sarcoma viral RNA in formamide: comparison with other viral and cellular RNA species. J. Virol. **12**:549–599
4. **Duesberg, P. H., and P. K. Vogt.** 1973. RNA species obtained from clonal lines of avian sarcoma and from avian leukosis virus. Virology **54**:207–219.
5. **Joho, R. H., M. A. Billeter, and C. Weissmann.** 1975. Mapping of biological functions on RNA of avian tumor viruses: location of regions required for transformation and determination of host range. Proc. Natl. Acad. Sci. U.S.A. **72**:4772–4776.
6. **Kawai, S., P. H. Duesberg, and H. Hanafusa.** 1977. Transformation-defective mutants of Rous sarcoma virus with *src* gene deletions of varying lengths. J. Virol. **24**:910–914.
7. **Lai, M. M. C., P. H. Duesberg, J. Horst, and P. K. Vogt.** 1973. Avian tumor virus RNA: a comparison of three sarcoma viruses and their transformation-defective derivatives by oligonucleotide fingerprinting and DNA-RNA hybridization. Proc. Natl. Acad. Sci. U.S.A. **70**:2266–2270.
8. **Lai, M. M. C., S. S. F. Hu, and P. K. Vogt.** 1977. Occurrence of partial deletion and substitution of the *src* gene in the RNA genome of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **74**:4781–4785.
9. **Lee, Y. F., and E. Wimmer.** 1976. "Fingerprinting" high molecular weight RNA by two dimensional gel electrophoresis: application to polio virus RNA. Nucleic Acid Res. **3**:1647–1658.
10. **Martin, G. S., and P. H. Duesberg.** 1972. A subunit in the RNA of transforming avian tumor viruses. I. Occurrence in different virus strains. II. Spontaneous loss resulting in nontransforming variants. Virology **47**:494–497.
11. **Owada, M., and K. Toyoshima.** 1973. Analysis on the reproducing and cell-transforming capacities of a temperature sensitive mutant (ts 334) of avian sarcoma virus B77. Virology **54**:170–178.
12. **Stehelin, D., R. V. Guntaka, H. E. Varmus, and J. M. Bishop.** 1976. Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. J. Mol. Biol. **101**:349–365.
13. **Stone, M. P., R. E. Smith, and W. K. Joklik.** 1975. 35S a and b RNA subunits of avian RNA tumor virus strains cloned and passaged in chick and duck cells. Cold Spring Harbor Symp. Quant. Biol. **39**:859–868.
14. **Vogt, P. K.** 1971. Spontaneous segregation of nontransforming viruses from cloned sarcoma viruses. Virology **46**:939–946.
15. **Wang, L.-H., P. Duesberg, K. Beemon, an P. K. Vogt.** 1975. Mapping RNase T_1-resistant oligonucleotides of avian tumor virus RNAs: sarcoma-specific oligonucleotides are near the poly(A) end and oligonucleotides common to sarcoma and transformation-defective viruses are at the poly(A) end. J. Virol. **16**:1051–1070.
16. **Yoshida, M., and Y. Ikawa.** 1977. Induction of some transformation-related properties by a transformation defective mutant of avian sarcoma virus. Virology **83**: 444–448.
17. **Yoshida, M., M. Owada, and K. Toyoshima.** 1975. Strain specificity of changes in adenylate cyclase activity in cells transformed by avian sarcoma viruses. Virology **63**:68–76.