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Generation of Nondefective Rous Sarcoma Virus by Asymmetric Recombination Between Deletion Mutants

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A replication-defective deletion mutant of Prague Rous sarcoma virus (RSV), which lacks functional *gag*, *pol*, and *env* genes, was crossed with a transformation-defective deletion mutant derived from Schmidt-Ruppin RSV. Transformation- and replication-competent viruses were generated in the cross. Characterization of one of these rescued viruses indicated that it was a nondefective recombinant containing the *src* gene of the replication-defective mutant plus the replicative genes of the transformation-defective virus. These results indicate that, contrary to previous reports, asymmetric recombination between RSV deletion mutants can result in the formation of nondefective RSV.

Recombination occurs at a high frequency between point mutants of Rous sarcoma virus (RSV) (16). Recombination also occurs at a high frequency between a temperature-sensitive *src* mutant of RSV and the envelope (*env*) deletion mutant N8 (5, 7) and between nondefective (*nd*) RSV and transformation-defective (*td*) *src* deletion mutants (5, 11, 14). In contrast, the *env*-defective Bryan RSV and the *env* deletion mutant N8 do not generate *nd* recombinants when grown in the presence of *td*RSV (3, 5, 6). The Bryan and N8 strains differ from *nd*RSV in the absence of *env* genes but share with *nd*RSV functional *gag*, *pol*, and *src* genes. Since *td*RSV contains a functional *env* gene, it would be expected that the deletion would be repaired by recombination, generating *nd*RSV. However, it is possible that the *env* deletion in the Bryan and N8 strains overlaps with the *src* deletion in the *td* mutants, so that recombination to generate nondefective RSV would not be possible.

Here, we have carried out crosses with a *gag*-, *pol*-, and *env*-defective RSV mutant and *td*RSV to determine whether asymmetric recombination can occur between deletion mutants of RSV to generate nondefective virus when there is a region of homology situated between the two deletions. The replication-defective (*rd*) mutant, *rd*BK303, used in these studies is a deletion mutant derived from a UV-irradiated stock of Prague RSV, subgroup A (PR-A). It is maintained as a provirus in a clone of transformed "nonproducer" quail fibroblasts, UV3-B3 (8). The DNA of this provirus contains a deletion of approximately two-thirds of the genome, and the clone does not contain detectable *gag*, *pol*, or *env* gene products. The defective provirus retains an intact *src* gene, which encodes a functional *src* gene product, plus the terminal repeats found in normal provirus (8). The precise extent of the deletion in *rd*BK303 has not yet been determined. However, the DNA of *rd*BK303 hybridizes to an *env*-specific complementary DNA (cDNA) probe, which does not hybridize to the RNA of Bryan RSV but which does hybridize to the RNA of transformation-defective virus, indicating that sequences of the *env* gene which are deleted in Bryan RSV are retained in *rd*BK303 (8). The other parent in the cross was a standard transformation-defective mutant, *td*NY106 derived from Schmidt-Ruppin RSV, subgroup A (SR-A). This mutant does not appear to retain any detectable *src* sequences (4). By infecting the *rd*BK303-transformed quail cells with *td*NY106, we have obtained a recombinant *nd*RSV, termed B8. The nature of the cross that generated B8 RSV is diagrammed in Fig. 1.

The cross was carried out by co-cultivating the nonproducer quail clone UV3-B3 with C/E *chf*-negative chick embryo fibroblasts infected with *td*NY106. The cell mixture was then treated with mitomycin C and plated for infectious centers on susceptible chick C/E indicator fibroblasts. These procedures have been described previously (8). One to ten infectious centers were obtained per 10^6 quail cells. Stocks prepared from foci picked from these infectious center assays were found to contain a large excess of *td* virus. Therefore, the transforming viruses were purified by successive agar colony isolation. Several of these stocks appeared to contain nondefective virus, and one of these, B8, was characterized in detail.

When the stock of B8 was plated on chicken embryo fibroblasts, all of the foci picked (10/10) yielded infectious virus. Dilutions of B8 beyond the endpoint for focus formation were tested for

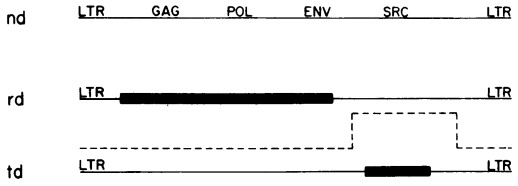

FIG. 1. *Genetic structure of the deletion mutants used in the cross. Top line: ndRSV; middle line: the rd mutant rdBK303; bottom line: the td mutant tdNY106. The dashed line indicates the exchange required to produce nd virus.*

an excess of *td* virus both by reverse transcriptase assays and by interference tests with *nd*SR-A. The endpoint dilution tests indicated that the B8 stock did not contain an excess of a *td* (helper) component. These results suggested that B8 was nondefective. To confirm the presence of *nd* virus, the size of the viral RNA of B8 was compared with that of a *td* virus by polyacrylamide gel electrophoresis. The results (Fig. 2) indicate that B8 contains RNA of size class *a* (about 10,000 nucleotides [2]), which is characteristic of the RNA of nondefective RSVs.

If B8 is a recombinant between *rd*BK303 and *td*NY106, it should contain the *src* gene of Prague RSV plus replicative genes derived from *td*NY106. (This would not be the case if it were derived from a contaminant in parental virus or cell stocks or by recombination with a cellular transforming gene). To determine the genetic origin of B8, the RNA was analyzed by fingerprinting RNase T_1-resistant oligonucleotides. ^{32}P-labeled viral RNA was subjected to polyacrylamide gel electrophoresis, and size class *a* RNA was eluted, digested with RNase T_1, and fingerprinted by published procedures (12). The fingerprints of B8 RNA, of *td*NY106 RNA, and of PR-A RNA are shown in Fig. 3A to C, and the compositions of the individual oligonucleotides determined by RNase A digestion are shown in Table 1. Four oligonucleotides (no. 4, 6, 9, and 18) are shared by *td*NY106 and PR-A, and their origin cannot be determined. All the remaining oligonucleotides of B8 have identical counterparts in *td*NY106 (identical numbers) with the exception of B8 oligonucleotides 51 and 52. The B8 oligonucleotides 51 and 52 are those previously identified as being *src*-specific in wild-type Prague RSV (12). To characterize the *src* RNA sequence of B8 further, the ^{32}P-labeled B8 RNA was hybridized to cDNA prepared by hybridizing PR-B RSV cDNA with *td*PR-B RSV RNA (9). The RNase T_1-resistant hybrids were isolated, and the RNA of the resulting hybrid was analyzed by fingerprinting its RNase T_1-resistant oligonucleotides. This procedure allows the resolution of many additional *src*-specific oligonucleotides. The fingerprint of the $cDNA_{src}$-protected RNA is shown in Fig. 3D, and the composition of the individual oligonucleotides is given in Table 1. The result indicated that the *src*-specific RNA sequence of B8 is indistinguishable from that of Prague RSV, which is distinct from *src*-specific RNA sequences of other RSV strains (Lee and Duesberg, manuscript in preparation).

These results indicate that the transforming virus B8 recovered from a cross of *rd*BK303 and *td*NY106 is a nondefective recombinant containing *src*-specific sequences from the replication-defective parent and replicative genes from the transformation-defective parent. We conclude therefore that nonadjacent deletions in retroviruses can be repaired by asymmetric recombination (Fig. 1). Since the transforming viruses resulting from the cross had to be purified from the original mixture before they could be characterized, we cannot determine the stage during the rescue procedure at which recombination takes place or estimate the frequency with which the recombination event occurs. Since only 1 to 10 infectious centers were obtained per 10^6 quail cells plated, it appears that the recombination event is relatively infrequent.

The finding that deletion mutants of RSV can recombine to generate *nd*RSV suggests that the

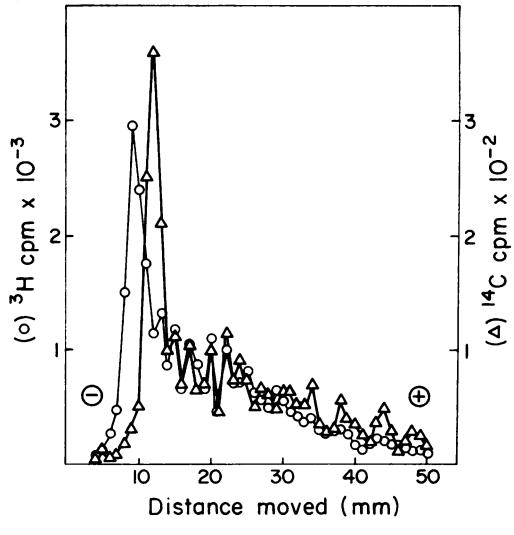

FIG. 2. *Polyacrylamide gel electrophoresis of 35S RNA of rescued RSV B8. ^3H-labeled 50 to 70S RNA of B8 (○) and ^{14}C-labeled 50 to 70S RNA of avian erythroblastosis-associated virus (△) were mixed, heat dissociated, and electrophoresed on 2% polyacrylamide gels as previously described (10). The avian erythroblastosis-associated viral RNA serves as a marker for size class b RNA (10).*

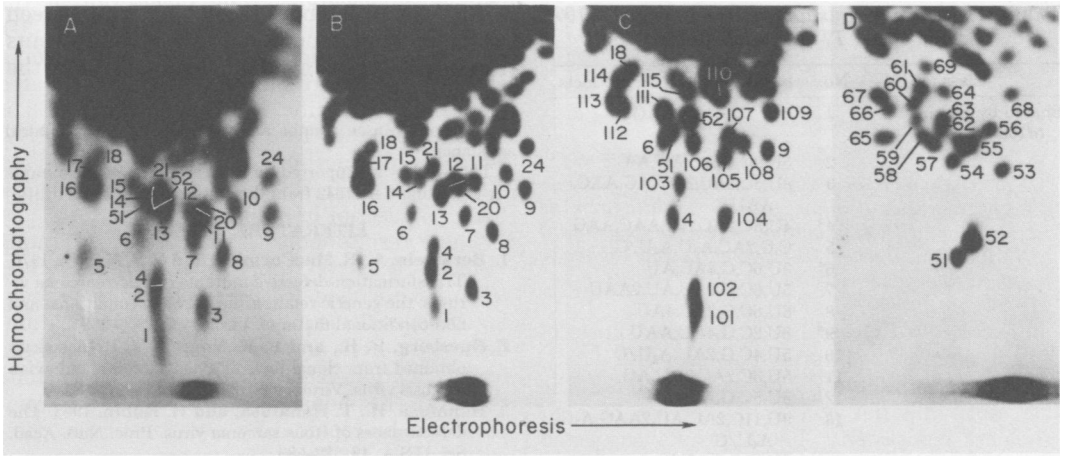

FIG. 3. *Electrophoretic-chromatographic analyses (fingerprints) and autoradiography of the RNase T_1-resistant ^{32}P-labeled oligonucleotides of (A) rescued RSV B8, (B) tdNY106, a transformation-defective src deletion of SR-A RSV, (C) PR-A RSV, and (D) the src-specific RNA sequence of B8. (A to C) Size class a RNA of B8 was eluted from a 2% polyacrylamide gel; the 60 to 70S RNAs of tdNY106 and PR-RSV were prepared by sucrose gradient centrifugation. The RNAs were digested with RNase T_1 and fingerprinted as described elsewhere (12). The src-specific RNA sequence of B8 was obtained as a hybrid formed between B8 RNA and src-specific cDNA following procedures described previously (9). In short, src-specific cDNA was prepared by hybridizing 1 μg of PR-B RSV cDNA with 5 μg of the RNA of tdL01, a src deletion of PR-B RSV (1) for 12 h at 40°C in 10 μl 70% formamide, 0.3 M NaCl, 0.03 M sodium citrate, and 0.01 M sodium phosphate (pH 7.0), to base pair all but the src-specific sequences of the cDNA. The src-specific cDNA was then hybridized for 2 h in 20 μl of the above buffer with 0.2 μg (1 × 10^6 cpm) of B8 [^{32}P]RNA. After digestion of nonhybridized RNA for 30 min at 40°C with 5 U of RNase T_1 in 200 μl of 0.15 M NaCl–0.015 M sodium citrate, pH 7.0, the hybrid was isolated by chromatography on a Bio-Gel (BioRad) P100 column (15 by 0.7 cm). Subsequently the hybrid was phenol extracted and melted, and the RNA was fingerprinted after digestion with RNase T_1 as described for (A to C).*

failure to observe formation of *nd*RSV by recombination between *td*RSV and Bryan or N8 RSV is a consequence of their particular genetic structures. If the *env* deletion in the Bryan and N8 strains is contiguous with or overlaps the *src* deletion in standard *td* viruses, illegitimate recombination would be required to generate nondefective virus from the two defectives. Since the deletion in the Bryan and N8 strains does not appear to affect the expression of the *src* gene, and since the deletion in *td*RSV does not affect the expression of the *env* gene, it is likely that the overlap of the two deletions occurs in the intercistronic region between the *env* and *src* genes.

It was previously reported that there are sequences present between the *env* and *src* genes that are shared by N8 and a *td* mutant of SR-RSV (13); however, the particular *td* mutant used in this work subsequently has been found to contain only a partial *src* defect (Wang, personal communication). Since we have shown here that recombination can occur between deletion mutants, we would expect recombination resulting in *nd*RSV to occur between *env*-defective Bryan or N8 RSV and mutants with partial *src* deletions such as those described by Wang et al. (13 and personal communication) and by Kawai et al. (4, 15).

The effects of sequence nonhomology on the formation of recombinants has been studied in detail in bacteriophage lambda (10). In this case the presence of regions of nonhomology (either deletions or insertions) in both parents reduces the recombination frequency by up to 50-fold as compared with point mutations separated by a similar interval. Recombination between point mutations and recombination between deletions appear to involve distinct processes. The majority of recombinants from crosses involving closely linked point mutations result from mismatch repair of hybrid heteroduplex structures. In contrast, extensive regions of nonhomology are excluded from such heteroduplex structures. Thus, only recombining structures which are both initiated and resolved within the interval separating the two deletions or insertions can give rise to recombinants (10). In the case of RSV, the molecular mechanism of recombination is unknown. However, it seems likely that

TABLE 1. *RNase A-resistant sequences of B8, td106, and PR-A T_1-oligonucleotides*

Class	No.	RNase A digestion products
Shared by B8 and *td*106	1	6U,7C,G,3AC,AAC
	2	5U,9C,G,AC,AU,A_3C
	3	8U,5C,2AC,2AU,AG,AAC, AAU
	4[a]	4U,8C,2AC,AU,AAC,AAG
	5	C,G,2AC,A_3U,A_4U/C
	6[a]	3U,6C,G,3AC,AU
	7	5U,6C,G,2AC,AU,2AAU
	8	6U,6C,G,2AC,4AU
	9[a]	8U,2C,G,4AU,AAU
	10	5U,4C,G,2AU,A_4U/C
	11	5U,3C,2AC,3AU,AG
	12	5U,8C,G,AC,4AU
	13	9U,11C,2AC,AU,2AAG,A_3C, A_4U/C
	14	6U,10C,G,2AC
	15	4U,6C,G,2AC,AU,AAC
	16	U,16C,G,7AC,2AU,2AG, AAC,AAU,A_3U,A_4U/C
	17	U,5C,3AC,AAG
	18[a]	3C,AU,AG,A_5U/C
	20	U,2C,G,AC,AU,AAC,AAU
	21	3U,5C,G,3AC,3AU
	24	7U,6C,G,AU
src oligonucleotides of B8 and PR-RSV	51	4U,5C,G,2AC,AU,A_3C
	52	7U,12C,G,4AC,2AU,AG
	53	4U,3C,G,AC,AAC
	54	2U,5C,G,AC,2AU
	55	4U,9C,AC,2AU,2AAG
	56	3U,3C,G,2AC
	57	U,4C,AC,AU,AG
	58	U,2C,G,AC,2AAC
	59	4C,G,A_3C
	60	4C,AC,AU,AG
	61	2U,4C,G,3AC,AU,AAG
	62	U,4C,AC,AU,AG
	63	U,3C,G,AU,AAC
	64	2U,4C,G,AAC
	65	9C,2G,3AC,A_4U/C
	66	2C,2AC,AAG
	67	7C,3AC,2AG,AAC
	68	4U,2C,G,AAC
	69	2C,AU,A_3G
PR-A specific	101	8U,8C,G,2AC,2AU,2AAU, A_3U
	102	10U,9C,G,2AC,AAC,AAU, A_3C
	103	3U,6C,G,AC,AU,A_3C
	104	4U,5C,G,3AC,3AU,A_3U
	105	4U,3C,G,AC,2AU,AAC,AAU
	106	4U,5C,AAG,A_4U/C
	107	4U,3C,2AC,3AU,AG
	108	4U,3C,G,AC,2AU,A_5U/C
	109	10U,8C,G,2AU
	110	8U,10C,G,AU
	111	3U,5C,2G,4AC,2AU,AAC, A_5U/C
	112	5C,2AC,AU,A_6G
	113	U,10C,G,4AC,AU,AG,AAC, A_5U/C
	114	8C,3AC,AG,AAU,AAG
	115	6U,7C,3AC,AU,A_4G

[a] Shared with PR-A.

in RSV, as in lambda, recombination between two deletion mutants requires exchange events restricted to a region of homology separating the two deletions.

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LITERATURE CITED

1. **Bernstein, A., R. MacCormick, and G. S. Martin.** 1976. Transformation-defective mutants of avian sarcoma viruses: the genetic relationship between conditional and non-conditional mutants. Virology **70**:206–209.
2. **Duesberg, P. H., and P. K. Vogt.** 1973. RNA species obtained from clonal lines of avian sarcoma and avian leukosis virus. Virology **54**:207–219.
3. **Hanafusa, H., T. Hanafusa, and H. Rubin.** 1963. The defectiveness of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **49**:572–580.
4. **Kawai, S., P. H. Duesberg, and H. Hanafusa.** 1977. Transformation-defective mutants of Rous sarcoma virus with *src* deletions of varying length. J. Virol. **24**: 910–914.
5. **Kawai, S., and H. Hanafusa.** 1972. Genetic recombination with avian tumor virus. Virology **49**:37–44.
6. **Kawai, S., and H. Hanafusa.** 1973. Isolation of defective mutant of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **70**:3493–3497.
7. **Kawai, S., and H. Hanafusa.** 1976. Recombination between a temperature-sensitive mutant and a deletion mutant of Rous sarcoma virus. J. Virol. **19**:389–397.
8. **Martin, G. S., K. Radke, S. Hughes, N. Quintrell, J. M. Bishop, and H. E. Varmus.** 1979. Mutants of Rous sarcoma virus with extensive deletions of the viral genome. Virology **96**:530–546.
9. **Mellon, P., A. Pawson, K. Bister, G. S. Martin, and P. H. Duesberg.** 1978. Specific RNA sequences and gene products of MC29 avian acute leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **75**:5876–5878.
10. **Sodergren, E. J., and M. S. Fox.** 1979. Effects of DNA sequence non-homology on formation of bacteriophage lambda recombinants. J. Mol. Biol. **130**:357–377.
11. **Vogt, P. K.** 1971. Genetically stable reassortment of markers during mixed infection with avian tumor viruses. Virology **46**:947–952.
12. **Wang, L.-H., P. H. Duesberg, K. Beemon, and 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.
13. **Wang, L.-H., P. H. Duesberg, S. Kawai, and H. Hanafusa.** 1976. Location of envelope-specific and sarcoma-specific oligonucleotides on RNA of Schmidt-Ruppin Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **73**: 447–451.
14. **Wang, L.-H., P. H. Duesberg, P. Mellon, and P. K. Vogt.** 1976. Distribution of envelope-specific and sarcoma-specific nucleotide sequences from different parents in the RNAs of avian tumor virus recombinants. Proc. Natl. Acad. Sci. U.S.A. **73**:1073–1077.
15. **Wang, L.-H., C. C. Halpern, M. Nadel, and H. Hanafusa.** 1978. Recombination between viral and cellular sequences generates transforming sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **75**:5812–5816.
16. **Wyke, J. A., and J. A. Beamand.** 1979. Genetic recombination in Rous sarcoma virus: the genesis of recombinants and lack of evidence for linkage between *pol*, *env*, and *src* genes in three factor crosses. J. Gen. Virol. **43**:349–364.