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Distribution of envelope-specific and sarcoma-specific nucleotide sequences from different parents in the RNAs of avian tumor virus recombinants

(recombinants of Rous sarcoma virus/mapping ribonuclease-T_1-resistant oligonucleotides/gene order of Rous sarcoma virus)

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ABSTRACT The distribution of leukosis-virus- and sarcoma-virus-specific oligonucleotide sequences was investigated in the RNAs of viral recombinants selected for an envelope gene (*env*) from a leukosis parent and a sarcoma gene (*src*) from a sarcoma parent. For this purpose 20 to 30 RNase-T_1-resistant oligonucleotides were chemically analyzed and mapped within the 10,000 nucleotides of each viral RNA relative to the 3′-poly(A) end. The resulting oligonucleotide maps were compared. Proceeding from the 3′ to the 5′ end, the maps of four recombinants contained: (*i*) in a segment of 2000 nucleotides, three to four *src*-specific oligonucleotides, so identified because they were shared only with the sarcoma parent; and (*ii*) in a segment of 8000 nucleotides, 20 oligonucleotides shared with the leukosis parent, of which six to seven were also shared with the sarcoma parent. Two other recombinants contained: (*I*) in a segment of 2000 (one) or 3000 (the other) nucleotides, three *src*-specific oligonucleotides; (*ii*) in a segment of 3000 (one) or 2000 (the other) nucleotides, five (one) or four (the other) oligonucleotides, all or some of which are *env*-specific, because they were shared with the leukosis parent; (*iii*) in a segment of 5000 nucleotides (both), 11 functionally unidentified sarcoma-virus-derived oligonucleotides, of which seven were also shared with the leukosis parent. The map locations of parental oligonucleotides were not changed in recombinants and all viral strains tested shared six to eight highly conserved oligonucleotides at equivalent map locations. The partial map -*env*-*src*-poly(A) emerged from the analyses of these recombinants.

Nondefective (nd) avian sarcoma viruses have been shown to contain at least four genetic elements (1): a gene for viral DNA polymerase, termed *pol;* a gene for the internal group-specific antigen, termed *gag;* a gene for the viral envelope glycoprotein, termed *env;* and a gene for sarcomagenic cell transformation, termed *src* (2). The genome of nd sarcoma viruses is thought to be a 60–70S RNA complex which is diploid (3) containing two identical 30–40S subunits of 10,000 nucleotides (4–6) with a poly(A) sequence at the 3′ end (7). We have recently mapped the *src* and *env* genes on the viral RNA and constructed the partial map [*gag-pol*]-*env-src*-poly(A) (2, 7–10). The method involved (*i*) the construction of oligonucleotide maps of viral RNAs by ordering 20 to 30 RNase-T_1-resistant oligonucleotides on the basis of their distance from the 3′-poly(A) end and (*ii*) functional identification of specific oligonucleotides of nd sarcoma virus RNA by their absence from the RNA of closely related deletion mutants with known functional defects.

Abbreviations: nd, nondefective; PR-A and PR-B, Prague strain nd Rous sarcoma viruses of subgroup A and B, respectively; RAV-2, Rous-associated leukosis virus of subgroup B, and RAV-3, of subgroup A. PR-A × RAV-2, PR-B × RAV-3 designate nd sarcoma virus recombinants selected for the subgroup marker of RAV-2 or RAV-3, respectively.

We investigate here an independent approach (9) to identify genes on viral RNA by correlating in recombinants the distribution of parental gene markers with parental oligonucleotide map segments. This should allow us to determine map locations of crossover points and of genes which participated in the cross. It should also shed light on the mechanism of virus recombination. We have analyzed here avian sarcoma virus recombinants selected for a *src* gene from an nd sarcoma parent and for an *env* gene from a leukosis virus parent. We have shown previously that these recombinants had been generated by crossing over (4, 6).

RESULTS AND DISCUSSION

Oligonucleotide Maps of Recombinant and Parental Avian Tumor Virus RNAs. Six recombinants are studied here. Three were selected for the subgroup B *env*-marker of the Rous-associated leukosis virus RAV-2 and the *src*-marker of the nd sarcoma virus Prague Rous of subgroup A (PR-A) and were termed PR-A × RAV-2 no. 1, no. 3, and no. 5 in previous studies (4, 6). Three were selected for the subgroup A *env*-marker of the leukosis virus RAV-3 and the *src*-marker of nd sarcoma virus Prague Rous of subgroup B (PR-B) and were termed previously PR-B × RAV-3 no. 1, no. 2, and no. 4 (4, 6). [A leukosis virus contains all genes of an nd sarcoma virus, except *src* (6, 8).] To construct oligonucleotide maps viral [^{32}P]RNA was randomly degraded by alkali. Poly(A)-tagged RNA fragments were selected by binding to oligo(dT)-cellulose, fractionated in sucrose gradients into different size classes, and fingerprinted (Fig. 1, I–IX) as described in detail (2, 7, 8). The map position of a given oligonucleotide relative to the poly(A) end of the RNA was deduced from the size of the smallest fragment from which it could be isolated (2, 7, 8). The large oligonucleotides of each fingerprint shown in Fig. 1 were numbered and most of them were chemically identified by determining their RNase-A-resistant fragments (Tables 1–4) as described previously (4, 6, 8). Each of the four tables lists the compositions of all oligonucleotides of a given parent virus and all those oligonucleotides of a recombinant shared with that parent. In some cases a fingerprint spot consisted of two or more overlapping oligonucleotides, particularly when 60–70S viral RNAs (Fig. 1, IA–IXA) or large poly(A)-tagged fragments were analyzed. Letter designations were used to indicate the number of oligonucleotides in such a spot. If the multiple oligonucleotides of such a spot had different map locations, the composition of the one mapping closest to the 3′-poly(A) end of the RNA could be obtained, free of that mapping further away, from the appropriate poly(A)-tagged RNA fragment (2). In other cases the composition of one oligonucleo-

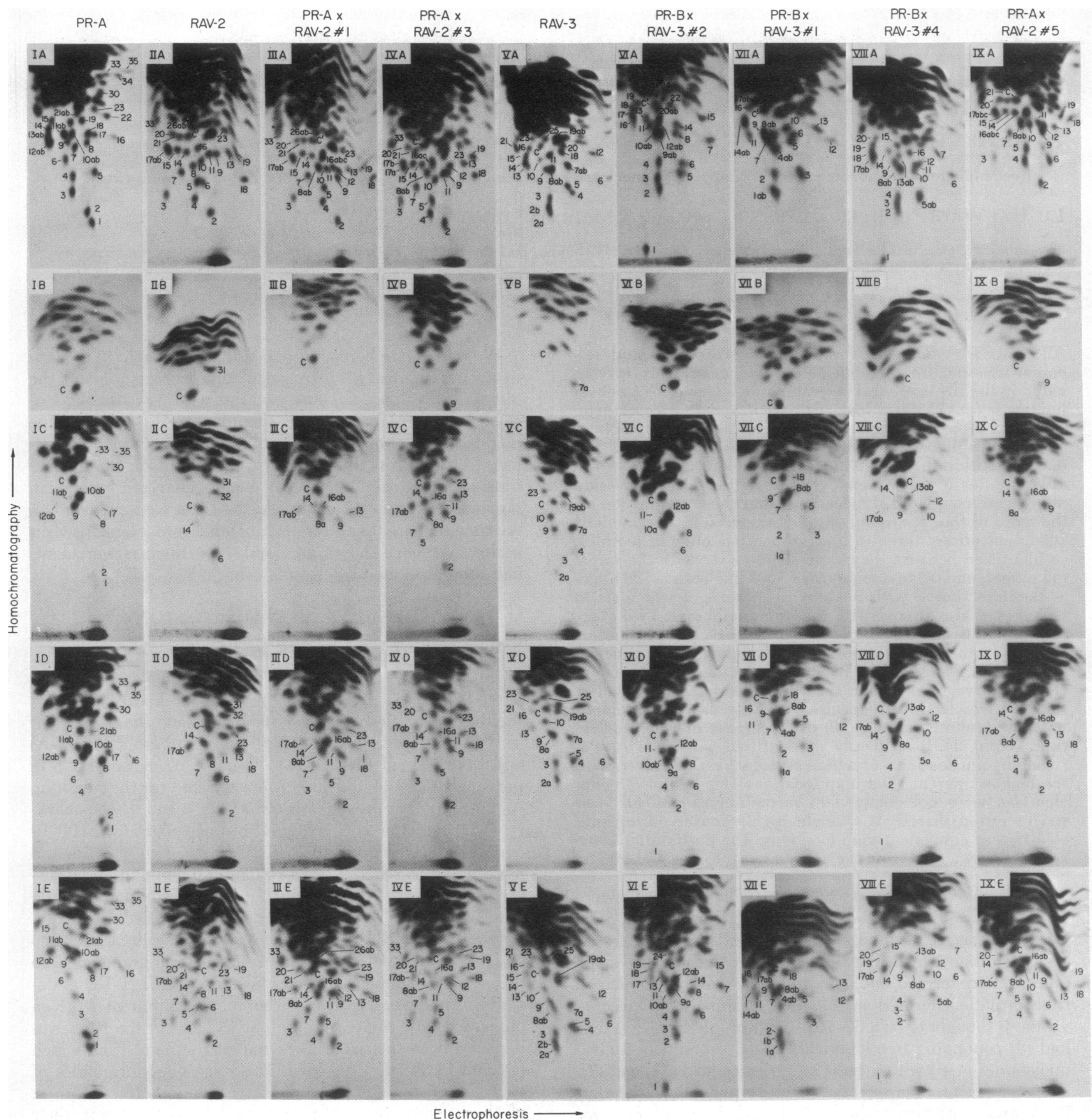

FIG. 1. Fingerprint patterns of RNase-T$_1$-resistant oligonucleotides of 60–70S viral [^{32}P]RNAs and of poly(A)-tagged [^{32}P]RNA fragments of wild-type and recombinant avian tumor virus RNAs: PR-A (I): 60–70S RNA after fragmentation and removal of poly(A) fragments (A), poly(A) fragments of 4–10 S (B), 15–19 S (C), 20–23 S (D), and 24–28 S (E). RAV-2 (II): 60–70S RNA (A), poly(A) fragments of 4–10 S (B), 11–15 S (C), 16–20 S (D), and 23–27 S (E). PR-A × RAV-2 #1 (III): 60–70S RNA (A), poly(A) fragments of 4–10 S (B), 15–19 S (C), 20–23 S (D), and 26–30 S (E). PR-A × RAV-2 #3 (IV): 60–70 S (A), poly(A) fragments of 10–15 S (B), 16–19 S (C), 20–23 S (D), and 26–30 S (E). RAV-3 (V): 60–70S RNA after fragmentation and removal of poly(A) fragments (A), poly(A) fragments of 5–11 S (B), 12–17 S (C), 18–23 S (D), and 24–30 S (E). PR-B × RAV-3 #2 (VI): 60–70 S (A), poly(A) fragments of 4–8 S (B), 18–22 S (C), 23–26 S (D), and 27–32 S (E). PR-B × RAV-3 #1 (VII): 60–70 S (A), poly(A) fragments of 2–8 S (B), 9–15 S (C), 16–20 S (D), and 26–31 S (E). PR-B × RAV-3 #4 (VIII): 60–70 S RNA after fragmentation and removal of poly(A) fragments (A), poly(A) fragments of 5–11 S (B), 12–17 S (C), 18–23 S (D), 24–30 S (E). PR-A × RAV-2 #5 (IX): 60–70 S (A), poly(A) fragments of 5–12 S (B), 13–18 S (C), 19–22 S (D), 23–28 S (E). Conditions for fingerprinting have been described in detail (2, 7, 8). All (B) panels show only the upper half of the respective fingerprints.

tide from a double spot could be estimated, because a counterpart with the same fingerprint and map location was found in a parental or sister virus (Fig. 2, Tables 1–4). By subtracting the composition of an oligonucleotide so identified from that of the mixture, we could estimate the composition of the remaining oligonucleotide(s) of a multiple spot.

Table 1. RNase-T_1-resistant oligonucleotides of PR-A and their homologous* counterparts in PR-A × RAV-2 recombinants

Spot no†				RNase A digestion products
PR-A	PR-A x RAV-2 #1	#3	#5	
1				7U,8C,G,2(AC),2(AU),2(AAU),(AAAU)
2				7-8U,8-9C,G,1-2(AC),(AAC),(AAU),(AAAC)
3	4	4	4	3U,8C,2(AC),(AU),(AAC),(AAG)
4	5	5	5	3U,6C,G,(AC),(AU),(AAAC)
5				4U,5C,G,3(AC),3(AU),(AAAN)
6				2U,5C,G,3(AC),(AU)
7	10	10	10	4U,5C,(AAG),(AAAAN)
8	9	9	9	3-4U,3-4C,G,(AC),2(AU),(AAC),(AAU)
9	8a	8a	8a	3U,4C,G,2(AC),(AU),(AAC)
10ab	16ab	~16a	16ab	6U,9C,G,4(AC),2(AU),(AG)
		16a		3U,4C,G,3(AC),(AU)
11ab	~14	~14	~14	3U,5C,2G,4(AC),2(AU),(AAC),(AAAAAAN)
12ab				10C,5(AC),2(AU),(AG),(AAC),(AAU),(AAAAAAG)
13ab				U,7-8C,G,3(AC),0.5(AG),0.5(AAU),(AAAAAN)
14				U,5C,3(AC),(AAG)
15	20	20	20	2-3C,(AU),(AG),(AAAAAAN)
16	18	18	18	6U,2C,G,4(AU),(AAU)
17				4-5U,2-3C,G,(AC),2(AU),(AAAAAN)
18	12	12	12	4U,3C,2(AC),3(AU),(AG)
21ab				4U,6C,G,2(AC),(AU),(AG),2(AAU)
22	19	19	19	8U,6C,G,(AC),(AU)
23				2U,C,G,2(AU),(AAU),(AAAC)
30				4-5U,2-3C,G,(AC),1.5(AU),0.5(AAU)
33				5U,C,G,(AC),(AU)
34				7U,C,(AU),(AG)
35				4U,G,2(AU)
C	C	C	C	G(AC)(AU)(AAU)(AAAN)

* Homologies between the oligonucleotides of parental viruses and recombinants were based on analyses of their RNase-A-resistant fragments determined as described (4, 6, 8). Those oligonucleotides whose identity could only be estimated by their fingerprint and map positions because they were not resolved from other overlapping oligonucleotides (see *text*) are indicated by the symbol ~.

† Numbers refer to oligonucleotide spots shown in Fig. 1. Letter designations are used if spots consisted of two or more overlapping oligonucleotides.

The oligonucleotide maps shown in Fig. 2 were constructed from the data shown in Fig. 1 and other data not shown here. The oligonucleotide map of PR-B was revised from a previous investigation (8). To facilitate comparisons between maps of a recombinant and its parents, the maps of each recombinant were placed between those of their parents (Fig. 2). Oligonucleotides shared by a recombinant with one or both of its parents are connected by lines in the diagrams of Fig. 2. In principle each oligonucleotide of a recombinant should have a homologous counterpart in one of its parents. This was true for PR-A × RAV-2 no. 1 and no. 3, but a few exceptions were observed in the other recombinants studied. This may be due to technical reasons such as uncertainties with the analyses of oligonucleotides from multiple spots or of oligonucleotides with insufficient radioactivity. In addition, crossing-over or postrecombinational mutations may have differentially altered the RNA of recombinants to generate different oligonucleotides (4, 6).

Four Recombinants Possibly Generated by a Single Cross Between Their Parents. The oligonucleotide maps of PR-A × RAV-2 no. 1, no. 3, and no. 5 and PR-B × RAV-3 no. 1 contained, proceeding from the 3′ to the 5′ end, (*i*) in a segment of about 2000 nucleotides a cluster of three to four sarcoma-virus-derived oligonucleotides and (*ii*) in a segment of about 8000 nucleotides a cluster of about 20 leukosis-virus-derived oligonucleotides, of which six to seven were also shared with the sarcoma parent (Fig. 2). The 3′-terminal segment is thought to include most or all of the *src* gene, because it contained the only oligonucleotides of these recombinants which were shared solely with the sarcoma parent. This is consistent with previous analyses which have identified in PR-B and in other nd sarcoma viruses (2, 7, 8, 11) two to three *src*-specific oligonucleotides with the same compositions and map locations as their respective counterparts in the recombinants analyzed here. The 5′-terminal segments must contain, among others, the leukosis-virus-specific *env* gene of these recombinants. However, its map location cannot be estimated directly because this RNA segment must also contain *gag* and *pol* genes. The genetic map [*gag*,-*pol*,*env*]-*src*-poly(A), in which the relative locations of *gag*, *pol*, and *env* are unknown, is consistent with the oligonucleotide maps of these recombinants. Considering that the *gag* as well as *pol* gene products of all avian tumor viruses are serologically and functionally related (12–16), it appears likely that those oligonucleotides which are shared by both parents (between 5000 nucleotides and the 5′ end) signal *gag*- and/or *pol*-specific sequences. The simplest interpretation of these maps suggests that these recombinants were generated by a single cross which linked the 3′-terminal 2000 nucleotides of the sarcoma parent to the 5′-terminal 8000 nucleotides of the leukosis parent. However, because these recombinants shared oligonucleotides with both parents near the 5′ ends and oligonucleotide C at the 3′ end of their RNAs, it cannot be excluded that further crosses occurred.

Table 2. RNase-T_1-resistant oligonucleotides of RAV-2 and their homologous* counterparts in PR-A × RAV-2 recombinants

Spot no†				RNase A digestion products
RAV-2	PR-A x RAV-2 #1	#3	#5	
2	2	2	2	5U,5-6C,G,(AC),2(AU),(AAC),2(AAU)
3	3	3	3	C,G,2(AC),(AAAU),(AAAAAN)
4	4	4	4	3U,8C,2(AC),(AU),(AAC),(AAG)
5	5	5	5	3-4U,7C,G,(AC),(AU),(AAAC)
6				U,C,(AC),(AU),(AG),(AAU),(AAAAAN)
7	7	7	7	2U,6C,2(AC),(AU),(AG),(AAC)
8	8b	8b	~8b	2U,4C,(AC),(AAAC),(AAAAG)
9	12	12	12	4U,3C,2(AC),3(AU),(AG)
10	10	10	10	4U,6C,(AAG),(AAAAN)
11	11	11	11	4U,6-7C,G,0.5(AC),2-3(AU)
13	13	13	13	3U,3C,G,2(AU),(AAAAN)
14	14	14	14	2U,4-5C,G,2(AC),(AU),(AAC)
15	15	15	15	U,5C,G,2(AC),(AU),(AAC)
16	16c	16c	16c	2U,5C,G,3(AC),2-3(AU)
17ab	17ab	17ab	17ab	U,7C,G,3(AC),(AU),(AG),(AAC),(AAAAN)
18	18	18	18	6U,3C,G,4(AU),(AAU)
19	19	19	19	7U,6C,G,(AC),(AU)
20	20	20	20	3C,0.5(AC),(AU),(AG),(AAAAAN)
21	21	21	21	U,4-5C,2(AC),(AU),(AAG)
23	23	23		5U,3C,G,(AAC),(AAU)
26ab	26ab			3U,6C,G,2(AC),2(AU),(AG),(AAC)
33	33	33		4C,G,4(AC)
C	C	C	C	G(AC),(AU),(AAU),(AAAN)

*, †, same as Table 1.

Two Recombinants Possibly Generated by a Double Cross. In the 3′ to 5′ direction the oligonucleotide map of PR-B × RAV-3 no. 2 and no. 4 contained: (*i*) in a segment of 2000 nucleotides a cluster of three PR-B-specific oligonucleotides, (*ii*) in a segment of 2000 to 3000 nucleotides a block of four to five RAV-3-specific oligonucleotides and, (*iii*) in a segment of about 5000 nucleotides a block of 11 PR-B-specific oligonucleotides, six of which were also shared

Table 3. RNase-T_1-resistant oligonucleotides of PR-B and their homologous* counterparts in PR-B × RAV-3 recombinants

Spot no†				RNase A digestion products
	PR-B x RAV-3			
PR-B	#1	#2	#4	
2		1	1	U,2C,G,3(AC),(AAU),3(AAAAC)
3				5U,7C,G,(AC),2(AU),(AAC),2(AAU)
4	~1b	3	3	3U,7C,2(AC),(AU),(AAC),(AAG)
5		5	5b	4U,4C,G,3(AC),3(AU),(AAAC)
6	2	4	4	5U,8C,G,(AC),(AU),(AAAC)
7	5	8	10	5U,3C,G,(AC),(AU),(AAC),(AAU)
8	~4ab	~9b	~8b	5U,6C,(AAG),(AAAAAC)
9	7	10a	~9	4U,6C,G,2(AC),(AU),(AAAC)
10				2U,6C,G,2(AC),(AU),(AAC)
11		15	7	8U,7C,G,(AU)
12ab	8ab	12ab	13ab	5U,8C,G,4(AC),2(AU),(AG)
13				6U,3C,(AC),(AAAC),(AAAG)
14	9	11	14	2U,4C,G,2(AC),(AU),(AAC)
15		13		U,2C,2(AC),(AU),(AG),(AAC)
16	10	22	16	6U,8C,G,(AU)
17		20	15	4U,4C,(AC),(AAAAG)
18	21			6U,3C,G,(AU),(AAU)
19ab			~17ab	U,7C,G,4(AC),(AU),(AG),(AAC),(AAAAC)
20a	~14	~18	19	U,5C,3(AC),(AAG)
20b	~16	~19	20	3C,(AU),(AG),(AAAAAN)
21	12	7	6	6U,2C,G,4(AU),(AAU)
22	6	14	11	5U,4C,2(AC),4(AU),(AG)
23			12	4U,4C,2-3(AU),(AAAG)
C	C	C	C	G,(AC),(AU),(AAU),(AAAN)
	‡17ab			3U,9C,G,3(AC),(AU),(AG),0.5(AAU)

*, †, same as Table 1. Data for PR-B are from ref. 8; the composition of spot 20 was revised.

‡ Recombinant oligonucleotides listed below the lower line are those for which no homologous counterparts were found in parental viruses (see *text*).

Table 4. RNase-T_1-resistant oligonucleotides of RAV-3 and their homologous* counterparts in PR-B × RAV-3 recombinants

Spot no†				RNase A digestion products
	PR-B x RAV-3			
RAV-3	#1	#2	#4	
2a	1a	2	2	6U,9C,G,3(AC),(AU),(AAAC)
2b	~1b	3	3	3U,8C,2(AC),(AU),(AAC),(AAG)
3	2	4	4	4U,7C,G,(AC),(AU),(AAAC)
4	3	6	5a	7U,5C,G,(AC),2(AU),(AAC),(AAU)
5				6U,5C,G,3(AC),3(AU)
6	12	7	6	6U,2C,G,4(AU),(AAU)
7a				3U,4C,2(AU),(AG),3(AAU)
7b	~6	14	11	5U,2C,3-4(AC),3(AU),(AG)
8a		9a	~8a	4U,5C,G,3(AC),(AU)
8b	~4ab	9b	8b	4U,5C,(AAG),(AAAAAC)
9		10b		3U,4C,2(AC),0.5(AU),(AAG),(AAAN)
10	9	11	14	3U,5C,G,2(AC),(AU),(AAC)
11				2U,4C,G,3(AC),2(AU)
12	13			8U,8C,G,(AC),(AU)
13	11			4C,2-3(AC),(AU),(AAAAAG)
14	14a	17	18	U,5C,G,3(AC),(AAAAAN)
15	14b	18	19	1-2U,5C,4(AC),(AAG)
16	16	19	20	3C,0.5(AC),(AU),(AG),(AAAAN)
18	10	22	16	5U,8C,G,1-2(AU)
19ab				5U,7C,G,2(AC),(AU),(AG),2-3(AAU)
20				2U,3C,G,(AC),2(AU),(AAU),(AAAC)
21				6C,G,5(AC),(AG)
23				2U,6C,G,3(AC)
C	C	C	C	G,(AC),(AU),(AAU),(AAAN)
		‡16		1-2U,5C,(AC),(AG),(AAU)
		17		U,4C,G,2(AC),(AAAAN)

*, †, same as Table 1, ‡ same as Table 3.

with RAV-3 (Fig. 2). As suggested above the 5′-map segments of these recombinants may include *gag*- and *pol*-specific sequences. Their 3′-segments are thought to include the *src* gene, because they contained a cluster of three oligonucleotides which were the same as the *src*-specific counterparts of PR-B (see above, and refs. 7, 8, and 11). Since their middle segments contained a cluster of oligonucleotides shared with RAV-3, it would follow that the RAV-3-specific *env* gene of these recombinants maps in these RNA segments. The genetic map [*gag,pol*]-*env*-*src*-poly(A) is consistent with the oligonucleotide maps of these recombinants. It appears plausible that these recombinants were generated by a double cross between their parents, one near the 3′ end (at 2000 nucleotides in PR-B × RAV-3 no. 2 and 3000 in PR-B RAV-3 no. 4) and the other near the 5′ end of the parental *env* genes (both at about 5000 nucleotides) (Fig. 2).

Genetic Maps Derived by Comparative Analyses of Recombinants and Their Parents or of Wild-Type Viruses and Their Deletion Mutants. The map *env*-*src*-poly(A) derived here from recombinants agrees with that derived earlier from deletion mutants (2, 7, 11). Moreover, the recombinant maps show that (*i*) parental oligonucleotides retain their parental map location in recombinant RNAs and (*ii*) six to eight highly conserved oligonucleotides map equivalent positions in most viral RNAs analyzed here and previously (Fig. 2 and ref. 2, 7, and 8). Hence gene orders in wild-type and recombinant RNAs are the same. The identification of the *env*-locus by the two methods is confirmed specifically because *env* of all viruses tested here and previously includes one highly conserved oligonucleotide, e.g. no. 6 in Pr-B (Fig. 2). This oligonucleotide was absent from an envelope-defective deletion mutant of Schmidt-Ruppin virus (e.g. no. 2c, ref. 2) and was suggested previously to be *env*-specific in sarcoma viruses Pr-B (no. 6, ref. 8), PR-C and B77 (no. 3b, ref. 8), because it was part of a map segment that differed most among viruses with different *env*-genes (8, 9). Exact definitions of gene borders from analyses of recombinants can only be obtained if many different crosses of a given pair of genes are analyzed because intragenic crosses would define gene borders too narrowly and extragenic crosses too broadly. Because intragenic crosses of *src* cannot occur between a nd sarcoma virus and a leukosis virus (which lacks *src*) we can expect only maximal definitions of the *src* gene from analyses of our recombinants. By contrast, mapping of sequences in wild-type RNA, which are missing in deletion mutants, is unambiguous. However, wild-type sequences so identified may not represent all sequences of the gene under analysis, because the genetic defect of the deletion mutant may be due to a partial rather than a complete deletion of the respective gene (2). Moreover, the method is limited to the genes for which a deletion mutant can be found (2). This is not the case if recombinants are used to map viral genes. Here every gene can be mapped provided a block of specific oligonucleotides can be identified that segregates during recombination with a biological marker of that gene.

Notes Added in Proof. Joho *et al.* have identified *env* in PR-B by oligonucleotides exchanged in PR-B recombinants selected for a different host range (18). Their intuitive assumption that the gene order of PR-B applies also to PR-B recombinants and the leukosis parent, whose oligonucleotides were not mapped, is confirmed by

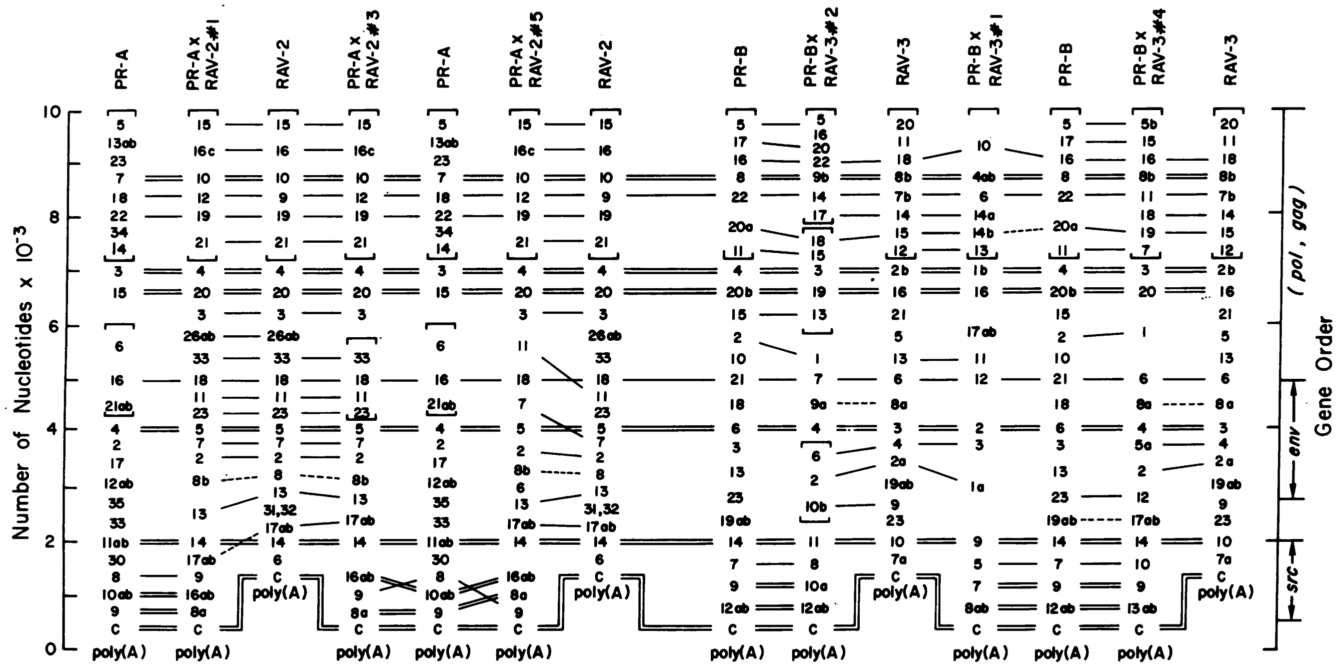

FIG. 2. Oligonucleotide maps of wild-type and recombinant viral 30–40S RNAs. RNase T_1-resistant oligonucleotides, numbered as in Fig. 1 and Tables 1–4, were ordered linearly on the basis of their distance from the 3′-poly(A) end of viral RNA (see *text* and refs. 2, 7, and 8). This distance, expressed in nucleotides, was calculated by Spirin's formula [molecular weight = 1550 × $S^{2.1}$, ref. 17] from the S values of the poly(A)-tagged RNA fragments (Fig. 1) used to map the oligonucleotides as described (2, 7, 8). Those oligonucleotides which could not be mapped accurately are put in brackets. The maps of PR-A, RAV 2, PR-A × RAV 2 #1, #2, and #5, RAV 3, PR-B × RAV-3 #1, #3, and #4 were derived from the data of Fig. 1 and others not shown here and of Tables 1–4 as described (2, 7, 8). The map of PR-B is from a previous report (8) with a revised map position for oligonucleotide no. 4. Oligonucleotides shared by different viruses are connected by lines. Broken lines connect oligonucleotides whose compositional identity could only be estimated indirectly by map position due to overlaps with other oligonucleotides on the same fingerprint (see *text* and Tables). Highly conserved oligonucleotides are connected by double lines and conserved oligonucleotide, by a single line. Their number designations for each of the following viruses, PR-B (here and ref. 8), SR-A (2), PR-C, and B77 (8) are in this order: C in all viruses; 14, 11, X (td PR-C), 12; 6, 2c, 3b, 3b; 21, 17, 18, absent from B77; 20b, 15, 15, 17; 4, 26, 3a, 3a; 22, 19, -, -; 8, 7, 6, 6. *src*-Specific oligonucleotides of PR-B and PR-A are also highly conserved species (2, 8). Approximate gene locations determined as described in the *text* are indicated on right ordinate.

our data. However, a comparison of their data with ours on cross-over points can only be made after their recombinants are mapped.

Recently we have mapped oligonucleotides of PR-C LA 337, which has a temperature-labile polymerase, and of several LA 337 recombinants. Oligonucleotides that were probably *pol*-specific mapped between 6000 and 8000 nucleotides from the 3′ end, suggesting that the most likely complete map of avian sarcoma viruses is *gag-pol-env-src* (19).

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