Sequences and Functions of Rous Sarcoma Virus RNA

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Abstract

A procedure has been developed to map the genetic elements of avian tumor virus RNA, which has a molecular weight of about 3 x 10^6 daltons and a poly(A) sequence at the 3' end. For this purpose, about 30 RNase T_1-resistant oligonucleotides were ordered relative to the 3'-poly(A) terminus of the RNA, to construct an oligonucleotide map of viral RNAs. A cluster of seven envelope gene (*env*)-specific oligonucleotides, identified by their absence from the otherwise very similar oligonucleotide map of an envelope-defective deletion mutant (which lacks the major viral glycoprotein), mapped at a distance of 0.9 to 1.6 x 10^6 daltons from the poly(A) end of sarcoma virus RNA. A cluster of three sarcoma gene (*src*)-specific oligonucleotides, identified by their absence from the otherwise nearly identical oligonucleotide map of a transformation-defective deletion mutant mapped at a distance of 0.2 to 0.6 x 10^6 daltons from the poly(A) end of sarcoma virus RNA. The oligonucleotide maps of sarcoma viruses and of related deletion mutants were the same from the poly(A) end up to 0.2 x 10^6 daltons and included one terminal oligonucleotide, termed C, which is found in all avian tumor viruses tested so far. Preliminary mapping experiments ordering the *src*-specific and *env*-specific oligonucleotides of recombinants, selected for sarcoma and envelope genes of different parents, agree with those obtained by comparing maps of wild type viruses and deletion mutants. A partial genetic map consistent with these results suggests that the *src* gene maps between the *env* gene and the 3'-poly(A) end of viral RNA. This map reads: poly(A)-*src*-*env*-(pol, gag).

a) *Introduction*

The genome of avian tumor viruses is probably a 60–70S RNA complex (1, 2). It consists of predominantly two 30–40S RNA subunits (3) with an approximate molecular weight of 3 x 10^6 daltons each (3, 4, 5, 6). Genetic (7) and biochemical (5, 6) studies suggest that these 30–40S subunits are very similar, perhaps identical. This implies that the viral genome is diploid.

The 30–40S RNA of nondefective (nd) avian sarcoma viruses carries at least

four different genetic elements (8): (i) a gene for cell transformation, termed *src* (17), [the previous term *onc* is replaced here by *src* since so far a transformation gene has only been found in sarcoma viruses (4, 7, 9, 10, 12, 13). By contrast oncogenicity is a more general term and applies to transformation by both sarcoma and leukemia viruses]. (ii) a gene for the viral envelope glycoprotein, termed *env*, (iii) a gene for the viral DNA polymerase, termed *pol* and (iv) a gene for the viral group-specific antigen, termed *gag*.

It is the purpose of our experiments to identify chemically RNA sequences of these genes on 30–40S tumor virus RNAs and to map these sequences on the viral RNA relative to its 3'-poly(A) terminus (9, 10).

b) *Chemical identification of src-specific and env-specific sequences of avian tumor virus RNA*

RNA sequences of the *src* and *env* genes of nondefective (nd) sarcoma viruses can be chemically identified, because they are missing from certain related deletion mutants. For example, transformation-defective (td) deletion mutants, which lack the *src* gene, were also shown to lack 15 % of the RNA present in corresponding nd sarcoma virus RNA (4, 10, 13). Chemical identification of these and other sequences of tumor virus RNA was based on the large RNase T_1-resistant oligonucleotides which they contain. These are prepared by exhaustive digestion of viral [^{32}P]RNA with RNase T_1, which cleaves only after G residues. These large RNase T_1-resistant oligonucleotides consist of 15–40 nucleotides, including C, A, and U, and have one G at the 3' hydroxyl end. They represent altogether about 5 % of the RNA (5). The large oligonucleotides form a unique pattern, which is termed a fingerprint, after two-dimensional resolution by electrophoresis and chromatography (11). Twenty to thirty such large oligonucleotides are found in avian tumor virus RNA. Since these oligonucleotides are rather evenly distributed over the RNA, an average 5 % segment of the RNA carries one such oligonucleotide and can be so identified. An RNA segment can be identified unambigously by two or more such oligonucleotides.

Autoradiographs of fingerprint analyses of the 60–70S RNAs of three nd sarcoma viruses, Rous sarcoma virus (RSV) Prague B (PR-B), Prague C (PR-C) and B77, and of their td counterparts are shown in Fig. 1. It can be seen that, in agreement with earlier studies (13), fingerprint patterns of each nd sarcoma virus and its corresponding td deletion mutant are very similar. Identical numbers were used to designate presumably homologous (same composition and size) spots in corresponding patterns of nd and td viruses. Because two fingerprint analyses of the same digest never run completely identically (11), visual identification of presumably homologous oligonucleotides, by triangulation with neighboring spots, is relatively subjective. Therefore definitive identification of oligonucleotides in different fingerprint patterns was based on partial sequence analyses of oligonucleotides. These were obtained by eluting oligonucleotides from the DEAE-cellulose thin layer and digesting them with RNase A. The resulting fragments of most oligonucleotides were then resolved by electrophoresis on DEAE paper (11) and have been described elsewhere (10).

Each nd virus tested in Fig. 1 contained two oligonucleotide spots (numbered 9 and 12 in PR-B, 8 and 10 in PR-C, and 8 and 10 in B77) which were not present

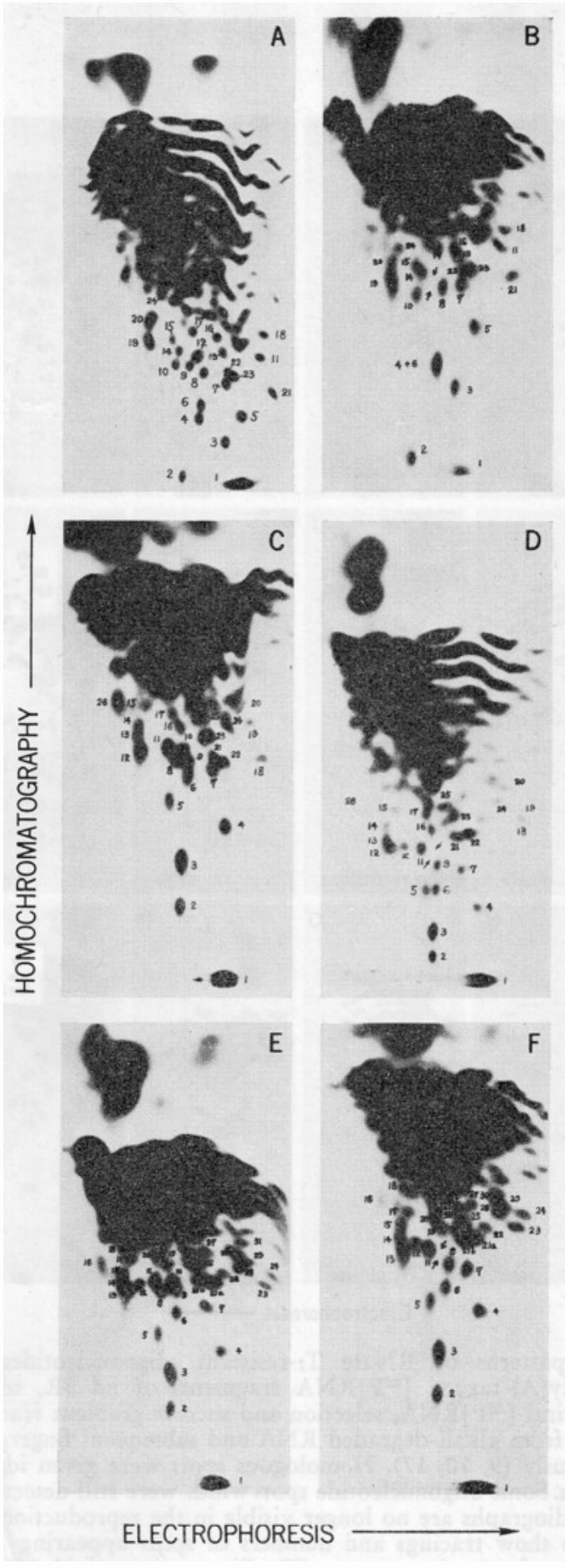


Fig. 1: Autoradiographs of two-dimensional fingerprint analyses of RNase T1-digested 60–70S [^{32}P]RNAs from different nd and td viruses (10): PR-B (A), td PR-B (B), PR-C (C), td PR-C (D), B77 (E), td B77 (F). Conditions for the preparation of viral [^{32}P]RNAs, digestion with RNase T1 and fingerprinting by electrophoresis and homochromatography were described (10). The arrows in B, D, F denote the locations where sarcoma spots would appear.

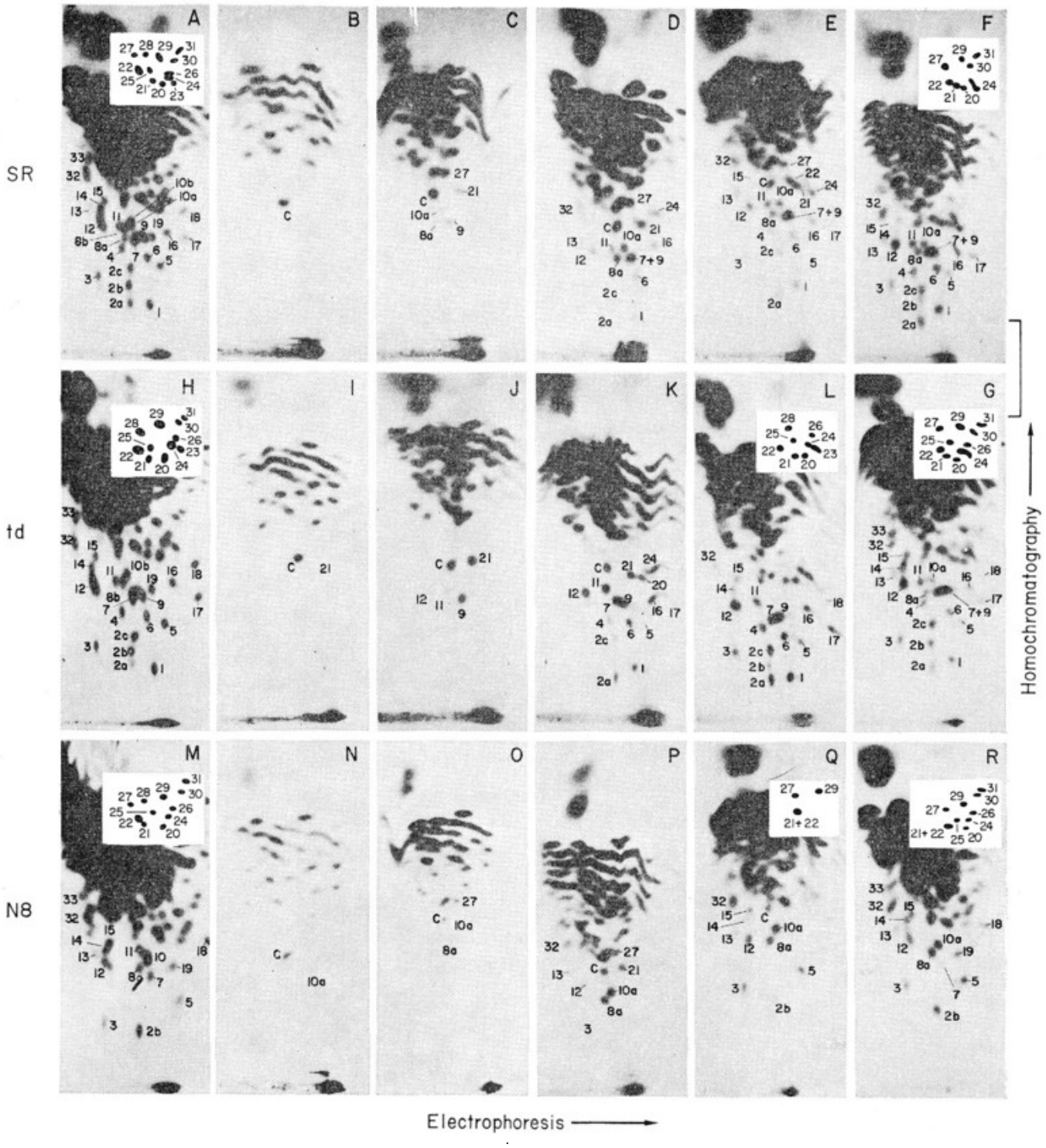


Fig. 2: Fingerprint patterns of RNase T1-resistant oligonucleotides of 60–70S viral [^{32}P]RNAs and poly(A)-tagged [^{32}P]RNA fragments of nd SR, td SR and SR N8 (17). Preparation of viral [^{32}P]RNA, selection and sucrose gradient fractionation of poly-(A)-tagged fragments from alkali-degraded RNA and subsequent fingerprint analysis have been described previously (9, 10, 17). Homologous spots were given identical numbers in all fingerprint patterns. Some oligonucleotide spots which were still detectable and numbered in the original autoradiographs are no longer visible in the reproductions shown here. The inserts in some panels show tracings and numbers of spots appearing in the center right region of the respective fingerprint pattern. SR: Fingerprints of 60–70S nd SR RNA (A) and poly(A)-tagged RNA fragments of 4–10S (B), 10–14S (C), 14–18S (D), 18–22S (E), 22–26S (F) and 26–30S (G). Approximately 35 x 10^6 cpm of [^{32}P]RNA was degraded at

in the fingerprint pattern of the corresponding td virus. These oligonucleotide spots are sarcoma-specific and are thought to come from sarcoma-specific sequences of nd virus RNA. They had homologous positions in each of the three sarcoma viruses compared here. Biochemical analyses indicated that they also contained the same RNase A-resistant fragments and that the faster chromatographing sarcoma-specific oligonucleotide spot of each nd sarcoma virus (no. 12 PR-B, no. 10 PR-C, no. 10 B77) consisted of two chromatographically overlapping oligonucleotides (10). We conclude that three out of about twenty-five large RNase T_1-resistant oligonucleotides of the nd sarcoma viruses are from sarcoma-specific sequences. Sarcoma-specific sequences of nd sarcoma viruses had been estimated previously to represent about 450,000 daltons or about 15 % of the mass of the 30–40S RNA (4, 10, 13).

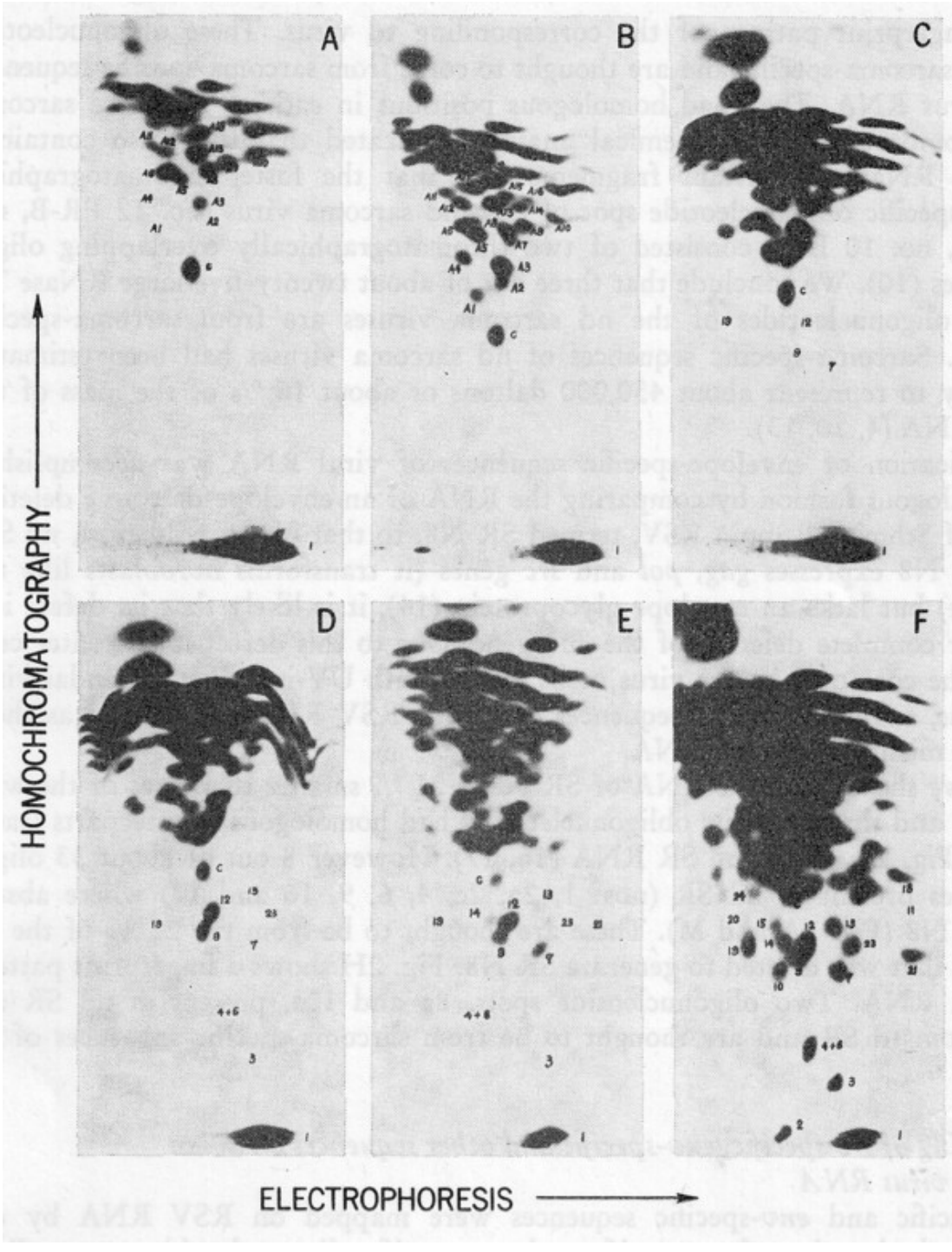
Identification of envelope-specific sequences of viral RNA was accomplished in an analogous fashion by comparing the RNA of an envelope-defective deletion mutant of Schmidt-Ruppin RSV, termed SR N8, to that of the wild type, nd SR. Since SR N8 expresses *gag*, *pol* and *src* genes (it transforms fibroblasts like the wild type) but lacks an envelope glycoprotein (14), it is likely that its defect is a partial or complete deletion of the *env* gene. Due to this defect it can enter cells only in the coat of a helper virus or by fusion with UV-inactivated Sendai virus (14). Thus, envelope-specific sequences of nd SR RSV RNA are defined as those which are missing in SR N8 RNA.

We have shown that the RNA of SR N8 is 21 % smaller than that of the wild type (16) and that all of its obligonucleotides had homologous counterparts (same numbers Fig. 2A, M) in nd SR RNA (16, 17). However 8 out of about 33 oligonucleotides present in nd SR (nos: 1, 2a, 2c, 4, 6, 9, 16 and 17) where absent from SR N8 (Fig. 2A and M). These are thought to be from the 21 % of the nd SR RNA that was deleted to generate SR N8. Fig. 2H shows a fingerprint pattern of td SR RNA. Two oligonucleotide spots, 8a and 10a, present in nd SR are absent from td SR and are thought to be from sarcoma-specific sequences of nd SR.

c) *Mapping of src-specific, env-specific and other sequences on avian tumor virus RNA*

Src-specific and *env*-specific sequences were mapped on RSV RNA by determining the location of *src*-specific and *env*-specific oligonucleotides on an oligonucleotide map. For this purpose viral RNA was randomly degraded by alkali.

pH 11 at 50° for 3 min. Sixteen percent of the starting RNA was recovered as poly(A)-tagged fragments after two cycles of binding and elution from oligo(dT)-cellulose (9, 10). These fragments had a broad distribution with a peak at about 21S after sucrose gradient fractionation (10) done to obtain the size cuts (B-G). td: Fingerprints of 60–70S td SR RNA (H) and of poly(A)-tagged RNA fragments of 4–10S (I), 10–15S (J), 15–20S (K) and 20–28S (L). About 7×10^6 cpm of 60–70S [^{32}P]RNA was fragmented for 6 min as described above, and approximately 11 % was recovered as poly(A)-tagged RNA fragments with an average sedimentation coefficient of 16S. SR N8: Fingerprints of 60–70S SR N8 RNA (M) and of poly(A)-tagged RNA fragments of 4–9S (N), 9–15S (O), 15–20S (P), 20–25S (Q) and 25–30S (R). About 5×10^6 cpm of 60–70S [^{32}P]RNA was degraded for 3 min as above, and 18 % was recovered as poly(A)-containing RNA fragments with an average sedimentation coefficient of 22S.



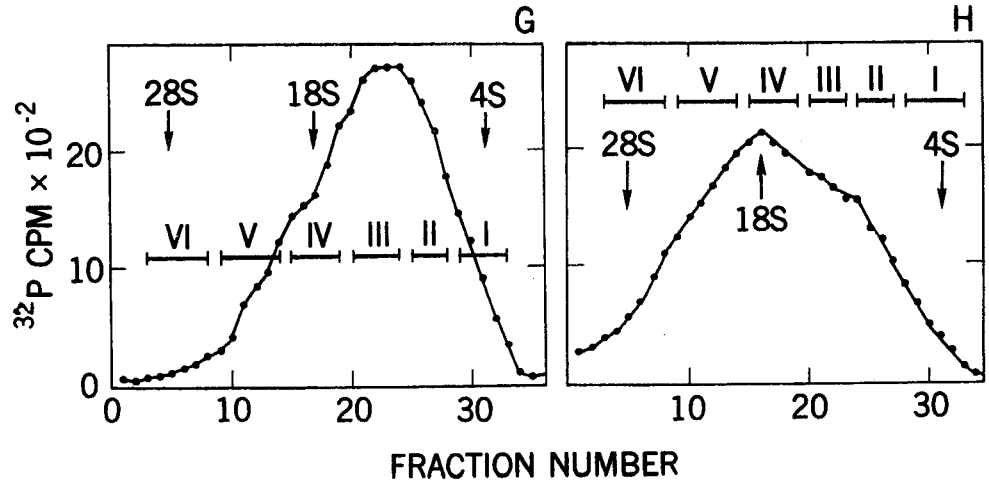


Fig. 3: Autoradiographic fingerprint analyses of poly(A)-tagged PR-B RNA fragments of

Poly(A)-tagged RNA fragments were selected by binding to oligo (dT)-cellulose, fractionated according to size and fingerprinted to detect their oligonucleotides (compare Figs. 2, 3, 5, 7). The map position of a given oligonucleotide relative to the poly(A) end of viral RNA was then deduced from the size of the smallest poly(A)-tagged RNA fragment from which it could be isolated. In this fashion, 20 to 30 large RNase T_1-resistant oligonucleotides were ordered on the basis of their distance grom the 3'-poly(A) end of the RNA to construct oligonucleotide maps (Figs. 2–6). The method has been described in detail (9, 10, 17). Examples of the fractionation of poly(A)-tagged fragments of PR-B RNA and their fingerprints are shown in Fig. 3, and the fingerprints of poly(A)-tagged fragments of nd SR RNA, td SR RNA and SR N8 RNA are shown in Fig. 2. These fingerprints demonstrated clearly that in all cases examined an approximately linear relationship existed between the size of poly(A)-containing fragments and the number of RNase T_1-resistant oligonucleotides they contained. We could deduce the approximate location of each oligonucleotide relative to the 3'-poly(A) end of the RNA from the size of the smallest poly(A)-tagged RNA fragment from which it could be isolated (9, 10). In this way the oligonucleotide maps of nd PR-B, td PR-B, nd PR-C, nd B77 and td B77, shown in Fig. 4, were derived.

It can be seen in Fig. 4 that the *src*-specific oligonucleotides of PR-B (no. 9 and 12), PR-C (no. 8 and 10) and B77 (no. 8 and 10), respectively, cluster together and map very near the poly(A) end of each viral RNA. The same appears to be true for spots 8a and 10a which are thought to be *src*-specific spots of nd SR (Fig. 6). It is concluded that the *src*-gene-specific sequences map near the poly(A) end of viral RNA. It can also be seen in Fig. 4 that the oligonucleotide maps of PR-B and td PR-B, as well as those of B77 and td B77, are the same as far as determinded with the exception of the *src*-specific oligonucleotides which are absent from the td viruses. This implies that the gene order of the nd and corresponding td viruses is the same. A few oligonucleotides of PR-B, PR-C and B77 were found to have homologous chromatographic locations and chemical compositions [compare fingerprints of Fig. 1 and biochemical analyses of Wang *et al.* (10)]. The

various sizes after exhaustive digestion with RNase T_1 as described (10). Approximately 25 x 10^6 to 30 x 10^6 cpm of 60 to 70S [^{32}P]PR-B RNA were partially degraded with Na_2CO_3 at pH 11. and 50 C for 8 min to yield RNA fragments with a peak at 12S (G) and for 4 min to yield RNA fragments with a peak at 18S (H). Poly(A)-tagged fragments were selected by two consecutive cycles of binding to and elution from membrane filters (Millipore Corp.) (10). About 7.3 % of the radioactive RNA was recovered as poly(A)-tagged fragments if degradation was for 4 min (H), and 5.2 % of the radioactive RNA was recovered if degradation was for 8 min (G). Poly(A)-tagged RNA fragments selected from the 8 min (G) and 4 min (H) degradation mixture were fractionated according to size by sedimentation in sucrose gradients as described (10). The radioactivity was determined from an appropriate aliquot of each gradient fraction (G, H). The poly(A)-tagged viral RNA fragments were divided into several discrete pools (I→VI) indicated by horizontal bars in (G) and (H). Sedimentation coefficients of these pools were estimated from the positions of 28S, 18S and 4S chicken cell RNA standards analyzed in a parallel gradient and indicated in (G) and (H) by arrows. Pools of RNA fragments from the two gradients with the same range of sedimentation coefficients were combined, and the RNA of each pool was fingerprinted. The fingerprints of pools I→VI are shown in A→F, respectively. Some oligonucleotide spots which were still detectable and were numbered in the original autoradiographs are no longer visible in the reproductions shown here and in Fig. 2 and Fig. 5.

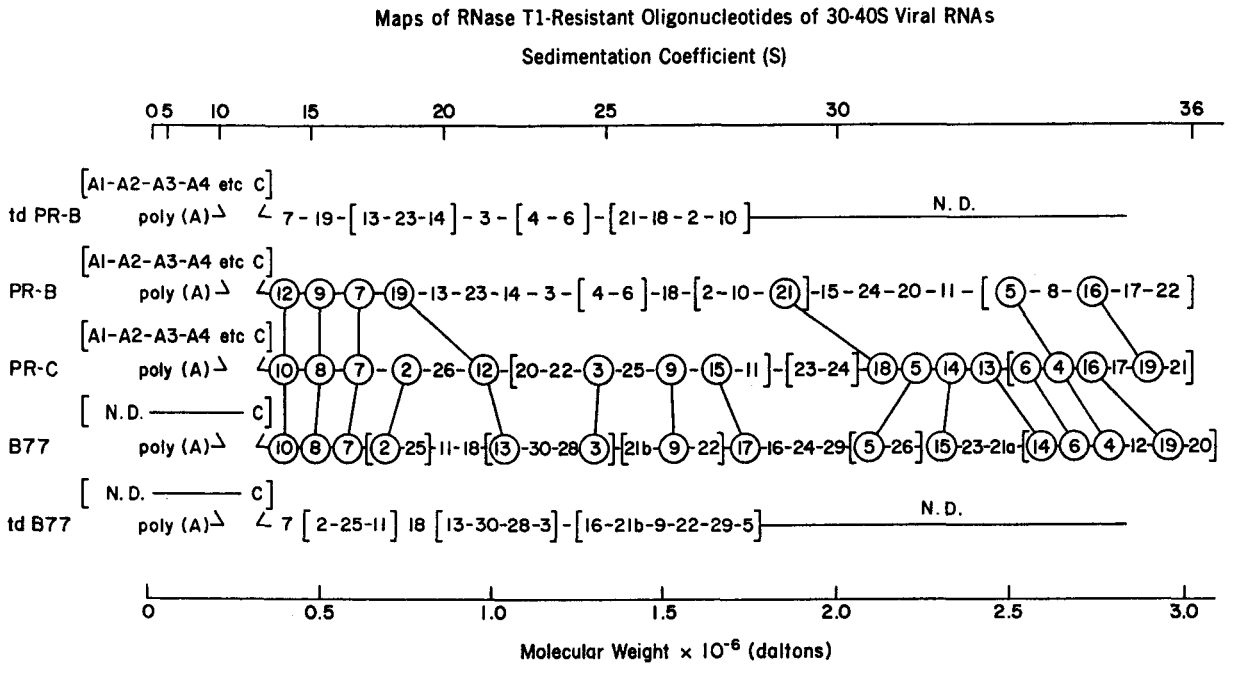
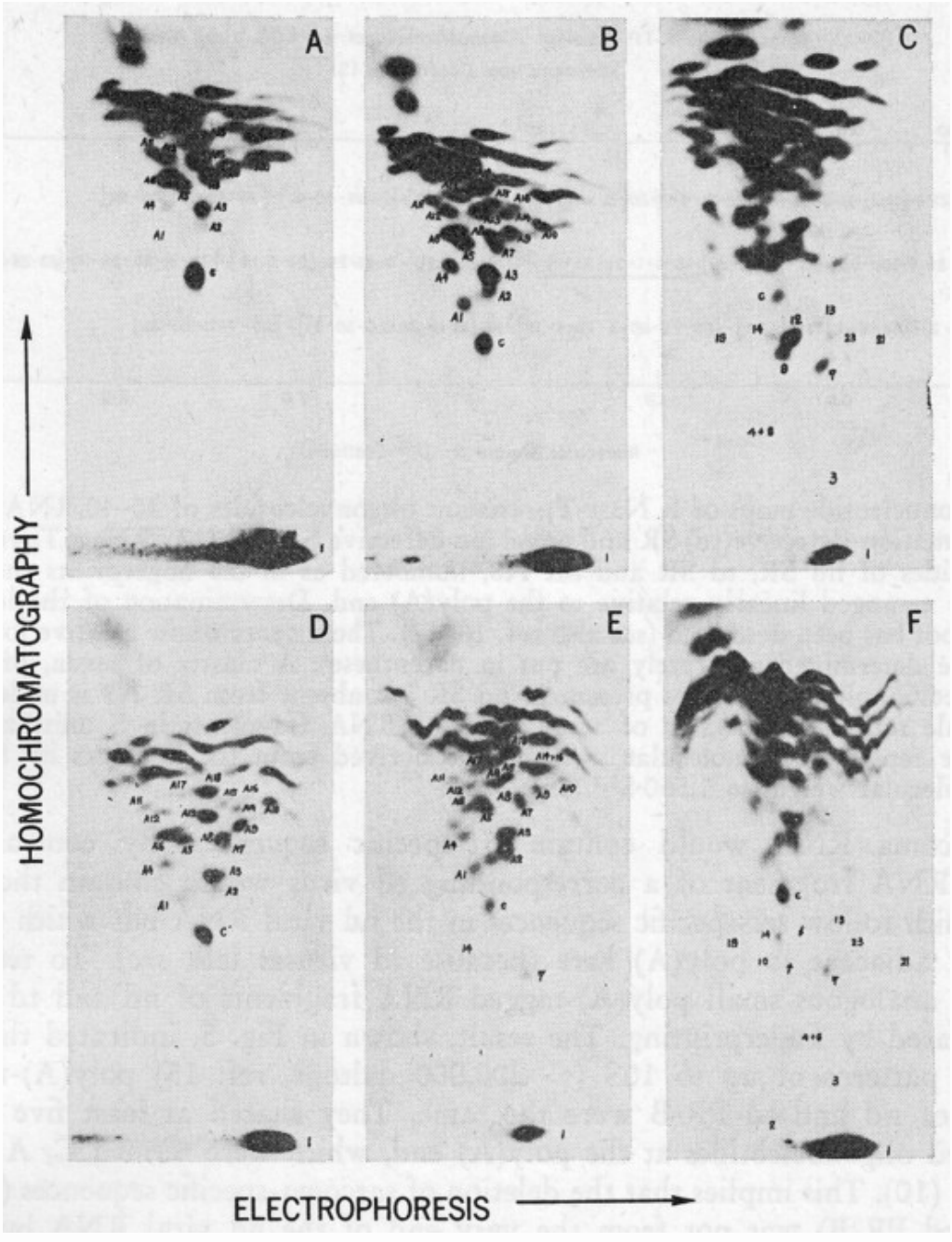


Fig. 4: Maps of RNase T1-resistant oligonucleotides of 30 to 40S viral RNAs (10). RNase T1-resistant oligonucleotides of td PR-B, PR-B, PR-C, B77, and td B77, numbered as in the fingerprints shown in Fig. 1, 3, 5 were arranged linearly relative to the poly(A) end. The map position of a given oligonucleotide was determined from the smallest poly(A)-tagged RNA fragment from which it could be obtained. The relative positions of several oligonucleotides, which first appeared together within a given 5 to 10S cut of poly(A)-tagged fragments (Fig. 3), were estimated by quantitating their molarities. The higher the molarity of a given oligonucleotide, the closer it was placed toward the poly(A) end of a given size cut (see text). Oligonucleotides whose relative map positions within a certain sedimentation range could not be estimated are in parentheses. ND: Not determined. The top scale represents the size of the viral RNA or RNA fragments in S values and the bottom scale represents a molecular weight scale derived from the respective S values by Spirin's formula: molecular weight = $1{,}550 \cdot S^{2.1}$ (15). Those oligonucleotides of the three nd sarcoma viruses that have very similar or identical compositions and sequences (Fig. 1, ref. 10) are circled and connected by vertical lines. The portions of these maps which include the poly(A) segments and the terminal heteropolymeric sequences of about 140,000 daltons, containing the oligonucleotides A1→A4 and C (in parenthesis), are not strictly proportional to the scale of the remaining RNA segments.

oligonucleotides shared by these viruses are circled in the maps of Fig. 4 and connected by lines. Again it can be seen that homologous oligonucleotides also have homologous map locations in the three viral RNAs compared in Fig. 4. This implies that the gene orders of these three different strains of viruses are also closely related.

Given the location of *src*-specific sequences near the poly(A) end of viral RNA, a simple mechanism could be suggested to explain the occurrence of td-deletion mutants from nd sarcoma viruses. This mechanism postulates that the deletion could result from late initiation during transcription from viral RNA to proviral DNA (2) or premature termination during transcription from proviral DNA to viral RNA. If this were correct, one would predict that the terminal heteropolymeric sequences at the 3'-poly(A) end of nd and corresponding td viruses should be different. A small poly(A)-tagged fragment, with the approximate size of the *src*-specific sequences (~ 450,000 daltons, see above and Fig. 4),



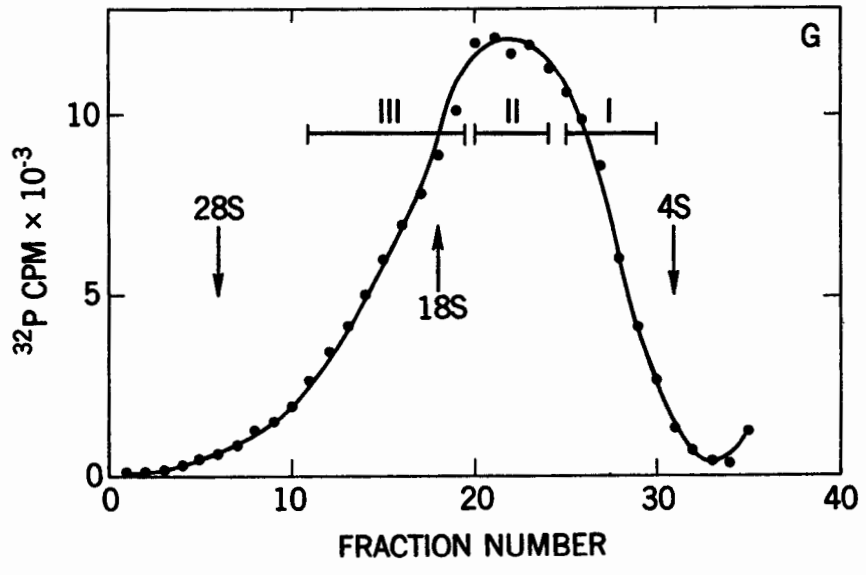


Fig. 5: Fingerprint patterns of RNase T1-resistant oligonucleotides of small poly(A)-tagged fragments of PR-B and td PR-B RNAs (10). 60–70S [^{32}P]PR-B RNA was degraded for

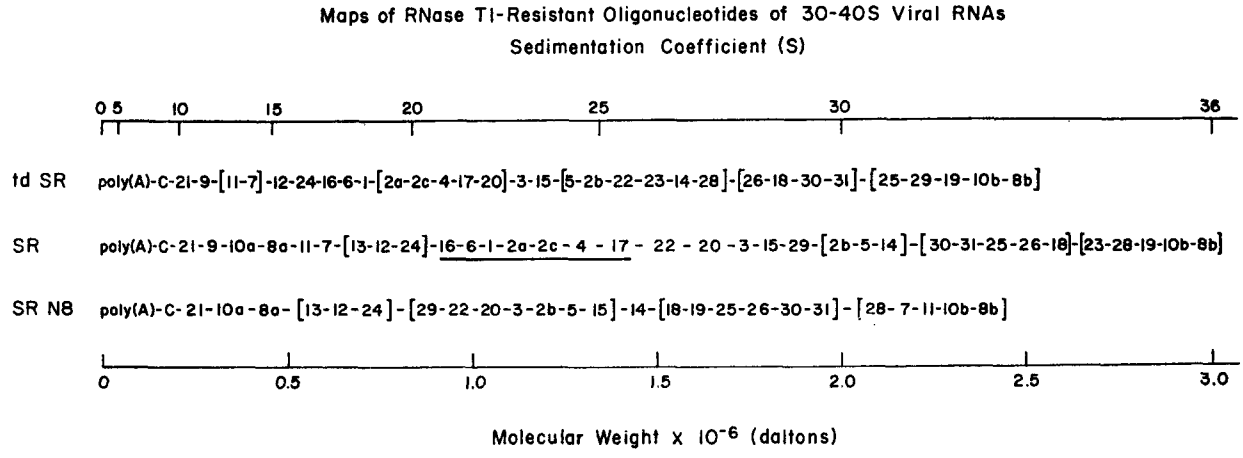


Fig. 6: Oligonucleotide maps of R Nase T_1-resistant oligonucleotides of 30–40 RNAs of nd SR, transformation-defective(td) SR and envelope-defective SR N8 (17). RNase T_1-resistant oligonucleotides of nd SR, td SR and SR N8, numbered as in the fingerprints shown in Fig. 2, were arranged linearly relative to the poly(A) end. Determination of the location of a given spot has been described (see also ref. 16, 17). Those spots whose relative locations could not be determined accurately are put in parentheses. A cluster of seven, probably envelope-specific, oligonucleotides present in nd SR but absent from SR N8 is underlined. The top scale represents the size of viral RNA or RNA fragments in S units and the bottom scale represents a molecular weight scale derived from the S values by Spirin's formula (molecular weight = $1{,}550 \cdot S^{2.1}$ (15).

of nd sarcoma RNA would contain *src*-specific sequences. By contrast, an analogous RNA fragment of a corresponding td virus would contain those sequences which follow *src*-specific sequences in the nd viral RNA but which would be directly adjacent to poly(A) here (because td viruses lack *src*). To test this hypothesis, analogous small poly(A)-tagged RNA fragments of nd and td PR-B were compared by fingerprinting. The result, shown in Fig. 5, indicated that the fingerprint patterns of up to 10S ($\sim$ 200,000 daltons, ref. 15) poly(A)-tagged fragments of nd and td PR-B were the same. They shared at least five intermediatesized oligonucleotides at the poly(A) end, which were termed C, A1, A2, A3 and A4 (10). This implies that the deletion of sarcoma-specific sequences (which generated td PR-B) was not from the very end of the nd viral RNA but was internal and that some 3'-terminal heteropolymeric sequences of the nd virus had been conserved in the deletion mutant. It would follow that the RNA of the nd sarcoma viruses studied here is arranged in this order: poly(A) of about 60,000 daltons (9), a heteropolymeric sequence of about 150,000 daltons which is shared by nd and corresponding td viruses (Fig. 4, 6), about 450,000 daltons of *src*-specific

8 min with Na_2CO_3 and analyzed as described for Fig. 3. Poly(A)-tagged fragments sedimenting between 4 to 7S (A), 7 to 10S (B), and 15 to 20S (C) were fingerprinted after digestion with RNase T_1. About 9×10^6 cpm of 60 to 70S td PR-B [^{32}P]RNA was partially degraded with Na_2CO_3 at 50° for 8 min. About 6.6 % of the starting radioactivity was recovered after two cycles of binding and elution from membrane filters (10). Sedimentation of the poly(A)-tagged fragments is shown in (G). Three pools of poly(A)-tagged fragments were prepared: I (4 to 7S), II (8 to 13S), and III (13 to 23S). Fingerprint analyses of RNase T_1-digested pools I→III are shown in D→F. The arrows in F denote the locations where the sarcoma-specific spots would appear. The homomixture b used here was preincubated at 60° for 24 hr (10).

Possible structures of recombinants between nondefective (nd) sarcoma and transformation-defective (td) leukosis viruses.*

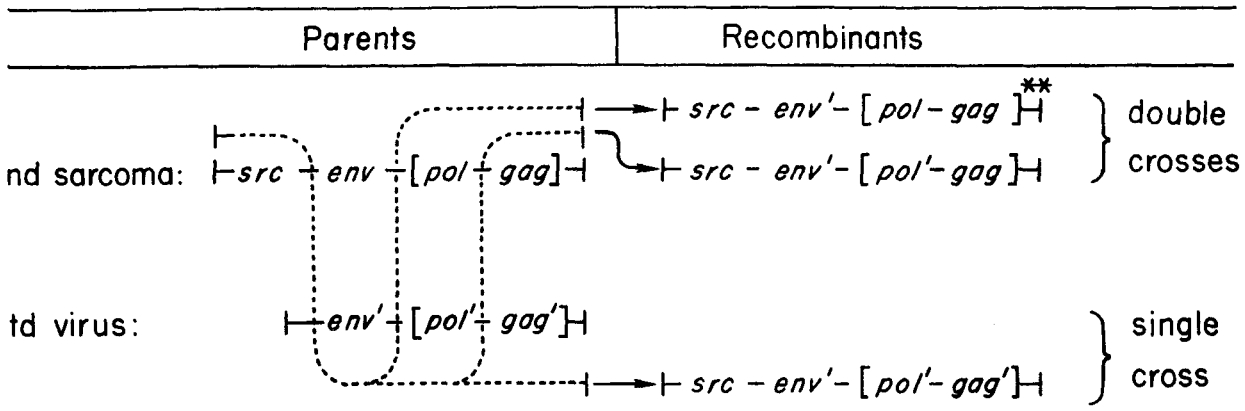


Fig. 7: *Recombinants were selected for the *src* gene of nd sarcoma viruses and the *env* gene of td viruses. Intragenic crossovers and multiple crossovers are not depicted. **Relative map location of *pol* and *gag* genes is unknown.

sequences (Fig. 4), and the remaining 2.4 x 10^6 daltons which should contain *env*, *pol*, and *gag* sequences.

We have used SR N8, the envelope-defective deletion mutant of nd SR, to map *env*-specific sequences in nd SR RNA. The method was the same as that described to map the *src*-specific sequences. Fingerprint patterns of poly(A)-tagged RNA fragments of nd SR, SR N8 and also of td SR of various sizes are shown in Fig. 2, and the resulting oligonucleotide maps are shown in Fig. 6. It can be seen in Fig. 6 that seven out of eight presumably *env*-specific oligonucleotides map together (nos. 16, 6, 1, 2a, 2c, 4 and 17) in a cluster that is about 0.9–1.6 x 10^6 daltons away from the poly(A) end. It may be concluded that *env*-specific sequences of nd SR map in this region of the RNA. In addition *src*-specific oligonucleotides of nd SR (nos. 8a, 10a in Fig. 2, 6), like those of other nd sarcoma viruses (Figs. 3, 4, ref. 10), appear to map near the poly(A) end of viral RNA. It would follow that *src*-specific sequences map between the poly(A) end and *env*-specific sequences of nd SR. Since we do not know whether SR N8 is a complete or perhaps only a partial deletion of the *env* gene, we can only define a minimal map segment for the *env* gene with this virus. Further deletion mutants or recombinants (see below) would be required to determine the exact map segment of the *env* gene. The remaining oligonucleotide (no. 9) which is present in nd SR but absent from SR N8 may be the result of a point mutation that set apart the two viruses after the original deletion had occurred (16, 17).

In summary, we may deduce the partial map, poly(A)-*src*-*env*-, for nd sarcoma virus RNAs by comparing their oligonucleotide maps to those of related deletion mutants.

Fingerprint analyses of short poly(A)-tagged RNA fragments of nd SR, SR N8 and td SR were found to look very similar, and all contained oligonucleotide spot C (compare Figs. 2B, I, N and C, J, O). This is analogous to the finding described above that nd sarcoma viruses and corresponding td viruses also have the same terminal heteropolymeric sequences (Fig. 4) (compare also refs 10 and 17).

d) *Distribution of env-specific and src-specific sequences from different parents in the RNAs of avian tumor virus recombinants*

Recently we have started to map genes of avian tumor virus recombinants (5) by comparing the oligonucleotide maps of recombinants to those of their parents. The recombinants used had inherited an *env* gene from a td or leukosis virus parent and a *src* gene from a nd sarcoma virus parent. *Src* and *env*-specific oligonucleotides of the recombinants can be identified by correlating parental and recombinant *src* or *env* markers with the respective segments of their oligonucleotide maps.

All of these recombinants must have at least one crossover point between the *src* gene (which is near the end of the nd virus) and the *env* gene which appears to be close to *src*, but is perhaps not exactly adjacent (Fig. 5, ref. 17). The crossover point between the *src* and the *env* genes should approximately define the 5' end of the *src* gene and the 3' end of the *env* gene. If double crosses occurred, the 5' end of the *env* gene could be approximately defined by analysis of the second crossover point as outlined in the scheme on Fig. 7.

Preliminary mapping experiments of several recombinants between Prague RSV-A and leukosis virus RAV-2, and Prague RSV-B and leukosis virus RAV-3 (5), suggest that all recombinants selected for the *src* gene of the nd virus and the *env* gene of the leukosis virus parent contain a cluster of three to four *src*-specific oligonucleotides mapping in about the first 0.6×10^9 daltons away from the 3' end of their RNAs. The remainder of the oligonucleotide map of some recombinants was completely colinear with that of the leukosis virus parent which had donated the *env* gene. Another recombinant RNA contained leukosis virus-specific oligonucleotides only between 0.6 and 1.5×10^6 daltons from the poly(A) end, its remaining oligonucleotides mapping between 1.5×10^6 daltons and the 5' end of the RNA were again derived from the sarcoma parent. It would appear that this recombinant was generated by a double cross between parents and that the leukosis virus-specific oligonucleotides between 0.6 and 1.5 daltons from the poly(A) end include the leukosis virus-specific *env* gene. Based on the analyses of these recombinants their *env* and *src* genes can be tentatively ordered to yield the partial map: poly(A)-*src*-*env*-. This map is in good agreement with that derived above from comparative analyses of nd viruses and their deletion mutants.

Discussion

A genetic map of avian tumor viruses: A tentative map emerges from the experiments described here for nd avian sarcoma viruses, beginning at the poly(A) coordinate in molecular weight units ($\times 10^{-5}$): 0–0.6, poly(A); 0.6–2, heteropolymeric sequences shared with corresponding td and envelope-defective viruses; 2–6.5, *src*-specific sequences; 6.5–9, a segment of RNA which may have a distinct function or may be part of the envelope gene (Nd SR and SR N8 share oligonucleotides (nos. 12 and 24, Fig. 6) in this map position, but SR N8 may not represent a complete deletion of the *env* gene.); 9–16, *env*-specific sequences; 16–30, *pol*-specific and *gag*-specific sequences which have not been mapped so far. Further experiments analyzing different deletion mutants and viral recombinants will be used to map the functions described more precisely and to map the two remaining genes, *pol* and *gag*, of tumor virus RNA.

Generation of deletion mutants: It was observed that the oligonucleotide maps of all td-deletion mutants and of the *env*-defective mutant SR N8 show deletions from within the RNA, rather than from the ends as would be expected for deletions arising from transcriptional errors. This suggests that the deletion mutants we have analyzed may be the products of recombinational events occurring at the level of proviral DNA. A proviral DNA intermediate could, by formation and subsequent elimination of loops, delete any sequences from the genome with equal probability.

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Note added in proof: More complete data on mapping *src* and *env*-genes in vival-recombinants have since been published by us (18, 19). In addition we have mapped the *pol*-gene of a temperature-sensitive polymerase mutant and of recombinants derived from it and have proposed poly(A)-*src-env-pol-gag* as the complete genetic map of nd RSV (19).

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