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# T1 Oligonucleotide Maps of N-, B-, and B → NB-Tropic Murine Leukemia Viruses Derived from BALB/c

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We previously described and characterized RNase T1 RNA fingerprints of an N-, a B-, and five B → NB-tropic murine leukemia viruses derived from BALB/c mice (Faller and Hopkins, J. Virol. **23**:188-195, 1977, and J. Virol. **24**:609-617, 1977). These viruses share the majority of their large RNase T1-resistant oligonucleotides, but each possesses some "unique" oligonucleotides relative to the others. We have ordered the large T1-resistant oligonucleotides of the N-, the B-, and one NB-tropic virus relative to the 3′ end of their genomes to obtain oligonucleotide maps. These maps indicate that (i) the large T1 oligonucleotides shared by the N-, B-, and NB-tropic viruses probably occupy the same relative positions on their genomes; (ii) the 14 T1 oligonucleotides that differ between the N- and B-tropic viruses are derived from regions scattered along the genomes; and (iii) an oligonucleotide that is present in five NB-tropic viruses but not in their B-tropic virus progenitors lies toward the 5′ end of the NB-tropic virus oligonucleotide map.

Both N- and B-tropic leukemia viruses can be obtained from BALB/c mice. N-tropic virus can be induced from cultured BALB/c fibroblasts with BUdR (1) or can be obtained from extracts of spleens of nondiseased mice (8, 9). B-tropic virus can be obtained from extracts of spleens of adult nondiseased mice and from spontaneous or chemically induced tumors of BALB/c (9, 20). Genetic evidence combined with hybridization studies indicates that a single locus specifies the inducibility of N-tropic virus in the BALB/c mouse and that this locus is probably integrated proviral DNA (22, 26). On the basis of further analysis of this type, Aaronson and Stephenson and their collaborators have recently suggested that the B-tropic virus of BALB/c may either be specified by genes linked to the N virus-inducing locus, or, alternatively, may be derived from the N-tropic virus, perhaps by mutation, recombination, etc. (22). Consistent with this hypothesis, the genomes of N- and B-tropic viruses of BALB/c were found to be >90% homologous by hybridization analysis (2, 22), and we have shown that fingerprints of the RNA of an N- and a B-tropic isolate derived from the spleen of an adult BALB/c mouse are very similar: they share approximately 30 of 36 to 38 large T1-resistant oligonucleotides (6).

NB-tropic viruses can be derived from B-tropic viruses by forced passage of the B-tropic virus through nonpermissive Fv-1$^n$ mouse cells in vitro (14, 18). We reported previously that five NB-tropic viruses obtained by forced passage of B-tropic virus of BALB/c through NIH Swiss fibroblasts are genetically very similar to their B-tropic virus progenitors as determined by RNA fingerprinting (5): the B- and NB-tropic viruses share 33 to 34 out of 35 to 36 of their large RNase T1-resistant oligonucleotides. However, these NB-tropic viruses possess a (common) new oligonucleotide relative to their B virus progenitor and have all lost one (and the same) B virus oligonucleotide. The possibility existed that these changes might be related to the genetically stable change from B- to NB-tropism.

Our motivations in using RNA fingerprinting to analyze the genomes of N-, B-, and NB-tropic viruses of BALB/c have been twofold. (i) Our first motive was to obtain physical markers for the phenotypic and biochemically defined differences that have been demonstrated between these viruses. The new oligonucleotide in NB-tropic viruses is potentially one such marker for NB-tropism. The particular N- and B-tropic isolates studied, designated SP-N (11) and LP-B (11, 15), respectively, differ in their tropism (9, 17, 21), XC plaque morphology (24), electrophoretic mobility of three virion proteins (p15, p30, and gp70) (25), and ability to induce the $G_{IX}$ antigen (27) on infected cells (12, 19). Because we have isolated recombinants between SP-N and LP-B (6, 12, 14, 25), the oligonucleotides that are unique to SP-N versus LP-B are poten-

tially useful physical markers for these differences. (ii) Our second motive was to investigate relationships between the genomes of endogenous viruses.

For these purposes it is necessary to know as precisely as possible the physical position on the genomes of the oligonucleotides that differ between N-, B-, and NB-tropic viruses. As a first step in this direction, we have ordered the large RNase T1-resistant oligonucleotides of these viruses relative to the 3′ end of the viral RNA by using methodology similar to that established by Duesberg and his collaborators (28) and by Coffin and Billeter (3) in analogous studies with avian type C viruses. Here we present the oligonucleotide maps obtained from these experiments and consider these maps in terms of the objectives outlined above.

## MATERIALS AND METHODS

**Cells and viruses.** The origin and methods of propagation of the SP-N (11), LP-B (14, 15), and NB-tropic (14, 5) viruses have been described. Briefly, SP-N and LP-B are clonal isolates derived, respectively, from N- and B-tropic viruses obtained from the spleen of a single nondiseased adult BALB/c mouse. SP-N virus used in these studies was obtained from chronically infected NIH/3T3 cells, LP-B and NB-tropic (NB-ES-clone 745) viruses from chronically infected BALB/3T3 cells. The particular cell line (NIH/3T3, BALB/3T3, or Sc-1) used to obtain $^{32}$P-labeled viral RNA has not been found to affect the T1 fingerprint of the N-, B-, or NB-tropic viral RNAs (5 and unpublished data).

**RNA preparation and fingerprinting.** These procedures have been described in detail previously, as have the techniques used to identify and characterize the oligonucleotides (5). Quantitation of the radioactivity in the excised gel disks containing the oligonucleotides was determined by Cerenkov counting of the disk or by scintillation counting of the disk after addition of 5 ml of 89% toluene-based scintillation fluid (Econofluor, New England Nuclear Corp.)–10% Protosol (New England Nuclear Corp.)–1% water and heating to 60°C for 4 h.

**Partial fragmentation of viral RNA.** The procedure described by Coffin and Billeter (3) was used. Briefly, RNA samples were adjusted to 400 μg of RNA/ml of water with carrier RNA and heated to 50°C. $Na_2CO_3$ was added to 0.05 M, and the solution was incubated at 50°C for the desired length of time. The hydrolysis was stopped by the addition of Tris-hydrochloride (pH 7.5) to 0.1 M. The solution was then either precipitated with 2 volumes of ethanol or layered directly on a 15 to 30% sucrose gradient as described previously (5) and centrifuged in a Beckman SW41 rotor for 5 h at 36,000 rpm, 20°C. Fractions (0.4 ml) of the gradients were collected, and the appropriate fractions were pooled. A 100-μg amount of carrier RNA was added to each sample of pooled $^{32}$P-labeled RNA along with 2 volumes of ethanol, and the samples were stored at −20°C.

**Selection of poly(A)-containing fragments.** For selection of polyadenylic acid [poly(A)]-containing fragments, chromatography on polyuridylic acid [poly(U)]-Sephadex was performed essentially as described by Coffin et al. (4). Briefly, columns of 0.2-ml bed volume were equilibrated with NaCl-Tris-sodium dodecyl sulfate (SDS) buffer (0.5 M NaCl–10 mM Tris-hydrochloride [pH 7.5]–0.2% SDS). RNA samples were dissolved in 250 μl of a buffer containing 20 mM Tris-hydrochloride (pH 7.5)–5 mM EDTA–0.2% SDS, heated in a boiling-water bath for 30 s, cooled, and adjusted to 0.5 M NaCl. The sample was allowed to flow into the column, and the column was washed with 2 ml of NaCl-Tris-SDS buffer. A 1-ml amount of 90% formamide–50 mM Tris-hydrochloride (pH 5.5)–0.5% SDS was used to elute the bound RNA. This bound fraction was precipitated by the addition of 100 μg of carrier RNA, EDTA to 50 mM, and 2 volumes of ethanol.

**Ordering of the oligonucleotides.** The technique used to order the oligonucleotides with respect to each other and to the [poly(A)-containing] 3′ terminus of the genome is essentially the same as described by Wang et al. (28). $^{32}$P-labeled 35S viral RNA was degraded into fragments of various sizes by treatment with alkali, or the natural radioactive decay of the $^{32}$P was allowed to fragment the RNA. The poly(A)-containing fraction of this fragmented RNA is enriched for those oligonucleotides that lie closer to the 3′, poly(A)-containing terminus. The radioactivity of each oligonucleotide was quantitated (see above) in fingerprints of fragmented poly(A)-terminated RNA, and oligonucleotides were ordered by plotting them according to their increasing relative molar yields as a function of increasing distance from the 5′ end of the map.

We found, however, that the slope of such a plot using fragmented RNA of a large heterogeneous size distribution was often too shallow to effectively order the oligonucleotides. More accuracy and steeper slopes were achieved by selecting smaller size distributions of the fragmented RNA by velocity sedimentation before poly(A) selection and fingerprinting each of these more homogeneous fractions separately.

Before determination of molar yield, the radioactivity of each oligonucleotide was normalized to correct for the effect of the oligonucleotide chain length on the total radioactivity present. By using a fingerprint prepared from 70S RNA, the normalization factor for each oligonucleotide was established by determining the ratio of the radioactivity in that oligonucleotide to the radioactivity in a "reference" oligonucleotide (usually oligonucleotide 14, see Fig. 1 and 6) that was known to lie near the 3′ terminus of the genome. The molar yield of each oligonucleotide from each fingerprint of size-selected, poly(A)-containing RNA was then determined by expressing the ratio of radioactivity in that oligonucleotide to the radioactivity of the reference oligonucleotide (which should always be present in molar amount) and dividing by the normalization factor. The molar yield was expressed as a percentage of one molar yield.

**Limitations of the methods of T1 RNA fingerprinting and oligonucleotide mapping in these studies.** T1 RNA fingerprinting has the disadvantage that the large RNase T1-resistant oligonucleotides

appearing in a fingerprint represent only a small fraction (approximately 5%) of the viral genome. Thus, considerable differences in sequence between viruses may go undetected, and it is quite possible that one may not have T1 oligonucleotide markers for biological properties of interest.

When considering the implications of the oligonucleotide maps presented here, the following limitations of the method of mapping employed should be kept in mind. (i) Comparison of the results obtained in independent experiments indicates that in general the relative positions of adjacent oligonucleotides should only be considered accurate to within ±1 to 2 oligonucleotides and that, as expected, the inaccuracy of oligonucleotide positions is greater for oligonucleotides located toward the 5′ end of the genome (±2 to 3 oligonucleotides). (ii) An oligonucleotide map does not necessarily reflect physical distances accurately: only if the oligonucleotides were distributed perfectly evenly along the genome would numbers of oligonucleotides accurately reflect lengths of RNA. However, it should be noted that experiments designed to detect extreme divergence from a random, even distribution of T1-resistant oligonucleotides along the genome (unpublished data; 27; and Coffin, personal communication) indicate that extreme deviations may not be common and that approximate physical positions of oligonucleotides based on the assumption of an even distribution are not, in general, misleading.

## RESULTS

**Background.** For the purpose of this discussion, we have reproduced the (previously published) fingerprints of SP-N, LP-B, and an NB-tropic virus derived from the B-tropic virus of BALB/c in Fig. 1 and 2 (5, 6). The diagrams of the fingerprints of SP-N and LP-B are drawn so as to indicate which oligonucleotides are shared by these two closely related viruses (open circles, Fig. 1C and D), which are present in SP-N but not LP-B (black circles, Fig. 1C) and which are present in LP-B but not SP-N (cross-hatched circles, Fig. 1D). Note that spot 12 of SP-N is represented as a half-black, half-open circle in

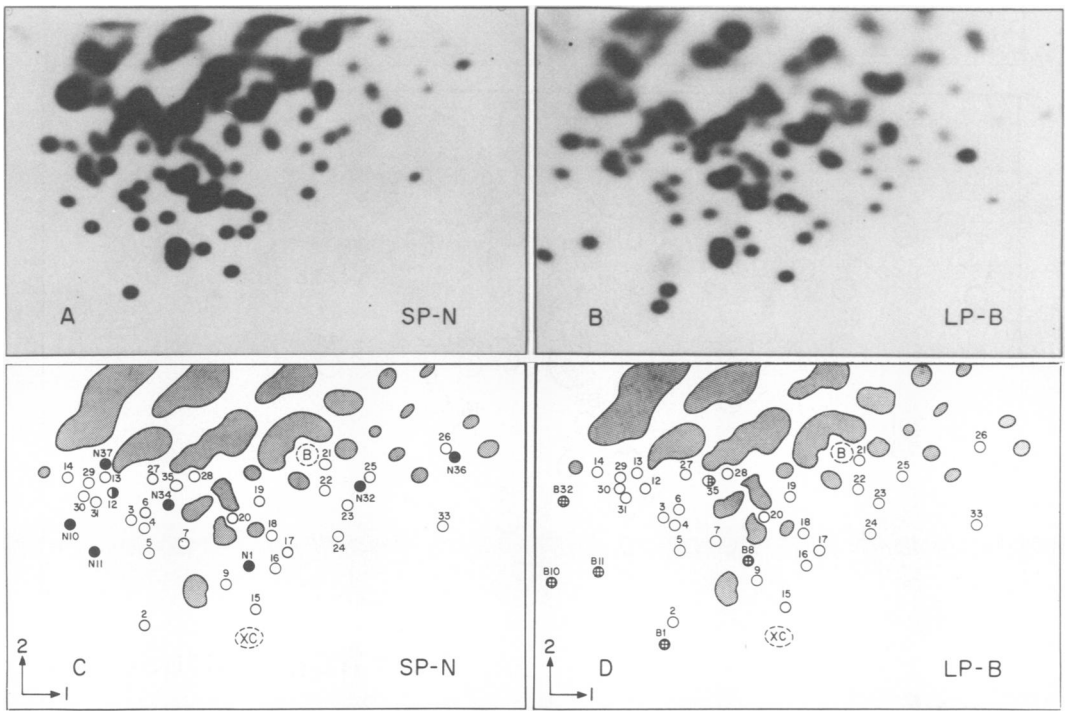


FIG. 1. *Autoradiograms (fingerprints) of RNase T1-resistant products from $^{32}$P-labeled 70S RNA of SP-N and LP-B after two-dimensional gel electrophoresis. T1 RNA fingerprints of (A) SP-N and (B) LP-B viral RNAs and diagrams of the fingerprints of (C) SP-N and (D) LP-B (6). Large T1-resistant oligonucleotides that are present in molar amount (except spots 12 of SP-N and 35 of LP-B, which are present in twice molar amount) are numbered in the diagrams (as in reference 6). Open circles represent oligonucleotides that are present in fingerprints of both SP-N and LP-B. Black circles in C represent oligonucleotides present in SP-N but not LP-B; cross-hatched circles in D represent oligonucleotides that are present in LP-B but not SP-N. See text for explanation of symbols representing spots 12 of SP-N and 35 of LP-B. Arrows in C and D indicate direction of migration in first and second dimensions of the gel electrophoresis. "XC" and "B" indicate positions of dye markers xylene cyanol FF and bromophenol blue, respectively.*

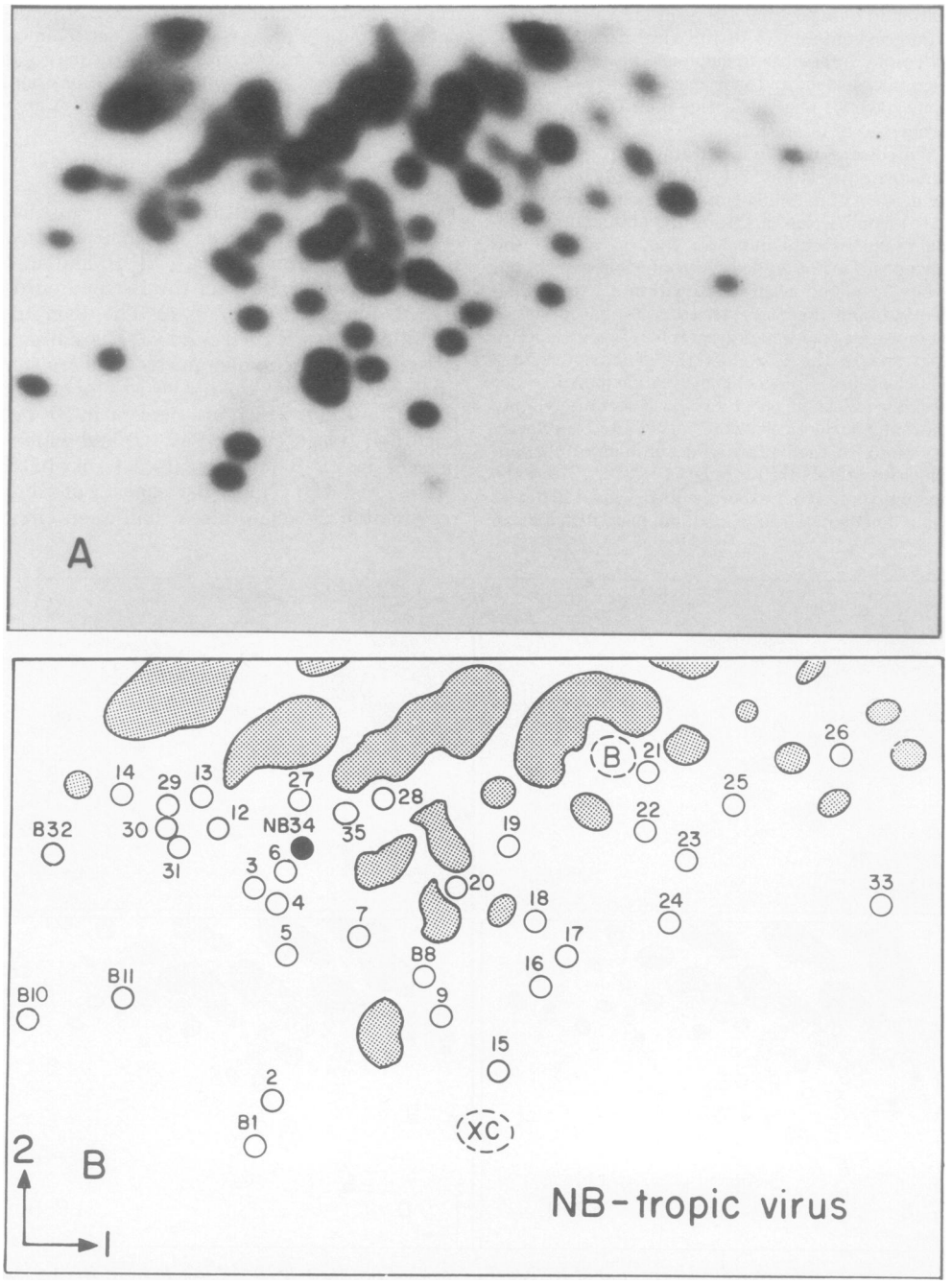


FIG. 2. *T1 RNA fingerprint of an NB-tropic virus derived from the B-tropic virus of BALB/c. Open circles in the diagram (B) of the fingerprint (A) represent oligonucleotides that are shared by the NB-tropic virus and LP-B virus. The black circle represents an oligonucleotide found in five NB-tropic viruses but not in their B-tropic virus progenitors (5). Arrows, XC, and B as in Fig. 1.*

Fig. 1C, and spot 35 of LP-B is represented by a half-open, half-cross-hatched circle. Each of these spots consists of two oligonucleotides that comigrate, one of which is common to SP-N and LP-B and one of which is found only in the virus possessing the "doublet" spot. (Thus, spot 12 of SP-N contains two oligonucleotides designated 12 and N-12, and spot 12 of LP-B contains only

oligonucleotide 12. Spot 35 of LP-B contains oligonucletoides 35 and B-35, and spot 35 of SP-N contains only oligonucleotide 35.)

The diagram of the NB-tropic virus is drawn so as to emphasize which oligonucleotides are shared with the B-tropic progenitor of this virus (open circles, Fig. 2B) and which oligonucleotide is new in the NB virus (black circle, Fig. 2B). (Note that the NB-tropic virus possesses oligonucleotide 35 but lacks B-35 [5].)

**Results of a typical experiment used to construct an oligonucleotide map. Oligonucleotide maps of SP-N, LP-B, and an NB-tropic virus.** The technique used to order the oligonucleotides with respect to one another and to the poly(A)-containing 3′ terminus of the genome is essentially the same as that described by Wang et al. (28) with the modifications described in detail above. The procedure is illustrated by the results of a typical experiment.

Approximately 20 × $10^6$ cpm of $^{32}$P-labeled 70S viral RNA was prepared from NIH/3T3 cells chronically infected with SP-N virus. One portion of RNA was retained for T1 digestion without further treatment. Remaining portions were denatured and then either layered on a sucrose gradient or subjected to alkaline hydrolysis and layered on gradients. Figure 3 shows typical sedimentation profiles of heat-denatured untreated (Fig. 3A) and alkali-treated (Fig. 3B) $^{32}$P-labeled RNA. The regions of the gradients designated fractions I, II, and III were pooled. Poly(A)-terminated RNA in each fraction was bound to a poly(U)-Sephadex column, eluted, and digested with RNase T1, and a fingerprint was prepared. Figure 4A, C, and D shows fingerprints of poly(A)-selected RNA from fractions I, II, and III, respectively. (Figure 4B is a fingerprint obtained from an independent mapping experiment. See legend to Fig. 4.) Figure 5A through C shows a plot of the relative molar yields of the oligonucleotides in the fingerprints of RNA obtained from fractions I, II, and III. It is apparent from Fig. 5 that only those oligonucleotides that fall on a steep gradient of molar yield (regions denoted by bars in Fig. 5) can be ordered relative to one another from this one experiment. (This includes most of the oligonucleotides between positions 1 and 6 in fraction I [Fig. 5A], 14 through 24 in fraction II [Fig. 5B], and 28 through 31 in fraction III [Fig. 5C].) If one repeats this experiment using different extents of alkaline hydrolysis and selects different size classes (sucrose gradient fractions) of RNA, then one can obtain a steep gradient of molar yield over nearly all regions of the genome and, thus, arrive at an oligonucleotide map. Data from four experiments were pooled to obtain the oligonucleotide map of SP-N (Fig. 6A). The

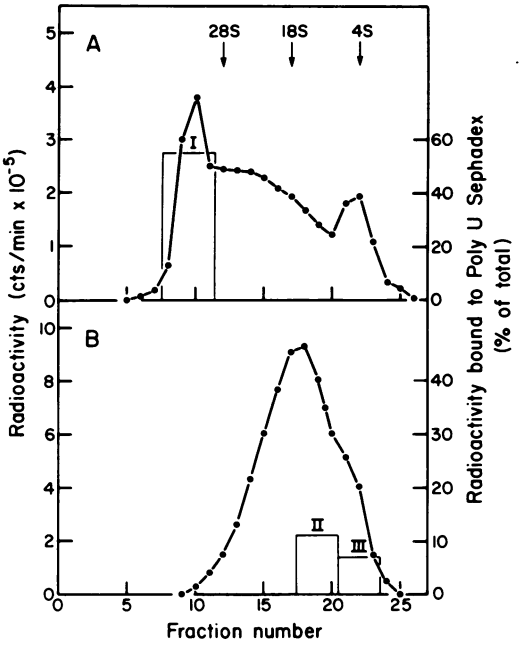


FIG. 3. *Velocity sedimentation profiles on sucrose gradients of untreated and alkali-degraded $^{32}$P-labeled heat-denatured SP-N RNA. $^{32}$P-labeled 70S RNA of SP-N virus was heat-denatured and divided into portions. One portion was layered on a sucrose gradient (A), and one was subjected to alkali-fragmentation (2.5 min, conditions as described in the text) and layered on a gradient (B). $^{3}$H-labeled cellular RNA species were used as markers. Fractions of 0.4 ml were collected from the bottom of the gradients, and their radioactivity was quantitated by Cerenkov counting. Indicated fractions were pooled to yield fraction I (A) and fractions II and III (B). Poly(A)-terminated RNA in fractions I, II, and III was selected by chromatography over poly(U)-Sephadex. The percentage of radioactivity in fractions I, II, and III that bound to poly(U)-Sephadex is indicated.*

same technique was used to establish an oligonucleotide map of NB-tropic virus (six experiments, three different hydrolysis times [Fig. 6B]). Because two independent experiments indicated that the oligonucleotides shared by the NB-tropic virus and LP-B lie in the same relative positions on the genomes of these viruses, we did not attempt to construct an accurate map of LP-B using multiple alkali fragmentation times for the RNA. The "crude" map of LP-B obtained from two experiments is shown in Fig. 6C.

We draw the following conclusions from Fig. 6; (i) oligonucleotides that are shared by the N-, B-, and NB-tropic viruses lie in the same positions on the genomes of these viruses; (ii) the new NB-oligonucleotide, NB-34, lies towards the

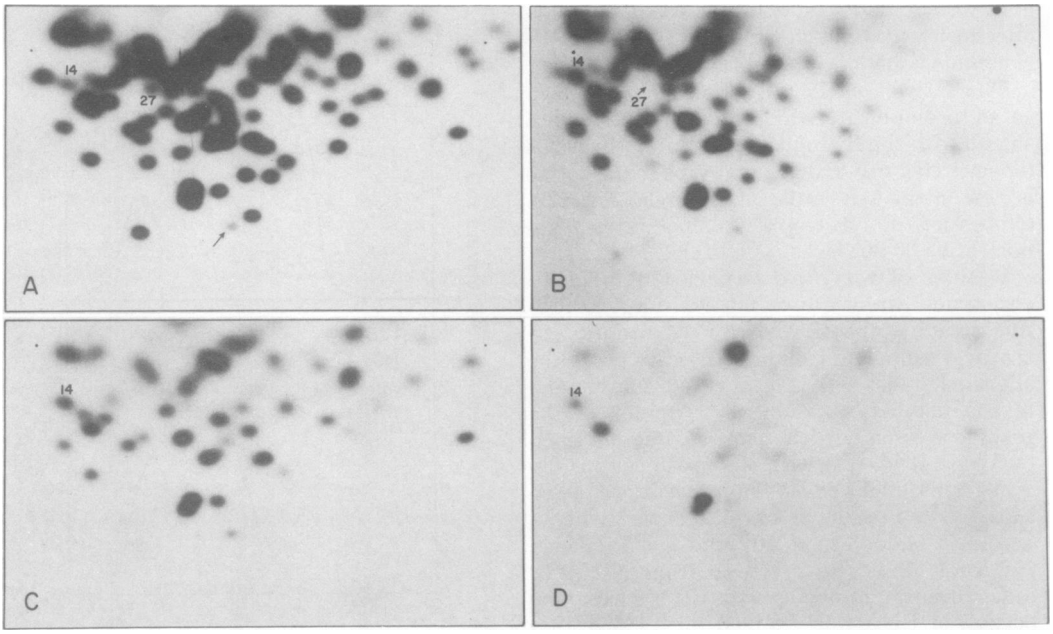


FIG. 4. *T1 RNA fingerprints of fragmented poly(A)-containing SP-N RNA. (A), (C), and (D) are fingerprints of poly(A)-containing SP-N RNA obtained from the fractions I, II, and III, respectively, indicated in Fig. 3A and B. The fingerprint in B was obtained in a different experiment from RNA whose average size was intermediate between that of fractions I and II. It is included to show how use of different size classes of RNA allows oligonucleotide mapping over different regions of the genome. Oligonucleotides that lie further from the 3' end of the genome can be seen to fade in intensity in fingerprints of smaller size classes of poly(A)-selected RNA: oligonucleotide 27 is relatively near the 5' end of the oligonucleotide map and has faded in (A) and disappeared in (B); oligonucleotide 14 is very near the 3' end of the oligonucleotide map and persists in D. Quantitation of the radioactivity present in each oligonucleotide of each fingerprint (see text) is necessary for accurate ordering of oligonucleotides but is in agreement with cruder visual estimations. (Note that when making visual estimations it is necessary to recall that oligonucleotides near the bottom of a fingerprint are larger and, thus, their spots are darker than those of oligonucleotides present in equal amount that lie near the top of the fingerprint.) Note that the fingerprint of poly(A)-selected fraction I RNA (A) appears almost indistinguishable from the fingerprint of 70S RNA of SP-N virus (Fig. 1A); however, careful visual observation and quantitation reveal that several spots have faded in this fingerprint. We noted previously the (variable) presence in fingerprints of 70S viral RNAs of spots that are not present in molar amount relative to the oligonucleotides presumed to be derived from the viral genome (6). One such spot can be seen in several of these fingerprints [see arrow in (A)]. The origin of these "contaminant" spots is unknown (6).*

5' end of the genome; and (iii) oligonucleotides unique to SP-N versus LP-B are derived from regions scattered along the length of the genome of these viruses.

**Determining approximate oligonucleotide map positions for oligonucleotides N-12 and B-35.** Because oligonucleotide mapping relies on accurate quantitation of the radioactivity present in each oligonucleotide, in general only oligonucleotides that appear as well-separated spots in a fingerprint are amenable to being ordered. However, because several lines of evidence have indicated that oligonucleotides N-12 and B-35 are associated with the phenotypes N-tropism and B-tropism (5, 7), it became of interest to attempt to map these oligonucleotides, each of which, as noted above, comigrates with a second oligonucleotide (designated 12 and 35, respectively) that is common to SP-N and LP-B. (The association of oligonucleotide B-35 with B-tropism is suggested by the facts that [i] B-35 is missing in fingerprints of NB-tropic viruses derived from the B-tropic virus of BALB/c [5] and [ii] B-35 was not present in fingerprints of 16 N-tropic recombinants between SP-N and LP-B [7]. The association of N-12 with N-tropism is suggested by the fact that N-12 was present in all the N-tropic recombinants between SP-N and LP-B [7].)This objective was feasible for two reasons. (i) The common oligonucleotides 12 and 35 can be mapped in the virus that possesses oligonucleotide 12 but not N-12 (LP-B or NB-tropic virus) or possesses 35 but not B-35 (SP-N or NB-tropic virus) (Fig. 6).

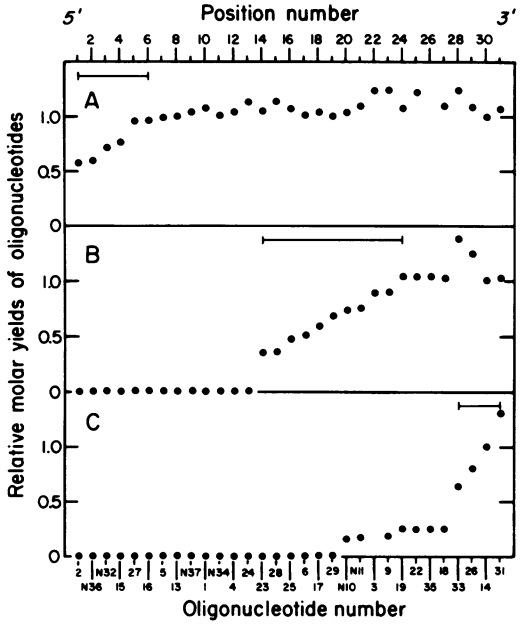


FIG. 5. *Relative molar yields of T1-resistant oligonucleotides in fingerprints of fragmented poly(A)-containing SP-N RNA. Relative molar yields of the large unique T1-resistant oligonucleotides in fingerprints of fragmented poly(A)-selected RNA of different size classes were calculated as described in the text and plotted in increasing amount from left (5' end) to right (3' end). (A), (B), and (C) show the data obtained in this way from the fingerprints shown in Fig. 4A, C, and D, respectively, and thus from the size classes of RNA designated fractions I, II, and III in Fig. 3A and B. The region of each graph indicated by a bar contains a relatively steep gradient of molar yields, and most oligonucleotides lying within these regions can be ordered with respect to one another. "Oligonucleotide number" is that assigned to each oligonucleotide in Fig. 1C (6).*

(ii) The products of pancreatic RNase digestion of oligonucleotide N-12 differ from those of oligonucleotide 12, and similarly, the digestion products of B-35 differ from those of 35. [The oligonucleotide represented by spot 12 of LP-B consists of U, 4C, 3(AC), ($A_2U$), and ($A_4G$), and spot 12 of SP-N consists of 2U, 7C, 6(AC), (AU), ($A_2U$), ($A_3G$), and ($A_4G$) (5, 6). This suggests that oligonucleotide 12 is common to SP-N and LP-B but that SP-N possesses in addition an oligonucleotide N-12 that comigrates with oligonucleotide 12 and has the inferred composition U, 3C, 3(AC), (AU), and ($A_3G$). The oligonucleotide at spot 35 of SP-N or of the NB-tropic virus yields the secondary digestion products 3U, 6C, 2($A_2C$), and (AG), whereas spot 35 of LP-B consists of 4-6U, 7C, 4-6(AC), (AU), 2($A_2C$), and 2(AG) (5, 6). The greater intensity (counts per minute) of spot 35 in fingerprints of LP-B and the presence in this spot of secondary digestion products not found in spot 35 of SP-N or the NB-tropic viruses implies that spot 35 of LP-B contains two oligonucleotides, 35 and B-35, that comigrate. Because of the presence of only one type of G-containing digestion product in spot 35 of LP-B and because of our inability to physically separate oligonucleotide 35 from the postulated B-35, the existence of B-35 had remained tentative (6). However, the experiment described below in which the (AC)-rich component of spot 35 of LP-B was enriched in non-poly(A)-containing RNA while the second-

| | POSITION | 5' 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 3' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | SP-N | ( | 2 | N 36 | N 32) | 21 | ← | ( | 27 | 15) | 5 | 16 | 13 | 7 | N 37 | N (1 | N 34) | ( | 4 | 24 | ( | 23 | 28) | 25 | 17 | | 6 | 29 | N 11 | N 10 | ( | 3 | 9) | 12 | | 22 | 19 | ( | 18 | 35) | 33 | 26 | 14 | 31 |
| | | | | | | | N12 ---------- → | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B | B→NB | ( | 2 | | | 21 | NB 34) | 27 | 15 | | 5 | 16 | 13 | 7 | | B (8 | | 4) | 24 | ( | 23 | 28) | 25 | 17 | B 11 | 6 | 29 | B 32 | B 10 | | 3 | 9 | 12 | B 1 | 22 | ( | 19 | 18 | 35) | 33 | 26 | 14 | 31 |
| C | LP-B | ( | 2 | | | 21 | | 27 | 15) | ( | 5 | 16 | 13 | 7 | | B 8) | | ( | 4 | 24 | ( | 23 | 28 | 25 | ( | 17 | B 11 | 6 | ( | 29 | B 32 | B 10) | ( | 3 | 9 | 12 | B 1 | ( | 22 | 19 | 18) | 35 | ( | 33 | 26 | 14 | 31) |
| | | | | | | | ← ---------- B35 ---------- → | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

FIG. 6. *T1 oligonucleotide maps of SP-N, LP-B, and B → NB-tropic viruses. Relative order of the T1 oligonucleotides of (A) SP-N, (B) B → NB, and (C) LP-B viruses with respect to each other and to the 3' end of the genome. "N," "B," and "NB" indicate oligonucleotides unique to SP-N, LP-B, and the NB-tropic virus, respectively (see Fig. 1 and 2). Those groups of oligonucleotides enclosed within parentheses could not be unambiguously ordered with respect to one another. The positions of oligonucleotides N12 [in (A)] and B35 [in (C)] could not be accurately determined, but data indicate that they lie within the regions enclosed by the arrows (see text). Because mapping experiments indicated that the order of T1 oligonucleotides of LP-B virus was the same as that of the corresponding oligonucleotides of the NB-tropic virus (or SP-N virus), we did not attempt to construct an accurate map of LP-B (see text). Thus, many of the oligonucleotides in (C) are enclosed within parentheses. Oligonucleotides shared by the N-, B-, and NB-tropic viruses were assigned the same map position number because they lie in the same relative order (± 2 to 3 oligonucleotides) on the genomes of these viruses.*

ary digestion products identical to those of the single oligonucleotide 35 were enriched in poly(A)-containing RNA would seem to provide good evidence for the existence of B-35 as well as for the presence of the common oligonucleotide 35 in spot 35 of LP-B.]

We first determined that oligonucleotides N-12 and B-35 lie considerably to the 5′ side of oligonucleotides 12 and 35 in the oligonucleotide maps of SP-N and LP-B by showing that the products of pancreatic RNase digestion of N-12 and B-35 were enriched relative to those of 12 and 35 in fingerprints of non-poly(A)-terminated RNA (5′ enriched) versus poly(A)-terminated RNA (3′ enriched) of SP-N and LP-B, respectively (see above). More accurate placement of oligonucleotides N-12 and B-35 could then be obtained by analyzing fingerprints of fragmented, poly(A)-terminated RNA in which spots 12 (of SP-N) and 35 (of LP-B) approach the intensity (counts per minute) expected if only the single oligonucleotides 12 and 35 are still present in the RNA. At this point, N-12 and B-35 must have disappeared from the doublets and thus be located to the 5′ side of the most 5′ oligonucleotides still retained in this size class of RNA. This analysis (data not shown) indicates that N-12 and B-35 lie within the 5′ third of the oligonucleotide maps of SP-N and LP-B, respectively. Recently (23a) it has become possible to infer the "accurate" oligonucleotide map position of oligonucleotide N-12. The N-tropic ecotropic viruses, Akv-1 and Akv-2, obtained from NIH Swiss mice inheriting either the *Akv-1* or *Akv-2* virus-inducing locus of the AKR mouse, yield identical T1 RNA fingerprints and share a majority of their large RNase T1-resistant oligonucleotides with the N- and B-tropic viruses of BALB/c (23). In particular, they appear to possess both oligonucleotides 12 and N-12 of SP-N. An N-tropic MCF virus (10), apparently a recombinant between Akv-1 or -2 and an as yet unidentified, presumably xenotropic-like virus, has lost oligonucleotide 12 (perhaps via a recombination event) but retains N-12. N-12 lies in the 5′ third of the oligonucleotide map of this MCF virus at a point corresponding to position 5 (±3 oligonucleotides) on the oligonucleotide map of SP-N shown in Fig. 6. This result is in agreement with the approximate position of N-12 determined as described above.

## DISCUSSION

**Consideration of the NB-tropic virus oligonucleotide map.** The previous observation that five NB-tropic viruses independently derived from the B-tropic virus of BALB/c acquire a common T1-resistant oligonucleotide, NB-34, and lose the B-tropic virus oligonucleotide, B-35, had raised the possibility that these changes might be related to the genetically stable change from B- to NB-tropism (5). Furthermore, consideration of the products of pancreatic RNase digestion of oligonucleotides NB-34 and B-35 suggested that the disappearance of oligonucleotide B-35 might be related to the appearance of NB-34 and that this conversion could conceivably occur by a single base change (5). Several lines of evidence, in conjunction with the oligonucleotide maps reported here, support the apparent association of oligonucleotides B-35 and NB-34 with B- and NB-tropism and are at least consistent with the possibility that B-35 may give rise to NB-34.

(i) Oligonucleotide B-35 was not inherited by any (of 16) N-tropic recombinants between SP-N and LP-B and thus might be linked to a (the) determinant of B-tropism (7). (We have not isolated B-tropic recombinants.)

(ii) Oligonucleotide NB-34 has not been found in fingerprints of N- or B-tropic viruses of BALB/c or in recombinants between SP-N and LP-B (25 different viruses examined in all) (5, 6, and unpublished data).

(iii) Oligonucleotides B-35 and NB-34 both lie within the 5′ third of the oligonucleotide maps of B- and NB-tropic viruses, and, thus, it is conceivable that these oligonucleotides are alleles.

Two interesting speculations relating this data to biological observations can be made. (i) If one assumes that B-35 gives rise to NB-34 and that this change reflects the genetic alteration responsible for the change from B- to NB-tropism, then the fact that conversion of B-35 to NB-34 might be accounted for by a single base change could explain the ease with which B-tropic viruses of BALB/c give rise to NB-tropic viruses in vitro after forced passage through Fv-1$^n$ cells (13, 18). (It should be noted that our data do not rule out a recombinational origin of NB-tropic viruses.) (ii) The results of Kashmiri et al. (16) suggest that NB-tropic viruses lack determinants of N- or B-tropism. The concomitant loss of oligonucleotide B-35 and the acquisition of NB-34 in fingerprints of NB-tropic viruses might be consistent with this observation if B-35 and NB-34 are associated with B- and NB-tropism, respectively.

The five NB-tropic viruses whose RNA fingerprints have been examined, as well as three other NB-tropic viruses derived from clones of WN1802B by forced passage through NIH/3T3 cells, all possess a p30 with altered electrophoretic mobility determined by SDS-polyacryl-

amide gel electrophoresis relative to the p30 of their B-tropic virus progenitors (13). Although it is possible that the new oligonucleotide NB-34 and/or the loss of B-35 reflect an alteration in the genome that results (directly or indirectly) in an altered p30 protein, because RNA fingerprinting reveals such a small percentage of the viral genome it is quite possible that the genetic changes responsible for the altered p30's of these NB-tropic viruses are not revealed in their large RNase T1-resistant oligonucleotides. (Evidence for such hidden alterations is provided by the finding that two NB-tropic viruses with identical RNA fingerprints possess p15 proteins with different electrophoretic mobilities [5].)

**Comparison of the oligonucleotide maps of SP-N and LP-B.** SP-N possesses eight large T1-resistant oligonucleotides not found in LP-B, whereas LP-B possesses six T1 oligonucleotides not found in SP-N. Figure 6 shows that these 14 oligonucleotides are derived from regions scattered along the length of the oligonucleotide maps (although there are insufficient data to indicate whether oligonucleotide differences between the two viruses occur preferentially at certain positions on the genome). We noted previously that SP-N and LP-B differ in a number of biological and biochemically defined properties. These include tropism (17, 21), XC plaque morphology (14, 24), the electrophoretic mobility of three virion proteins (p15, p30, and gp70) (25), and the presence of $G_{TX}$ antigen on infected cells (12, 19, 27). From analysis of the RNA fingerprints of recombinants between SP-N and LP-B for the inheritance of these 14 N- and B-specific oligonucleotides, and from the oligonucleotide maps reported here, it has been possible to assign approximate oligonucleotide map positions to some of these phenotypic and biochemically defined differences (7).

It is interesting to note the possibility that some of the oligonucleotide differences between SP-N and LP-B could be the result of single base changes or other minor alterations that occurred during the long tissue culture history of these viruses. We noted previously that three B-specific oligonucleotides, B8, B10, and B32, might be related to three N-specific oligonucleotides, N1, N10, and N11, respectively (6). This possibility was based on two observations. (i) The products of pancreatic RNase digestion of B8 and N1, of B10 and N10, and of B32 and N11 are similar and related in such a way that single base changes could conceivably cause B8, for example, to disappear from a fingerprint and to reappear as N1, etc. (ii) The inheritance of B8 and N1, etc., is mutually exclusive among 16 recombinants between SP-N and LP-B. The possible relationship of these pairs of oligonucleotides receives further (essential) support from the fact that the members of each pair lie at similar, and thus potentially allelic, positions on the oligonucleotide maps. We also noted previously that three other N-specific oligonucleotides (N32, N34, and N36) were not present in the RNA fingerprint of N-tropic virus induced by BUdR from BALB/c cells in culture and, thus, conceivably could have arisen during tissue culture passage of SP-N (6). These considerations suggest that RNA fingerprints of some N- and B-tropic isolates from BALB/c might differ by even fewer oligonucleotides than the fingerprints of SP-N and LP-B.

Robbins et al. have obtained evidence that suggests that B-tropic virus of BALB/c may be derived from the BALB/c N-tropic virus (22). A priori it might seem that consideration of the data in Fig. 6 could rule out at least some mechanisms and possibly suggest mechanisms for how the proposed conversion from N- to B-tropic virus might occur. However, further considerations, including, for example, those raised in the previous paragraph, indicate the difficulties in trying to draw conclusions concerning this hypothesis from the data available to us at present. Analysis of the RNA fingerprints of a number of de novo independent isolates of N- and B-tropic viruses of BALB/c would seem to be a minimum requirement for such speculations. However, if the proposal of Robbins et al. (22) is correct, our data may impose one requirement on its mechanism. As noted above, we have obtained genetic evidence that a (the) determinant(s) of N-tropism resides in the 5′ third of the oligonucleotide map of SP-N (7). Thus, a necessary and perhaps sufficient requirement for conversion from N- to B-tropism might be an alteration in the viral genome at a position corresponding to this portion of the map.

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