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Structure of Products of the Moloney Murine Leukemia Virus Endogenous DNA Polymerase Reaction

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We have investigated the process by which the single-stranded RNA genome of Moloney murine leukemia virus is copied into DNA in vitro. DNA synthesis is initiated near the 5′ end of the genome, and the elongation of the growing chain occurs by a jumping mechanism whereby the DNA synthesized at the 5′ end of the genome is elongated along the 3′ end. Unique DNA fragments synthesized beyond the 5′ end of the genome in vitro have, at their 5′ and 3′ ends, copies of unique sequences from the 5′ and 3′ ends of the genome. These flank a copy of the 49- to 60-nucleotide terminally redundant sequence. These results indicate that the terminal redundancy serves as a "bridge" to allow a DNA molecule synthesized at the 5′ end of the genome to serve as a primer for synthesis from the 3′ end.

An early step in the replication of retroviruses (RNA tumor viruses) is the transcription of the single-strand genomic RNA into DNA by the reverse transcriptase packaged in the virions (16). To understand the process by which the first strand of DNA complementary to the viral genome is synthesized, we have studied this process in vitro with purified Moloney murine leukemia virus (Mo-MuLV). These studies and those of others have shown that DNA synthesis of Mo-MuLV is initiated with a tRNA primer, proline tRNA, that is found near the 5′ end of the genome (3, 8, 12).

The initial major DNA product made in vitro by detergent-disrupted virions is called strong-stop DNA and is a transcript of the 135 ± 10 nucleotides between the primer binding site and the 5′ end of the genome (3, 8). DNA products longer than strong-stop DNA are transcripts of a single RNA sequence (8); that is, the sequences of the shorter DNA products synthesized in the in vitro reactions are subsets of the sequence of the longer molecules, and all the DNA products have the 135 ± 10 nucleotides of the strong-stop DNA at the 5′ ends.

The observation that DNA synthesis is initiated near the 5′ end of the genome means that some mechanism must exist for the elongation of the strong-stop DNA product along the 3′ end of the genome if the entire genome is to be copied. The first suggestion that such a mechanism must exist was made by Taylor and Illmen-see (15) when they demonstrated the 5′ location of the tRNA primer of Rous sarcoma virus.

We have shown elsewhere (3) that the genome of Mo-MuLV contains a terminally redundant sequence 49 to 60 nucleotides long. The genomes of Rous sarcoma virus and avian myeloblastosis virus are also terminally redundant, although the redundant sequences are substantially shorter (4, 9, 13, 14). It is clear that the terminally redundant sequence could be used to elongate the DNA chain past the 5′ end of the genome. The nascent DNA chain synthesized at the 5′ end of the genome could become displaced from the template and pair with the repeated sequence at the 3′ end. This DNA, annealed to the 3′ end, would then serve as a primer for subsequent elongation. Such models have been proposed by a number of workers (2, 6, 7, 10, 14).

These models predict that the DNA products longer than strong-stop DNA are derived by extension of the 5′ sequence along the 3′ end of the genome. Here, we present experiments that test this prediction.

MATERIALS AND METHODS

A 56-mg amount of purified Mo-MuLV virions was used in an endogenous DNA polymerase reaction. The reaction contained 50 mM Tris-hydrochloride (pH 8.3); 60 mM KCl; 0.015% Nonidet P-40; 2 mM dithiothreitol; 6 mM magnesium acetate; 50 μg of actinomycin D per ml; 1 mM dATP, dTTP, and dGTP; and 0.2 mM [α-^{32}P]-dCTP (1.8 Ci/mmol). The virus was added last, to a final concentration of 2 mg/ml. The reaction was kept at 20°C for 90 min.

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The reaction was terminated by the addition of sodium dodecyl sulfate to 0.1% and proteinase K to 0.2 mg/ml. After 30 min the mixture was chromatographed on Sephadex G-50 and eluted with 0.3 M sodium acetate–1 mM EDTA. Fractions containing the ^{32}P which eluted with the void volume were pooled and precipitated with ethanol. The sample was dissolved in 0.3 M sodium acetate and extracted twice with one volume of phenol-chloroform (1:1). The nucleic acid was precipitated with ethanol, and the RNA was hydrolyzed in 0.3 M NaOH–1 mM EDTA at 37°C overnight. After ethanol precipitation, the DNA was purified on a 7.5% polyacrylamide gel, pH 8.3. The DNA fragments were located by autoradiography, cut out, and eluted.

To size the DNA products, 0.01-μg samples were labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The labeled fragments were run on a 20% polyacrylamide gel containing 7 M urea. An *Hae*III digest of φX174 DNA (available from New England Biolabs) was denatured and labeled in a similar way and used as molecular weight markers. The products and yield of the reaction were 1.2 μg of strong-stop DNA (sized at 140 ± 5 bases) and approximately 0.1 μg of each of the following fragments (size in bases): 157, 175, 183, 197, 240, 282, and 470. The amount of DNA of each size class was calculated by determining the amount of ^{32}P (derived from the [α-^{32}P]ATP) of each size class and comparing this value with the total amount of radioactivity in the original reaction. In addition, there were three bands shorter than strong stop, each consisting of approximately 0.1 μg, and approximately 0.7 μg of unresolved material longer than 470 bases. The individual DNA products were judged to be greater than 90% pure. They were visible as distinct bands upon autoradiography of the gel. In similar experiments, the bands were of sufficient purity to permit determination of the sequences (unpublished data).

Mo-MuLV [^{32}P]RNA. 70S RNA was prepared from virus pelleted from the supernatant of virus-producing cells labeled with $^{32}PO_4$ (10 mCi/100 mM culture dish) as described (3). Such preparations had measured specific activities of 5×10^6 to 10×10^6 cpm/μg. To prepare 3′-terminal fragments, the procedure of Coffin and Billeter (2) was followed. A sample of the [^{32}P]-RNA containing 100 μg of repurified yeast RNA was incubated in 0.05 M Na_2CO_3 at 50°C for the indicated times. It has previously been shown that incubation of oncovirus RNA under these conditions yields two breaks per molecule per min (2). We reconfirmed this by polyacrylamide gel electrophoresis of a sample of Mo-MuLV [^{32}P]RNA incubated for 15 min (data not shown). After incubation, the mixture was neutralized and precipitated with ethanol. To isolate 3′ ends, the dried ethanol precipitate was dissolved in 20 mM Tris-hydrochloride (pH 7.5)–5 mM EDTA–0.2% sodium dodecyl sulfate, heated at 100°C for 30 s, and adjusted to 0.5 M NaCl. The sample was applied to a small (approximately 0.1-ml) column of polyuridylic acid–Sephadex-G-10, the column was washed with 0.5 ml of 10 mM Tris-hydrochloride (pH 7.5)–0.5 M NaCl–0.2% sodium dodecyl sulfate, and the polyadenylic acid [poly(A)]-containing RNA was eluted with 90% formamide–10% mM Tris-hydrochloride (pH 7.5)–0.5% sodium dodecyl sulfate. The eluted RNA was precipitated with ethanol.

RNA-DNA hybridizations were performed in 100 μl of 0.5 M NaCl–10 mM Tris-hydrochloride (pH 7.5) containing 20 μg of yeast RNA and the indicated labeled RNA and unlabeled DNA fragments. After annealing at 66°C for 16 to 20 h, the nonhybridized RNA was digested by adding 5 μg of RNase A (Worthington Biochemicals) and 5 U of RNase T_1 and incubating the mixture for 30 min at 37°C. Sodium dodecyl sulfate was added to 0.2%, and the samples were incubated at 66°C for 10 min to denature large nonspecific aggregates. Hybrids were isolated by chromatography on Sephadex G-100 (11).

Fingerprinting of RNA samples by RNase T_1 digestion and two-dimensional gel electrophoresis and analysis and quantitation of oligonucleotides were as described (1, 2).

RESULTS

The strategy of these experiments was to isolate Mo-MuLV DNA products of specific lengths synthesized in vitro by using detergent-disrupted virions and to determine the location on the Mo-MuLV genome of nucleotide sequences complementary to these DNA fractions. It has previously been shown that such DNA fractions fall into discrete size classes all of which share a common 5′-terminal sequence adjacent to the proline tRNA primer (8). These DNA molecules are therefore all elongation products of one another and can be used to monitor the progress of in vitro DNA synthesis along the viral genome.

For the preparation of such a set of Mo-MuLV DNA products of various lengths, purified Mo-MuLV virions were incubated in an endogenous DNA polymerase reaction, and the DNA synthesized was fractionated by polyacrylamide gel electrophoresis. Three fractions of the DNA were taken from the gel and used for all further experiments. These fractions had molecular lengths of 135 ± 10 (strong stop), 175 ± 10, and 240 ± 20 nucleotides.

To localize the sites of synthesis of these DNA products, each was annealed with Mo-MuLV [^{32}P]RNA in slight DNA excess. The annealed mixtures were treated with RNases A and T_1 and the hybrids were isolated by chromatography on Sephadex. The RNA-DNA hybrids so obtained were denatured, and the RNA component was fingerprinted by digestion with RNase T_1 followed by two-dimensional gel electrophoresis (Fig. 1). The conditions of electrophoresis were such that all oligonucleotides 4 or more nucleotides long were present. Oligonucleotides shorter than 4 migrated off the end of the gel. All numbered oligonucleotides were identified both by electrophoretic mobility and by

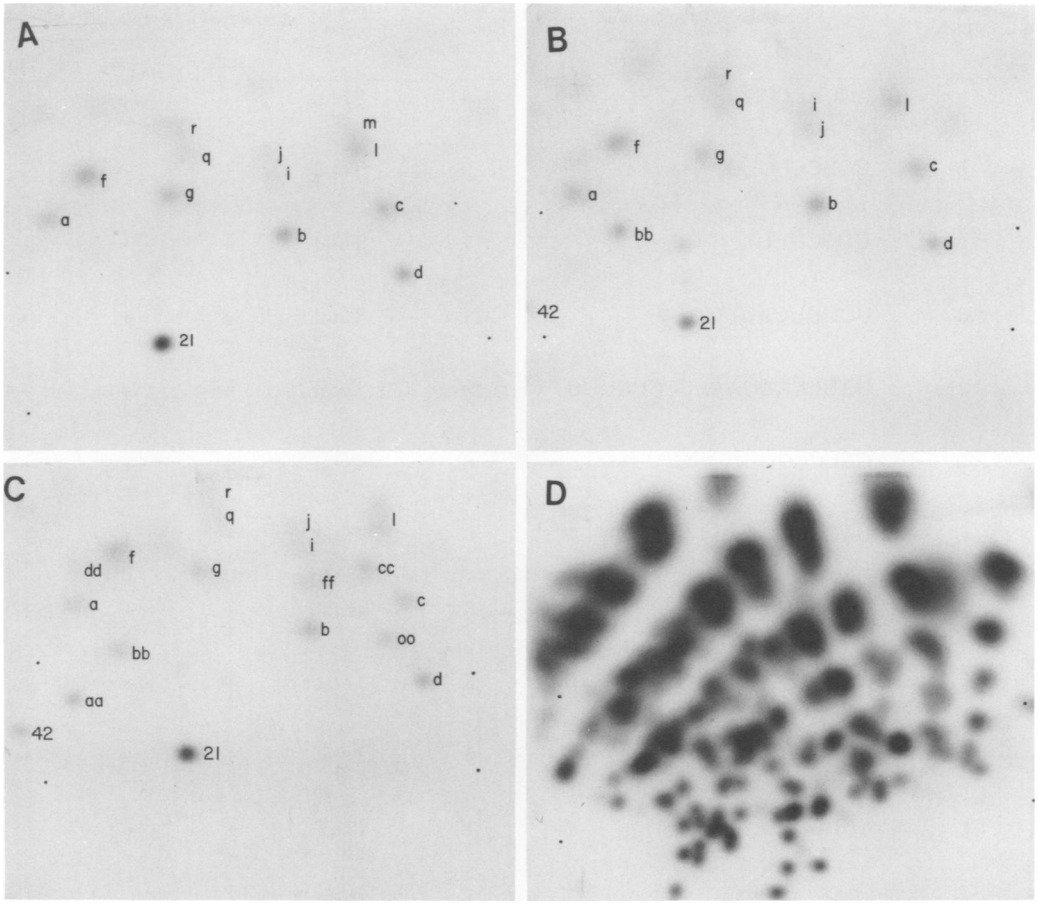

FIG. 1. *Hybridization of Mo-MuLV DNA fragments to total Mo-MuLV RNA. A 0.015-μg amount of each of the DNA fragments was annealed with 0.5 μg (2.5 × 10⁶ cpm) of Mo-MuLV 70S [³²P]RNA, and the resulting hybrids were isolated after treatment with RNases A and T₁ as described previously (3). Each was digested with RNase T₁ and fingerprinted by two-dimensional gel electrophoresis. The hybrids prepared (and percentages of total ³²P recovered in the hybrid fractions) were: (A) with strong-stop DNA (2.2%), (B) with the 175-nucleotide fragment (4.7%), and (C) with the 250-nucleotide fragment (4.7%). For a key to the locations of oligonucleotides, see Fig. 3. All oligonucleotides visible in the autoradiographs were further analyzed by digestion with RNase A (Table 1). (D) Fingerprint of total Mo-MuLV 70S [³²P]RNA prepared under the same conditions. The unmarked oligonucleotides above oligonucleotide 21 are partial digestion products of oligonucleotide 21 (see Fig. 3).*

their products after digestion with RNase A (Table 1). The RNA hybridized to strong-stop DNA (Fig. 1A) contained a set of oligonucleotides previously identified as being within the 5′-terminal 135 ± 10 nucleotides of the Mo-MuLV genome (3). A subset of these oligonucleotides (a, b, c, d, f_1, i, j, and l) is present only near the 5′ end of the genome and is derived from the region between the primer binding site and the terminally redundant sequence. Oligonucleotides 21, f_2, g, and q are derived from the redundant sequence (3). The f_1 and f_2 oligonucleotides are isomers that have the same electrophoretic mobility, and this mixture is labeled f in Fig. 1 through 3. Hybrids with the 175-nucleotide fragment contained the strong-stop oligonucleotides plus two more (42 and bb). Hybrids with the 240-nucleotide-long fragment yielded the same 5′ oligonucleotides as did those with the 175-nucleotide fragment plus 5 more, aa, cc, dd, ff, and oo (Fig. 1C). For comparison, a parallel fingerprint of total Mo-MuLV 70S RNA is also shown (Fig. 1D).

The results of the experiment shown in Fig. 1 are consistent with the previous observation that DNA products of increasing length isolated from endogenous RNA polymerase reactions are elongation of one another (8). The locations on the

TABLE 1. *Oligonucleotides in the Mo-MuLV genome complementary to Mo-MuLV DNA fractions*

Oligonucleotide[a]	Sequence or composition[b]	Length (nucleotides)	RNA fraction[c]	Presence in RNA protected by DNA fraction (no. of nucleotides):		
				135	175	240
a	ACUACCCG	8	Total	+	+	+
			3′	−	−	−
b	UCUCCUCUG	9	Total	+	+	+
			3′	−	−	−
c	UUCCUUG	7	Total	+	+	+
			3′	−	−	−
d	UCUUUCAUUUG[d]	11	Total	+	+	+
			3′	−	−	−
21	UAUCCAAUAAACCCUCUUG	19	Total	+	+	+
			3′	+	+	+
f_1	CAUCCG	6	Total[e]	+	+	+
			3′	−	−	−
f_2	UACCCG	6	Total[e]	+	+	+
			3′	+	+	+
g	CCCUUG	6	Total	+	+	+
			3′	+	+	+
i	ACUUG	6	Total	+	+	+
			3′	−	−	−
j	UCUCG	5	Total	+	+	+
			3′	−	−	−
l	AUUG	4	Total	+	+	+
			3′	−	−	−
m	m^7GpppG_mCG	4	Total	+	−	−
			3′	−	−	−
q	ACUG	4	Total	+	+	+
			3′	+	+	+
r	$(C_2, U)G + UCAG$	4	Total	+	+	+
			3′	−	−	−
aa	A_2U, 2AC, C_{2-3}, U_{1-2}, AG	13–15	Total	−	−	+
			3′	−	−	+
bb	A_4, A_2U, C_{3-4}, U_{1-3}, G	13–15	Total	−	+	+
			3′	−	+	+
cc	U_3, C_2, G	6	Total	−	−	+
			3′	−	−	+
dd	U, C_4, G	6	Total	−	−	+
			3′	−	−	+
ff	U_3, C_3, G	7	Total	−	−	+
			3′	−	−	+
oo	AU, U_4, C_2, G	9	Total	−	−	+
			3′	−	−	+
42	A_2C, 2AC, 9C, 2U, G	18	Total	−	+	+
			3′	−	+	+

TABLE 1—continued

[a] Numbering corresponds to Fig. 3.

[b] From Coffin et al. (3). In all cases the identities of the oligonucleotides from the experiments shown in Fig. 1 and 2 were confirmed by digestion with RNase A.

[c] Total 70S RNA from the experiment shown in Fig. 1; 3'-terminal RNA fractions from the experiment shown in Fig. 2.

[d] This oligonucleotide overhangs the 5' end of strong-stop DNA and is partially cleaved with RNase A in the preparation of the hybrid (3).

[e] These oligonucleotides are isomers and were not resolved in the fingerprints.

genome of the oligonucleotides shown in Fig. 1B and C but not in 1A should, therefore, give the location on the genome of this elongation. Among the oligonucleotides in the RNA protected by the 175- and 240-nucleotide-long fragments of DNA but not by strong-stop DNA is one, identified by mobility and RNase A digestion as oligonucleotide 42, which is long enough to be unique in the genome and therefore resolved in a fingerprint of the total RNA. The oligonucleotide has previously been localized near the poly(A) end of the Mo-MuLV genome (3). These results, therefore, suggest that elongation of the DNA in vitro occurs by copying sequences near the 3' end of the genome.

To confirm this conclusion, we characterized the RNA immediately adjacent to the poly(A) as the 3' end of the genome and determined how much of this RNA was complementary to the DNA products synthesized in vitro.

For these experiments Mo-MuLV 70S [^{32}P]-RNA was partially digested with alkali to pieces expected to average 500 nucleotides in length, and fragments containing the original 3' ends were isolated by chromatography on polyuridylic acid-Sephadex. The preparation was divided into four equal portions. A fingerprint of one portion of this preparation is shown in Fig. 2A. Comparison with the fingerprint in Fig. 1D shows that only 2 of 43 large oligonucleotides were present in significant amounts, a result approximately consistent with the estimated length of this fragment. The remaining portions were annealed with the three DNA fractions, and the hybrids were isolated and fingerprinted exactly as in the experiment shown in Fig. 1. As before (3), the hybrid with strong-stop DNA (Fig. 2B) yielded only the 4 oligonucleotides characteristic of the 3' complement of the terminally redundant sequence (21, f_2, g, and q). The 175-nucleotide (Fig. 2C) and 240-nucleotide (Fig. 2D) DNA fractions protected RNA containing the same oligonucleotides plus 2 (42 and bb) and 7 (42, aa, bb, cc, dd, ff, and oo) additional oligonucleotides, respectively. These oligonucleotides had electrophoretic mobilities and RNase A digestion products identical to those of the corresponding oligonucleotides in Fig. 1. To confirm their identities, all oligonucleotides in the experiments shown in Fig. 1 and 2 were eluted, and their compositions were determined by digestion with RNase A (Table 1). In all cases, this analysis confirmed the identities of these oligonucleotides, as shown schematically in Fig. 3.

To establish that hybridization to the DNA fragments was yielding a subset of the total sequence in the 3' terminal fragments, the sequence complexity of hybridized RNA relative to that of nonhybridized RNA was determined (Table 2). This was done by dividing the amount of radioactivity in oligonucleotide 21 in each of the four fingerprints in Fig. 2 by the input radioactivity to each fingerprint and the normalizing this value to that of nonhybridized RNA. Less than one-third of the amount of oligonucleotide 21 initially present (i.e., 165 cpm) was lost during the hybridization procedure (Table 2), indicating that the hybridization was nearly complete. The specificity of the hybridization is demonstrated by the substantially lower sequence complexities of the hybridized RNA as compared with the nonhybridized RNA. Assuming a complexity of 500 nucleotides for the nonhybridized RNA (based on the previously calculated rate of alkaline hydrolysis under the conditions used for this experiment [2]), the hybridized RNA fractions had complexities of 65, 100, and 140 nucleotides. These values are close to the expected values of approximately 55, 95, and 160 nucleotides derived from the lengths of the DNA fragments minus the unique sequence (80 nucleotides) adjacent to the primer near the 5' end of the genome. [The poly(A) was lost in the course of preparing the hybrids, as judged by its presence in the fingerprint of the nonhybridized RNA and its absence in the fingerprints of the hybrids (not shown).]

Several oligonucleotides (labeled 21', 21'', and 21''' in Fig. 3) were found in low yield in some of these hybridization experiments. These oligonucleotides yielded products after digestion with RNase A consistent with their being partial fragments of oligonucleotide 21, with 21' and 21'' deriving from the 3' portion and 21''' deriving from the 5' portion. These probably resulted

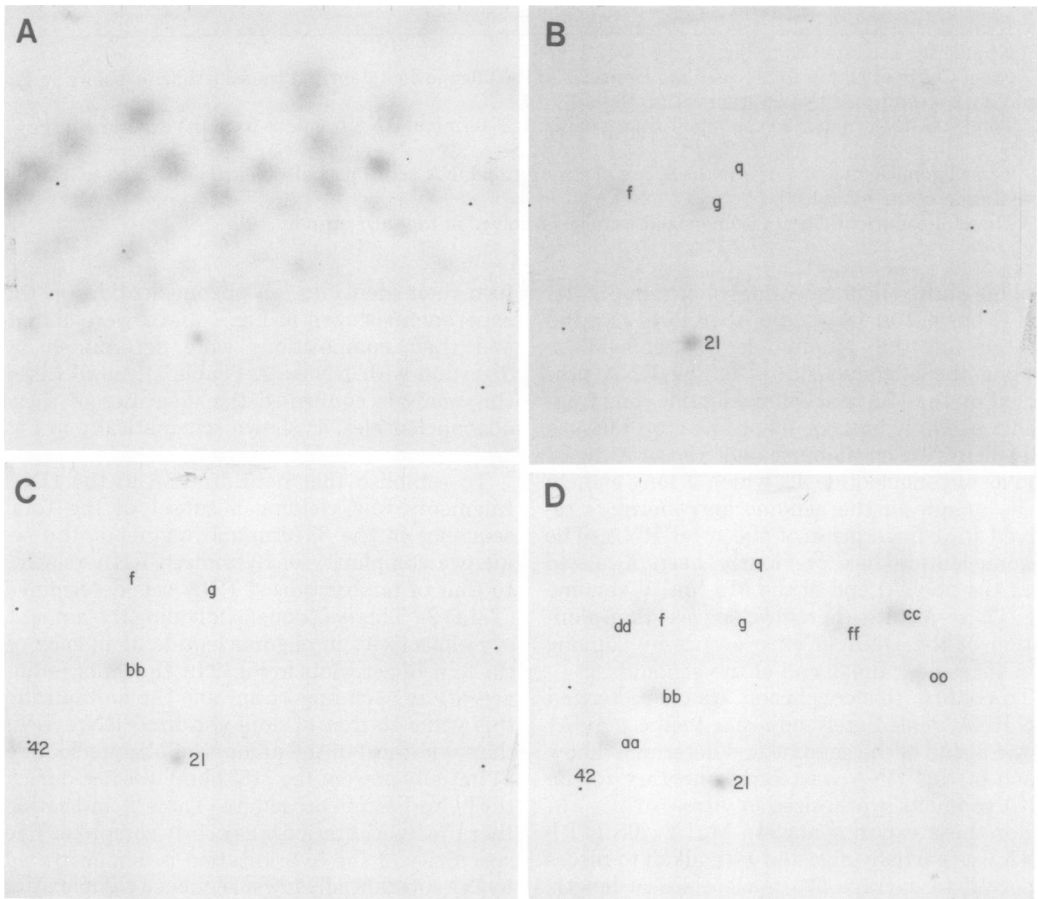

FIG. 2. *Hybridization of Mo-MuLV to 3'-terminal fragments of Mo-MuLV RNA. A preparation of Mo-MuLV [^{32}P]RNA (specific activity, 5 × 10^6 cpm/μg) was partially digested with 0.05 M Na_2CO_3 at 50°C for 10 min to yield pieces with an expected average length of about 500 nucleotides (2), and the fragments representing the original 3' terminus were isolated as described in the text. The 3' fragment preparation was divided into four equal portions, each derived from 0.5 μg of total RNA. One portion (A) was digested with RNase T_1 and fingerprinted. The remaining portions were annealed with 0.015 μg of each DNA fraction as in Fig. 1, and the hybrids were subsequently fingerprinted. The hybrids prepared (and percentages of total RNA in the hybrids) were: (B) with strong-stop DNA (8.7%), (C) with the 175-nucleotide fragment (14.3%), and (D) with the 250-nucleotide fragment (20.1%). The oligonucleotide labeled f in this figure had the composition of f_2 only.*

from slight overdigestions of the hybrids in some experiments. A similar phenomenon has also been seen in hybrids between Rous sarcoma virus strong-stop DNA and RNA (5).

The oligonucleotides derived from near the 3' end in the RNA protected by 175- and 240-nucleotide fragments (Fig. 2C and D) formed a subset of the oligonucleotides present before hybridization (Fig. 2A). To locate these oligonucleotides more precisely, a somewhat shorter poly(A)-containing fragment (expected to be about 300 nucleotides long) was isolated by chromatography on polyuridylic acid–Sephadex and fingerprinted (Fig. 4). No oligonucleotides of more than 6 nucleotides in length were found which were not also in the RNA protected by the 250-nucleotide fragment. To confirm the order of oligonucleotides near the 3' end of the genome predicted by these experiments, still shorter 3'-terminal fragments of Mo-MuLV RNA were prepared. Figure 4 also shows fingerprints of the 3' ends of partially digested RNA separated by polyacrylamide gel electrophoresis into fragments of 250 to 300 (Fig. 4B) and 150 to 175 (Fig. 4C) nucleotides. Only oligonucleotides 21, 42, aa, and bb were visible in the fingerprint of the longer fragment, and only 21 (and, more prominent, 21″) were present in the shorter one. Thus, the redundant sequence must lie to the 3' side of the unique oligonucleotides in the ge-

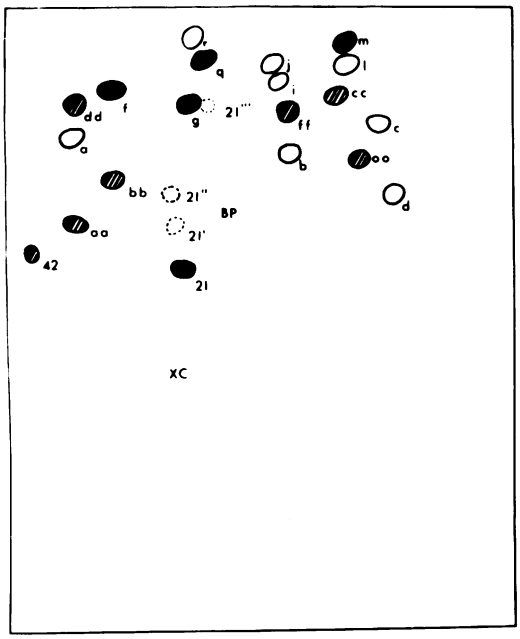

FIG. 3. *Oligonucleotides protected by Mo-MuLV DNA fragments. All the oligonucleotides taken for further analysis in Fig. 1 and 2 are shown. Symbols: open, oligonucleotides unique to the 5' end; hatched, oligonucleotides unique to the 3' end; closed, oligonucleotides found at both ends. XC and BP show the positions of the xylene cyanol FF and bromophenol blue dye markers, respectively.*

nome and immediately adjacent to or very near the poly(A).

We conclude that the elongation products of strong-stop DNA are complementary at their 5' ends to sequences present at the 5' end of the Mo-MuLV genome, complementary at their 3' ends to sequences very near the poly(A) at the 3' end of the genome, and complementary in the middle to the redundant nucleotide sequence found at each end of the genome.

DISCUSSION

The results presented in this paper provide direct evidence that in vitro DNA synthesis by the endogenous polymerase of tumor viruses proceeds from the 5' end of the genome across the discontinuity between 5' and 3' ends and continues elongation from the 3' end of the viral genome. This conclusion is based on the results that the 135 ± 10-nucleotide-long strong-stop DNA isolated as the major product of Mo-MuLV in vitro polymerase reactions is a copy of the 5' end of the genome (3) and that longer DNA products, previously shown to be elongation products at the 3' end of strong-stop DNA, contain sequences complementary to both the 5' and the 3' ends. The observations of Junghans et al. (10) suggest that a similar phenomenon is obtained with Rous sarcoma virus. They found that hybrids between the viral genome and DNA products synthesized in vitro were often circular.

The mechanism used to jump the gap between the ends in the course of DNA synthesis can also be inferred from these results, which show that the terminally redundant sequence is copied only once. The fragments of Mo-MuLV used in these experiments were 175 ± 10 and 240 ± 20 nucleotides long. These fragments were complementary to 135 nucleotides at the 5' end and at least 30 and 71 nucleotides (the sums of the lengths of the 3' oligonucleotides in Table 1), respectively, of unique sequence at the 3' end of the Mo-MuLV genome. Therefore, they were both too short to include also the 60 or so nucleotides of the redundant sequence at the 3' end and must contain a copy of this sequence only once.

A second argument is based on the previously published oligopyrimidine patterns of these DNA fragments. Oligonucleotide 21, which is present at both ends of the genome, contains the sequence UAAAC (Table 1). The complement of this sequence yields the oligopyrimidine d(G)TTT(A), which is present once in Mo-MuLV strong-stop DNA. This sequence is also present only once in a 250-nucleotide-long elongation product of this DNA (2). Similarly, a dCCCG sequence of DNA that does not yield a large oligonucleotide in the RNA but that is within the sequence spanned by the redundancy (4) is present only once both in the strong-stop DNA and in a 250-nucleotide-long elongation product. In a strict sense these data only exclude a complete duplication. However, a mechanism whereby a partial duplication could occur is

TABLE 2. *Sequence complexity of hybrids between Mo-MuLV RNA and DNA fragments*[a]

DNA fragment	Radioactivity in oligonucleotide 21[b]		Relative complexity[c]
	cpm	% of input[d]	
Strong stop	109	2.1	0.13
175 nucleotides	110	1.4	0.20
250 nucleotides	124	1.0	0.28
None	165	0.28	

[a] From the experiment shown in Fig. 2.

[b] Includes 21', 21'', and 21''', where these were present.

[c] Derived by dividing the values in the third column for hybridized RNA into the value obtained for the nonhybridized RNA (0.28%).

[d] [(Counts per minute in oligonucleotide 21 − background [25 to 28 cpm]) ÷ counts per minute in input to fingerprint] × 100.

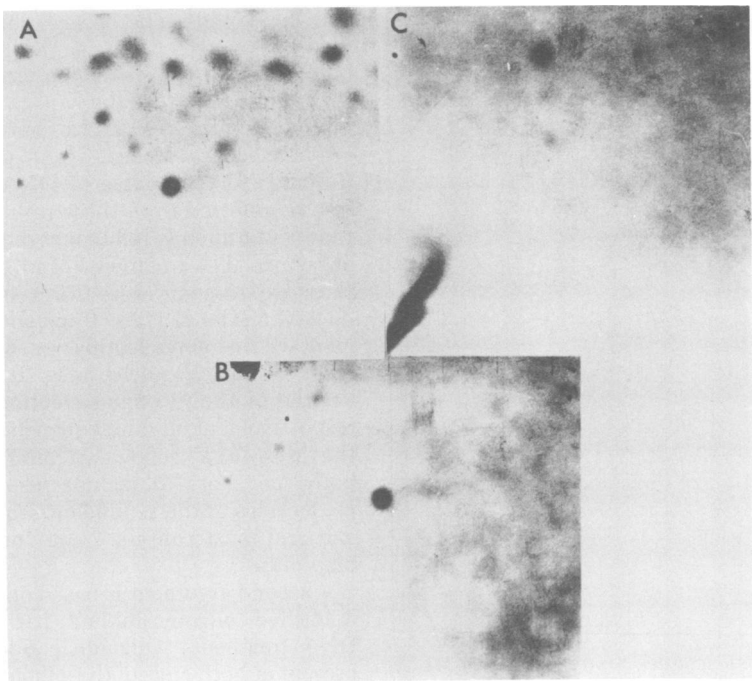

FIG. 4. *Oligonucleotides nearest the 3' end of Mo-MuLV RNA. A preparation of Mo-MuLV [^{32}P]RNA was randomly degraded as described in the legend to Fig. 2 except that digestion was for 15 min to yield pieces with an expected average length of about 300 nucleotides. The poly(A)-containing fraction was fingerprinted directly (A) or further fractionated by electrophoresis on a 5% polyacrylamide gel of the type described by Haseltine et al. (8). Size markers were 5S rRNA and tRNA. RNA fractions 250 to 300 (B) nucleotides and 150 to 175 (C) nucleotides long were eluted from the gel. The fractions were digested with RNase T_1 and fingerprinted. Oligonucleotides shorter than about 6 nucleotides were run off the second-dimension gel and are not visible.*

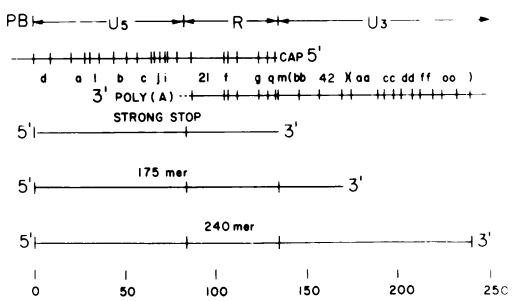

FIG. 5. *Structure of strong-stop DNA and its elongation products. The top line shows the designation of various regions of the Mo-MuLV genome; from left to right: the primer binding site, unique sequence near the 5' end, the terminally redundant sequence, and unique sequence near the 3' end. The next two lines show the 5'- and 3'-terminal regions of the genome with the terminally redundant sequences juxtaposed. The locations of oligonucleotides a through q is from Coffin et al. (3). The orders of oligonucleotides in parentheses were not determined. The lower lines show portions of the genome copied into strong-stop DNA and its elongation products. The scale, in nucleotides, is shown at the bottom.*

difficult to envision, and we therefore suggest that the elongation products have the structure shown in Fig. 5.

Since still longer products (up to least 1,000 nucleotides) contain the same 5' sequence (8), these most likely represent the continuation of synthesis from the 3' end of the genome toward the 5' end. The fact that such products probably contain the complement of redundant sequence only once provides strong evidence that this sequence serves as a "bridge" to facilitate transfer of the growing DNA chain from the 5' end to the 3' end of the genome.

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