Reverse Transcription of 7S L RNA by an Avian Retrovirus

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Linial et al. isolated a quail cell line, SE21Q1b, that is transformed by a single integrated provirus of Rous sarcoma virus. Virus particles are released from these cells, but because of a provirus defect, cellular rather than viral RNA is packaged. When these virus particles are disrupted with melittin in the presence of an appropriate reaction mixture containing actinomycin D, there is significant reverse transcription of packaged cellular RNA species. We have shown that (i) cellular 7S L RNA is an efficient template; (ii) initiation is on a unique tRNA-like primer; (iii) synthesis produces a 135-base strong-stop DNA product; and (iv) after synthesis, RNase H acts to remove the 135 bases of the 7S L RNA which acted as the template. A possible facilitator of such specific transcription may be that, in the virus particles but not in the cell, the majority of the 7S L RNA species exist complexed with the tRNA, even before the disruption of the virus. From the size and sequence features of the reverse transcript of 7S L RNA, we speculate that such events may have participated in the process by which animal cell genomes have, in the course of evolution, accumulated multiple copies of *Alu*-like elements.

There is now a body of circumstantial evidence that additional copies of a cellular gene can arise via reverse transcription of the corresponding RNA, followed by integration of that reverse transcript (1, 9, 21). One class of examples of this comprises the so-called processed genes, for which the RNAs that are postulated to be reverse transcribed are RNA polymerase II transcripts. Another class of examples includes repetitive sequences, such as *Alu*-like elements, that are RNA polymerase III transcripts (9).

In an attempt to study the reverse transcription of cellular RNAs in a potentially biologically relevant situation, we have characterized the endogenous reaction products of a defective retrovirus particle, SE21. As first shown by Linial et al. (12), these virions are defective in that, as they are assembled and released from the host cell, they package predominantly cellular RNAs rather than viral RNAs. The defect may be due to the deletion of a packaging signal located near the 5' end of the provirus (20). Nevertheless, SE21 virions retain a level of endogenous RNA-directed DNA synthesis activity that is comparable to that of wild-type virus. Earlier, we reported that a significant fraction of the reverse transcripts from the endogenous reaction of SE21 are initiated with tRNA-sized primers (24). Now we present evidence that some of the reverse transcription events of these virions utilized cellular 7S L RNA as the template. This was not too surprising, since 7S L RNA is one of the three major RNAs (70S, 7S, and 4S RNA) packaged by retroviruses (2). However, there were, in addition, strong similarities between this reverse transcription of 7S L RNA and the typical synthesis of minus-strand DNA from a retroviral RNA genome as it occurs in a wild-type retrovirus particle. Both used a unique preassociated tRNA as primer and came to a strong stop at the 5' terminus of the template RNA; in addition, template RNA was specifically degraded by RNase H activity (29). Others have noted that the specific region of the 7S L RNA that we found to be reverse transcribed has significant homology to the consensus *Alu* sequence (27). Our findings thus raise the possibility that retrovirus-mediated reverse transcription of

7S L RNA (which is an RNA polymerase III transcript) may be a step in the generation of certain *Alu*-like elements.

MATERIALS AND METHODS

Virus. The Prague A strain of Rous sarcoma virus was derived from the transformed quail clone Q-PrA-4 (24). The transformed quail clone SE21Q1b (12) was provided by M. Linial. The virions harvested from the tissue culture fluids of these cells are referred to as SE21. The psi-2 cell line was provided by T. Martin. This line was originally isolated from NIH/3T3 cells transfected by a provirus of Moloney murine leukemia virus whose putative packaging signal sequences had been deleted (15). The released psi-2 virions, like the SE21 virions, contain less than 1% retroviral RNA.

Plasmids. Two recombinant cDNA clones of human 7S L RNA were provided by E. Ullu. Plasmid p7L1 includes the entire 7S L RNA sequence, whereas plasmid p7SL1 contains only a central *Sau*3a fragment located between 105 and 231 nucleotides from the 5' terminus. This fragment thus avoids the *Alu*-like sequences at nucleotides 1 to 95 and 236 to 303 (27).

Endogenous DNA synthesis. The endogenous reaction of melittin-disrupted virions was carried out in the presence of actinomycin D as previously described (24). To isolate viral RNA or endogenous reaction products, virions were digested with 0.5 mg of pronase per ml in 0.5% sodium dodecyl sulfate at 37°C for 45 min and then extracted with phenol and precipitated with salt and ethanol. Alkali treatment of extracted nucleic acids was carried out at 37°C for 1 h in 0.6 N NaOH. Samples were then neutralized with Tris-hydrochloride and precipitated with ethanol.

Gel electrophoresis and transfer. Electrophoresis of samples in gels of agarose or acrylamide was performed either in E buffer (24) or with glyoxalated samples in 10 mM phosphate buffer with recirculation (25). Transfer to nitrocellulose filters was essentially by the method of Thomas (25). In certain cases (see Fig. 6), nondenatured samples were transferred by the method of Southern, except that transfer was begun immediately after electrophoresis without any treatment of the gel (22).

Prehybridization and hybridization. After transfer, the filters were baked at 80°C for 2 h. They were prehybridized,

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hybridized, washed, and autoradiographed as described previously (24). The hybridization solutions contained 0.5×10^6 cpm of either nick-translated plasmid DNA or endogenous reaction product per ml.

Filter selection of cDNA. The method for filter selection of cDNA was essentially that of Maniatis et al. (14). Briefly, DNA of plasmid p7L1 was first denatured and spotted onto nitrocellulose filters, and then alkali-treated endogenous reaction products of SE21 were dissolved in an appropriate volume of hybridization solution and incubated with the filters. The cDNA that bound to the filters was eluted and collected by precipitation with ethanol.

DNA sequencing. Reverse transcripts of 7S L RNA were separated from other endogenous reaction products of SE21 by the filter selection method. Subsequent 5'-end labeling, gel purification, and sequencing reactions were done by the method of Maxam and Gilbert (16). One modification was that the elution was not by pulverization but by rocking the gel slice in 2 M ammonium acetate at 37°C for 16 h. Also, potassium permanganate was used for the T-specific reaction as described by Schmid (18). To identify the 5'-terminal nucleotide, the samples were electrophoresed into a 20% sequencing gel with ^{32}P as a marker (16). The final DNA sequence was derived by combining results from sequencing gels of 20 and 6% acrylamide.

RNA sequencing method. The RNA primers of Rous sarcoma and SE21 virions were end labeled by a modified endogenous reaction. The available deoxynucleotides were limited to ^{32}P TTP with or without cold dATP as described previously (24). Such tagged primers were released by heat denaturation and purified by electrophoresis into a 6% acrylamide gel. They were located, eluted, extracted once with phenol and twice with ether, and collected by precipitation with salt and ethanol in the presence of 20 μg of yeast tRNA as carrier.

RNA sequencing was by partial digestion with base-specific ribonucleases (7). The enzymes used were RNase T₁ (7), U2 (7), Phy M (6), *Bacillus cereus* (13) (Pharmacia P-L Biochemicals, Milwaukee, Wis.), and RNase chicken liver III (10) (Bethesda Research Laboratory, Rockville, Md.). The procedures, buffers, and reaction conditions were essentially as recommended by the manufacturers, except that for successful Phy M digestion, the amount of RNase Phy M had to be increased from 1 to 10 U as previously observed by others (17). Electrophoresis was carried out in the presence of 8 M urea in gels of 8 or 20% acrylamide.

RESULTS

Linial et al. have shown that the SE21 virions have an endogenous DNA polymerase reaction with an activity comparable to that of wild-type virus (12), even though they contain cellular rather than viral RNAs. The following experiment was undertaken to determine whether in that endogenous reaction there were any preferred cellular RNA templates. To do this, the labeled endogenous reaction products of SE21 virions were treated with alkali and used as a source of cDNA probe to hybridize to virion RNAs that had been glyoxalated, separated by gel electrophoresis, and transferred to a nitrocellulose filter.

The probe hybridized predominantly to a single cellular RNA species of ca. 300 bases (b) in length (Fig. 1A, lane 1). A similar RNA species was also detected in the wild-type avian retrovirus (lane 2) and in a murine retrovirus, psi-2 (lane 3).

Others have shown that retroviruses normally contain only three major RNA components: 70S, 7S, and 4S RNA

species (2). The 70S RNA is a dimer of the retroviral genomic RNA, and the 4S RNAs are a specific subset of the cellular tRNAs. The 7S RNA has recently been identified as 7S L RNA, an abundant cellular species of ca. 300 b in length (27). Therefore, we reasoned that the 300-b RNA species we detected by hybridization might be packaged cellular 7S RNA. To test this hypothesis, the filter used in Fig. 1A was stripped of probe and then rehybridized with a probe made from a cDNA clone of human 7S L RNA (27). As shown in Fig. 1B, lanes 1 to 3, the same major bands were in fact labeled. These results were thus consistent with the interpretation that cellular 7S L RNA was reverse transcribed in the endogenous reaction of SE21.

The sequence and possible structure of both the human and rat 7S L RNAs have been elucidated (11, 27). In the 300-b sequence of human 7S RNA, the 95 nucleotides at the 5' end and the 67 nucleotides at the 3' end show strong homology to a family of eucaryotic repetitive sequences, the *Alu*-like elements. However, the central 140 nucleotides are unique and highly conserved during evolution (26). The probe used in Fig. 1B was p7L1, a cDNA clone containing all of the human 7S L RNA sequences. To determine whether or not the observed hybridization was through *Alu*-homologous regions, we rehybridized the filter with a probe made with plasmid p7SL1 which contained only the middle unique sequence. The results were essentially identical to those shown in Fig. 1B (data not shown). These findings further support the interpretation that, in the endogenous reaction of SE21, cellular 7S L RNA is a major RNA template.

To pursue this further, we attempted to identify those particular endogenous cDNA products of SE21 which hybridized to 7S L RNA. To do this, we used a hybridization selection procedure (14). The total products of a labeled

FIG. 1. Analysis of the RNA species reverse transcribed in the endogenous reaction of SE21. (A) RNAs extracted from SE21 virions (lane 1), Rous sarcoma virus (lane 2), and psi-2 virions (lane 3) were glyoxalated, subjected to electrophoresis in 1.5% agarose gel, transferred to nitrocellulose, and then hybridized with labeled alkali-treated endogenous reaction products of SE21. (B) The filter of (A) was stripped to remove the probe and then rehybridized with nick-translated DNA of plasmid p7L1.

endogenous reaction were treated with alkali and hybridized to DNA of plasmid p7L1 immobilized on a nitrocellulose filter. The hybridized cDNA was eluted, denatured by glyoxalation, and then analyzed on a gel of 6% polyacrylamide. We observed a single major DNA species which, relative to size markers, was ca. 135 b in length (Fig. 2, lane 2). This species was a significant fraction of the total endogenous reaction product (lane 1). With a control filter containing pBR322 DNA, no hybridized species was selected (lane 3). These results are consistent with the interpretation that the reverse transcription was both initiated and terminated at discrete positions on the 7S L RNA template.

Previously, we reported that most reverse transcripts synthesized in the endogenous reaction of SE21 used tRNA-sized primers (24). It was possible that the synthesis of this 135-b cDNA also began with a unique tRNA-sized primer. We therefore examined the total endogenous reaction products without any alkali treatment. We then found a major species not of 135 b but of ca. 210 b in length (lane 4). To confirm the relationship between these two, the 210-b species was gel purified and analyzed further (lane 5). When this species was treated with alkali, glyoxalated, and then run into the same gel, it migrated to the same position as the filter-selected 135-b cDNA (lane 6). These data were thus consistent with a unique tRNA-sized species being the primer for the reverse transcription of 7S L RNA which yielded a 135-b DNA species covalently attached to a 75-b RNA primer.

In the following experiments, rather than studying the total endogenous reaction products of SE21, we focused specifically on events involving 7S L RNA. To do this, we extracted total nucleic acids from SE21 virions both before and after an unlabeled endogenous reaction and used a probe specific for 7S L sequences to assay the RNA templates, the reaction intermediates, and the DNA products. Before the endogenous reaction, one major band of 300 b was detected among the total nucleic acids of SE21 (Fig. 3A, lane 1). (The broad minor band at ca. 200 b was not always present and

FIG. 2. Analysis of nucleic acid species generated in the endogenous reaction of SE21. The product was treated with alkali and selected by hybridization to a filter containing DNA of plasmid p7L1. Samples were glyoxalated before separation in a gel of 6% polyacrylamide, followed by autoradiography. Lane 1, Total reaction product; lane 2, reaction products as hybrid selected to p7L1 DNA; lane 3, reaction product as hybrid selected to pBR322 DNA; lane 4, total endogenous reaction products, but without prior alkali treatment; lanes 5 and 6, the 210-b species eluted from lane 4 and resubmitted to electrophoresis without and with prior alkali treatment, respectively.

FIG. 3. Analysis of the 7S L RNA-related nucleic acid species generated by the endogenous reaction of SE21. Nucleic acids were extracted from virions, glyoxalated, separated in a 6% acrylamide gel, transferred, and then hybridized with specific probes. Lane 1, Without prior endogenous reaction; lane 2, after endogenous reaction; lane 3, as in lane 2, but treated with alkali before glyoxalation. The probe used in A and B was nick-translated DNA of plasmid p7L1; in C, the probe was the DNA of p7SL1. After the autoradiography for A, the probe was removed from the filter by heat denaturation, and then hybridization was carried out with the p7SL1 probe to obtain the data of C. The filter was then treated with alkali to not only remove the probe but also remove filter-bound RNA. It was then rehybridized with the p7L1 probe to obtain the data of B.

was interpreted as due to limited degradation of the 7S L RNA present in the virus.) After the endogenous reaction, the number of bands increased to four (lane 2), with sizes relative to the markers of ca. 300, 210, 165, and 135 b. When the total reaction products were treated with alkali before electrophoresis, we detected only the 135-b band (lane 3). The 300- and 135-b species were interpreted as 7S L RNA template and the cDNA product, respectively. A clue to the identity of the 210- and 165-b species was obtained when the filter was treated with alkali and rehybridized with the same probe. As shown in panel B, the 300- and 165-b species disappeared (cf. lane 2 of Fig. 3A and B). Therefore, the 165-b species, like the 300-b template, was RNA. Considering its ability to hybridize with p7L1 probe, we inferred that it was derived from 7S L RNA during the endogenous reaction. Because the 210-b species persisted even after the filter was treated with alkali, it should have DNA components. But since the species migrated faster when pretreated with alkali (cf. lanes 2 and 3 of Fig. 3B), it must also have contained RNA. Thus, combining the two observations, we concluded that the 210-b species was the 135-b cDNA with covalently linked tRNA-sized primer.

To understand which region of 7S L RNA was reverse transcribed and which part gave rise to the 165-b RNA, we used the subclone p7SL1 as the probe. As mentioned earlier, this subclone was useful because it contained only 120 b from the conserved central sequences of 7S L RNA but neither the 105 b at the 5' end nor the 75 b at the 3' end. The filter as used in Fig. 3A was therefore stripped and rehybridized with p7SL1. This probe hybridized only to the 300- and 165-b species (Fig. 3C, lane 2). It did not hybridize to the 135-b DNA species, the reverse transcript of 7S L RNA (cf. lane 3 of Fig. 3A and C). Therefore, the 135-b DNA species must be predominantly reverse transcribed not from the central region but from sequences at either the 5' or 3' end of 7S L RNA. Several lines of evidence supported the former

FIG. 4. Model for the reverse transcription of 7S L RNA.

interpretation that the synthesis was initiated from tRNA-sized primer bound at a unique central region of 7S L RNA and proceeded out to its 5' terminus (Fig. 4). The model explained the unique start and stop sites needed to define the discrete 135-b DNA species and the generation of the 165-b RNA species as a consequence of RNase H action (29) on the reverse-transcribed sequence of 7S L RNA. Furthermore, as presented in the following section, the nucleotide sequence of the 135-b DNA species confirmed the model. However, it did not explain why the 135-b DNA species failed to hybridize to the p7SL1 probe, whose sequence it overlapped for ca. 25 b. Our interpretation was that this was probably due to divergence in this region between human and quail 7S L RNAs.

To determine the sequence of the 135-b cDNA, we used filter selection to isolate this cDNA from the total products of an endogenous reaction. This cDNA was labeled at the 5' end with polynucleotide kinase. With the procedures of Maxam and Gilbert (16), the complete nucleotide sequence was determined (Fig. 5). The complement of this sequence was compared with the available sequence of the human 7S L RNA (27). There was 85% agreement with the 5' terminus of 7S L RNA. Our studies thus localized the initiation site as being 135 b from the 5' terminus of 7S L RNA. Furthermore, the termination sites were located at the 5' end of 7S L RNA. There was a limited microheterogeneity at the 3' terminus of the cDNA, presumably because in some cases transcription stopped as many as several nucleotides earlier. Also, we

noted that the 7S L sequence immediately 3' to the initiation site was 5'-UGG-3'. Such a sequence would be able to pair with the universal 3'-end sequence of tRNAs. This observation added support to an earlier suggestion that tRNA was used as the primer in this reverse transcription event.

For the endogenous reaction of Rous sarcoma virus, it has been shown that, with an appropriate subset of available labeled deoxyribonucleotides, it is possible to end label the primer molecules (4). We used a similar strategy to label the putative primer for the transcription of 7S L RNA as a preliminary to RNA sequencing.

From the sequence of the reverse transcript of 7S L RNA, we know that the first deoxyribonucleotide added onto the primer is thymidine (Fig. 5). Therefore, we supplied only [³²P]TTP in the endogenous reaction of SE21. The products were extracted, released by heat denaturation, and subjected to acrylamide gel electrophoresis. The appropriate band was located and eluted; to obtain the nucleotide sequence, samples were subjected to partial digestions with five base-specific RNases. These digests were resolved on sequencing gels. To get a reliable comparison, the tRNA^{Trp} primer of Rous sarcoma virus was tagged with the trinucleotide 5'-AAT-3' (4) and examined by the same sequencing procedure.

From the migration of the tagged SE21 primer relative to that of the Rous sarcoma virus primer in a sequencing gel of 8% polyacrylamide, we deduced the size of primer of 7S L RNA to be 75 ± 2 b. We were unable to obtain the full sequence of the primer. We obtained the following features: (i) some regions were resistant to all five RNases (Fig. 6A; see asterisk) which, as others have shown, is an indication of modified bases (5); (ii) the sequence at the 3' end was 5'-CCCA-3' (Fig. 6A); (iii) two other stretches of consecutive sequences were deduced, 5'-ACGCACGGCGC-3' (Fig. 6B) and 5'-GUGCUNAUNNNAGNCAA-3' (data not shown). Our interpretation of these size and sequence features was that the primer was unique in sequence and probably a tRNA. The primer however was not the same as the tRNA^{Trp} primer of wild-type virus.

The efficient reverse transcription of 7S L RNA relative to that of other cellular RNAs suggested that a facilitating factor is present, such as the prior association that occurs between a retrovirus genome and the tRNA, that acts as the primer (3, 4). To explore such a possibility, [³²P]TTP tagging reaction products were extracted and analyzed by electrophoresis into a gel of 5% acrylamide, with and without a

SE21 cDNA	3'	NNNNAGCCC	GCGCCACCGC	GTGCGGACAT	CAGGGTCGAT	GAGCCCTCCG
	5'	NNNNTCGGG	CGCGGTGGCG	CACGCCTGTA	GTCCCAGCTA	CTCGGGAGGC
Human 7S L	5'	AGGCGCCGGG	CGCGGTGGCG	CGTGCCTGTA	GTCCCAGCTA	CTCGGGAGGC
		10	20	30	40	50
SE21 cDNA		ACTCGGGCGG	CCTAGCGAAC	TCGGGTCTCT	AAGACCCGAC	GTACGCGCAT
		TGAGCCCGCC	GGATCGCTTG	AGCCAGGAG	TTCTGGGCTG	CAGTGCCTTA
Human 7S L		TGAGGCTGGA	GGATCGCTTG	AGTCCAGGAG	TTCTGGGCTG	TAGTGCCTTA
		60	70	80	90	100
SE21 cDNA		ACGGCTCGCC	NGCAGGCGCG	ATTCGGGCGG	TAGTTAT	
		TGCCGAGCGG	NCGTCCGCGC	TAAGCCCGCG	ATCAATA	
Human 7S L		TGCCGATCGG	GTGTCCGCAC	TAAGTTCGGC	ATCAATATGG	TGACCTCCCG
		110	120	130	140	150

FIG. 5. Sequence of the 135-b reverse transcript of 7S L RNA and the comparison between its complement and the sequence at the 5' terminus of human 7S L RNA.

FIG. 6. Sequence of the RNA primer. The RNA primer was end labeled with [32 P]TTP, dATP, or both. Samples of these products were submitted to partial digestion with the indicated RNases or to alkali. The digestion products were then resolved on a sequencing gel of 8% polyacrylamide, followed by autoradiography. A, Sequence ladder of [32 P]TTP- and dATP-tagged primer of 7S L RNA to show the sequence of the 3'-end terminus; B, sequence ladder of [32 P]TTP-tagged primer.

prior heat denaturation. After denaturation, only one species was labeled in the tagging reaction; this was the primer (species C; Fig. 7, lane 2). However, without this denaturation, the migration of the tagged primer was significantly slower (lane 2; species A). Our interpretation was that it was associated with another RNA species. To test the hypothesis that this second species was 7S L RNA, we carried out a similar reaction but with unlabeled TTP. Then, rather than assay the tagged primer, we assayed the 7S L RNA by using the Southern procedure and the specific probe. We found that, without prior denaturation, the 7S L RNA migrated in the same manner (lane 3; species A) as did the nondenatured tagged tRNA. Furthermore, after heat denaturation, the mobility of the 7S L RNA increased (lane 4; species B). Our interpretation was that species A consisted of one molecule of 7S L RNA (species B) and one molecule of tRNA (species C).

As discussed above, we have referred to the 7S L RNA as an efficient template for reverse transcription in the endogenous reaction of SE21 virions. To quantitate this efficiency, the data of Fig. 3C, lane 2, was subjected to densitometric analysis. We deduced that, in a 1-h reaction, 29% of the 7S L RNA was reverse transcribed and then specifically digested by RNase H. In three independent experiments, the utilization ranged from 25 to 90%.

DISCUSSION

In our study of the reverse transcription of cellular RNAs in the SE21 virion system, we discovered that packaged 7S

L RNA was efficiently reverse transcribed. Although these reverse transcripts were not a major fraction of the total mass of endogenous reaction product (actually ca. 0.5%; data not shown), the 7S L RNA was an efficient template in that 25 to 90% of the total virion 7S L RNA was utilized as a template.

7S L RNA is an abundant cellular RNA and recently has been shown to be an essential component of the signal recognition particle (30). The sequences of the mammalian 7S L RNA (human and rat) have been previously reported (11, 27). They are highly conserved and show the following similar characteristics: terminal sequences, 95 b at the 5' end and 67 b at the 3' end, that are homologous to the repetitive *Alu*-like sequence, with a central sequence of 140 b that is almost unique (27).

The 7S L RNA is an important cellular RNA, but it was actually first found within avian retrovirus particles (2) and has since been found to be present in all retroviruses studied (29). It remains to be determined whether 7S L RNA is accidentally packaged into virions or plays an unknown function in the life cycle of a retrovirus. In terms of the latter possibility, our observation that 7S L RNA was reverse transcribed in SE21 virions may be potentially relevant.

The present studies showed that the reverse transcription of 7S L RNA was initiated from a preformed template-primer complex and used a unique tRNA-like species as the primer. DNA synthesis stopped at the 5' terminus of the template, and then the RNA template was degraded by RNase H activity, which left a residual 165-b fragment. All of these aspects of the scheme are qualitatively the same as

FIG. 7. The association between 7S L RNA and its tRNA primer. The tRNA primer was end labeled in an endogenous reaction containing dATP and [32 P]TTP. The total product was extracted, and samples were analyzed without (lane 1) or with (lane 2) prior heat denaturation. A similar reaction was carried out but with [32 P]TTP replaced by TTP. This product was similarly analyzed, but after electrophoresis the nucleic acids were transferred to nitrocellulose and hybridized with a probe to plasmid p7L1 (lane 3, without heat; lane 4, with heat). Lanes 5 and 6 are as for lanes 3 and 4 but with SE21 virions not submitted to an endogenous reaction.

that by which minus-strand strong-stop DNA is reverse transcribed from a retrovirus genome (29).

In retroviruses, the genomic RNA is not associated with primer tRNA until the virions are released and mature (3). Then, the template-primer complex forms and serves as an efficient initiator for reverse transcription. The association of tRNA and template cannot be reconstructed by using physiological conditions of temperature and salt concentration (23). Therefore, facilitating factors are present within the virus. We suggest that, in SE21 virions, the same factors could be also responsible for associating the 7S L RNA and its primer.

From the partial RNA primer sequence obtained, we concluded that the tRNA-like primer of 7S L RNA was different from the tRNA^{Trp} primer which is used by wild-type Rous sarcoma virus (29). Also, the T_m for 7S L RNA and its primer was determined to be $59 \pm 4^\circ\text{C}$, significantly lower (11°C) than that of Rous sarcoma virus RNA and tRNA^{Trp} (24). Hence the base-paired region between 7S L RNA and its primer was probably less than 18 base pairs (29).

The similarity between the reverse transcription of 7S L RNA and the initial phase of reverse transcription of wild-type retroviral RNA was interesting for another reason. It raised the possibility that the reverse transcripts of 7S L RNA could integrate into the cell genome in a manner similar to the insertion of a provirus. The speculation was further supported by the observation that, in eucaryotic cell genomes, there are many copies of sequences that are partially homologous to 7S L RNA. They are the *Alu*-like elements (19). Such elements have already been postulated to be generated by a process involving reverse transcription (9, 21). In addition to this, some investigators, noting that 7S L RNA was more conserved through evolution than *Alu*-like elements, suggested that the latter were generated from the

former (8, 28). The identity and source of the enzyme responsible for such putative reverse transcription of a cellular RNA copy is not known. Our observation that 7S L RNA was reverse transcribed in SE21 virions was not only consistent with their hypothesis but also suggested detailed aspects of this process, such as the priming and the generation of discrete DNAs. In our case, 7S L RNA was packaged inside the virions and was readily accessible to the reverse transcriptase. Furthermore, it is worth noting that all of the characterized mammalian *Alu*-like elements consist of a basic unit of ca. 130 b in length (19), very similar in size and sequence to the 135-b reverse transcript that we detected. Combining these considerations, we propose that retrovirus-mediated reverse transcription of 7S L RNA and its subsequent integration may be the missing link in the generation of eucaryotic *Alu*-like elements. One way to test this hypothesis will be to isolate and sequence the *Alu*-like elements of the quail cell line and compare them with the observed reverse transcripts of 7S L RNA. Another test of this model, at least for this system, will be to determine whether, as a consequence of "infection" with SE21, new 7S L RNA-related elements can be introduced into cultured quail cells.

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