Further Characterization of Virus-like 30S (VL30) RNA of Mice: Initiation of Reverse Transcription and Intracellular Synthesis

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SUMMARY

We have studied the virus-like 30S (VL30) RNA sequences of mice. Previous work has shown that these sequences are coded in the mouse genome, expressed in some normal cells and released as pseudotypic particles from cells producing murine C-type retroviruses. VL30 sequences have some similarities to standard retrovirus RNA, but differences also exist. To further assess the similarities and differences, several aspects of VL30-specific metabolism were investigated. We studied the initiation of VL30-specific DNA synthesis during an endogenous reverse transcriptase reaction. Short initial VL30-specific cDNA transcripts were covalently attached to RNA as measured by equilibrium banding in caesium sulphate density gradients. Therefore, reverse transcription of VL30-specific cDNA is initiated by an RNA primer. The intracellular synthesis of VL30 RNA was investigated by pulse labelling uninfected JLS-V9 cells with 3H-uridine. Hybridization of the pulse-labelled nuclear RNA indicated that the major VL30-specific RNA evident after a 15 min label was the same size as the mature VL30 RNA. Thus, VL30 RNA is apparently not synthesized via a higher mol. wt. precursor. Both of these results demonstrate similarity of VL30 RNA sequences to standard retroviruses. One unique feature of VL30 RNA was detected. JLS-V9 cells contained both the monomeric VL30 RNA and a hydrogen-bonded 38S form which yielded the monomer when denatured. This contrasts with standard murine leukaemia virus which is only found as a monomer within cells.

INTRODUCTION

Most, if not all, higher vertebrate species carry endogenous sequences complementary to retroviruses. In the mouse, sequences complementary to C-type [murine leukaemia virus (MuLV); Gelb et al., 1971] and B-type (murine mammary tumour virus; Varmus et al., 1973) viruses have been identified. Endogenous C-type MuLVs can be induced from mouse cells by a variety of agents, and they have been well studied (Besmer et al., 1974). More recently, another class of murine virus-like sequences has been identified, the virus-like 30S (VL30) sequences. These sequences were first identified as 30S RNA present as pseudotypes in MuLV preparations from a wide range of mice (Besmer et al., 1979; Hawk et al., 1978; Sherwin et al., 1978). In our studies, we found that bromodeoxyuridine (BrdUrd) induction of the Balb/c-derived JLS-V9 cell line resulted in the production of virions which contained VL30 RNA sequences rather than endogenous MuLV 38S RNA as the main component.
although xenotropic MuLV was the major biological constituent (Besmer et al., 1979). This has provided a convenient source of VL30 RNA sequences and facilitated biochemical experiments.

Mouse VL30 sequences resemble retrovirus sequences in several ways: (i) they can be packaged into MuLV particles by pseudotyping with MuLV proteins; (ii) in virus particles, they are templates for the endogenous MuLV reverse transcriptase; (iii) in virus particles, they exist in a 50S to 55S (presumably dimeric) form; (iv) their synthesis can be increased by treatment of cells with agents that induce endogenous MuLV transcription; and (v) the DNA sequences complementary to VL30 RNA are present in multiple copies (between 20 and 50 per genome) (Besmer et al., 1979; Howk et al., 1978).

On the other hand, several properties also serve to distinguish mouse VL30 sequences from standard murine retroviruses: (i) they share no nucleic acid sequence homology with C-type MuLVs (Besmer et al., 1979; Howk et al., 1978; Sherwin et al., 1978) or B-type viruses (Sherwin et al., 1978); (ii) cells which contain VL30 RNA on polyribosomes do not synthesize proteins which are antigenically related to MuLV proteins (Fan & Mueller-Lantzsch, 1976); (iii) uninduced cells which contain complete copies of VL30 RNA do not produce virus particles (Besmer et al., 1974); and (iv) VL30 sequences are only poorly infectious, even when pseudotyped with infectious MuLVs (Scolnick et al., 1979). Thus, it is possible that VL30 sequences are considered virus-like only because they can be induced and packaged into C-type virus particles.

In the present study we have used biochemical techniques to further assess the similarities or differences of VL30 sequences to those of standard murine retroviruses. In particular, we have tested for some of the properties which are characteristic of retroviruses to determine if they occur for VL30 sequences.

**METHODS**

**Cells and viruses.** All cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum (Gibco). The JLS-V9 cell line is a line of Balb/c bone marrow-derived cells (Wright et al., 1967). Mink lung cells productively infected with Balb/c xenotropic virus were prepared according to Besmer & Baltimore (1977) by infecting mink lung cells with serial dilutions of BU-V9 virus (see below). The infected culture obtained from the highest dilution of infecting BU-V9 virus was used.

Virus particles containing VL30 sequences were induced from JLS-V9 cells by BrdUrd treatment (BU-V9 virus) (Besmer et al., 1974). The JLS-V9 cells were treated with 20 μg/ml BrdUrd for 24 h in normal growth medium. After removing BrdUrd, fresh medium was added and tissue culture supernatants were collected at 24 h intervals for 3 days. Virus was purified as described previously (Fan & Baltimore, 1973).

**Synthesis of cDNAs.** For high specific activity cDNA, BU-V9 virions were incubated in an endogenous reverse transcriptase reaction, as described previously for Moloney MuLV virions (M-MuLV), (Fan & Verma, 1978). The reaction contained exogenously added oligodeoxynucleotide primers (Taylor et al., 1976), as well as labelled deoxynucleoside triphosphate. The specific activity of the cDNA was between 1.5 × 10⁷ and 2 × 10⁷ ct/min/μg, as calculated from the specific activity of the labelled triphosphate.

Large quantities of BU-V9 cDNA were synthesized as described previously for M-MuLV cDNA (Fan, 1977), and the purified cDNA was attached to nitrocellulose filters also as described by Fan (1977).

**Synthesis of short initial transcripts.** Short initial transcripts of VL30-specific cDNA were made from endogenous reverse transcriptase reactions of BU-V9 virions. Reactions contained 2 mg/ml BU-V9 virus, 50 mM-tris buffer pH 7.4, 10 mM-dithiothreitol, 6 mM-magnesium
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acetate, 60 mM-NaCl, 0.015% Nonidet P40 (NP40), 50 µg/µl actinomycin D, 400 µM-dCTP, 400 µM-dATP, 20 µM-dGTP and 20 µM-dTTP. In addition, α-32P-dTTP was used to label the cDNA. Reactions were incubated at 37 °C for the times indicated. After incubation, the reactions were terminated by addition of 1/10 vol. of 10% SDS and 4 mM-NaCl and unincorporated deoxynucleoside triphosphates removed by passage over a Sephadex G-75 column. The cDNA, which eluted in the void volume, was then extracted twice with phenol–chloroform, twice with chloroform, and precipitated with 2 vol. ethanol. The ethanol precipitates were collected by centrifugation and suspended in 10 mM-tris pH 7.4, 1 mM-EDTA. For pilot reactions vol. were 0.1 ml containing 10 µCi α-32P-dTTP (final sp. act. 4.8 Ci/mmol). For the large scale reaction used in Fig. 2, reaction vol. was 1 ml, and 770 µCi α-32P-dTTP was used (final sp. act. of dTTP was 37 Ci/mmol).

Extraction of cytoplasmic RNA. Extraction of cytoplasmic RNA has been described previously (Fan & Besmer, 1975). The cells were removed from monolayer surfaces by trypsinization and lysed in reticuloeyte standard buffer (RSB) containing 1% NP40 and 50 µg/ml dextran sulphate. Nuclei were removed by centrifugation. The cytoplasmic fraction was adjusted to 0.2 M-NaCl, 1% SDS, 10 mM-EDTA and phenol–chloroform extracted.

Centrifugation techniques. Size analysis of DNA in 5 to 20% alkaline sucrose gradients (Verma, 1978) and of RNA in neutral sucrose gradients (Fan, 1977) have been described previously. Buoyant density analysis of DNA and RNA molecules in equilibrium Cs2SO4 density gradients was performed as described previously (Verma et al., 1971). A 400 µl sample of DNA in 0.01 M-tris, 1 mM-EDTA was boiled for 5 min, rapidly cooled in ice and combined with 2.5 molal Cs2SO4 (Gallard-Schlesinger, New York, U.S.A.) to give a final density of 1.55 g/ml. The sample was placed in a 10 × 60 mm polypropylene centrifuge tube, and centrifugation was for 72 h at 32000 rev/min in a Beckman SW60 swinging bucket rotor. Fractions were collected by puncturing the bottom of the centrifuge tube, and the density of each fraction was determined from the refractive index. Radioactively labelled 3H Sindbis virus 49S RNA (a gift of B. Sefton) and 3H AKR MuLV cDNA (digested with alkali) were analysed in a parallel gradient to identify the densities of RNA and DNA. Before density-gradient centrifugation, some DNA samples were first made alkaline by adding 1/10 vol. 3 M-NaOH for 5 min, rapidly cooled and neutralized with 3 M-HCl.

Hybridization techniques. Hybridization across sucrose gradients with high specific activity cDNA and identification of hybrids by digestion with S1 single-strand-specific nuclease have been described previously (Fan & Baltimore, 1973). Hybridization of pulse-labelled nuclear RNA to filters containing excess cDNA has also been described by Fan (1977). Each nitrocellulose filter contained 0.2 µg BU-V9 cDNA.

Modified conditions were used in hybridization across Cs2SO4 density gradients. In this case, the labelled cDNA in the gradient fractions was hybridized to saturation with excess cellular RNA containing VL30 or Balb/c xenotropic MuLV RNAs. Portions (15 µl) of each fraction were adjusted to 1 M-NaCl, 0.1% SDS, 5 mM-EDTA, 5 mM-TES, 0.5% diethylidioxydiformate and 5 mg/ml of the appropriate cellular RNA in a final vol. of 40 µl. The samples were then incubated at 68 °C for 24 h. The presence of Cs2SO4 did not interfere with either the kinetics or extent of annealing (not shown). After annealing, each sample was diluted 10-fold with S1 nuclease buffer (0.25 M-potassium acetate pH 4.5, 10 mM-ZnSO4, 20 µg/ml denatured calf thymus DNA) and digested with S1 nuclease (8 units/ml) for 60 min at 45 °C. The S1-resistant hybridized material was determined by precipitation with trichloroacetic acid, filtration and scintillation counting. As controls, portions of each fraction were incubated without RNA and digested in the same manner. The S1-resistant radioactivity (representing approx. 10% of input radioactivity) from these samples was subtracted from the values in which RNA had been added to give the corrected values for the amount hybridized.
RESULTS

Endogenous reverse transcription of VL30 RNA utilizes an RNA primer

A principal characteristic of retroviruses is the transcription of viral genomic RNA into DNA by the virus-coded reverse transcriptase. This process was demonstrated by incubating purified virus particles with detergent and deoxynucleoside triphosphate precursors (the endogenous reaction) (Baltimore, 1970; Temin & Mitzutani, 1970). The initial product of the endogenous reaction was found to be a DNA molecule covalently linked to RNA. This was demonstrated by its intermediate density between RNA and DNA in a Cs$_2$SO$_4$ density gradient (Manly et al., 1971; Verma et al., 1971). The intermediate density could be converted to DNA density upon alkali degradation of the attached RNA, but not by heating alone (Verma et al., 1971).

We used the same approach to investigate the nature of the primer of VL30-specific reverse transcription in virus particles. Induced virus from BrdUrd-treated JLS-V9 cells (BU-V9 virus; Besmer et al., 1979) was prepared as starting material. We had previously shown that BU-V9 virions contain large amounts of VL30 RNA as well as smaller amounts of xenotropic MuLV 38S RNA (Besmer et al., 1979). The BU-V9 virus was incubated in an endogenous reverse transcriptase reaction for various times under conditions of limiting deoxynucleoside triphosphate precursors, with some triphosphates radioactively labelled. It has been shown for standard retroviruses that such conditions result in the synthesis of small DNA transcripts which represent a limited extension of the RNA primer (Faras et al., 1973).

Size analysis of the reaction products in alkaline sucrose gradients is shown in Fig. 1. The sedimentation positions of two restriction enzyme fragments of polyoma virus DNA (390 and 112 nucleotides) are also indicated. For subsequent experiments, a reaction time of 60 min was chosen since (i) sufficient amounts of labelled product could be prepared and (ii) the size of the DNA extension was relatively small. A small DNA product would be expected to maximize the difference in buoyant density between a covalent RNA–DNA hybrid molecule and pure DNA.

Fig. 2(a) shows Cs$_2$SO$_4$ density-gradient analysis of the BU-V9 endogenous reaction product synthesized as in Fig. 1. After heat denaturation, the labelled DNA had a density intermediate between DNA and RNA, and after alkali degradation of the RNA it had a density close to pure DNA. This indicates that most if not all of the DNA synthesized in these reactions was covalently linked to RNA, and suggests that reverse transcription of VL30 RNA may also occur from an RNA primer. However, since BU-V9 virus contained both VL30 RNA and 38S xenotropic RNA (as mentioned above), it is possible that the majority of the radioactivity incorporated in Fig. 2(a) was synthesized from the minority xenotropic 38S RNA component. Therefore, it was necessary to assay the labelled DNA for VL30-specific and xenotropic MuLV-specific sequences.

In Fig. 2(b) portions of gradient fractions from Fig. 2(a) were annealed to saturation with excess amounts of uninduced JLS-V9 cell cytoplasmic RNA. We have previously shown that uninfected JLS-V9 cell cytoplasmic RNA contains complete copies of VL30-specific sequences in relatively high concentration (Fan & Besmer, 1975; Besmer et al., 1979), and lacks xenotropic MuLV-specific RNA. Furthermore, there is no nucleic acid cross-homology between VL30-specific RNA and xenotropic MuLV RNA. Therefore, the annealings in Fig. 2(b) would identify the distribution of the VL30-specific DNA in the density gradient. As shown in the figure, the hybridizations confirm that VL30-specific DNA sequences have intermediate density in the endogenous reaction product which are converted to DNA density upon alkali treatment. This establishes that endogenous synthesis of VL30 RNA in BU-V9 virus particles is initiated from an RNA primer.

In Fig. 2(c), the distribution of xenotropic MuLV-specific DNA in the same density
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Fig. 1. Size analysis of small BU-V9 cDNA transcripts. Virions induced from BrdUrd-treated JLS-V9 cells (BU-V9 virions) were incubated in an endogenous reverse transcriptase reaction containing α-32P-dTTP under conditions which produce short initial transcripts. Samples were taken at 30 (○), 60 (●) and 90 (△) min and sedimented in 5 to 20% alkaline sucrose gradients. Centrifugation was for 5 h at 59000 rev/min and 4 °C in a Beckman SW60 swinging bucket rotor. Afterwards the gradients were fractionated and the amount of radioactivity in each fraction was measured. Two HpaII restriction fragments of 3H polyoma virus DNA (390 and 112 nucleotides in length) were sedimented in a parallel gradient, and their positions are shown (arrows).

Fig. 2. Buoyant density analysis of initial BU-V9 cDNA transcripts. BU-V9 virions were incubated in an endogenous reverse transcriptase reaction containing α-32P-dTTP for 1 h as in Fig. 1. After incubation, the cDNA was purified and divided in half. One half (●) was heated at 100 °C for 5 min; the other half (○) was brought to 0.3 M NaOH and heated at 100 °C for 5 min, chilled and neutralized. The two samples were then sedimented to equilibrium in Cs2SO4 density gradients. Centrifugation was at 32 000 rev/min for 72 h and 22 °C in a Beckman SW60 swinging bucket rotor. Radioactivity in aliquots of each gradient fraction was measured by Cerenkov counting and the distribution of total radioactivity for the two gradients is shown in (a). The density (g/ml) of every fifth fraction was determined by measuring the refractive index, and is also shown in (a). Portions of fractions were hybridized to saturation with excess amounts of VL30-specific RNA (b) or with xenotropic MuLV-specific RNA (c). The source of the VL30-specific RNA was uninduced JLS-V9 cell cytoplasmic RNA, and the source of xenotropic MuLV-specific RNA was cytoplasmic RNA from mink cells productively infected with xenotropic MuLV. After annealing, the hybridized cDNA was measured by digesting unhybridized cDNA with S1 nuclease, and the amount of S1-resistant radioactivity was plotted. Purified 3H Sindbis virion RNA and 3H AKR MuLV cDNA were included in the gradients as markers for the homopolymers and their locations are indicated (arrows).

density gradient was determined by annealing portions of fractions with cytoplasmic RNA from mink cells productively infected with xenotropic MuLV. Analogous to Fig. 2 (b), this hybridization would detect xenotropic MuLV-specific DNA, but not VL30-specific DNA (Besmer et al., 1979). The results indicate that xenotropic MuLV-specific DNA in the reaction was also attached to an RNA primer, as expected.
Pulse-labelling of VL30 RNA sequences

The nature of the synthesis of VL30 RNA in cells was also investigated. Most cellular mRNA is transcribed in the form of large precursor molecules which are then processed by removal of intervening sequences to the smaller mature form (Harpold et al., 1979; Crick, 1979). However, the initial transcript of M-MuLV RNA in productively infected cells appears to be the same size as M-MuLV 38S genomic RNA (Fan, 1977), and recent results indicate that the long terminal repeat (LTR) sequences of retroviruses may themselves contain the promoter signals for viral RNA synthesis (Hughes et al., 1979; Sabran et al., 1979; Sutcliffe et al., 1980; Van Beveren et al., 1980). It was of interest, therefore, to examine VL30 RNA synthesis to determine if it resembles retrovirus transcription or host mRNA transcription.

In Fig. 3, JLS-V9 cells were pulse-labelled for 15 min with $^3$H-uridine and nuclear RNA was extracted. After extraction, the labelled nuclear RNA was denatured and separated according to size by sedimentation in a neutral sucrose gradient, and the gradient was fractionated. RNA from each fraction was then concentrated and hybridized to saturation with nitrocellulose filters containing excess amounts of VL30 cDNA. The filters were washed and treated with pancreatic ribonuclease to remove unhybridized RNA, and the hybridized radioactivity was measured. As shown in Fig. 3, a major peak of pulse-labelled VL30-specific RNA with a sedimentation value of 27S was detected. There were also smaller amounts of larger RNA of heterogeneous size. It should be noted that the size of 27S in Fig. 3 is the same

Fig. 3. Size analysis of pulse-labelled VL30-specific RNA. JLS-V9 cells ($6 \times 10^6$) were labelled with $^3$H-uridine for 15 min and nuclear RNA was extracted. The RNA was then denatured in dimethyl sulfoxide and sedimented in a 15 to 30% sucrose gradient containing SDS. Centrifugation was for 18 h at 16000 rev/min and 25 °C in a Beckman SW41 rotor. The gradient was fractionated and a portion of each fraction was assayed to determine distribution of total radioactivity (O). The remainders of the fractions were concentrated and hybridized with excess amounts of BU-V9 cDNA attached to nitrocellulose filters. After annealing unhybridized $^3$H RNA was removed by digestion of the filters with ribonuclease. The amount of hybridized $^3$H RNA bound to the nitrocellulose filters is shown (●). Sedimentation positions of 45S rRNA precursor and 28S and 18S rRNAs are also shown (arrows).
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as that of monomeric VL30 RNA from induced virus particles: it would appear that this difference (27S versus 30S) is due to the fact that VL30 RNA has an apparent S value of 30S when measured by gel electrophoresis, but a value of 27S when measured in sucrose gradients. The size distribution of pulse-labelled VL30 RNA resembles in principle that for pulse-labelled M-MuLV-specific RNA in infected cells. The major discrete pulse-labelled species is the same size as mature virion RNA, and a lower amount of larger heterogeneous RNA is also present (Fan, 1977). In our kinetic and size analyses of M-MuLV-specific RNA synthesis, we found no evidence for a larger precursor to the genomic-sized RNA, and concluded that the initial transcript was the same size as virion RNA. The pattern of pulse-labelling of VL30 RNA therefore suggests that, like M-MuLV RNA, the primary transcript may be the same size as the mature VL30 RNA.

Intracellular VL30 RNA in higher mol. wt. structures

Standard C-type retrovirus RNA exists in virions as a 70S dimer of two 38S subunits. The 70S dimer is not present inside cells, but is formed shortly after the virus is released from the cell (Canaani et al., 1973). In BU-V9 virus, VL30 RNA sequences exist in a 50S (presumably dimeric) hydrogen-bonded structure, and some may be in higher mol. wt. structures (Besmer et al., 1979). In early studies, we identified two sizes of RNA in JLS-V9 cells which would hybridize with BU-V9 cDNA (38S and 27S) (Fan & Besmer, 1975). In retrospect, these species represented VL30-specific RNA, although the 38S size initially suggested a standard C-type MuLV RNA. In subsequent studies of denatured JLS-V9 cytoplasmic RNA, only the monomeric form of VL30 RNA was found (Besmer et al., 1979).

We therefore re-examined the nature of both the intracellular 27S and 38S VL30-related sequences. Cytoplasmic RNA was prepared from JLS-V9 cells and sedimented in a sucrose gradient. The distribution of VL30 RNA sequences was assayed by hybridizing portions of sucrose gradient fractions with high specific activity BU-V9 cDNA. As described above, the only hybridization that would take place is to VL30 RNA sequences. As shown in Fig. 4 (b), both the 38S and 27S VL30-specific RNA species were evident, as reported previously. When JLS-V9 cytoplasmic RNA was denatured with dimethyl sulphoxide and sedimented in a parallel sucrose gradient, we observed only the monomeric 27S species, as also shown in Fig. 4 (b). Therefore, the 38S VL30-specific RNA does represent the VL30 monomeric RNA hydrogen bonded in a higher mol. wt. 38S form. Based on its sedimentation characteristics, this 38S form does not represent the dimer that is present in BU-V9 virus particles. For comparison, the sedimentation properties of 32P-labelled BU-V9 virion are shown in Fig. 4 (a). The sedimentation profile shows a major peak of 70S and a minor peak of 50S to 55S. The latter peak contains the dimers of VL30 RNA, while the former contains both VL30 RNA and xenotropic MuLV 38S RNA (Besmer et al., 1979). It is clear from Fig. 4 (a, b) that the 38S intracellular form of VL30 RNA has a sedimentation value different from the 50S to 55S form in induced BU-V9 virions. Evidence comes from the fact that the virion 50S to 55S form sedimented faster than a Sindbis 49S RNA marker, while the intracellular 38S form sedimented more slowly.

An experiment was also performed to clarify whether or not the 38S intracellular form of VL30 RNA appeared as an aggregation artefact. A whole cell lysate of JLS-V9 cells was prepared by disruption of monolayer cultures with SDS. After lysis, protease K was added to further deproteinize the nucleic acid, and high mol. wt. DNA was sheared to reduce viscosity by a very brief sonication. The extract was then layered directly on to a sucrose gradient and sedimentation analysis was performed as in Fig. 4 (b). Both the 38S and 27S forms of VL30 RNA were observed (not shown). This extraction did not include procedures which might promote aggregation of RNA (such as phenol extraction and ethanol precipitation) and indicates that the 38S form did not occur by aggregation during extraction.
Fig. 4. Size analysis of undenatured and denatured VL30-specific RNA. (a) 32P-labelled BU-V9 virus was prepared from BrdUrd-induced JLS-V9 cells which had been labelled for 12 h with 100 μCi 32P-orthophosphate. Viral RNA was extracted and sedimented in a 15 to 30% sucrose gradient containing SDS. Centrifugation was for 3.5 h at 30000 rev/min and 25 °C in the SW41 rotor. The gradient was fractionated and the amount of 32P radioactivity in each fraction was measured. 3H-labelled Sindbis virus 49S genomic RNA was also included in the sample, and its sedimentation position is indicated. The 32P BU-V9 RNA contained RNA complexes of 70S and 50S as indicated. (b) Cytoplasmic RNA from uninduced JLS-V9 cells was prepared and sedimented as in (a) except that centrifugation was for 7 h. The gradient was fractionated and portions of each fraction were hybridized with high specific activity 3H BU-V9 cDNA (1000 cts/min assay). Percentage hybridization was determined by S 1 nuclease digestion, and the amount of hybridization is shown (O). An equivalent amount of JLS-V9 cytoplasmic RNA was denatured with dimethyl sulphoxide before sedimentation in a parallel sucrose gradient, and hybridization across this sucrose gradient is also shown (D). 3H Sindbis virus 49S RNA was sedimented in a third gradient and its sedimentation position is shown. The positions of 28S and 18S rRNA are also indicated (arrows).

DISCUSSION

In the results reported here, VL30 sequences were found to exhibit two other properties similar to standard murine retroviruses. As shown in Fig. 2, the reverse transcription of VL30 RNA in induced virus particles was initiated on an RNA primer, similar to standard MuLV. We have not investigated the nature of the RNA primer for VL30-specific DNA synthesis, although the primer for M-MuLV reverse transcription has been identified as tRNApro (Peters et al., 1977). Another feature of the RNA-primed reverse transcription of standard MuLV is that the location of the tRNA primer is at a specific position near the 5' end of the 38S RNA. This results in synthesis of a small, discrete 'strong-stop' cDNA when endogenous reactions are performed under conditions of limiting substrate. We examined endogenous reverse transcriptase reactions of BU-V9 virions by acrylamide gel electrophoresis in an effort to
identify a similar strong-stop sequence for VL30 transcription. Discrete DNA fragments of small size were synthesized. However, they were identical in size to M-MuLV strong-stop cDNA and its related fragments, and appeared to represent transcripts of the xenotropic MuLV RNA also present in BU-V9 virus preparations (D. Dolberg, I. M. Verma & H. Fan, unpublished results). No other discrete fragments specific to VL30 DNA could be detected; thus, we could not identify a discrete primer site on VL30 RNA, although such a site might have been undetected by this procedure.

If a specific RNA primer binding site exists on VL30 RNA, this site might have homology to the primer binding site of MuLV 38S RNA. We therefore also compared the nucleic acid sequence homology between M-MuLV strong-stop cDNA and VL30 RNA, since strong-stop cDNA represents those sequences directly adjacent to the M-MuLV primer binding site and it is quite conserved among different strains of M-MuLV (Hazeltine & Kleid, 1978). The lack of homology between total M-MuLV cDNA and VL30 RNA has been reported previously (Besmer et al., 1974; Howk et al., 1978; Keshet et al., 1980), and M-MuLV strong-stop cDNA also showed no homology to VL30 RNA (D. Dolberg, I. M. Verma & H. Fan, unpublished results).

The second similarity between VL30 sequences and MuLVs was that the pattern of intracellular pulse-labelling of VL30 RNA also resembled virus-specific RNA labelling in M-MuLV-infected cells. As discussed in Results, the finding that the only major species of pulse-labelled VL30-specific RNA is the same size as mature VL30 RNA suggests that the primary transcript is the same size as the VL30 RNA.

Keshet et al. (1980) have recently isolated lambda phage recombinant DNA clones containing copies of VL30 sequences. They have recently identified terminally repeated regions within the VL30 sequences, similar to the LTR sequence present in standard retrovirus proviral DNA (E. Keshet, Y. Shaul, J. Kaminchik & H. Aviv, personal communication). The pulse-labelling results presented here suggest that, like standard retroviruses, the terminal repeat regions of genomic VL30 DNA sequences may also carry a promoter signal for transcription.

The VL30 sequences showed one difference from MuLV in these experiments, and that was the presence of an intracellular hydrogen-bonded form of 38S. No intracellular higher mol. wt. forms have been observed for MuLV-specific DNA in infected cells. The intracellular 38S form is different from the presumed dimeric 50S to 55S form of VL30 RNA present in induced BU-V9 virus, and might represent a dimeric form with different secondary structure. Alternatively, the VL30 RNA may hydrogen bond with some other intracellular RNA to form the 38S structure.

In summary, these experiments indicate that the intracellular synthesis of VL30 RNA and initiation of its reverse transcription in induced virus particles occurs in the same fashion as for MuLV, reinforcing the hypothesis of the retrovirus-like nature of these sequences. There was one difference, i.e. the occurrence of an intracellular hydrogen-bonded form of VL30 RNA.

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