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Characterization of a Unique Retroviral Recombinant Containing 7S L Sequences

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Using a recently described system to generate recombinants between avian leukosis viruses (ALV) and cellular *neo* mRNA (A. M. Hajjar and M. L. Linial, J. Virol. 67:3845–3853, 1993), we isolated a recombinant containing 7S L sequences. Analysis of this recombinant revealed that it most likely arose during reverse transcription of three copackaged RNAs: 7S L RNA, *neo* RNA, and ALV genomic RNA. Reverse transcription appears to have initiated on the 7S L RNA. A model for the generation of this recombinant is described.

Avian retroviruses have been shown to package small RNAs (for a review, see reference 5). One such RNA which is present in large amounts in virions is the 7S L RNA, the ca. 300-nucleotide RNA component of the signal recognition particle (7). It is not known what role, if any, the 7S L RNA plays in the retroviral life cycle. The quail packaging cell line *SE*21Q1b randomly packages nonviral messages (4). Chen et al. have used melittin-disrupted virions isolated from *SE*21Q1b cells and have shown that the 7S L RNA is reverse transcribed (1). In their work, it was demonstrated that reverse transcription initiated at a specific internal location on the 7S L RNA, and the cDNA was found to be covalently attached to a 75-nucleotide RNA. This RNA presumably hybridized to the 7S L RNA just downstream of the site of initiation and acted as a primer for reverse transcription.

We have recently described a system to study nonhomologous retroviral recombination (2). Use of the *SE*21Q1b packaging line allows us to package a *neo* RNA that is devoid of retroviral sequences. This packaging, however, is not sufficient to confer G418 resistance on QT35 cells following infection. Addition of avian leukosis virus (ALV) allowed selection of G418-resistant cells, presumably by generating virions that copackaged the *neo* RNA with a helper viral genomic RNA. Recombinant proviruses, with a long terminal repeat driving expression of *neo*, which can be generated during reverse transcription, are then selected for in this assay. The 5′ recombination junctions of 11 clones were identified by sequencing polymerase chain reaction products that were generated by using a sense primer in U3 and an antisense primer in the *neo* open reading frame as previously described (2). One of the recombinants we isolated (1A6) was found to contain 7S L sequences inserted between the long terminal repeat and *neo* (Fig. 1). This recombinant was generated from virus harvested from CN3BP-1A cells. The single cell clone CN3BP-1A was derived from *SE*21Q1b cells, containing a single integrated copy of pCMVneo, that had been previously transfected with pRCAS-BP, a plasmid encoding ALV (2, 3). Therefore, generation of the 1A6 recombinant provirus required recombination between three different RNAs (7S L, *neo*, and a genomic ALV RNA).

In the experiments of Chen et al. (1) described above, initiation of reverse transcription occurred at a specific internal position on the 7S L RNA (Fig. 2). Surprisingly, the 3′ breakpoint in the 7S L sequences in 1A6 occurs at the exact nucleotide that was described as the site for initiation of reverse transcription. This observation and the absence of a primer binding site in 1A6 suggest that the 1A6 recombinant was generated during reverse transcription that was initiated on the 7S L RNA.

The structure of the 5′ end of the 1A6 DNA leads to the following model. Following initiation of reverse transcription on the 7S L RNA at an internal location (Fig. 3A), reverse transcriptase (RT) jumps to the viral genomic RNA and reverse transcribes U5 and R (Fig. 3B). There are two identical nucleotides at this junction, between U5 and 7S L (Fig. 1). The minus-strand DNA then jumps to the 3′ end of the helper virus RNA (Fig. 3C), allowing reverse transcription of the viral RNA to continue. At some point during the synthesis of the minus strand of the *env* or *pol* coding sequences, RT jumps to the copackaged *neo* RNA and reverse transcribes it to its 5′ end (Fig. 3C). Plus-strand synthesis initiates normally at the polypurine tract (PPT), generating a plus-strand primer containing U3, R, U5, and 7S L sequences (Fig. 3D). RT jumps to the 3′ end of the minus-strand DNA before reverse transcribing the tRNA-like primer that initiated reverse transcription. Such prematurely terminated plus-strand primers were seen in other 5′ junctions (2). This jump occurs without any homology at the breakpoint, perhaps facilitated by a conformation that juxtaposes the ends of the minus-strand DNA, allowing plus-strand DNA synthesis to continue and completing double-stranded DNA synthesis (Fig. 3E and F).

This model predicts the presence of 7S L sequences at the 3′ end of the recombinant. Polymerase chain reaction was performed on cell DNA isolated from 1A6 cells, with DNA from 1A4 cells (another recombinant that does not contain 7S L sequences at the 5′ junction) as a negative control, to amplify the predicted 3′ long terminal repeat-7S L sequences. An antisense primer which hybridizes to 7S L RNA (5′-GCTCTA GATATTGATGCCGGCCT-3′; the *Xba*I cloning site is underlined) was used with two different sense primers. The first set of reactions, with a sense primer contained within the PPT (5′-AGGGAGGGGGAAATG-3′), was performed by using a 5-min denaturing step at 95°C followed by 30 cycles of a 1-min denaturation at 95°C, a 1-min annealing step at 62°C, and a 3-min extension at 72°C. The second set of reactions, with a sense primer corresponding to the 3′ end of *env* (5′-GGCCT GTGGGCAGCCTG-3′), was performed by using a 5-min

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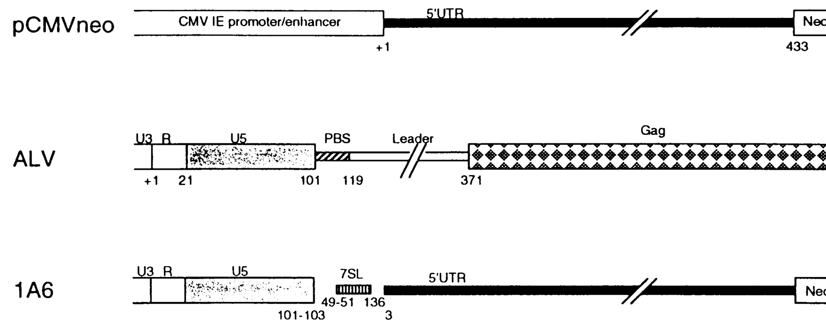

FIG. 1. Alignment of the 5′ recombination junction of 1A6 with parental sequences (pCMVneo and ALV). +1 indicates the start of transcription for each parental RNA. The numbers at 1A6 recombination sites correspond to the numbers from each parental RNA. PBS, primer binding site.

denaturation step at 95°C followed by 30 cycles of a 1-min denaturation at 95°C and a 2-min annealing-extension step at 72°C. Reaction conditions were as previously described (2) except that the reaction mixes with the 3′ *env* primer contained 200 μM each deoxynucleoside triphosphate. Appropriately sized bands (450 bp for the PPT-α7S L and 630 bp for the 3′ *env*-α7S L reactions) were gel isolated and purified with a QIAEX kit (Qiagen, Chatsworth, Calif.) and were cloned into the pCRII vector by using the TA cloning system (Invitrogen, San Diego, Calif.). Dideoxy-chain termination DNA sequencing with the Sequenase kit (U.S. Biochemical Corp., Cleveland, Ohio) revealed that the U5-7S L junction was the same as that found at the 5′ end, supporting our model that shows that initiation of reverse transcription on the 7S L RNA generates a minus-strand primer with 7S L sequences. If RT had jumped to the 7S L RNA following reverse transcription of *neo* during minus-strand DNA synthesis, 7S L sequences would not be present at the 3′ end of the provirus. If RT had jumped to the 7S L RNA during plus-strand strong-stop DNA synthesis, the 7S L sequences would have been in the antisense orientation at the 5′ end and would have been absent from the 3′ end.

We conclude that copackaging of 7S L RNA, *neo* RNA, and ALV RNA led to the generation of clone 1A6. Reverse transcription initiated on 7S L at the precise position previously shown to be generated by reactions using permeabilized virions (1). This is the first in vivo demonstration that 7S L RNA can play a role in retroviral reverse transcription. It is not known which sequences in 7S L allow its specific encapsidation. However, it appears that the sequences retained in clone 1A6 are not sufficient for packaging, since virions isolated from 1A6 cells, which contain ALV, did not package *neo* RNA (data from Northern [RNA] blots not shown). 1A6 RNA also lacks retroviral packaging sequences (ψ, [5]). It will be of interest to add ψ sequences into the 1A6 recombinant to see whether it can be packaged and reverse transcribed during subsequent rounds of infection.

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FIG. 2. Alignment of partial quail 7S L sequences with the complete human RNA. The sequence of human 7S L RNA is from reference 6. The quail sequences were obtained from endogenous RT reactions (1) and are incomplete. The complete sequence of avian 7S L RNA has not yet been determined. The 3′ ends of the quail and 1A6 sequences are identical and therefore may have been primed by tRNA-like primers which hybridized to the 7S L RNA just downstream of the 3′ ends of the partial RNAs depicted. b, bases.

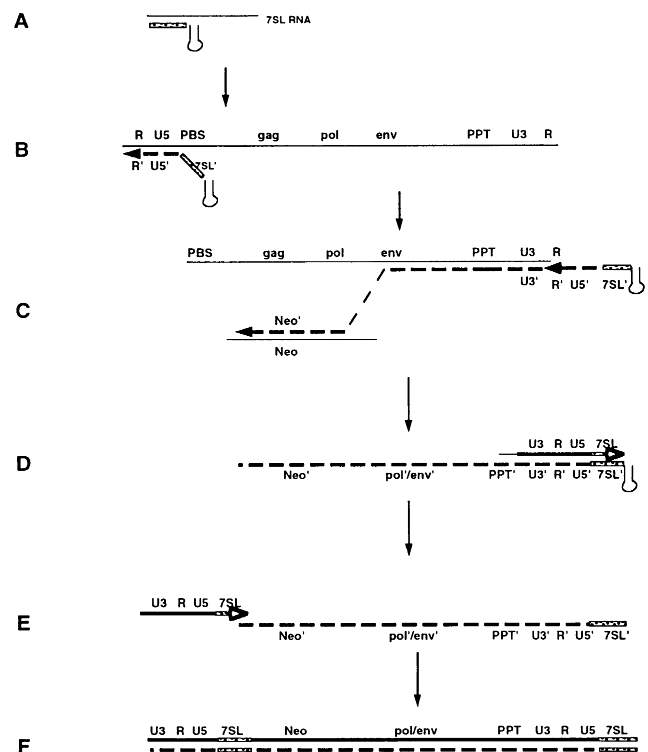

FIG. 3. Model for the generation of 1A6. (A) Initiation of reverse transcription of the 7S L RNA by a tRNA-like molecule (stem-loop structure) occurs internally within the 7S L molecule, as previously proposed (1). (B) RT jumps to the 5′ end of the genomic RNA. (C) Minus-strand translocation with an RT jump to *neo* RNA. (D) Plus-strand initiation at the PPT. (E) Plus-strand translocation. (F) Recombinant provirus. PBS, primer binding site. Thin solid line, RNA; broken line, minus-strand DNA; thick solid line, plus-strand DNA; shaded bar, 7S L sequences.

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