RNase H-Mediated Release of the Retrovirus RNA Polyadenylate Tail During Reverse Transcription

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By examining enzymatic reactions *in vitro*, we found that an early event during reverse transcription of the avian myeloblastosis virus RNA genome is the release of the 3' polyadenylate tail from the viral RNA template. By using specially constructed molecules containing minus-strand, strong-stop DNA and the 3' end of the viral RNA genome, we found that the reverse transcriptase-associated RNase H is responsible for the endonucleolytic release of polyadenylate.

Reverse transcription of the single-stranded RNA genome into double-stranded DNA is an essential feature of retrovirus replication (reviewed in references 17 and 39). This process is dependent upon a retrovirus reverse transcriptase which has both DNA polymerase (2, 38) and RNase H (26) activities. To define specific events of retrovirus double-stranded DNA synthesis, we reconstructed the process by using extensively purified reverse transcriptase and the genomic 35S RNA-tRNA^{Trp} template-primer complex from avian myeloblastosis virus (AMV) (27, 28). It is known that AMV DNA synthesis is initiated at the 3' terminus of a tRNA^{Trp} primer located 100 nucleotides from the 5' end of the viral 35S RNA (15, 35). The 5'-terminal 100 bases of the 35S RNA subunit are then copied, resulting in the synthesis of strong-stop (–) DNA (16, 35). After a transcriptional jump which involves base pairing of the 3' end of the strong-stop (–) DNA with the short repeat (R) sequence adjacent to the polyadenylate [poly(A)] tract, strong-stop (–) DNA is elongated into larger (–) DNA molecules (30, 36). This transcriptional jump is thought to be facilitated by the reverse transcriptase-associated RNase H activity which has been shown to remove the 5'-terminal sequences of the viral 35S RNA subunit subsequent to the synthesis of strong-stop (–) DNA (7, 8, 11). After the transcriptional jump, synthesis of the second DNA strand [(+) DNA] begins at a discrete location on the (–) DNA (20, 24, 28, 31, 34, 40). The synthesis of this (+) DNA species requires that the 3' end of the viral RNA genome be displaced from the (–) DNA, perhaps by an unwinding process or by degradation (e.g., by RNase H) after it is copied. We decided to test the possibility that genomic RNA is degraded during reverse transcription near the 3' end. We reasoned that if viral RNA is degraded at its 3' end during the elongation of strong-stop (–) DNA, then the release or degradation of the 3' poly(A) tail would be an early event in this process. We describe such an event in this communication and, furthermore, present evidence that the reverse transcriptase-associated RNase H activity is responsible for mediating this poly(A) release. A similar phenomenon has recently been described for the reverse transcription of globin mRNA (3).

Release of poly(A) during reconstructed reverse transcription. We used molecular hybridization with excess ³H-labeled polyuridylyate [poly(U)] (4) to determine the fate of the 3'-terminal poly(A) tail during reverse transcription of AMV 35S RNA-tRNA^{Trp} by purified reverse transcriptase. In this study, 35S RNA-tRNA^{Trp} was purified from the genomic 60S RNA complex, and the αβ form of reverse transcriptase was purified as previously described (18, 28) from virus purified from the plasma of leukemic chicks (33). The standard reverse transcriptase reaction conditions were those which we have previously shown to allow both the synthesis of genome-length (–) DNA transcripts and extensive (+) DNA synthesis, including the synthesis of strong-stop (+) DNA (28). In preliminary studies (data not shown), we found that during the course of 90-min reconstruction reactions, the poly(A) of 35S RNA was not digested to acid-soluble nucleotides, as measured by hybridization. It was also found that all of the material hybridizing to [³H]poly(U) was alkali sensitive, demonstrating that only RNA was detected by this assay. Although the poly(A) tail was not extensively hydrolyzed, the possibility still existed that it was released from the viral RNA by an internal break introduced into the template. For example, once the transcriptional jump with strong-stop (–) DNA occurs, an RNA-DNA hybrid, which could act as a substrate for RNase H, is formed adjacent to the poly(A) tail. In this case, the 100- to 200-nucleotide-long poly(A) tail would remain large enough to be acid insoluble. To test this possibility, we fractionated the products of a 20-min reverse transcriptase reaction with 35S RNA-tRNA^{Trp} as the template-primer by glycerol gradient centrifugation and hybridized the fractions to excess [³H]poly(U) (Fig. 1). In a parallel control reaction, DNA synthesis was prematurely terminated by the omission of dCTP. In both samples, the poly(A) tail of intact 35S RNA was detected by hybridization to [³H]poly(U). However, it was found that in the reaction containing all four deoxynucleoside triphosphates (dNTP) substrates, an additional peak sedimented at ca. 4S. Similar results were obtained when the samples were heated to 60°C for 3 min and quickly cooled just before centrifugation (data not shown). These results suggest that the poly(A) tail was being released and that DNA synthesis was required. The release of the poly(A) tract began within 5 min of DNA synthesis and continued for at least 40 min (Fig. 2). Again, in the absence of DNA synthesis no poly(A) release was observed. In an earlier study in which identical reaction conditions were used, we could not detect (+) DNA synthesis within 5 min after the start of a reconstruction reaction

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FIG. 1. Release of poly(A) during DNA synthesis. Standard 35S RNA-tRNA^{Trp}-directed reactions (20 μ l; 20 min) with the four unlabeled dNTPs (●) or without dCTP (○) were terminated by the addition of a solution containing 0.18 ml of 10 mM Tris-hydrochloride (pH 7.4), 40 mM NaCl, 2 mM EDTA, and 0.2% sodium dodecyl sulfate; reaction mixtures were then fractionated by centrifugation in nondenaturing 10 to 30% (vol/vol) glycerol gradients containing the same buffer with a Beckman SW50.1 rotor at 49,000 rpm for 150 min at 20°C. Fractions were collected and hybridized to [³H]poly(U) to detect poly(A) as described by Bishop et al. (4). The procedure for the synthesis of ³H-labeled homopolymers has been previously described (42).

(28). Thus, poly(A) release appears to begin before the initiation of (+) DNA synthesis.

The size of the released poly(A), as measured by sedimentation through glycerol gradients (Fig. 3A), was ca. 4.5S. This is similar to or slightly smaller than the size of the poly(A)-containing T₁ oligonucleotide derived from the total digestion of 35S RNA by RNase T₁ (Fig. 3B). We have been unable to reproducibly observe poly(A)-containing cleavage products sedimenting between 4.5S and 35S. As the poly(A)-containing T₁ oligonucleotide contains 16 to 19 nucleotides on the 5' side of the poly(A) tail and includes the short R sequence (35), these results suggest that the primary cleavage site is located in the vicinity of or on the 3' side of the R sequence. We have not specifically determined the cleavage site.

Role of reverse transcriptase-associated RNase H in the mediation of poly(A) release. To investigate the mechanism of poly(A) release, we prepared a defined model substrate to mimic the natural RNA-DNA hybrid formed during the transcriptional jump with strong-stop (–) DNA. This RNA-DNA hybrid, called 15S polyadenylated [poly(A)⁺] RNA-strong-stop (–) DNA, was prepared by annealing

FIG. 2. Kinetics of poly(A) release from 35S RNA-tRNA^{Trp}. The fraction of poly(A) appearing in the 4S to 5S peak fractions from glycerol gradients of variously timed, 20- μ l standard 35S RNA-tRNA^{Trp}-directed reactions in the presence (●) or absence (○) of the four unlabeled dNTPs was determined by hybridization to [³H]poly(U).

purified strong-stop (–) DNA (28) in at least a threefold molar excess to presized (~15S) poly(A)⁺-containing fragments derived from 35S RNA subunits (37). Purified 15S poly(A)⁺ RNA-strong-stop (–) DNA was found to be an efficient template-primer for DNA synthesis and, moreover, directed the early synthesis of the strong-stop (+) DNA species that we have previously reported to be synthesized in the endogenous reconstruction reactions directed by AMV 35S and 60S RNAs (28; data not shown). Thus, we conclude that the 15S poly(A)⁺ RNA-strong-stop (–) DNA hybrid is a valid model substrate with which to study the detailed events of reverse transcription at the 3' end of the viral genome. To test for poly(A) release, we prepared a model hybrid substrate in which the 15S RNA had been labeled at either the 5' end with [γ -³²P]ATP (2,900 Ci/mmol; New England Nuclear Corp.) and polynucleotide kinase (PL Biochemicals) or the 3' end with cytidine 3',[5'-³²P]-bis(phosphate) (2,900 Ci/mmol; New England Nuclear) and T4 RNA ligase (PL Biochemicals) in labeling reactions as described elsewhere (9, 23). Table 1 summarizes the results when 5'-³²P-labeled 15S poly(A)⁺ RNA-³H-labeled strong-stop (–) DNA was incubated with purified reverse transcriptase in the absence of dNTPs and then fractionated with oligodeoxythymidylate [oligo(dT)]-cellulose. Only 59% of the 5'-³²P-labeled 15S poly(A)⁺ RNA hybridized to the strong-stop (–) DNA bound to the column after incubation with reverse transcriptase, indicating the breakdown of some of the RNA. A corresponding amount (60%) of the ³H-labeled strong-stop (–) DNA was also found in the poly(A)⁺ fraction. In a control experiment, the binding of 5'-³²P-labeled 15S poly(A)⁺ RNA alone to oligo(dT)-cellulose was found to be unaffected by prior incubation with

reverse transcriptase (Table 1). This suggests that the RNA breakdown, as measured by this assay, is dependent upon an RNA-DNA hybrid. The structural integrities of the $[5'-^{32}\text{P}]\text{RNA}$ and ^3H -labeled strong-stop (–) DNA in this experiment were examined by polyacrylamide gel electrophoresis performed under denaturing conditions. The $[5'-^{32}\text{P}]\text{RNA}$ in the nonpolyadenylated $[\text{poly}(\text{A})^-]$ fraction migrated with the same mobility as or only slightly faster than the $[5'-^{32}\text{P}]\text{RNA}$ in the $\text{poly}(\text{A})^+$ fraction (data not shown), suggesting that the cleavage of the RNA in this experiment was limited to the 3' region. The ^3H -labeled strong-stop (–) DNA recovered from the $\text{poly}(\text{A})^+$ and $\text{poly}(\text{A})^-$ fractions after alkaline hydrolysis to destroy the $[^{32}\text{P}]\text{RNA}$ was found to be intact (data not shown), suggesting that the nucleolytic activity measured in this experiment was confined to RNA. The incubation of reverse transcriptase with $3'-^{32}\text{P}$ -labeled 15S $\text{poly}(\text{A})^+$ RNA alone or hybridized to strong-stop (–) DNA indicated the release of a 3' RNA species 100 to 200 nucleotides long only from the hybrid structure (Fig. 4).

The above results suggest that the loss of binding to oligo(dT)-cellulose by the 15S $\text{poly}(\text{A})^+$ RNA–strong-stop (–) DNA hybrid is due to the removal of the $\text{poly}(\text{A})$ tail of

FIG. 3. Sedimentation rates of $\text{poly}(\text{A})$ released during reverse transcription and of $\text{poly}(\text{A})$ released after RNase T_1 treatment. (A) Glycerol gradient centrifugation of $\text{poly}(\text{A})$ released during reverse transcription of 35S $\text{RNA-tRNA}^{\text{Trp}}$. Centrifugation of the reaction mixture (10 min; standard 50- μl reaction) was done for 10.5 h in an SW50.1 rotor. (B) Glycerol gradient centrifugation of a $\text{poly}(\text{A})$ -containing T_1 oligonucleotide. The $\text{poly}(\text{A})$ -containing T_1 oligonucleotide from AMV 35S RNA was prepared as described by Stoll et al. (35). In this experiment, 6.5 ng was centrifuged in parallel with the sample in (A). Nonlabeled *Escherichia coli* 5S RNA and chicken myeloblast 4S RNA were used as internal sedimentation markers.

TABLE 1. Effect of reverse transcriptase on the binding of $5'-^{32}\text{P}$ -labeled 15S $\text{poly}(\text{A})^+$ RNA– ^3H -labeled strong-stop (–) DNA to oligo(dT)-cellulose

Sample	Reverse transcriptase	% Bound to oligo(dT)-cellulose	
		$[5'-^{32}\text{P}]\text{RNA}$	^3H -labeled strong-stop (–) DNA
$5'-^{32}\text{P}$ -labeled 15S $\text{poly}(\text{A})^+$ RNA ^a	–	98.2	
	+	98.5	
$5'-^{32}\text{P}$ -labeled 15S $\text{poly}(\text{A})^+$ RNA– ^3H -labeled strong-stop (–) DNA hybrid ^b	–	97.4	98.0
	+	59.0	60.3

^a $5'-^{32}\text{P}$ -labeled 15S $\text{poly}(\text{A})^+$ RNA (0.24 μg ; 6,150 cpm) was incubated for 10 min at 37°C in standard reactions (50 μl) without dNTPs in the presence or absence of reverse transcriptase. The reaction was terminated by the addition of sodium dodecyl sulfate and EDTA to 1% and 10 mM, respectively, and the mixture was loaded in high-salt buffer (1) on a prewashed oligo(dT)-cellulose column (1). The column was then washed with 10 volumes of high-salt buffer containing 0.2% (wt/vol) sodium dodecyl sulfate, and the $\text{poly}(\text{A})^+$ material was eluted as described previously (1). The amount of radioactivity in the $\text{poly}(\text{A})^+$ and $\text{poly}(\text{A})^-$ fractions was determined by liquid scintillation counting.

^b $5'-^{32}\text{P}$ -labeled 15S $\text{poly}(\text{A})^+$ RNA (0.49 μg ; 12,560 cpm) was hybridized to 1.0 μg of ^3H -labeled strong-stop (–) DNA (850,000 cpm) in 16 μl of 10 mM KPO_4 (pH 7.5)–0.5 M NaCl–1 mM EDTA for 30 min at 53°C. ^3H -labeled strong-stop DNA was synthesized and purified from polyacrylamide gels as described previously (28). The resulting hybrid was purified by oligo(dT)-cellulose chromatography. Approximately 35 ng of $[^3\text{H}]\text{DNA}$ bound to the $\text{poly}(\text{A})^+$ RNA. The purified 15S $\text{poly}(\text{A})^+$ RNA–strong-stop (–) DNA hybrid was concentrated by ethanol precipitation after NaCl was added to 0.4 M. The precipitate was collected by centrifugation at $20,000 \times g$ for 30 min at 1°C, washed once with 0.5 ml of 70% ethanol in 40 mM NaCl, and dissolved in 10 μl of a solution containing 10 mM Tris-hydrochloride (pH 7.4), 40 mM NaCl, and 0.1 mM EDTA. The sample was divided in equal portions and included in reactions as described above. The values for ^3H -labeled strong-stop (–) DNA have been corrected for ^{32}P crossover into the ^3H channel.

the RNA by a nucleolytic activity associated with the purified reverse transcriptase. The requirement of an RNA-DNA hybrid for $\text{poly}(\text{A})$ release suggests the involvement of RNase H .

To document that RNase H was responsible for the nucleolytic release of $\text{poly}(\text{A})$ from the 15S $\text{poly}(\text{A})^+$ RNA–strong-stop (–) DNA hybrid, the effects of the RNase H -specific inhibitor polydeoxycytidylate-oligodeoxyguanylate₁₂₋₁₈ (7, 25) were examined under conditions in which the reverse transcriptase-associated DNA polymerase activity was only partially inhibited. It was found that polydeoxycytidylate-oligodeoxyguanylate₁₂₋₁₈ inhibited $\text{poly}(\text{A})$ release almost completely (Table 2), further suggesting that RNase H is responsible for the cleavage.

Studies were also performed with the purified α subunit from the AMV reverse transcriptase $\alpha\beta$ complex. The purified α subunit retains both DNA polymerase activity and RNase H activity (13). To determine whether the purified α subunit also had the ability to cleave the $\text{poly}(\text{A})$ tail, we tested it with the 15S $\text{poly}(\text{A})^+$ RNA–strong-stop (–) DNA hybrid. The α subunit, based upon RNase H activity with $[^3\text{H}]\text{poly}(\text{A})$ -polydeoxythymidylate [$\text{poly}(\text{dT})$], released a lower (60%), yet significant, amount of $\text{poly}(\text{A})$ than did an equivalent amount of the $\alpha\beta$ complex (Table 2). This further implicates RNase H as being responsible for $\text{poly}(\text{A})$ release.

DNA synthesis was required for $\text{poly}(\text{A})$ release when the 35S $\text{RNA-tRNA}^{\text{Trp}}$ complex was used as the substrate (Fig. 1 and 2) but not when the 15S $\text{poly}(\text{A})^+$ RNA–strong-stop (–) DNA hybrid was used as the substrate (Table 1 and Fig. 4). We suggest that this reflects the requirement for the formation of a suitable substrate in the case of the 35S

FIG. 4. Release of [$3'$ - 32 P]RNA from the $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA complex. An oligo(dT)-cellulose-purified $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA hybrid (0.25 μ g; RNA-DNA molar ratio, $\sim$ 10:1) or $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA alone was incubated with or without AMV reverse transcriptase in 8- μ l standard reactions in the absence of dNTPs for 20 min at 37°C. The reactions were terminated by the addition of 24 μ l of a solution containing 84% (vol/vol) deionized formamide, 12 mM EDTA, and 0.6% (wt/vol) Sarkosyl. After being heated at 55°C for 3 min, the samples were quickly cooled in ice, subjected to electrophoresis in a 4.8% polyacrylamide gel containing 7 M urea (22), and analyzed by autoradiography. In this electrophoretic system, single-stranded RNA and DNA of equal chain length migrate with the same mobility (22). Lanes: a, denatured *Hae*III-digested ϕ X174 DNA markers (nucleotide length indicated at left); b, $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA hybrid without reverse transcriptase; c, $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA hybrid with reverse transcriptase; d, $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA without reverse transcriptase; e, $3'$ - 32 P-labeled 15S poly(A) $^{+}$ RNA with reverse transcriptase.

RNA-tRNA^{Trp} complex, which includes the synthesis of strong-stop (-) DNA and the formation of a hybrid consisting of the 3'-terminal region of strong-stop (-) DNA and the 3' R sequence of 35S RNA. In the absence of DNA synthesis when the 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA hybrid was used as the substrate, hydrolysis appeared to be limited to poly(A) release. In other studies (J. C. Olsen, Ph.D. thesis, University of Montana, Missoula, 1982), it was found that in the presence of DNA synthesis, RNA hydrolysis at the 3' end of the viral RNA genome was more extensive than the hydrolysis of the poly(A) segment and was limited by the elongation of (-) DNA.

Recent *in vitro* studies have demonstrated that a short, discrete, purine-rich oligoribonucleotide derived from the viral RNA genome acts as a primer for the initiation of strong-stop (+) DNA synthesis (10, 31, 32, 34). These studies strongly implicate the reverse transcriptase-associated RNase H activity in both the generation and the subsequent removal of this primer (5, 10, 31, 32, 34). The events leading up to the generation of the strong-stop (+) DNA primer are not completely clear. Our results demonstrating poly(A) release favor a model in which the formation of a hybrid consisting of the 3'-terminal region of strong-stop (-) DNA and the 3' R region of the RNA genome during reverse transcription would generate a substrate suitable for the endonucleolytic activity of RNase H. This would generate a free RNA end. Elongation of (-) DNA followed by 3',5'-exonuclease action by RNase H would progressively

degrade the viral RNA and eventually form the primer for strong-stop (+) DNA. If this model is correct, then it must be explained how the DNA polymerase activity of reverse transcriptase can discriminate between the various RNA degradation products and choose the specific RNA fragment used to prime the precise (+) DNA initiation event observed in this region. Alternatively, the primer for (+) DNA initiation might be formed by precise RNase H-mediated endonucleolytic nicks, and poly(A) release may reflect RNase H specificity in cleavage near polypurine-rich regions.

Early studies (14, 19, 21, 41) suggested that the reverse transcriptase-associated RNase H behaves as an exonuclease requiring a free RNA end, as exemplified by the hydrolysis of 5'-terminal sequences from the viral RNA template after the synthesis of strong-stop (-) DNA (7, 8, 11). More recent studies, however, suggest that RNase H might behave as an endonuclease with some substrates. For example, Gerard (12) showed that either AMV RNase H or the RNase H activity associated with purified Moloney leukemia virus reverse transcriptase is able to degrade the poly(A) in the homopolymer hybrid poly(A)-poly(dT), in which the poly(A)

TABLE 2. Properties of reverse transcriptase-mediated poly(A) release with the 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA hybrid as the substrate

Expt	Reaction conditions ^a	DNA polymerase activity ^b	RNase H activity ^c	% Poly(A) released ^d
1	Complete Plus polydeoxycytidylate-oligodeoxyguanylate	492	21	69
		283	0.7	4
2	Complete Minus the $\alpha\beta$ form, plus the α form	109	72	47
		288	68	28

^a Reaction mixtures (50 μ l) were incubated for 10 min in experiment 1 and for 30 min in experiment 2. In experiment 1, polydeoxycytidylate-oligodeoxyguanylate (molar ratio, 1:1) was added to the reaction mixture last to a final concentration of 0.8 μ g/ml. In experiment 2, purified reverse transcriptase from the glycerol gradient step was further fractionated on a phosphocellulose column to separate the α and $\alpha\beta$ enzyme forms (13). The purity of the α subunit was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; the α subunit was found not to contain detectable β subunit.

^b Values represent picomoles of [3 H]TTP incorporated with poly(A)-oligo(dT)₁₂₋₁₈ as the template-primer. Reactions (50 μ l) contained the following: 50 mM Tris-hydrochloride (pH 8.1), 8 mM MgCl₂, 40 mM NaCl, 10 mM dithiothreitol, 0.1 mM [3 H]TTP (50 cpm/pmol), 5 μ g of poly(A)-oligo(dT)₁₂₋₁₈ per ml, and purified reverse transcriptase. The poly(A)-oligo(dT)₁₂₋₁₈ template-primer was prepared as described previously (42). Reaction mixtures were incubated at 30°C, the reactions were terminated by pipetting the mixtures onto Whatman DE-81 filters, and the mixtures were processed for analysis by liquid scintillation spectrophotometry as described previously (42). The amount of enzyme used in these experiments incorporated [3 H]TTP into DNA in a linear manner, at least through the indicated incubation times.

^c Values represent picomoles of [3 H]poly(A) solubilized with [3 H]poly(A)-poly(dT) as the substrate. Reaction mixtures were incubated at 30°C in experiment 1 and at 37°C in experiment 2. Reactions (50 μ l) contained the following: 50 mM Tris-hydrochloride (pH 8.1), 8 mM MgCl₂, 40 mM NaCl, 10 mM dithiothreitol, 1.4 μ g of [3 H]poly(A)-poly(dT) hybrid per ml, and purified reverse transcriptase. The [3 H]poly(A)-poly(dT) hybrid was prepared exactly as described previously (42). The extent of degradation of [3 H]poly(A) was measured by determining the amount of radioactivity rendered acid soluble (6). Reaction mixtures were mixed with 1 volume of calf thymus DNA (1 mg/ml) in 0.15 M NaCl-0.015 M sodium citrate and 2 volumes of 2 M HCl. The acid-insoluble polynucleotides were allowed to precipitate at 4°C for 15 min and were then collected by centrifugation at 8,700 $\times$ g for 5 min. A portion of the supernatant was removed and counted in Aquasol-2 (New England Nuclear).

^d Total poly(A) released in the oligo(dT)-cellulose assay with purified 15S poly(A) $^{+}$ RNA-strong-stop (-) DNA as the substrate, as described in Table 1.

is covalently linked to polycytidylate at its 5' end and to polydeoxyadenylate at its 3' end. Omer and Faras (29) demonstrated the involvement of AMV RNase H in the endonucleolytic removal of the tRNA^{Trp} primer from (−) DNA during reverse transcription. Our results demonstrating the release of the poly(A) tail are also best explained by endonucleolytic cleavage by RNase H. Further studies are required to precisely define the substrate specificity of RNase H. It may be, for example, that the specificity of the endonucleolytic activity of viral RNase H allows the cleavage of the RNA moiety in an RNA-DNA hybrid containing unmatched RNA and DNA tails. Alternatively, RNase H may exhibit limited sequence-specific endonucleolytic activity. At any rate, it appears that the reverse transcriptase-associated RNase H mediates a number of important events during the synthesis of retrovirus double-stranded DNA.

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