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Mechanism of Action of Ribonuclease H Isolated from Avian Myeloblastosis Virus and *Escherichia coli**

(RNA-dependent DNA polymerase/processive exonuclease/*E. coli* endonuclease)

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ABSTRACT **Purified preparations of RNA-dependent DNA polymerase isolated from avian myeloblastosis virus contain RNase H activity. Labeled ribohomopolymers are degraded in the presence of their complementary deoxyribopolymer, except [^{3}H]poly(U)·poly(dA). The degradation products formed from [^{3}H]poly(A)·poly(dT) were identified as oligonucleotides containing 3′-hydroxyl and 5′-phosphate termini, while AMP was not detected. The nuclease has been characterized as a processive exonuclease that requires ends of poly(A) chains for activity. Exonucleolytic attack occurs in both 5′ to 3′ and 3′ to 5′ directions.**

RNase H has also been purified from *E. coli*. This nuclease degrades all homoribopolymers tested in the presence of their complementary deoxyribopolymers to yield oligonucleotides with 5′-phosphate and 3′-hydroxyl termini. *E. coli* RNase H has been characterized as an endonuclease.

RNA tumor viruses appear to replicate via a DNA intermediate since virus infection is blocked by inhibitors of DNA synthesis (1–3), and since an enzyme capable of transcribing viral RNA into DNA, RNA-dependent DNA polymerase, is found in virions (4, 5). The products formed by purified RNA-dependent DNA polymerase are RNA·DNA hybrids, in which the DNA is covalently linked to RNA. Recently, Mölling *et al.* (6) reported that RNase H, an enzyme that specifically degrades polyribonucleotides in RNA·DNA hybrids, copurifies with the avian myeloblastosis virus (AMV) polymerase. These authors proposed that RNase H activity, in conjunction with the AMV polymerase, played an important role in the generation of free DNA from RNA·DNA transcript products. The presence of RNase H activity in preparations of purified RNA-dependent DNA polymerase has been confirmed in several laboratories (7–9). The function of RNase H, if any, in the transcription of viral RNA is not clear. In the present paper, we characterize the AMV RNase H as a processive exonuclease, and compare its properties with RNase H activity purified 2000-fold from *Escherichia coli*. Evidence is presented that the latter enzyme acts endonucleolytically.

MATERIALS AND METHODS

AMV was a generous gift of Dr. J. Beard, Duke University. *E. coli* B was purchased from General Biochemicals; polynucleotide phosphorylase (*Micrococcus luteus*) (10) and RNase II (11) were generous gifts of Dr. Maxine Singer, National Institutes of Health. T4 RNA ligase (12) and polynucleotide kinase (13) were prepared by Drs. R. Silber and V. G. Malathi. [γ-^{32}P]ATP was purchased from New England Nuclear; labeled polynucleotides and other nucleotides were from Schwarz/Mann Research or Miles Laboratories, Inc. *N*-cyclohexyl-*N*′-β(4-methylmorpholinium)ethyl carbodiimide *p*-toluene sulfonate was from Aldrich Chemical Co. All other reagents were described (14).

Assay for RNase H. Reaction mixtures (0.05 ml) containing 1 μmol of Tris·HCl (pH 8.0), 0.5 μmol of $MgCl_2$, 0.3 μmol of dithioerythritol, 2 μg of albumin, 0.52 nmol of poly(dT), 0.56 nmol of [^{3}H]poly(A), and enzyme were incubated for 30 min at 38°. Reactions were stopped by the addition of 0.1 ml of cold 0.1 M sodium pyrophosphate, 0.4 mg of albumin, 52 nmol of denatured salmon-sperm DNA, and 0.5 ml of 5% Cl_3CCOOH. The reaction mixtures were centrifuged at 7000 x *g* in an International centrifuge, and the supernatant was collected and counted in 10 ml of Bray's scintillation fluid (15).

Preparation of Circular Poly(A). Circular poly(A) was prepared with [5′-^{32}P]poly(A) (1420 cpm/pmol) and T4 RNA ligase by the method of Silber *et al.* (12). Unreacted poly(A) was removed by exhaustive digestion with RNase II (0.2 unit) (11), and the reaction mixture was heated at 65° for 4 min; 20% of the ^{32}P remained acid-insoluble after RNase II treatment.

Synthesis of [5′-^{32}P], [3′-^{3}H]Poly(A). 5′-Hydroxyl-terminal poly(A) (0.18 nmol of nucleotide, chain length 500–1000), prepared by treatment with bacterial alkaline phosphatase (2.8 units), was labeled with ^{32}P in a reaction mixture (1 ml) containing 50 μmol of Tris·HCl (pH 8.0), 3 μmol of sodium phosphate, 3 μmol of dithioerythritol, 75 μg of albumin, 5 μmol of $MgCl_2$, [γ-^{32}P]ATP (3240 cpm/pmol, 12 nmol), and polynucleotide kinase (13 units). The mixture was incubated at 38° for 15 min, after which time an additional 13 units of kinase, 12 nmol of [γ-^{32}P]ATP, and 3 μmol of dithioerythritol were added. Quantitative phosphorylation was achieved after a total of 30 min of incubation. The reaction was stopped by one extraction with phenol [saturated with 1 M Tris·HCl (pH 7.5)] and three with ether. Unreacted ATP was removed by gel filtration through Sephadex G-50. [^{3}H]AMP was added to the 3′ end of [5′-^{32}P]poly(A) by treatment with polynucleotide phosphorylase as follows: [5′-^{32}P]poly(A) dried under reduced pressure, was dissolved in a reaction mixture (0.05 ml) containing 5 μmol of Tris·HCl (pH 8.0), 0.5 μmol of $MgCl_2$, 3.7 μg of albumin, 1.2 μmol of [^{3}H]ADP (34 cpm/pmol), and

Abbreviations: AMV, Avian myeloblastosis virus.

* This paper is no. V in the series "RNA-Dependent DNA Polymerase Activity of RNA Tumor Viruses." The preceding paper is ref. 14.

0.07 unit of polynucleotide phosphorylase (10). The reaction was stopped after incubation for 20 min at 38° by heating at 80° for 4 min, and unreacted [^{3}H]ADP was removed by gel filtration through Sephadex G-50.

Synthesis of Cellulose–[5′-^{32}P], [^{3}H]Poly(A). Uniformly labeled [^{3}H]poly(A) (9 cpm/pmol, 0.8 μmol) was treated with [γ-^{32}P]ATP and polynucleotide kinase as described above, dialyzed overnight at 4° against 500 ml of 0.04 M sodium 2-(*N*-morpholino)ethanesulfonate (pH 6.0), concentrated to 0.4 ml under reduced pressure, and covalently linked to cellulose (Munktell, no. 410 powder) with *N*-cyclohexyl-*N*′-β-(4-methylmorpholinium)ethyl carbodiimide *p*-toluene sulfonate as described by Gilham (16). Unreacted poly(A) was removed from the cellulose by washing with 50 mM sodium phosphate (pH 7.5) overnight, followed by washing with 2 M NaCl–50 mM sodium phosphate (pH 7.5) at 4°, also overnight, and finally washing with water until the salt concentration of the eluate was less than 0.02 M. About 70% of the poly(A) was retained on the cellulose; less than 3% of the remaining ^{32}P label was released by incubation with 3 units of alkaline phosphatase for 60 min. Digestion with micrococcal nuclease (5 μg) released 80% of the [^{3}H]poly(A) in 60 min. Treatment with a large excess of RNase II (0.4 unit) released only 45% of the poly(A) after 60 min.

Synthesis of [^{3}H]Poly(A)–(dAMP)$_n$. A reaction mixture (0.05 ml) containing 5 μmol of Tris·HCl (pH 8.0), 0.15 μmol of $MnCl_2$, 2 μg of albumin, 28 nmol of [^{3}H]poly(A) (32 cpm/pmol), 0.86 μmol of dADP, and 0.14 unit of polynucleotide phosphorylase was incubated for 2 hr at 38°. The reaction was stopped by heating at 75° for 4 min, and unreacted poly(A) was removed with RNase II as described in the preparation of circular poly(A). About 10% of the poly(A) remained acid-insoluble after RNase II treatment. This procedure resulted in the addition of variable amounts of dAMP residues at the 3′-OH end of poly(A) (17).

Preparation of [^{3}H]poly(A)–Poly(C)·poly(G). A reaction mixture (0.05 ml) containing 5 μmol of Tris·HCl (pH 8), 2 μg of albumin, 0.4 μmol of $MgCl_2$, 1.1 μmol of CDP, 20 nmol of [^{3}H]poly(A) (9 cpm/pmol), and 0.07 unit of polynucleotide phosphorylase was incubated for 30 min at 38°. The reaction was stopped by heating at 80° for 4 min, and 0.13 μmol of poly(G) was added. Unreacted poly(A) was removed with RNase II as described above. 8–20% of the poly(A) remained acid-insoluble after RNase II treatment.

Synthesis of Poly(C)–[^{3}H]Poly(A). Poly(C)–[^{3}H]poly(A) was prepared with polynucleotide phosphorylase as described for the synthesis of [^{3}H]poly(A)–poly(C), except that 107 nmol of 5′-OH poly(C) (40–50 nucleotides in length) and 1.13 μmol of [^{3}H]ADP (54 cpm/pmol) were added instead of [^{3}H]poly(A) and CDP. The reaction was stopped after 15 min of incubation by heating at 80° for 4 min. Under these conditions, some unprimed synthesis of poly(A) occurred, since [^{32}P]AMP was detected on electropherograms after treatment of the reaction mixture with alkaline phosphatase, [γ-^{32}P]ATP, and polynucleotide kinase, followed by exhaustive digestion with RNase II and venom diesterase.

Synthesis of Cellulose–[5′-^{32}P], [^{3}H]Poly(A)–Poly(C)·Poly(G). Cellulose–[5′-^{32}P], [^{3}H]poly(A) was treated with polynucleotide phosphorylase and CDP, as described above for the synthesis of [^{3}H]poly(A)–poly(C).

Purification of AMV Polymerase and Stimulatory Protein were described (14). Units of polymerase are expressed as nmol of d(AT) copolymer synthesized in 30 min. Since the amount of RNase H activity varies with degree of purification of enzyme, RNase H units in nmol of poly(A) rendered acid-soluble in 30 min are also indicated.

Purification of E. coli RNase H. RNase H has been purified 2000-fold from extracts of *E. coli* B as will be detailed elsewhere. The final enzyme fraction cleaved 80 μmol of [^{3}H]-poly(A) per mg of protein in 20 min at 30° in the presence of poly(dT). These preparations are free of detectable DNase activity (endo and exo) and virtually free of detectable RNase activity with poly(A) in the absence of poly(dT). No acid-soluble tritium was released when circular [^{32}P] poly(A) [in the absence of poly(dT)] was incubated with *E. coli* RNase H and RNase II.

RESULTS

RNase H Activity Associated with AMV Polymerase. AMV polymerase, purified as described (14), has detectable RNase H activity, as measured by acid-solubilization of [^{3}H]poly-(A) in the presence of poly(dT). In addition, AMV polymerase preparations chromatographed on IRC-50 or DNA–agarose also contain RNase H activity. Although RNase H is closely associated with polymerase activity, as measured by DNA synthesis primed by AMV 60S RNA or d(AT) copolymer synthesis, the two activities are not always coincidental. The ratio of DNA synthesis to poly(A) degradation varies as much as 4-fold across the enzyme peak eluted from phosphocellulose (8). These results, as well as observations that the two activities are differentially inactivated by heat or *N*-ethylmaleimide, suggest that although the two enzyme activities have not been physically separated they may reside on different enzyme molecules or on different subunits of the same enzyme molecule.

AMV RNase H activity is independent of DNA synthesis, since it does not require dNTPs for activity. A requirement for dTTP is observed when poly(dT) is replaced by oligo(dT) in the reaction. With poly(A) (300–400 nucleotides in length) + poly(dT), nuclease action is linear until about 60% of the poly(A) is rendered acid-soluble, after which the rate decreases markedly. However, the reaction continues until more than 95% of the poly(A) is degraded. Removal of poly(dT) at any time during the incubation with pancreatic DNase results in an immediate cessation of poly(A) degradation, indicating that poly(dT) is required for continual action of the AMV RNase H. Stimulatory protein, which enhances DNA synthesis catalyzed by the AMV RNA-dependent DNA polymerase, increased the rate of attack on [^{3}H]poly(A)·poly(dT) by AMV RNase H as much as 4-fold. The stimulatory protein, in the absence of AMV polymerase, was free of detectable RNase H activity. As observed for DNA synthesis, the stimulatory protein specifically affected AMV RNase H activity and had no effect on RNase H isolated from *E. coli* under identical conditions. The acid-soluble degradation products of poly(A)·poly(dT) were identified as oligonucleotides 2–8 nucleotides long containing 3′-OH and 5′-phosphate termini (7, 8). AMP was not detected among the reaction products. The acid-soluble products formed with *E. coli* RNase H have been similarly characterized and consist of oligonucleotides 2–6 nucleotides long containing 3′-OH and 5′-phosphate termini with 4% AMP.

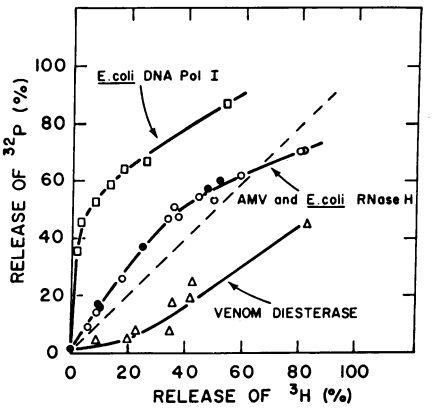

FIG. 1. Degradation of poly(A) labeled differentially at each end. [5′-^{32}P], [3′-^{3}H]poly(A) (1.1 nmol) and poly(dT) (1.04 nmol) were incubated for various lengths of time with each of the following enzymes: AMV polymerase (0.2 unit polymerase, 0.05 unit RNase H) and stimulatory protein (22.8 ng), *E. coli* RNase H (0.15, 0.3, or 0.6 unit), snake venom phosphodiesterase (0.015–0.15 unit) treated by the method of Sulkowski and Laskowski (20), or *E. coli* DNA polymerase I [0.12–1.2 units of Fraction VII, Jovin *et al.* (21)], and the amount of acid-soluble ^{3}H and ^{32}P was measured. The *broken line* is the expected curve if the rate of ^{32}P and ^{3}H release were identical.

Specificity of Action of AMV and E. coli RNase H. Various homoribopolymers are degraded in the presence of the corresponding complementary homodeoxyribopolymer by either enzyme. These include [^{3}H]poly(A)·poly(dT), [^{3}H]poly(I)·poly(dC), and [^{3}H]poly(C)·poly(dG). In the absence of complementary DNA strands or in the presence of the complementary RNA strands there is limited hydrolysis of ribopolymers. The only difference in specificity between the two enzymes is that AMV RNase H does not cleave [^{3}H]-poly(U)·poly(dA) (7). This specificity of the AMV enzyme is not understood, but it may have important consequences when the enzyme reaches regions rich in uridine. In addition to the synthetic homopolymers listed above, RNA polymerase products formed with single-stranded circular fd DNA are attacked by both nucleases. The RNA in hybrid structures was susceptible to digestion (85–90%) by either RNase H of AMV or *E. coli*. The RNase H-resistant RNA was susceptible to digestion by pancreatic RNase in the presence of 0.25 M NaCl.

Mode of Action of RNase H. In order to determine whether RNase H acts as an exo- or endonuclease, poly(A) was labeled at the 5′-terminus with [γ-^{32}P]ATP and polynucleotide kinase, and the 3′-end was extended by treatment with [^{3}H]-ADP and polynucleotide phosphorylase. If either RNase H is an exonuclease, then preferential release of label from one end or the other should occur. However, if radioactivity from both ends is released at the same rate, then the enzyme could act either endonucleolytically or processively from an end. The results with double-labeled poly(A)·poly(dT) are summarized in Fig. 1. As controls, doubly-labeled poly(A)·poly(dT) was treated with snake venom diesterase and DNA polymerase I. Venom phosphodiesterase, a 3′ to 5′ exonuclease, preferentially released ^{3}H from the 3′-end. *E. coli* DNA polymerase I contains a 5′ to 3′ RNase H activity (7, 8, 18). This activity preferentially released ^{32}P from the 5′-end of poly(A). The RNase H activity associated with DNA polymerase I was noted by us during the purification of RNase H from *E. coli* Pol A^{+} strains. The activity is insensitive to *N*-ethylmaleimide, coincides chromatographically with DNA polymerase activity measured with activated salmon-sperm DNA, and is neutralized by antibodies to DNA polymerase I. In contrast to these two enzymes, both *E. coli* and AMV RNase H preparations released ^{3}H and ^{32}P at the same rate. We conclude from these experiments that these RNase H activities might act endonucleolytically or processively.

Degradation of Circular Poly(A). These two mechanisms can be differentiated by use of RNA chains without ends. [5′-^{32}P]Poly(A) (40 nucleotides long) was treated with T4 RNA ligase, an enzyme that generates circular poly(A) products (12) in which the ^{32}P is not susceptible to attack by alkaline phosphatase or RNase II. *E. coli* RNase H degrades circular poly(A) to an acid-soluble form in the presence of poly(dT), while AMV RNase H does not (Table 1). Since circular poly(A) has no available ends, we conclude that *E. coli* RNase H is an endonuclease and that the AMV enzyme is probably an exonuclease.

AMV RNase H Is a Processive Exonuclease. A processive nuclease, once bound to a polynucleotide chain, completely degrades the chain before it is released. Thus, if excess [^{3}H]-poly(A)·poly(dT) is incubated with a limiting amount of RNase H so that all of the enzyme is bound, the addition of unlabeled poly(A)·poly(dT) to the incubation mixture should have no effect on the acid-solubilization of the labeled poly(A). As shown in Fig. 2, the addition of a 16-fold excess of unlabeled poly(A)·poly(dT) 1 min after the start of the reaction had no effect on the rate of degradation of [^{3}H]poly-(A)·poly(dT) by AMV RNase H. On the other hand, when unlabeled poly(A)·poly(dT) was added to the incubation before RNase H, the rate of reaction was markedly reduced (Fig. 2). We conclude from this experiment that RNase H associated with the AMV RNA-dependent DNA polymerase acts as a processive exonuclease. Consistent with this interpretation is the following experiment: [^{3}H]poly(A)·poly(dT) was treated with AMV RNase H until 0, 15, and 60% of the poly(A) was rendered acid-soluble. The reaction mixtures were treated with 10% HCHO at 70° for 10 min, then

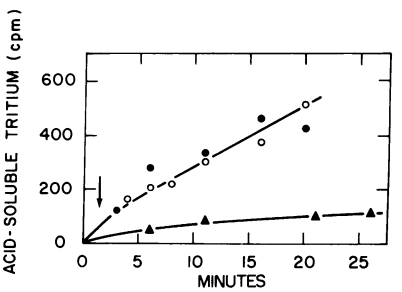

FIG. 2. Effect of adding excess poly(A)·poly(dT) on the rate of degradation of [^{3}H]poly(A)·poly(dT) by AMV RNase H. Purified AMV RNase H (0.07 unit polymerase, 0.015 unit RNase H) was incubated with [^{3}H]poly(A) (16.9 pmol, 475 cpm/pmol, prepared with *E. coli* RNA polymerase) and poly(dT) (18.8 pmol) for various lengths of time. Unlabeled poly(A) (276 pmol) and poly(dT) (376 pmol) were added where indicated. AMV RNase H, O——O; unlabled poly(A)·poly(dT) added 1 min after start of incubation (indicated by *arrow*), ●——●; unlabeled poly(A)·poly(dT) added before enzyme, ▲——▲.

analyzed on HCHO–sucrose gradients. The labeled poly(A) sedimented either as untreated poly(A) or as oligonucleotides at the top of the gradient. No large oligonucleotide intermediates were observed in the gradients.

Direction of Exonucleolytic Attack by AMV RNase H. Since AMV RNase H requires ends of RNA chains for activity, we investigated the direction of attack by modifying each end of poly(A), and determining the effect of such modifications on poly(A) degradation. The 3′-hydroxyl end of [^{3}H]poly(A) was blocked by addition of dAMP residues or extension of the poly(A) chain with poly(C). In both cases, poly(A) chains that were not extended were removed by digestion with RNase II. Both templates were rendered acid-soluble in the presence of poly(dT) by AMV RNase H (Table 1). The 5′-end of poly(A) was blocked by covalently linking it to cellulose, as described in *Methods*. 45% of the covalently linked poly(A) was released from the cellulose by the action of RNase II. 75% of the poly(A) (relative to the amount released by RNase II) was released by AMV RNase H (Table 1). The 5′-end of poly(A) was also blocked by synthesis of poly(C)–[^{3}H]poly(A) with polynucleotide phosphorylase. The [^{3}H]poly(A) in this polymer was rendered acid-soluble by AMV RNase H. These results indicate that AMV RNase H preparations act in both 5′ to 3′ and 3′ to 5′ directions. Consistent with the above conclusion is the observation that the ^{32}P and ^{3}H from [5′-^{32}P], [3′-^{3}H]poly(A) were released at the same rate, even when a large molar excess of AMV RNase H to ends of poly(A) was present. The experiment with the circular poly(A) indicated that ends were required for reaction. This conclusion was further confirmed by the observation that when the 3′-OH end of cellulose–[5′-^{32}P]-poly(A) was linked with poly(C), no degradation of the poly(A) was observed with AMV RNase H in the presence of poly(dT) (Table 1). *E. coli* RNase H, an endonuclease, degraded all poly(A) derivatives tested in the presence of poly-(dT) (Table 1).

TABLE 1. *Influence of substitutions at ends of poly(A) on the action of RNase H*

Polymer added	Activity (% degradation) by RNase H of: AMV +Poly-(dT)	AMV −Poly-(dT)	*E. coli* +Poly-(dT)	*E. coli* −Poly-(dT)
Circular [^{32}P]poly(A)	<1	<1	81	5
[^{3}H]Poly(A)–(dAMP)$_n$	89	0	84	0
[^{3}H]Poly(A)–poly(C)·poly(G)	85	0	96	10
Poly(C)–[^{3}H]poly(A)	88	6	95	4
Cellulose–[5′-^{32}P], [^{3}H]poly(A)	75	0	100	0
Cellulose-[5′-^{32}P], [^{3}H]poly(A)–poly(C)·poly(G)	4.6	0	100	4.6

Poly(A), modified as described, was treated as follows: (*A*) Circular [^{32}P]poly(A) (2000 cpm, 0.35 nmol)·poly(dT) (1 nmol) was incubated with purified AMV polymerase (0.4 unit polymerase, 0.1 unit RNase H) and stimulatory protein (22.8 ng) or *E. coli* RNase H (0.66 unit) for 30 min at 38°; acid-insoluble radioactivity was determined as described (14). (*B*) [^{3}H]poly(A)–(dAMP)$_n$ (14.1 pmol, 32 cpm/pmol) was incubated with or without poly(dT) (0.25 nmol) with either AMV RNase H (0.38 unit polymerase, 0.084 unit RNase H) or *E. coli* RNase H (0.44 unit) for 30 min at 38°, as described in part *A*. (*C*) [^{3}H]poly(A)–poly(C)·poly(G) (129 pmol, 9 cpm/pmol) was incubated with or without poly(dT) (1 nmol) with either AMV RNase H (0.38 unit polymerase, 0.084 unit RNase H) and stimulatory protein (17 ng) or *E. coli* RNase H (0.44 unit) for 30 min at 38°. (*D*) Poly(C)–[^{3}H]poly(A) (141 pmol, 9 cpm/pmol) was incubated with or without poly(dT) (0.2 nmol) with either AMV RNase H (0.38 unit polymerase, 0.084 unit RNase H) and stimulatory protein (17 ng) or *E. coli* RNase H (0.44 unit) for 30 min at 38°. (*E*) A suspension (0.1 ml) of cellulose–[5′-^{32}P], [^{3}H]poly(A) (1800 cpm ^{3}H, 9 cpm/pmol) was centrifuged at 3000 rpm for 2 min in an International centrifuge, and the supernatant was discarded. The cellulose was suspended in a reaction mixture (0.05 ml) containing AMV RNase H (1.9 units polymerase, 0.42 unit RNase H) and stimulatory protein (17 ng) or *E. coli* RNase H (3.3 units) with or without poly(dT) (2 nmol); tubes were mixed every 5 min during the incubation. After 60 min at 38°, the reaction was stopped by the addition of 0.2 ml of cold water and centrifuged in an International centrifuge for 2 min at 3000 rpm. The supernatant was counted in 10 ml of Bray's scintillation fluid. The cellulose was suspended in 0.2 ml of water and also counted in 10 ml of Bray's scintillation fluid. The amount of poly(A) released from the cellulose by RNase II was assumed to be all of the poly(A) accessible to attack by a processive nuclease. (*F*) Cellulose–[5′-^{32}P], [^{3}H]poly(A)–poly(C)·poly(G) (1320 cpm ^{3}H, 9 cpm/pmol) was treated as described in *E*.

DISCUSSION

Preparations of AMV polymerase purified in our laboratory contain detectable RNase H activity, as described by Mölling *et al.* (6), Baltimore and Smoller (7), and Keller and Crouch (9). Whether these two activities are part of one enzyme molecule composed of different subunits or present in physically separated proteins remains to be elucidated.

The products formed from poly(A)·poly(dT) by action of AMV RNase H are oligonucleotides with 3′-OH and 5′-phosphate termini. Despite this fact, the nuclease has an absolute requirement for ends of RNA chains for activity, as shown by its inability to digest circular poly(A) or poly(A) blocked at both 3′ and 5′ ends. Furthermore, the rate of degradation of [^{3}H]poly(A)·poly(dT) is unaffected by the addition of excess unlabeled poly(A)·poly(dT) after start of the reaction. These results can be explained if AMV RNase H acts as a processive exonuclease. The exonucleolytic cleavage can occur in both 5′ to 3′ and 3′ to 5′ directions, as shown by the ability of the nuclease preparation to degrade poly(A) blocked either at the 5′- or 3′-end. In contrast, *E. coli* RNase H acts endonucleolytically and attacked all of the poly(A) substrates used.

The role that AMV RNase H plays in the mechanism of RNA transcription to DNA is unclear. Our understanding of the action of RNA-dependent DNA polymerase has been hampered by the nature of the oncornaviral RNA copied. The RNA of these viruses is a 60–70S complex composed of 35S RNA and smaller segments hydrogen-bonded in a partly duplex structure (19). It is generally thought that the 35S RNA segments represent intact subunits, while the heterogeneous RNA material sedimenting between 4 and 30 S is degraded or nicked RNA molecules generated by action of the endogeneous RNase of virions.

The DNA products of RNA-dependent DNA polymerase

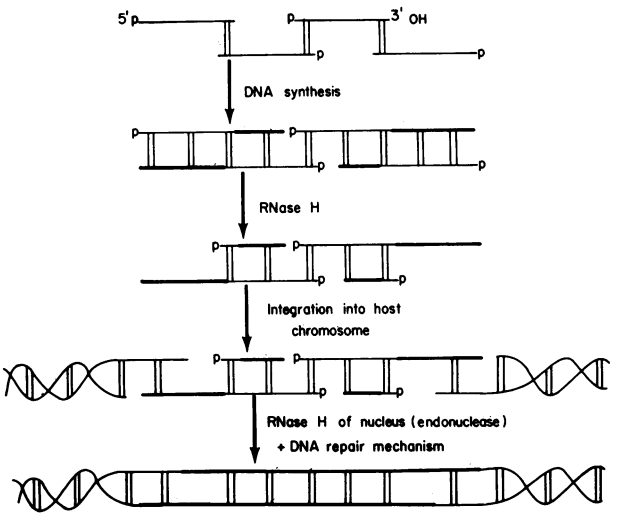

FIG. 3. Postulated role of RNA-dependent DNA polymerase and RNase H in virus replication. *Thin lines* represent viral RNA; *thick lines* represent newly synthesized DNA, and the *helical structure* represents the host chromosome.

acting on 60–70S segmented RNA template are small (5–8 S), as shown by alkaline sucrose gradient sedimentation and by label transfer experiments (14). Furthermore, isopycnic banding in Cs_2SO_4 after formaldehyde denaturation shows covalent attachment of the newly synthesized DNA to RNA primer 3'-OH ends. Since segmented 60–70S RNA has many such 3'-OH ends, the action of RNA-dependent DNA polymerase on this RNA would generate short covalently-linked DNA segments as RNA·DNA hybrids throughout the RNA (Fig. 3) instead of the viral length DNA expected for replication.

The mechanism of action of AMV RNase H reported here does not suggest to us a role for this activity in the release of DNA as postulated by Mölling *et al.* (6). Detailed studies show that RNA in internal RNA·DNA hybrids will *not* be cleaved by the AMV RNase H, because the enzyme is a processive exonuclease that requires hybrid RNA ends for activity. However, AMV RNase H would specifically remove RNA from both ends to create single-stranded DNA regions. These single-stranded DNA ends could be used by the host cell recombination machinery to incorporate the hybrid structure directly into the host chromosome. In this way, it may not be necessary to transcribe all RNA regions into DNA before integration. Once this structure is integrated, RNase H of the nucleus [an endonuclease (9)] could remove internally situated RNA in hybrid structures and the host DNA-repair machinery could convert these structures into DNA. This mechanism would obviate the necessity to integrate multiple small pieces of DNA into the host chromosome. A diagrammatic summary of the postulated reaction is presented in Fig. 3.

The mechanism proposed in Fig. 3 predicts that after infection, integration of part of the oncornaviral RNA into the host chromosome should occur. In addition, it suggests that RNA-dependent DNA polymerase may be responsible for converting virus RNA of suitable structure (partly duplex in structure, with a primer–template relationship) into RNA·DNA hybrids, rather than catalyzing the transcription of the entire RNA genome into DNA.

The mechanism of virus RNA replication is unknown. Since the genetic information of the virus proposed above would reside within the host chromosome, a second prediction of this model is that the synthesis of oncornaviral RNA is catalyzed by the DNA-dependent RNA polymerase of the host.

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