Defects in Primer-Template Binding, Processive DNA Synthesis, and RNase H Activity Associated with Chimeric Reverse Transcriptases Having the Murine Leukemia Virus Polymerase Domain Joined to *Escherichia coli* RNase H†

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ABSTRACT: The RNase H domain of murine leukemia virus (MuLV) reverse transcriptase (RT) was replaced with *Escherichia coli* RNase H, and the effect on RNase H activity and processive DNA synthesis was studied, using RNA−DNA hybrids containing sequences from the MuLV polypurine tract (PPT). Two chimeric RTs, having the entire polymerase domain or all but the last 19 amino acids, were expressed. In both cases, these RTs made multiple cuts in PPT-containing substrates, whereas wild-type cleavages occurred primarily at sites consistent with the distance between the polymerase and RNase H active sites. Primer extension assays performed with the chimeric RTs, an RNase H-minus RT, and wild-type showed that the presence of a wild-type viral RNase H domain facilitates processive DNA synthesis. When wild-type RT was bound to primer-template, two retarded bands could be detected in band-shift assays. In the absence of primer extension, a high proportion of enzyme-bound primer-template was associated with the faster-migrating band, whereas with DNA synthesis, more of the bound radioactivity was in the super-shifted complex. This suggests that the super-shifted complex contains the active form of RT. The mutant RTs were deficient in formation of this complex, but the chimeric RTs were somewhat less defective than the RNase H-minus mutant. Our results demonstrate that in the wild-type enzyme, the RNase H domain is required to stabilize the interaction between RT and primer-template.

Reverse transcriptase (RT)[1] is a virion-associated enzyme encoded by the retroviral *pol* gene and is synthesized from a large Gag-Pol precursor which is cleaved by the viral protease at a late stage in virus assembly (Levin et al., 1993). The role of RT is to make a double-stranded DNA copy of the viral RNA genome (Gilboa et al., 1979), a process which is essential for virus replication. The enzyme is multifunctional and has RNA- and DNA-dependent DNA polymerase activities as well as RNase H activity, which degrades the RNA moiety of an RNA−DNA hybrid (Varmus & Swanstrom, 1984; Hostomsky et al., 1993). RNase H has several roles in the process of reverse transcription (Champoux, 1993). One of its functions is to generate the RNA primer for initiation of plus-strand viral DNA synthesis by selective cleavage at the PPT, a short, highly conserved, purine-rich region near the 3′-end of the viral genome (Champoux, 1993).

Studies on MuLV and HIV RTs have established that RT has two functional domains: an N-terminal polymerase domain and a C-terminal RNase H domain (Johnson et al., 1986; Hansen et al., 1988; Hizi et al., 1988, 1991; Kotewicz et al., 1988; Levin et al., 1988; Tanese & Goff, 1988; Prasad & Goff, 1989). This arrangement of the domains has been confirmed by analysis of the X-ray crystal structures of the isolated HIV-1 RNase H domain (Davies et al., 1991) and HIV-1 RT (Kohlstaedt et al., 1992; Jacobo-Molina et al., 1993). Despite the similarity in RT domain organization, there are also differences in the structure and subunit composition of RTs from different retroviruses (Katz & Skalka, 1994). For example, MuLV RT is isolated from virions as a monomer (Moelling, 1974; Gerard & Grandgenett, 1975; Verma, 1975; Gerwin & Levin, 1977), whereas the avian retroviral RTs (Varmus & Swanstrom, 1984) and HIV-1 RT (diMarzo Veronese et al., 1986; Lightfoote et al., 1986) are isolated as heterodimers. The HIV-1 heterodimer consists of two subunits: a 66-kDa subunit (p66) and a 51-kDa subunit (p51), which lacks the RNase H domain, but has the same N-terminus as p66 (diMarzo Veronese et al., 1986; Lightfoote et al., 1986). Dimerization is a prerequisite for HIV-1 and HIV-2 RT activity (Restle et al., 1990, 1992; Becerra et al., 1991; Müller et al., 1991). Recently, it has been proposed that MuLV RT also functions as a dimer during DNA synthesis (Telesnitsky & Goff, 1993a).

Several investigators working with purified RTs have presented evidence suggesting that the polymerase and RNase H activities are not strictly coupled (Huber et al., 1989; DeStefano et al., 1991; Kati et al., 1992), while others (Oyama et al., 1989; Wohrl et al., 1990; Luo & Taylor, 1990; Schatz et al., 1990; Furfine & Reardon, 1991; Fu & Taylor, 1992; Gopalakrishnan et al., 1992; Ben Artzi et al., 1993; Post et al., 1993) have demonstrated coordination of these activities. We have also characterized RNase H cleavages catalyzed by wild-type RT as 3′-OH-dependent (i.e., cleav-

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[1] Abbreviations: RT, reverse transcriptase; PPT, polypurine tract; MuLV, murine leukemia virus; HIV, human immunodeficiency virus; EIAV, equine infectious anemia virus; kDa, kilodalton(s); nt, nucleotide(s); PCR, polymerase chain reaction; dNTPs, deoxyribonucleoside triphosphates; ddNTPs, 2′,3′-dideoxyribonucleoside triphosphates.

ages which occur when the 3′-OH of the DNA strand is bound to the polymerase active site and which are determined by the distance between the two active sites) or 3′-OH-independent (Post et al., 1993).

In the present study, we have continued to investigate the functional relationship between the two domains of RT and have used RNA−DNA hybrids containing sequences from the MuLV PPT to determine how replacement of the MuLV RNase H domain with a heterologous, cellular RNase H affects RT function. The cellular RNase H chosen for this work was *Escherichia coli* RNase H. X-ray crystallographic studies of *E. coli* RNase H (Katayanagi et al., 1990; Yang et al., 1990) and the RNase H domain of HIV-1 RT (Davies et al., 1991; Kohlstaedt et al., 1992; Jacobo-Molina et al., 1993) have demonstrated that these two structures are very similar; one striking difference, however, is that the HIV RNase H has a deletion in the connecting region between the α-B and α-D helices (consisting of the α-C helix and an adjacent loop region), which is thought to be important for substrate binding (Hostomsky et al., 1993). The isolated HIV-1 RNase H domain is not enzymatically active (Hostomsky et al., 1993), but insertion of the connecting region of *E. coli* RNase H generates a functional protein (Stahl et al., 1994). In contrast, the MuLV RNase H domain has the amino acids corresponding to the α-C helix and loop region of *E. coli* RNase H (Yang et al., 1990; Nakamura et al., 1991) and, like *E. coli* RNase H, can function in the absence of a polymerase domain (Tanese & Goff, 1988). Moreover, on the basis of sequence comparison and modeling studies, it has been predicted that the structures of MuLV and *E. coli* RNases H are very much alike (Yang et al., 1990; Nakamura et al., 1991).

Previously, we described a novel mutant, having the MuLV polymerase domain fused to the complete sequence of *E. coli* RNase H (Post et al., 1993). Although the *E. coli* RNase H substitution might be expected to have some effect on the overall structure of MuLV RT, it is important to note that the chimeric RT is a stable protein which is expressed as efficiently as wild-type (Post et al., 1993). Similarities in the RNase H structures of MuLV and *E. coli* cited above as well as the ability of the MuLV polymerase (Levin et al., 1984; Kotewicz et al., 1988; Tanese & Goff, 1988) and RNase H (Tanese & Goff, 1988) domains to function independently are factors which probably contribute to formation of a stable chimeric enzyme. We now have 2 versions of the chimeric construct, having either the entire polymerase domain or all but the last 19 amino acids. (These 19 amino acids form the C-terminus of the connection subdomain.) The RNase H activity, primer-template binding, and processive DNA synthesis catalyzed by the chimeric RTs are compared with the corresponding activities of wild-type and an RT which is missing the entire RNase H domain. We demonstrate that a wild-type viral RNase H domain facilitates processive DNA synthesis by stabilizing binding of RT to primer-template and provide further evidence for the cooperative interaction between the polymerase and RNase H domains in the wild-type enzyme.

MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP (3000 Ci/mmol), [α-^{32}P]ATP (3000 Ci/mmol), and [α-^{32}P]dATP (3000 Ci/mmol) were purchased from Amersham. T7 RNA polymerase and pGEM-3Z were purchased from Promega. The dNTPs, ddNTPs, poly(rA), and $(dT)_{12-18}$ were purchased from Pharmacia LKB Biotechnology Inc. To make the poly(rA)−oligo(dT) hybrid, poly(rA) and oligo(dT) were mixed in a 1:1 base ratio, using the A_{260} value supplied by the manufacturer to calculate the picomoles of oligo(dT). The Sequenase kit was obtained from U. S. Biochemical Corp. RNase T1, RNase U2, and DH5α competent cells (library efficiency) were from GIBCO-BRL. DC600 Hydrolink gel was purchased from AT Biochem. DNA oligonucleotides were synthesized on a Biosearch Synthesizer Model 8750 (New Brunswick Scientific Co.). A phosphorimager (Molecular Dynamics) was used to quantitate the amount of radioactivity in gels. All aqueous solutions were prepared with water treated with diethyl pyrocarbonate.

Bacterial Strains and Plasmids. Derivation of plasmids pP_AH_A, $pP_A\Delta H$, and pP_AH_{EC}-1 (previously designated pP_AH_{EC}) from the parent clone pRT24 (Hu et al., 1986) has been described (Post et al., 1993). All of the plasmids with MuLV RT sequences were isolated in *E. coli* strain DC519 (Hu et al., 1986; Sisk et al., 1986) and were expressed in *E. coli* strain DC520 (Hu et al., 1986; Sisk et al., 1986). The pGEM plasmid pPPT-A, where A refers to AKR MuLV (see below), was isolated in *E. coli* strain DH5α.

Construction of Plasmids. The chimeric clone pP_AH_{EC}-2 contains the entire AKR MuLV polymerase domain fused to sequences encoding *E. coli* RNase H (Kanaya & Crouch, 1983), in contrast to pP_AH_{EC}-1 which is missing the last 19 amino acids in the connection subdomain (Post et al., 1993). The new clone was generated by first digesting pP_AH_{EC}-1 at a unique *Xho*I site (nt 4098) in the AKR MuLV RT coding region (Herr, 1984). A 75-nt double-stranded oligonucleotide with *Xho*I sites at both ends and whose coding strand contained the RT sequence from nt 4098 to 4154 (Herr, 1984) was also digested with *Xho*I. The resulting 57-nt oligonucleotide was separated from uncut 75-mer in a 7% DC600 Hydrolink gel and then ligated to the *Xho*I-digest of pP_AH_{EC}-1. Sequence analysis was used to obtain clones with the correct orientation of the insert. The cloning strategy resulted in the addition of two amino acids (encoded by the 6 nt from the second *Xho*I site) to the C-terminus of the polymerase domain. The expressed P_AH_{EC}-2 RT had the predicted molecular size of 76 kDa, as shown by analysis in protein gels, and its specific polymerase and RNase H activities were the same as those of P_AH_{EC}-1 (data not shown; Post et al., 1993).

Clone pPPT-A contains the PPT and surrounding 5′ and 3′ sequences; it was constructed by using clone 623P-2, which has AKR MuLV sequences from the *Sal*I site to the 3′-end of the viral genome (Levin et al., 1984), as a template for PCR. The PCR fragment, containing nt 7602 to nt 8033 in the AKR MuLV coding strand (Herr, 1984), was inserted between the *Eco*RI and *Xba*I sites in pGEM-3Z. The insert and surrounding regions were sequenced using a Sequenase kit.

Enzyme Expression and Purification. The procedures used for expression of the RT clones and purification of the enzymes were carried out as described by Post et al. (1993). Multiple preparations of each of the enzymes gave reproducible specific activity values and essentially one band in Coomassie blue-stained polyacrylamide gels.

Preparation of Labeled RNA and Primers. The plasmid pPPT-A was digested with *Hin*dIII prior to transcription with T7 RNA polymerase; transcription reactions were carried out

according to the conditions specified by Promega. The resulting MuLV transcript contains 467 nt and includes at its 3′-end the 18 nt between the *Xba*I and *Hin*dIII sites of the vector. The sequence of the RNA was verified by analysis of the reverse transcript and comparison with the DNA sequence. Uniformly labeled RNA was obtained from reactions containing [α-^{32}P]ATP; 5′-end labeled RNA was obtained by incubating unlabeled RNA, dephosphorylated at its 5′-end, with [γ-^{32}P]ATP and T4 polynucleotide kinase, as described (Sambrook et al., 1989). RNA samples were purified by electrophoresis in 6% sequencing gels, except where noted otherwise. Primers used for RNase H cleavage and DNA synthesis reactions were purified by electrophoresis in 15% sequencing gels. Where specified, primers were labeled with ^{32}P at their 5′-ends using the same incubation conditions as those described for RNA (see above); labeled primer was separated from unincorporated ATP using a Stratagene NucTrap Push column, following instructions provided by the manufacturer.

Preparation of Single-Stranded Plus-Strand MuLV DNA. To prepare single-stranded plus-strand MuLV DNA, pPPT-A was digested with *Hin*dIII and subjected to PCR amplification with the foward primer used to make the clone. The 469-nt PCR fragment was purified by electrophoresis in a 3% low-melt agarose gel. Before addition to reactions, the purified fragment was heated at 95 °C for 10 min and immediately placed in ice to prevent formation of high molecular weight aggregates. The sequence of the single-stranded DNA was determined and shown to be the same as that of the coding strand of the double-stranded plasmid DNA (data not shown).

Polymerase and RNase H Assays. Definition of polymerase (P) and RNase H (H) units and assays of polymerase activity with poly(rA)−oligo(dT) and RNase H activity with poly(rA)−poly(dT) are described in Post et al. (1993). To assay RNase H cleavage with substrates containing the PPT region, 0.5 pmol of 5′-[^{32}P]RNA (~20 000 cpm) or 0.4 pmol of uniformly labeled [^{32}P]RNA (~70 000 cpm) was hybridized to 1−2 pmol of a 40-nt DNA oligonucleotide (see Figure 1) in 4 μL of buffer containing 125 mM Tris-HCl, pH 8.0, 250 mM KCl, and 25 mM $MgCl_2$ at 65 °C for 5 min followed by slow cooling at room temperature for 30 min. The hybrid was incubated in a final volume of 10 μL in reactions containing 50 mM Tris-HCl, pH 8.0, 75 mM KCl, 8 mM $MgCl_2$, 1 mM DTT, and RT, as indicated, for 1 h at 37 °C. Where cleavage was measured under conditions which permit limited extension of the DNA oligonucleotide primer, reactions also contained 0.25 mM each of 1 ddNTP and 3 dNTPs (ddNTP mixture). Assay of DNA synthesis in the presence of 1 ddNTP and 3 dNTPs was carried out under the same conditions as the corresponding RNase H cleavage reactions, except that 0.6 pmol of unlabeled RNA was hybridized to 0.2 pmol of 5′-^{32}P-labeled oligonucleotide (~140 000 cpm). Reactions were terminated by adding 4 μL of STOP solution from the Sequenase kit and were heated at 70 °C for 5 min; a 3-μL aliquot was loaded on a 6% sequencing gel.

Primer Extension with RNA and Single-Stranded Plus-Strand DNA Templates in the Presence of Four dNTPs. Assay of DNA synthesis in the presence of all four dNTPs was carried out with primer at a final concentration of 5 nM; the template to primer ratio was 2:1. The sequence of the primer is complementary to nt 7896−7912 in the AKR MuLV coding strand (Herr, 1984). Unlabeled T7 transcript or single-stranded plus-strand DNA from clone pPPT-A was hybridized to 0.05 pmol of 5′-^{32}P-labeled DNA oligonucleotide in buffer containing 50 mM Tris-HCl, pH 8.0, 75 mM KCl (RNA template) or 25 mM KCl (DNA template), and 2.5 mM DTT at 65 °C for 5 min followed by slow cooling at room temperature for 30 min. Five μL of hybrid was preincubated with 1 μL of RT (as indicated) for 5 min at 37 °C to allow the enzyme to bind to the primer-template and then incubated in a final volume of 10 μL for an additional 10 min at 37 °C in a prewarmed mixture (1−2 min at 37 °C) containing the same buffer used for hybridization, 8 mM $MgCl_2$, 0.25 mM each of the 4 dNTPs, and, where indicated, 16.5 pmol of a competitor, poly(rA)−oligo(dT). Reactions were terminated as described above under Polymerase and RNase H Assays; a 2-μL aliquot was loaded on a 6% sequencing gel.

Band-Shift Assay. Two procedures were used to assess enzyme binding to a short DNA duplex. (i) Binding to the duplex and extension of the primer by 1 nt: The template (2.5 pmol) consisting of a 56-nt DNA oligonucleotide [nt 7677−7732 in the AKR MuLV coding strand (Herr, 1984)] was hybridized to an equimolar amount of a 43-nt DNA oligonucleotide (primer) whose sequence begins at its 5′-end with the complement of nt 7732 in the AKR MuLV coding strand (Herr, 1984). Hybridization was carried out as described above for the single-stranded plus-strand DNA template except that in this case $MgCl_2$ was present in the buffer. Five microliters of hybrid was preincubated with 1 μL of RT (as indicated) for 5 min at 37 °C; 4 μL of a mixture (prewarmed at 37 °C for 2 min) containing 1 pmol of [α-^{32}P]-dATP and 25 pmol of poly(rA)−oligo(dT), where indicated, was then added, and the entire reaction was incubated for an additional 5 min at 37 °C. A control to verify that the primer was extended by only 1 nt was carried out as follows. Samples containing released product, present in the reaction or in phenol extracts of the reaction, were obtained by eluting material from bands migrating to the bottom of the gel and were analyzed together with an aliquot taken directly from the reaction on a 15% sequencing gel; in all cases, the radioactivity migrated to a position +1 nt behind the 5′-labeled 43-nt primer (data not shown). (ii) Binding to the DNA duplex containing 5′-end-labeled primer without primer extension: The reactions were the same as those described above except that the primer was labeled at its 5′-end with [γ-^{32}P]ATP and dATP was omitted from the reaction. After incubation, all reactions were put on ice and 2 μL of 50% (v/v) glycerol was added to each sample. A 2-μL aliquot was loaded on a 5% polyacrylamide gel [5% acrylamide: 0.13% bis(acrylamide)] and electrophoresis was carried out at room temperature for 90 min at 120 V (constant voltage) in a buffer containing 25 mM Tris-HCl and 162 mM glycine in a final pH of 8.0.

RESULTS

RNase H Cleavage of PPT-Containing Substrates in the Absence of DNA Synthesis. Because selective cleavage at the 3′ boundary of the PPT constitutes an essential step in plus-strand viral DNA synthesis (Champoux, 1993), it was of interest to investigate the RNase H activity of MuLV RT with substrates containing the PPT and surrounding sequences. Four substrates were made, each consisting of a 5′-[^{32}P]467-nt T7 AKR MuLV RNA transcript hybridized

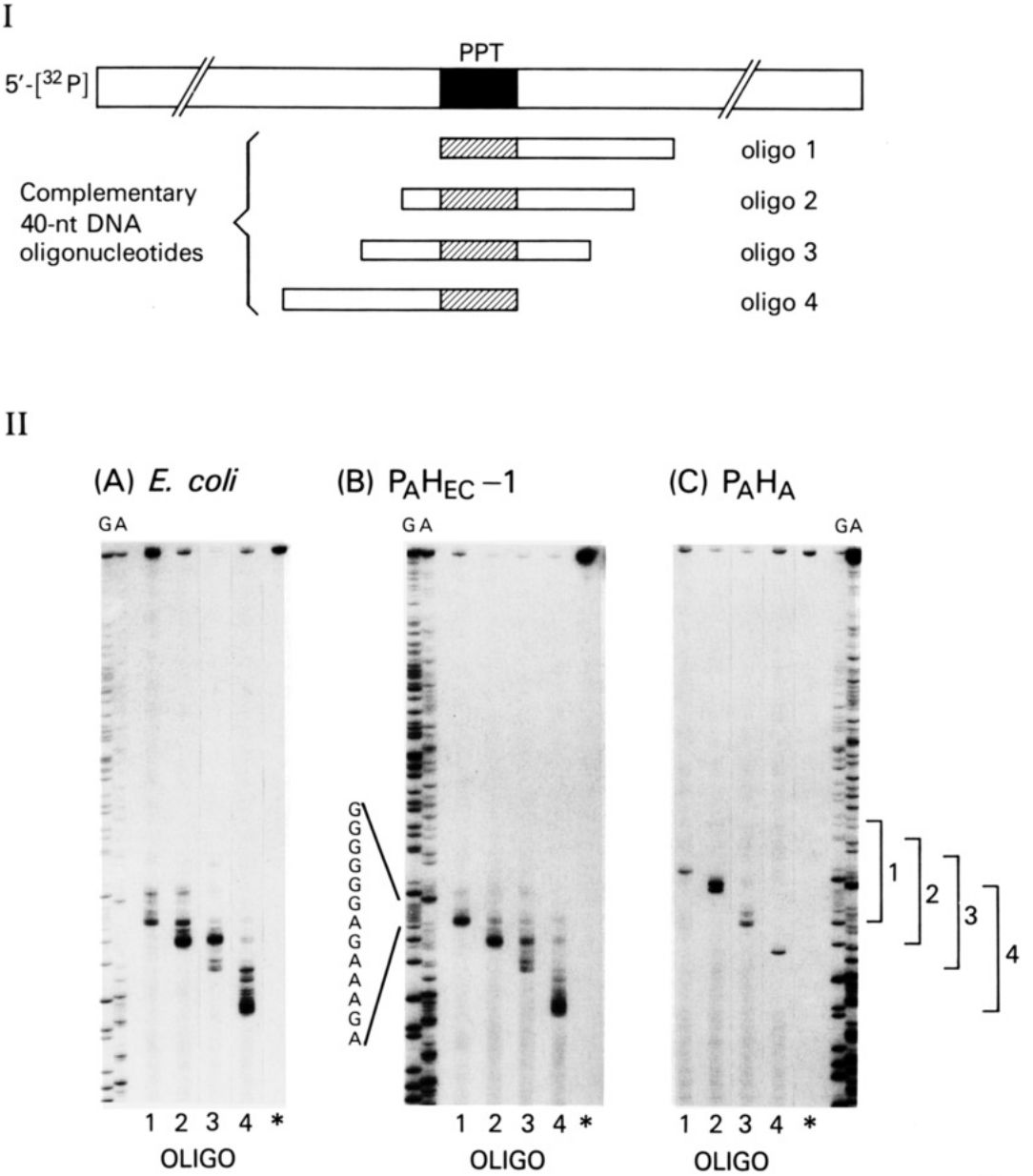

FIGURE 1: RNase H cleavage products generated from substrates containing the PPT region. (I) Schematic diagram of 5′-^{32}P-labeled MuLV RNA and complementary 40-nt DNA oligonucleotides. The oligonucleotides were complementary to the following nucleotides in the AKR MuLV coding strand (Herr, 1984): oligo 1, nt 7812−7851; oligo 2, nt 7805−7844; oligo 3, nt 7798−7837; oligo 4, nt 7785−7824. The PPT sequence in the RNA is represented by the filled-in box; the complementary PPT sequence in each of the oligonucleotides is represented by the box with diagonal lines. The diagram is not drawn to scale. (II) Gel analysis. (A) *E. coli* RNase H, 0.51 unit (H), 0.37 fmol (6.7 pg) of protein. (B) P_AH_{EC}-1 RT, 0.15 units (H), 0.008 fmol (0.6 pg) of protein. (C) P_AH_A RT, 0.00152 units (H), 0.83 fmol (63 pg) of protein. Lanes 1−4 are labeled in accordance with the oligonucleotide present in the RNA−DNA hybrid. The lane labeled with an asterisk is a control without enzyme or oligonucleotide. The marker lanes are the partial digestion products of the T7 MuLV RNA transcript with RNase T1 (G) and RNase U2 (A). The nucleotide sequence of the PPT (beginning with an A at the 5′-end) is shown to the left of the marker lanes in panel (B).

to one of four different 40-nt DNA oligonucleotides (oligos 1−4) having sequences complementary to the PPT and regions upstream and/or downstream of the PPT (see schematic diagram in Figure 1, panel I).

To study the effect of replacing the RNase H domain of RT with a cellular RNase H, we carried out reactions with wild-type RT and P_AH_A as well as with the chimeric RT P_AH_{EC}-1, a mutant in which *E. coli* RNase H has been substituted for the MuLV RNase H domain (Post et al., 1993), and purified *E. coli* RNase H. Limiting concentrations of enzyme were used. With both *E. coli* RNase H and P_AH_{EC}-1 RT (Figure 1, panels IIA,B, respectively), there were numerous cleavage products, resulting from mulitple cuts in the RNA within the PPT and in the surrounding regions. In fact, the cleavage patterns of the chimeric RT were virtually indistinguishable from those of the bacterial enzyme, in accord with results obtained earlier with the nonviral RNA_{160} substrate (Post et al., 1993). Similar findings were made with P_AH_{EC}-2, which contains the entire MuLV polymerase domain (data not shown). In contrast, with the wild-type RT (Figure 1, panel IIC), there were fewer cleavage products.

A detailed analysis of the cleavage sites used by the wild-type enzyme is shown in Figure 2. Substrates containing oligo 1 or oligo 4 (Figure 2A,D, respectively) were cleaved at a single site (+5 and −22, respectively), corresponding

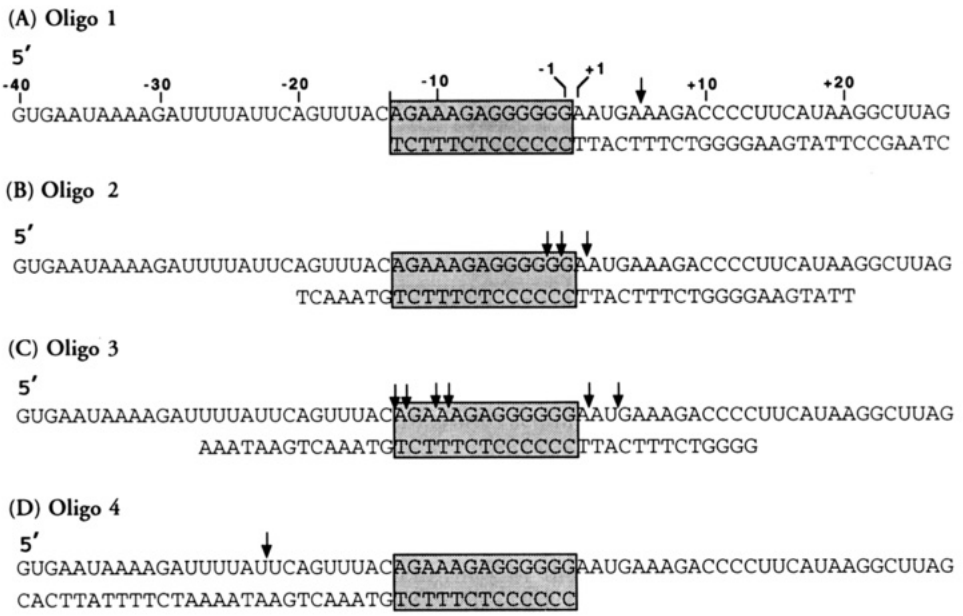

FIGURE 2: RNase H cleavage sites used by P_AH_A RT in reactions with PPT-containing substrates. A portion of the sequence of the 5′-^{32}P-labeled 467-nt MuLV RNA [nt 7785−7851 (Herr, 1984)] is shown together with the entire nucleotide sequence of each of the complementary 40-nt DNA oligonucleotides. In the RNA sequence in (A), nucleotides downstream of the PPT are numbered with a plus sign, while those in the PPT and upstream of the PPT are numbered with a minus sign; +1 refers to the A residue which is the site of initiation of MuLV plus-strand DNA synthesis (Finston & Champoux, 1984), and −1 refers to the 3′ G in the PPT. The nucleotides present in the PPT region are boxed. The arrows indicate cleavage sites in reactions with no dNTPs.

to positions in the RNA 18 nt from the 3′-end of the oligonucleotide. These sites are at the positions expected for the 3′-OH-dependent mode of cleavage (Post et al., 1993). At higher concentrations of enzyme, additional cleavages were observed (data not shown); these cuts result from the 3′-OH-independent mode of cleavage, which occurs when the 3′-end of the primer is no longer bound to the polymerase active site (Post et al., 1993). In the case of the substrates containing oligo 2 or oligo 3, the 18-nt position is located within the PPT at −3 (Figure 2B) and −10 (Figure 2C), respectively. Interestingly, in contrast to the situation in vivo, where the PPT is resistant to RNase H cleavage (Champoux, 1993), the synthetic oligo 2 and oligo 3 substrates were cut at the 18-nt position as well as at other sites, within and downstream of the PPT.

To determine whether RNase H and polymerase activities are coordinated in reactions with a PPT-containing RNA−DNA hybrid, RNase H cleavage catalyzed by the P_AH_A and P_AH_{EC}-1 RTs was examined under conditions of limited DNA synthesis. Reactions were performed in the presence or absence of three dNTPs and one ddNTP with uniformly labeled [^{32}P]RNA hybridized to oligo 4 (Figure 3A); parallel reactions to measure DNA extension contained unlabeled RNA and 5′-end-labeled oligo 4 primer (Figure 3B). In the absence of dNTPs, cleavage by the wild-type RT gave two products, a 211-nt 5′-product and a 256-nt 3′-product, whose sum equals the size of the 467-nt RNA in the RNA-DNA hybrid (Figure 3A, lane 6, and Figure 3C). When the ddNTP mixtures were added to the wild-type reactions, there was a correspondance between the number of nucleotides cleaved and the number of nucleotides by which the primer was extended. For example, with the ddCTP mixture, the primer was extended by 9 nt (Figure 3B, lane 10) and new 5′- and 3′-cleavage products, 202 nt and 265 nt, respectively, were observed (Figure 3A, lane 10). These findings are in agreement with our previous results with a nonviral RNA−DNA hybrid (Post et al., 1993).

Since the chimeric RT cleaves the substrate at many sites (Figure 1, IIB), the cleavage products generated in the absence of dNTPs (Figure 2A, lane 1) included the 5′- and 3′-products of 195−198 nt and 233−236 nt, respectively, as well as small internal fragments which are not detected on a 6% sequencing gel (Figure 3C). Addition of the ddNTP mixtures caused little or no change in the cleavage pattern (compare Figure 3A, lane 1 with lanes 2−5). This observation reflects the fact that the chimeric RT did not extend the primer efficiently, even though relatively high amounts of enzyme were added. In all cases, the products terminated before reaching the position where the ddNTP would be expected to terminate DNA synthesis (Figure 3B, lanes 2−5). In accord with our earlier conclusions (Post et al., 1993), the results presented in Figure 3 demonstrate that despite the presence of the MuLV polymerase domain, the chimeric RT is deficient in polymerase activity with a heteropolymeric (PPT-containing) template and is unable to catalyze 3′-OH-dependent cleavages.

RNA-Dependent DNA Polymerase Activity. The defect in polymerase activity exhibited by the chimeric RT suggested that the polymerase domain by itself may not be sufficient for efficient DNA synthesis and raised the possibility that the RNase H domain plays a role in this process. To investigate this question, we tested the ability of the P_AH_{EC}-1 and P_AH_{EC}-2 RTs to catalyze processive DNA synthesis in response to an RNA template, and we compared their activities with those of wild-type RT and an RNase H-minus RT, $P_A\Delta H$, which is missing the entire RNase H domain (Post et al., 1993). Reactions were carried out with the PPT-containing 467-nt RNA (Figure 1, panel I) in the presence or absence of a competitor, poly(rA)−oligo(dT).

As may be seen from Figure 4, the wild-type enzyme made full-length DNA (321 nt) under all conditions, but much less when the competitor was present (compare lane 1 with lane 2 and lane 3 with lane 4). This observation indicates that MuLV DNA polymerase activity is not completely proces-

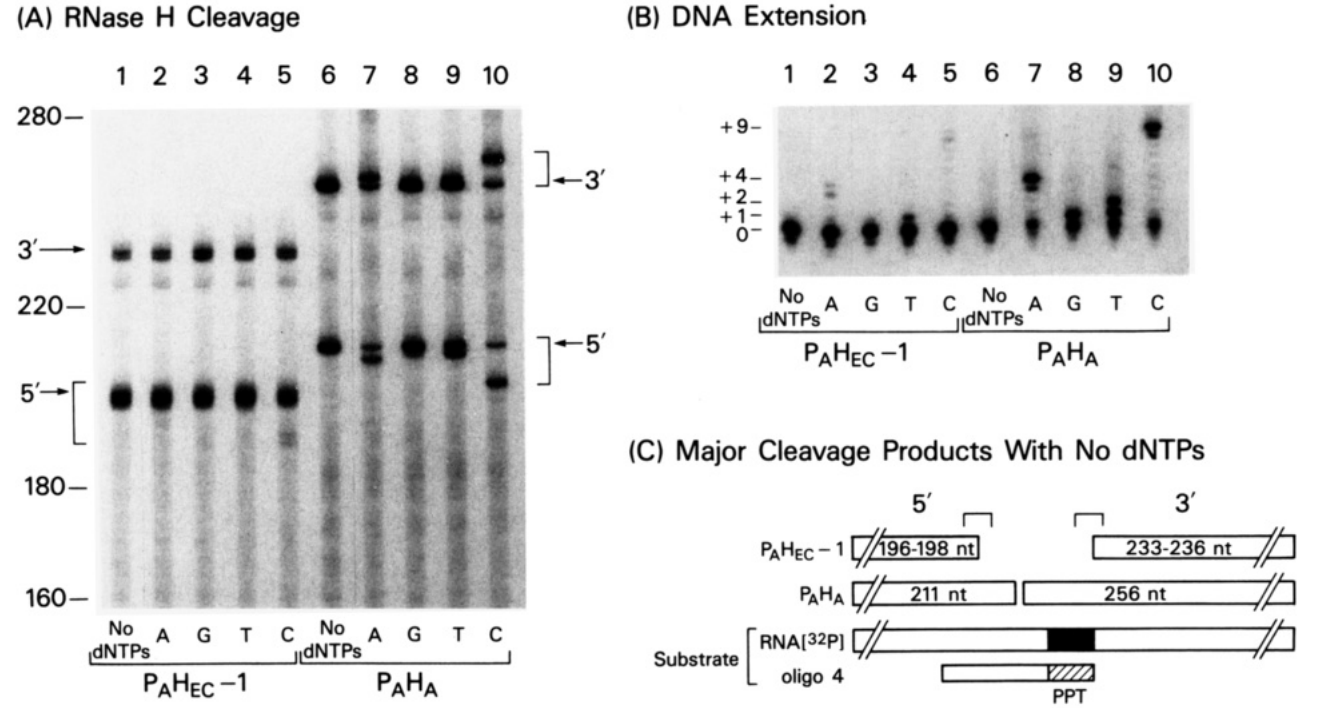

FIGURE 3: RNase H cleavage and DNA extension products generated in reactions containing the 467-nt MuLV RNA and the DNA primer, oligo 4, under conditions of limited primer extension. (A) RNase H cleavage. The substrate consisted of uniformly labeled [^{32}P]RNA hybridized to unlabeled primer. The following amounts of enzyme were added to each reaction: P_AH_{EC}-1, 400 units (P), 4500 units (H), 0.24 pmol of protein (lanes 1−5); and P_AH_A, 400 units (P), 0.46 unit (H), 0.25 pmol of protein (lanes 6−10). It should be noted that in this experiment it was necessary to have sufficient units of polymerase activity to detect DNA synthesis with both RTs; since the activity of P_AH_{EC}-1 was low in this assay, the amount of RNase H units added here turns out to be higher than that used in the experiment shown in Figure 1, panel II. Lanes 1 and 6, no dNTPs; lanes 2 and 7, ddATP mixture (A); lanes 3 and 8, ddGTP mixture (G); lanes 4 and 9, ddTTP mixture (T); and lanes 5 and 10, ddCTP mixture (C). 3′- and 5′-cleavage products generated by P_AH_{EC}-1 are indicated by arrows on the left and those generated by P_AH_A, by arrows on the right. RNA sizes are indicated on the left. (B) DNA extension. Unlabeled MuLV RNA template was hybridized to 5′-^{32}P-labeled oligo 4 primer. The amounts of enzyme added to each reaction and the description of the lanes are the same as in (A). The numbers on the left indicate the positions of DNA extensions of +1, +2, +4, and +9, which are predicted for reactions with 3 dNTPs and ddGTP (G), ddTTP (T), ddATP (A), and ddCTP (C), respectively; 0 refers to the unextended primer. (C) Major cleavage products with no dNTPs. Schematic diagram showing the PPT-containing substrate and the major RNase H cleavage products generated by P_AH_A and P_AH_{EC}-1 in reactions with no dNTPs. The PPT sequence in RNA is indicated by a filled-in box and the complementary sequence, by a box with diagonal lines in oligo 4. The diagram is not drawn to scale.

sive. Both the P_AH_{EC}-1 (lanes 9−12) and P_AH_{EC}-2 (lanes 13−16) RTs made very small products (50−60 nt) regardless of whether the competitor was added. Presumably, the potent RNase H activity of these enzymes (Post et al., 1993) degrades the RNA template before significant DNA synthesis can occur. The RNase H-minus mutant made full-length DNA, but approximately 3-fold less than wild-type in the absence of competitor (compare lane 2 with lane 6 and lane 4 with lane 8). When competitor was added to reactions where the enzyme:primer-template ratio was 100:1, $P_A\Delta H$ synthesized 10-fold less 321-nt DNA than wild-type (compare lanes 1 and 5), and at the lower enzyme concentration (lane 7), the 321-nt product could not be detected.

It is of interest to note that both the wild-type and $P_A\Delta H$ RTs made a significant number of small DNAs (pause/termination products), particularly in the region preceding the PPT. The products were the same for both enzymes, but the amounts synthesized by the RNase H-minus RT were greater than those made by the wild-type. In the case of the wild-type enzyme, the small DNAs were more prominent at the lower enzyme concentration (Figure 4, compare lanes 1 and 2 with lanes 3 and 4) and when the competitor was present (Figure 4, lanes 1 and 3). A major DNA intermediate made by the two enzymes (indicated in Figure 4 by an arrow) is one occurring at a C residue in the RNA template, 13 nt downstream of the PPT; this C is the first residue in a run of four C's which is encountered by RT as it traverses the RNA template prior to reaching the PPT. In one of the wild-type reactions containing the competitor (Figure 4, lane 1), this DNA represented 50% of the total DNA products. The sites at which the small DNAs were evident usually occurred at G and C residues, in agreement with similar observations on endogenous viral DNA synthesis by AKR and Moloney MuLVs (Messer et al., 1985) and RNA-dependent DNA synthesis by purified HIV-1 RT (Dudding et al., 1991; Klarmann et al., 1993; Volkmann et al., 1993).

DNA-Dependent DNA Polymerase Activity. The results described above with the RNA template demonstrate that the presence of an RNase H domain facilitates processive DNA synthesis. To further investigate this effect, we examined DNA synthesis in response to a DNA template. In this case, since a DNA duplex is not a substrate for RNase H, the template will not be degraded by the chimeric RTs. The DNA template was a 469-nt plus-strand DNA, having the same viral sequence as the RNA template.

In the absence of competitor (Figure 5A), wild-type RT made full-length DNA at all three concentrations of enzyme (lanes 1−3), although synthesis was most efficient with a 10:1 molar ratio of enzyme/primer-template. This was also true for all three mutant RTs (lanes 5, 8, and 11). However, the relative amount of full-length product made by the wild-type RT was 67% of the total radioactivity (i.e., in all the

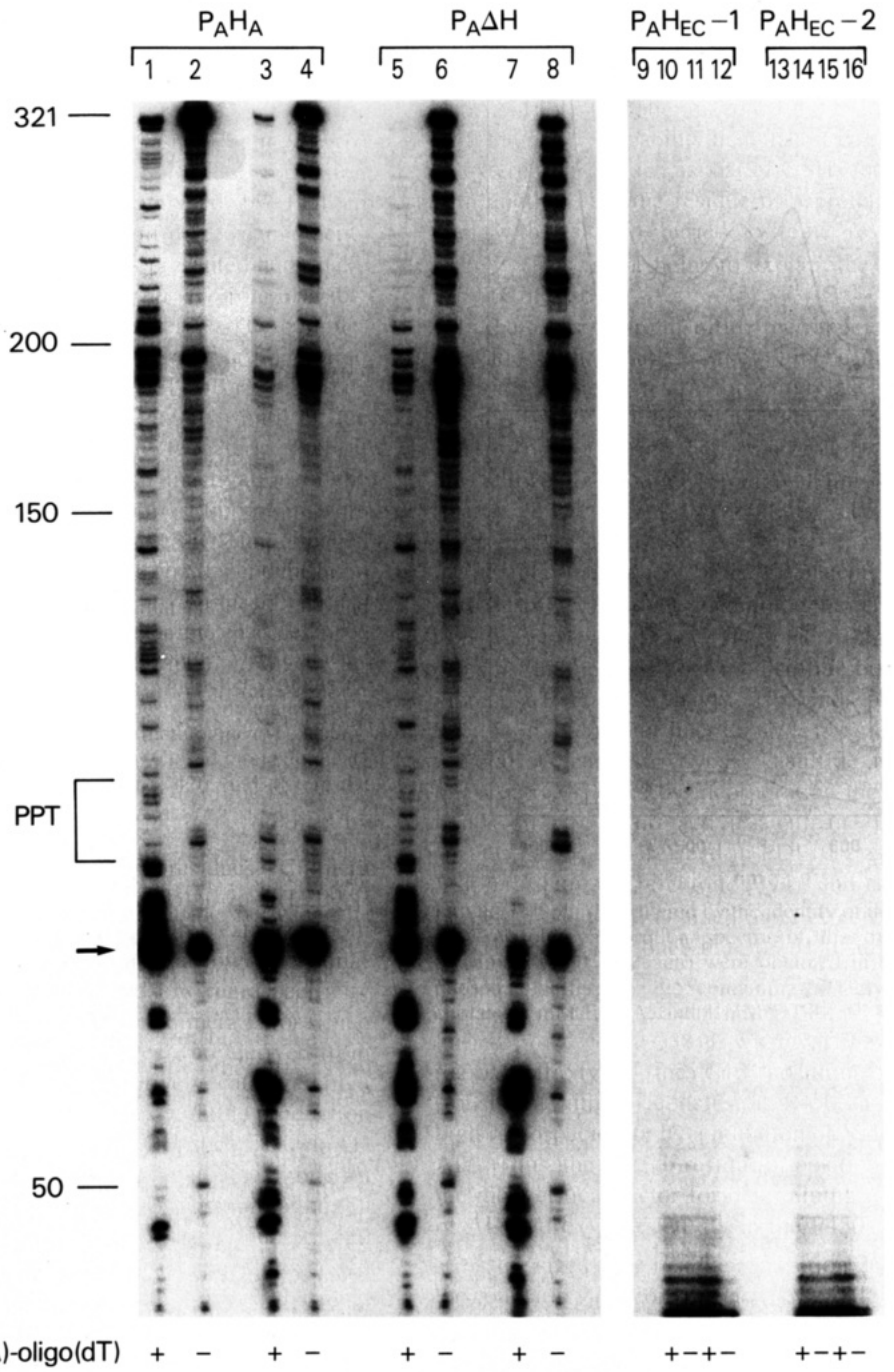

FIGURE 4: Primer extension directed by an MuLV RNA template in the presence and absence of competitor, poly(rA)−oligo(dT). The RNA template was purified by electroelution from a 3% Nusieve (3:1 agarose) gel. Reactions contained either 5 pmol of enzyme (lanes 1, 2, 5, 6, 9, 10, 13, 14) or 0.5 pmol of enzyme (lanes 3, 4, 7, 8, 11, 12, 15, 16); the enzyme:primer-template ratios were 100:1 and 10:1, respectively. The following enzymes were added: P_AH_A, lanes 1−4; $P_A\Delta H$, lanes 5−8; P_AH_{EC}-1, lanes 9−12; P_AH_{EC}-2, lanes 13−16. Where indicated (+), reactions contained the poly(rA)−oligo(dT) competitor. DNA fragment sizes and the location of the PPT region are shown to the left of each panel. The arrow on the left indicates a major pause/termination product. The full-length product is 321 nt.

DNA products) compared with 5% for $P_A\Delta H$ (lane 5), 10% for P_AH_{EC}-1 (lane 8), and 20% for P_AH_{EC}-2 (lane 11). In contrast to the wild-type, at the 1:1 molar ratio, none of the mutants was able to synthesize full-length DNA (compare lane 3 with lanes 6, 9, and 12).

Figure 5B shows the same experiment as that illustrated in Figure 5A, except that the poly(rA)−oligo(dT) competitor was present. The wild-type RT made full-length DNA at each concentration of enzyme tested (lanes 1−3); however, as already shown for reactions containing an RNA template (Figure 4), the amount of full-length DNA was significantly less in reactions containing competitor. None of the mutants could make full-length DNA under these conditions, and DNA synthesis was very inefficient. Thus, at each enzyme concentration tested, $P_A\Delta H$ made products which were less than 50 nt. The chimeric RTs, P_AH_{EC}-1 and P_AH_{EC}-2, had more activity than $P_A\Delta H$, but even at the highest enzyme concentration (lanes 7 and 10, respectively), the largest significant product was one terminating at the two dA residues which abut the PPT. Other studies have also shown that with DNA templates, DNA pause/termination sites often occur at dA residues (Huber et al., 1989; Williams et al., 1990; DeStefano et al., 1992; Klarmann et al., 1993). When the enzyme concentration was lowered, most of the DNA

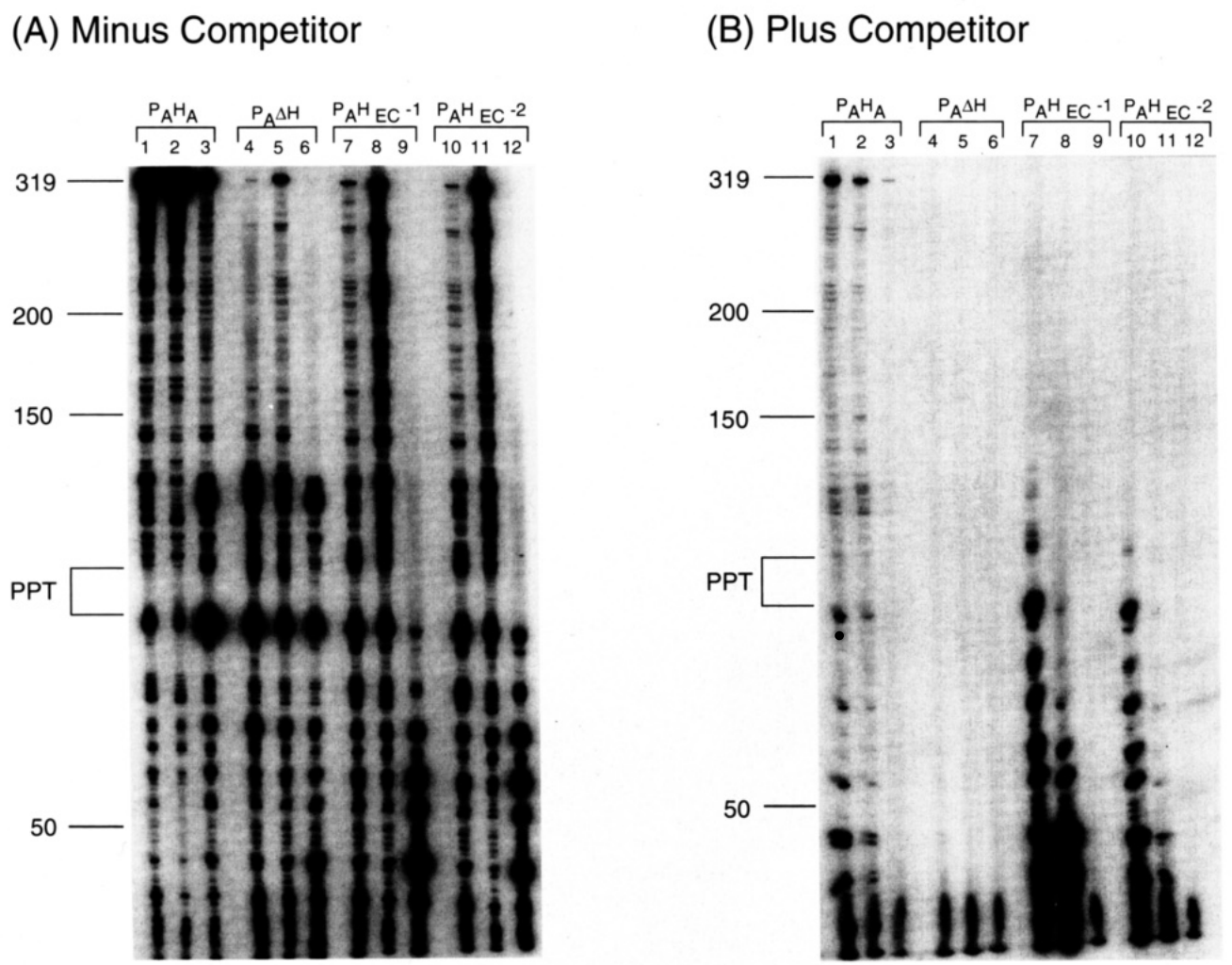

FIGURE 5: Primer extension directed by a single-stranded MuLV DNA template in the presence or absence of competitor, poly(rA)−oligo(dT). Reactions contained either 2.5 pmol of enzyme (lanes 1, 4, 7, 10), 0.5 pmol of enzyme (lanes 2, 5, 8, 11), or 0.05 pmol of enzyme (lanes 3, 6, 9, 12); the enzyme:primer-template ratios were 50:1, 10:1, and 1:1, respectively. The following enzymes were added: P_AH_A, lanes 1−3; $P_A\Delta H$, lanes 4−6; P_AH_{EC}-1, lanes 7−9; and P_AH_{EC}-2, lanes 10−12. (A) Minus competitor. (B) Plus competitor. DNA fragment sizes and location of the PPT region are shown to the left of each panel. The full-length product is 319 nt. This value differs from that of the 321-nt full-length product made with the RNA template because the 5′-terminus of the single-stranded DNA template has 2 nt upstream of the 5′ G of the *Eco*R1 site, whereas the 5′-terminus of the RNA has 4 nt in the corresponding position.

products were less than 50 nt (lanes 8, 9 and lanes 11, 12). These results, like those obtained with the RNA template (Figure 4), highlight the importance of the RNase H domain for processive DNA synthesis.

RT Binding to Primer-Template with and without Primer Extension. The deficiency in processive DNA synthesis exhibited by the chimeric RTs and the RNase H-minus mutant may result from an inability of these mutants to bind primer-template as efficiently as wild-type. This possibility was initially tested in a band-shift assay in which binding to the DNA duplex and extension of the primer by 1 nt must occur in order to detect a signal (Figure 6A). The band-shift data presented below are representative of results obtained in repeated experiments.

Figure 6A, lanes a−c, shows that the wild-type enzyme formed two complexes, one slowly-migrating (super-shifted) (band 1) and the other faster-migrating (band 2), which are retarded in a nondenaturing polyacrylamide gel. Similar observations have been reported by Telesnitsky and Goff (1993a). Bands 1 and 2 were present over a 10-fold range of enzyme concentration (data not shown), and when the primer was in excess, the band 1:band 2 ratio was approximately 2.3:1 (lanes a−c). [In repeated experiments, this ratio varied from 2:1 to 3:1 (data not shown)].

To determine whether there is a difference in the intrinsic binding activity of the wild-type RT and the mutants, we compared binding of primer-template to RT under two sets of conditions: (i) with primer extension, (Figure 6A); and (ii) without primer extension i.e., having the primer prelabeled at its 5′-end with ^{32}P and omitting dATP from the reaction (Figure 6B). The data shown are for reactions with an enzyme:primer-template ratio of 1:10.

In the absence of competitor, the relative amount of band 1 in the wild-type sample was reproducibly higher if primer extension was permitted to proceed; the ratio of band 1 to band 2 was 2:1 in this case (Figure 6A, lane 1) and 1:1 in the absence of nucleotide incorporation (Figure 6B, lane 1). This observation indicates that formation of the super-shifted complex is more efficient when DNA synthesis occurs. Most of the enzyme-bound radioactivity in the mutant RT samples (70−85%) was found in band 2, regardless of whether there was primer extension (Figure 6A,B, lanes 2−4); moreover, the total amount of radioactivity in bands 1 and 2 was consistently lower with the mutants than with wild-type RT

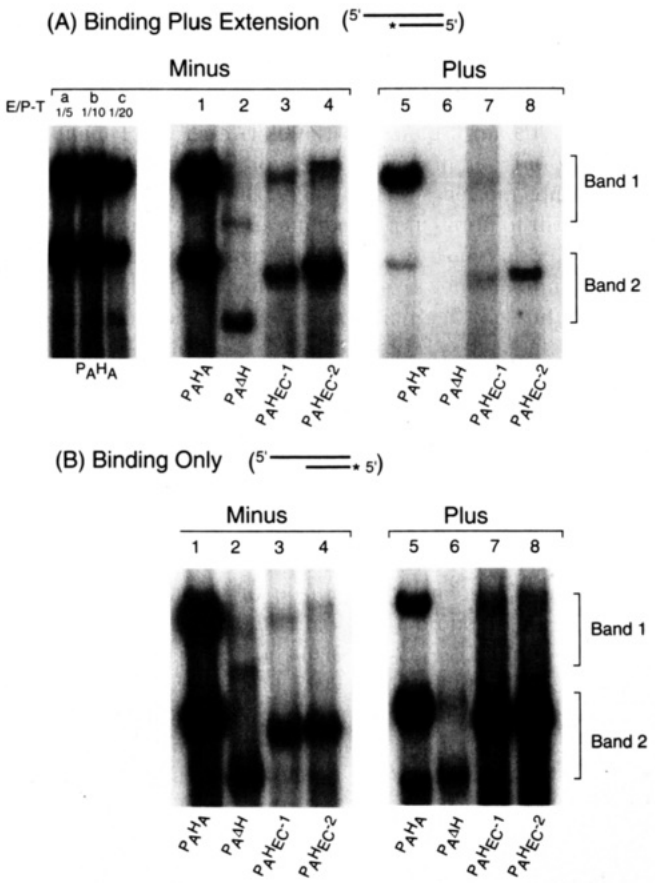

FIGURE 6: Comparison of binding of wild-type and mutant RTs to a short DNA duplex in the presence or absence of primer extension. Band-shift assays of reactions with (A) unlabeled primer and [α-^{32}P]-dATP (binding plus extension) or (B) 5′-end labeled primer and no dATP (binding only), respectively, were carried out as described under Materials and Methods. (A) Lanes a, b, c, P_AH_A; enzyme: primer-template (E/P-T) ratios are shown above each lane. (A and B) Lanes 1 and 5, P_AH_A; lanes 2 and 6, $P_A\Delta H$; lanes 3 and 7, P_AH_{EC}-1; lanes 4 and 8, P_AH_{EC}-2. The enzyme:primer-template ratio was 1:10. Minus or plus refers to the absence or presence of competitor, respectively. The positions of bands 1 and 2 are indicated to the right of the gels and are bracketed because of differences in the mobilities of complexes formed by each of the enzymes. The released products which migrate to the bottom of the gel are not shown (see Materials and Methods). In the schematic diagrams shown in parentheses, the long and short lines represent the template and primer, respectively, and the asterisk refers to the position of the radioactive label; the diagrams are not drawn to scale.

(Figure 6A,B, compare lanes 1 with lanes 2−4). Analysis of mutant reactions with enzyme:primer-template ratios of 1:5 or 1:20 gave similar results (data not shown). It should be noted that because the molecular mass of the RNase H-minus mutant (56 kDa) is lower than that of the wild-type (76 kDa) and chimeric RTs (74 and 76 kDa, respectively), the complexes formed by this mutant migrate ahead of bands 1 and 2 of the other enzymes (compare lanes 2 with lanes 1, 3, and 4). Interestingly, if primer extension occurred, the total radioactivity in bands 1 and 2 of the $P_A\Delta H$ sample was lower (range of 2−3-fold) than that of the chimeric RT samples (Figure 6A, compare lane 2 with lanes 3 and 4), but in the absence of primer extension, the level of binding was comparable for all three mutants (Figure 6B, compare lane 2 with lanes 3 and 4).

To make a further comparison between binding efficiency and processive DNA synthesis (Figure 5B), we examined the effect of competitor on binding of RT to primer-template under the two assay conditions. When competitor was added to primer extension reactions containing wild-type RT, approximately 90% of the radioactivity in bands 1 and 2 was now found in band 1 (Figure 6A, compare lane 5 with lane 1). This result indicates that when the enzyme is engaged in DNA synthesis, it dissociates more readily from the complex in band 2, thereby allowing the poly(rA)−oligo(dT) to compete more effectively with the primer bound in that complex. In sharp contrast to these results, in the absence of primer extension, 70% of the total enzyme-bound radioactivity in the wild-type sample was found in band 2 (Figure 6B, lane 5). This observation lends further support to the idea that under conditions where there is no nucleotide incorporation, the enzyme is less stably bound to the primer-template in the slowly-migrating complex.

Almost all of the radioactivity in the chimeric RT samples remained in band 2 in the presence of the competitor, regardless of whether DNA synthesis had occurred (Figure 6A and Figure 6B, compare lanes 7 and 8, respectively). In the primer extension reactions, the total amount of radioactivity in bands 1 and 2 was 5−10-fold lower than that in the wild-type sample; however, in the absence of primer extension, the amount of labeled primer bound by these two enzymes was approximately the same as the total amount of radioactivity in bands 1 and 2 of the wild-type (compare Figure 6A,B, lanes 3 and 4 with lane 1). With $P_A\Delta H$, no radioactivity could be detected in regions in the gel corresponding to bands 1 and 2 under DNA synthesis conditions (Figure 6A, lane 6). This result is not entirely unexpected since in polymerase reactions containing a DNA template and competitor, the RNase H-minus RT made only very short products, even at high enzyme concentrations (Figure 5B, lanes 4−6). In the absence of DNA synthesis, binding of the enzyme to primer-template was detected, but only in band 2 (Figure 6B, lane 6).

In summary, the data illustrated in Figure 6 correlate with the results on processive DNA synthesis (Figure 5) and strongly suggest that the active form of RT during DNA synthesis is contained in the slowly-migrating complex (band 1). The mutant RTs are deficient in their ability to form this complex and, in general, do not bind primer-template as efficiently as the wild-type enzyme.

DISCUSSION

In the present study, we have compared the activities of chimeric RTs (P_AH_{EC}-1 and P_AH_{EC}-2) having the MuLV polymerase domain fused to *E. coli* RNase H with the activities of wild-type RT (P_AH_A) and an RT ($P_A\Delta H$) which is missing the entire RNase H domain, using primer-templates containing sequences from the PPT region. We have addressed the following questions: (i) What is the nature of the RNase H cleavage pattern generated with PPT-containing substrates with or without concomitant DNA synthesis? (ii) What factors influence the ability of MuLV RT to catalyze processive RNA- and DNA-dependent DNA synthesis? (iii) Does the RNase H domain have a structural as well as catalytic role, and, if so, how does this affect primer-template binding and processive DNA synthesis? Our results demonstrate that despite the predicted similarities between the structures of *E. coli* RNase H and the RNase H domain of MuLV RT (Yang et al., 1990; Nakamura et al.,

1991), the chimeric RTs behave very differently from wild-type RT in RNase H, primer extension, and primer-template binding assays. We also show that the presence of the wild-type RNase H domain stabilizes binding of RT to primer-template, which facilitates formation of the active form of RT and processive DNA synthesis.

The experiments with PPT-containing substrates (Figure 1, panel I) are a striking example of differences in the RNase H activities of the chimeric RTs and wild-type enzyme. Cleavages generated by the chimeric RTs resulted in numerous cuts in the PPT and surrounding region (Figure 1, panel IIB; Figure 3A,C; data not shown) and were virtually identical to those generated by *E. coli* RNase H (Figure 1, panel IIA). Nonspecific cleavage of PPT-containing substrates by *E. coli* RNase H has also been described by others (Luo et al., 1990; Randolph & Champoux, 1994). The cleavage activity of P_AH_{EC}-1 was not significantly affected by limited primer extension (Figure 3A,B); i.e., RNase H activity was 3′-OH-independent and was not coordinated with DNA synthesis. These findings are in accord with our earlier results using a substrate with a nonviral sequence (Post et al., 1993) and demonstrate that the intrinsic activity of the *E. coli* RNase H domain (i) is not modified by the presence of a functional MuLV polymerase domain and (ii) is independent of the sequence of the RNA−DNA substrate.

In contrast to the results with the chimeric RTs, wild-type cleavages were coordinated with DNA synthesis (Figure 3A,C) and were determined primarily by the distance between the polymerase active site, to which the 3′-terminus of the oligonucleotide is bound, and the RNase H active site, i.e., 18 nt (Figure 1, panel IIC, Figure 2; Kohlstaedt et al., 1992; Jacobo-Molina et al., 1993). Interestingly, if the 18-nt position occurred within the PPT, the wild-type RT cut at that position (Figure 1, panel IIC; Figure 2), even though sites within the PPT are normally resistant to RNase H (Champoux, 1993); additional cleavages were also observed in this case, perhaps because under the conditions of the experiment the enzyme was forced to cut in a region it usually avoids. Similar observations have been reported in studies with HIV-1 RT (Wohrl & Moelling, 1990; Wohrl et al., 1991) and, recently, in an independent study with an MuLV system, which came to our attention while this paper was in preparation (Randolph & Champoux, 1994).

The present experiments demonstrate that within RT, the presence of a wild-type RNase H domain is a major determinant of processive RNA- and DNA-dependent DNA synthesis. Thus, despite the apparent wild-type polymerase activity of the chimeric RTs and $P_A\Delta H$ in assays with poly(rA)−oligo(dT) (Post et al., 1993), these enzymes are impaired in their abilities to synthesize full-length DNA (Figures 3B, 4, and 5; Post et al., 1993). Interestingly, the additional amino acids in P_AH_{EC}-2 (the first 19 of which form the C-terminus of the connection subdomain) do not significantly influence the polymerase activity or other enzymatic properties of the chimeric RTs (Figures 5 and 6; data not shown). Deficiencies in DNA synthesis are also associated with other MuLV (Gerwin et al., 1979; Levin et al., 1984; Messer et al., 1985; Tanese et al., 1991; Telesnitsky et al., 1992; Telesnitsky & Goff, 1993a) and HIV-1 (Dudding et al., 1991; Dudding & Mizrahi, 1993; Volkmann et al., 1993) RTs with mutations in RNase H. Furthermore, it has been shown that the p51 homodimers of the HIV-1 (Bavand et al., 1993) and EIAV (Wohrl et al., 1994) RTs are less processive than the corresponding p66/51 heterodimers.

How can we rationalize the participation of the RNase H domain in the polymerase function of RT? The fact that the chimeric RTs and the RNase H-minus mutant bind primer-template less efficiently than wild-type, especially when primer extension has occurred (Figure 6), strongly suggests that the wild-type RNase H domain promotes processive DNA synthesis by stabilizing the interaction between RT and primer-template. This conclusion is consistent with the idea that the RNase H domain plays a role in maintaining the enzymatically active structure of RT (Hostomsky et al., 1993). A similar conclusion can be inferred from kinetic data showing that HIV-1 RT has a greater affinity for poly(rA)−oligo(dT) when the length of the dT primer spans the two active sites (i.e., 16−20 nt) and there is a possibility for the RNase H domain to contribute to binding (Reardon et al., 1991; Beard & Wilson, 1993).

At present, it is not known how the polymerase and RNase H domains contact each other in the three-dimensional structure of MuLV RT. Enhancement of primer-template binding and processivity by wild-type RNase H may result from a close association of portions of the two domains, as in HIV RT (Kohlstaedt et al., 1992; Jacobo-Molina et al., 1993). Alternatively, since the MuLV domains are relatively independent of one another (Tanese & Goff, 1988), they may have fewer contacts than are present in HIV RT, and in this case, increased processivity could be a result of having two more or less independent nucleic acid binding sites. The small, but reproducible increase in processivity of the chimeric RTs compared with $P_A\Delta H$ could reflect an independent binding activity of the substituted *E. coli* RNase H or, possibly, a weak interaction between the domains.

In addition to the quantitative deficiency in primer-template binding exhibited by the chimeric RTs and $P_A\Delta H$, we also observe a major qualitative effect which is associated with the RNase H mutations. Thus, wild-type MuLV RT forms two complexes which are detected in band-shift assays: a slowly-migrating, super-shifted complex (band 1) and a faster-migrating complex (band 2) (Figure 6; Telesnitsky & Goff, 1993a); the predominant species in the mutant reactions is band 2, regardless of whether nucleotide incorporation has taken place (Figure 6). In contrast, formation of the super-shifted complex by wild-type RT is enhanced by conditions which promote primer extension (Figure 6, lanes 1 and 5), suggesting that the super-shifted complex contains the active form of the enzyme.

Recently, it has been proposed that HIV-1 RT complexed with primer-template and a dNTP undergoes a conformational change before actual incorporation of the nucleotide takes place (Kati et al., 1992; Hsieh et al., 1993). This type of change could lead to greater stability of the RT-primer-template complex and may also account for the fact that with the wild-type enzyme there is less band 1 relative to band 2 in the absence of primer extension (Figure 6). In addition, it is also possible that the super-shifted complexes formed by the mutant MuLV RTs are less stable compared with that of wild-type because the RNase H domain is involved in this conformational change.

One question of special interest concerns the composition of the super-shifted and faster-migrating complexes. Several possibilities are consistent with the data of Figure 6. (i) The two bands represent conformational variants of RT, both of which bind DNA. (ii) Band 1 contains monomeric RT bound to one molecule of DNA. (A complex with two DNA

molecules will migrate faster than a complex with only one DNA, due to an increase in negative charge.) (iii) Bands 1 and 2 contain dimeric or monomeric RT, respectively, bound to one molecule of DNA. (A complex with two protein molecules will migrate more slowly than a complex with only one protein molecule, due to the higher molecular mass.) We favor the third possibility for the following reasons. The functional activities of HIV and, MuLV RTs are remarkably similar and, taken together, the numerous examples cited above indicate that the same overall strategy is used by both viruses to synthesize DNA. Moreover, the finding that only the dimeric form of HIV-1 or HIV-2 RT is active (Restle et al., 1990, 1992; Becerra et al., 1991; Müller et al., 1991) provides a powerful biological precedent for the closely related MuLV RT, particularly when viewed in light of our evidence that band 1 contains the active form of the enzyme (Figure 6). Thus, it is attractive to consider that MuLV RT also functions as a dimer when it is engaged in DNA synthesis. A similar suggestion has been made by Teletsnitsky and Goff (1993a).

If this interpretation of the band-shift data is correct, MuLV RT would presumably form a homodimer in our experiments. It is intriguing to speculate about possible formation of a heterodimer between full-length MuLV RT and a small 50-kDa RT fragment which is detected in virions (Hu et al., 1986; Tanese & Goff, 1986). Like the HIV p51 subunit (di Marzo Veronese et al., 1986; Lightfoote et al., 1986), the 50-kDa MuLV protein is an N-terminal fragment of the full-length enzyme (J. G. Levin and M. Zweig, unpublished observations); it is also coprecipitated in a complex containing full-length RT and IN by mono-specific antisera directed against either MuLV RT or IN (Hu et al., 1986; J. G. Levin and M. Zweig, unpublished observations). Although further experiments are clearly needed to determine whether MuLV RT functions as a dimer, additional support for this idea comes from the finding that certain MuLV polymerase and RNase H mutants can complement each other *in trans* during virus replication (Telesnitsky & Goff, 1993b).

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REFERENCES

Bavand, M. R., Wagner, R., & Richmond, T. J. (1993) *Biochemistry 32*, 10543−10552.

Beard, W. A., & Wilson, S. H. (1993) *Biochemistry 32*, 9745−9753.

Becerra, S. P., Kumar, A., Lewis, M. S., Widen, S. G., Abbotts, J., Karawya, E. M., Hughes, S. H., Shiloach, J., & Wilson, S. H. (1991) *Biochemistry 30*, 11707−11719.

Ben-Artzi, H., Zeelon, E., Amit, B., Wortzel, A., Gorecki, M., & Panet, A. (1993) *J. Biol. Chem. 268*, 16465−16471.

Champoux, J. J. (1993) in *Reverse Transcriptase* (Skalka, A. M., & Goff, S. P., Eds.) pp 103−117, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Davies, J. F., II, Hostomska, Z., Hostomsky, Z., Jordan, S. R., & Matthews, D. A. (1991) *Science 252*, 88−95.

DeStefano, J. J., Buiser, R. G., Mallaber, L. M., Myers, T. W., Bambara, R. A., & Fay, P. J. (1991) *J. Biol. Chem. 266*, 7423−7431.

DeStefano, J. J., Buiser, R. G., Mallaber, L. M., Fay, P. J., & Bambara, R. A. (1992) *Biochim. Biophys. Acta 1131*, 270−280.

DiMarzo Veronese, F., Copeland, T., DeVico, A. L., Rahman, R., Oroszlan, S., Gallo, R. C., & Sarngadharan, M. G. (1986) *Science 231*, 1289−1291.

Dudding, L. R., & Mizrahi, V. (1993) *Biochemistry 32*, 6116−6120.

Dudding, L. R., Nkabinde, N. C., & Mizrahi, V. (1991) *Biochemistry 30*, 10498−10506.

Finston, W. I., & Champoux, J. J. (1984) *J. Virol. 51*, 26−33.

Fu, T.-B., & Taylor, J. (1992) *J. Virol. 66*, 4271−4278.

Furfine, E. S., & Reardon, J. E. (1991) *J. Biol. Chem. 266*, 406−412.

Gerard, G. F., & Grandgenett, D. P. (1975) *J. Virol. 15*, 785−797.

Gerwin, B. I., & Levin, J. G. (1977) *J. Virol. 24*, 478−488.

Gerwin, B. I., Rein, A., Levin, J. G., Bassin, R. H., Benjers, B. M., Kashmiri, S. V. S., Hopkins, D., & O'Neill, B. J. (1979) *J. Virol. 31*, 741−751.

Gilboa, E., Mitra, S. W., Goff, S., & Baltimore, D. (1979) *Cell 18*, 93−100.

Gopalakrishnan, V., Peliska, J. A., & Benkovic, S. J. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 10763−10767.

Hansen, J., Schulze, T., Mellert, W., & Moelling, K. (1988) *EMBO J. 7*, 239−243.

Herr, W. (1984) *J. Virol. 49*, 471−478.

Hizi, A., McGill, C., & Hughes, S. H. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1218−1222.

Hizi, A., Tal, R., & Hughes, S. H. (1991) *Virology 180*, 339−346.

Hostomsky, Z., Hostomska, Z., & Matthews, D. A. (1993) *in Nucleases* (Linn, S. M., Lloyd, R. S., & Roberts, R. J., Eds.) 2nd ed., pp 341−376, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hsieh, J.-C., Zinnen, S., & Modrich, P. (1993) *J. Biol. Chem. 268*, 24607−24613.

Hu, S. C., Court, D. L., Zweig, M., & Levin, J. G. (1986) *J. Virol. 60*, 267−274.

Huber, H. E., McCoy, J. M., Seehra, J. S., & Richardson, C. C. (1989) *J. Biol. Chem. 264*, 4669−4678.

Jacobo-Molina, A., Ding, J., Nanni, R. G., Clark, A. D. Jr., Lu, X., Tantillo, C., Williams, R. L., Kamer, G., Ferris, A. L., Clark, P., Hizi, A., Hughes, S. H., & Arnold, E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6320−6324.

Johnson, M. S., McClure, M. A., Feng, D. -F., Gray, J., & Doolittle, R. F. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7648−7652.

Kanaya, S., & Crouch, R. J. (1983) *J. Biol. Chem. 258*, 1276−1281.

Katayanagi, K., Miyagawa, M., Matsushima, M., Ishikawa, M., Kanaya, S., Ikehara, M., Matsuzaki, T., & Morikawa, K. (1990) *Nature 347*, 306−309.

Kati, W. M., Johnson, K. A., Jerva, L. F., & Anderson, K. S. (1992) *J. Biol. Chem. 267*, 25988−25997.

Katz, R. A., & Skalka, A. M. (1994) *Annu. Rev. Biochem. 63*, 133−173.

Klarmann, G. J., Schauber, C. A., & Preston, B. D. (1993) *J. Biol. Chem. 268*, 9793−9802.

Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A., & Steitz, T. A. (1992) *Science 256*, 1783−1790.

Kotewicz, M. L., Sampson, C. M., D'Alessio, J. M., & Gerard, G. F. (1988) *Nucleic Acids Res. 16*, 265−277.

Levin, J. G., Hu, S. C., Rein, A., Messer, L. I., & Gerwin, B. I. (1984) *J. Virol. 51*, 470−478.

Levin, J. G., Crouch, R. J., Post, K., Hu, S. C., McKelvin, D., Zweig, M., Court, D. L., & Gerwin, B. I. (1988) *J. Virol. 62*, 4376−4380.

Levin, J. G., Hatfield, D. L., Oroszlan, S., & Rein, A. (1993) in *Reverse Transcriptase* (Skalka, A. M., & Goff, S. P., Eds.) pp 5−31, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Lightfoote, M. M., Coligan, J. E., Folks, T. M., Fauci, A. S., Martin, M. A., & Venkatesan, S. (1986) *J. Virol. 60*, 771−775.

Luo, G., & Taylor, J. (1990) *J. Virol. 64*, 4321−4328.

Luo, G., Sharmeen, L., & Taylor, J. (1990) *J. Virol. 64*, 592−597.

Messer, L. I., Currey, K. M., O'Neill, B. J., Maizel, J. V., Jr., Levin, J. G., & Gerwin, B. I. (1985) *Virology 146*, 146−152.

Moelling, K. (1974) *Virology 62*, 46−59.

Müller, B., Restle, T., Kühnel, H., & Goody, R. S. (1991) *J. Biol. Chem. 266*, 14709−14713.

Nakamura, H., Katayanagi, K., Morikawa, K., & Ikehara, M. (1991) *Nucleic Acids Res. 19*, 1817−1823.

Oyama, F., Kikuchi, R., Crouch, R. J., & Uchida, T. (1989) *J. Biol. Chem. 264*, 18808−18817.

Post, K., Guo, J., Kalman, E., Uchida, T., Crouch, R. J., & Levin, J. G. (1993) *Biochemistry 32*, 5508−5517.

Prasad, V. R., & Goff, S. P. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 3104−3108.

Randolph, C. A., & Champoux, J. J. (1994) *J. Biol. Chem. 269*, 19207−19215.

Reardon, J. E., Furfine, E. S., & Cheng, N. (1991) *J. Biol. Chem. 266*, 14128−14134.

Restle, T., Müller, B., & Goody, R. S. (1990) *J. Biol. Chem. 265*, 8986−8988.

Restle, T., Müller, B., & Goody, R. S. (1992) *FEBS Lett. 300*, 97−100.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, 2nd ed., Section 5, pp 68−69, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schatz, O., Mous, J., & LeGrice, S. F. J. (1990) *EMBO J. 9*, 1171−1176.

Sisk, W. P., Chirikjian, J. G., Lautenberger, J., Jorcyk, C., Papas, T. S., Berman, M. L., Zagursky, R., & Court, D. L. (1986) *Gene 48*, 183−193.

Stahl, S. J., Kaufman, J. D., Vikic-Topic, S., Crouch, R. J., & Wingfield, P. T. (1994) *Protein Eng. 7*, 1103−1108.

Tanese, N., & Goff, S. P. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1777−1781.

Tanese, N., Roth, M. J., & Goff, S. P. (1986) *J. Virol. 59*, 328−340.

Tanese, N., Telesnitsky, A., & Goff, S. P. (1991) *J. Virol. 65*, 4387−4397.

Telesnitsky, A., & Goff, S. P. (1993a) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1276−1280.

Telesnitsky, A., & Goff, S. P. (1993b) *EMBO J. 12*, 4433−4438.

Telesnitsky, A., Blain, S. W., & Goff, S. P. (1992) *J. Virol. 66*, 615−622.

Varmus, H., & Swanstrom, R. (1984) in *Molecular Biology of Tumor Viruses, RNA Tumor Viruses* (Weiss, R., Teich, N., Varmus, H., & Coffin, J., Eds.) 2nd ed., pp 369−512, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Verma, I. M. (1975) *J. Virol. 15*, 843−854.

Volkmann, S., Wohrl, B. M., Tisdale, M., & Moelling, K. (1993) *J. Biol. Chem. 268*, 2674−2683.

Williams, K. J., Loeb, L. A., & Fry, M. (1990) *J. Biol. Chem. 265*, 18682−18689.

Wohrl, B. M., & Moelling, K. (1990) *Biochemistry 29*, 10141−10147.

Wohrl, B. M., Volkmann, S., & Moelling, K. (1991) *J. Mol. Biol. 220*, 801−818.

Wohrl, B. M., Howard, K. J., Jacques, P. S., & LeGrice, S. F. J. (1994) *J. Biol. Chem. 269*, 8541−8548.

Yang, W., Hendrickson, W. A., Crouch, R. J., & Satow, Y. (1990) *Science 249*, 1398−1405.

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