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RNA-primed DNA Synthesis: Specific Catalysis by HeLa Cell DNA Polymerase α

(RNA–DNA linked molecules/DNA initiation/natural primer-templates)

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ABSTRACT We have analyzed and compared the responses of the three major HeLa cell DNA polymerases (α, β, and γ) to a HeLa DNA template with short RNA or DNA primers hybridized to it. Only DNA polymerase α is able to synthesize DNA covalently bonded to the RNA primer via a $3' \rightarrow 5'$ phosphodiester bond. ^{32}P transfer experiments showed that all combinations of ribo- and deoxyribonucleotides are represented in the RNA–DNA linkages but their distribution is nonrandom. The RNA–DNA linked molecules base-paired to a HeLa DNA template strand represent a possible "natural" *in vitro* primer-template for DNA polymerases and can be extended by all three DNA polymerases (α, β, and γ). These findings indicate that DNA polymerases β and γ are capable of DNA-primed but not RNA-primed DNA synthesis, while DNA polymerase α is capable of both RNA-primed and DNA-primed DNA synthesis.

Animal cells possess several DNA polymerase activities whose role in DNA replication is still unknown. Unlike RNA polymerases, DNA polymerases do not seem to initiate new chains along templates but instead catalyze the extension of preexisting deoxyribo- or ribopolynucleotide chains. Such priming sites may be provided *in vitro* by using DNA activated with DNase I or oligodeoxynucleotides hydrogen-bonded to long homopolymers. Keller (8) has reported that DNA synthesis *in vitro* on single-stranded circular ϕX DNA can be started by a hydrogen-bonded RNA primer with KB cell DNA polymerase α as well as with *Micrococcus luteus* DNA polymerase I. Chang and Bollum (9) have reported that oligoadenylates, but not oligouridylates, are effective primers for DNA polymerase α from calf thymus. We have recently reported (10) that DNA-dependent (α and β) and RNA-dependent (γ) DNA polymerases from HeLa cells can utilize the oligonucleotide primer $(rA)_{12-18}$ on a poly(dT) template, suggesting that they are all able to covalently extend oligo(adenylic acid) with deoxyribonucleotide. DNA replication in bacteria (11–15) and mammalian cells (16–18) seems to occur by a discontinuous mechanism, and it has been proposed that RNA synthesis might be involved in the initiation of synthesis of the DNA fragments at specific sites on the template. Therefore, it became of interest to examine "natural" RNA, rather than synthetic oligoribonucleotides, as a primer for the various HeLa cell DNA polymerases.

Since single-stranded DNA by itself is poorly, if at all, utilized by mammalian DNA polymerases, we have examined how the three mammalian DNA polymerases (α, β, and γ) would work if concomitant RNA synthesis by *Escherichia coli* DNA-dependent RNA polymerase (coupled reaction) were allowed on a DNA strand, or if RNA chains previously synthesized on and still hybridized to a HeLa single-stranded DNA chain were used (uncoupled reaction) as a template. A profound difference was found in the ability of various DNA polymerases to utilize such a natural RNA-primed DNA template. Only DNA polymerase α is able to synthesize DNA covalently bonded to the RNA primer. Once formed, the newly synthesized deoxyribonucleotide chains can then be extended by all three DNA polymerases (α, β, and γ).

MATERIALS AND METHODS

Enzyme Fractions. HeLa cell DNA polymerases α, β, and γ were hydroxylapatite fractions and were purified as described (10). All polymerases were free of RNase H activity, as assayed with a [^{32}P]-labeled RNA·DNA hybrid synthesized by *E. coli* RNA polymerase. These enzymes contained no demonstrable deoxyribonuclease activity, as judged by failure to solubilize double- or single-stranded HeLa [^{3}H]DNA or failure to cleave T5 DNA as determined by agarose gel electrophoresis. A unit of DNA polymerase activity in each case is defined as 1 nmol of total deoxynucleoside triphosphate incorporated into an acid-insoluble form in 30 min at 37° with an activated DNA template (19). *E. coli* RNA polymerase was a gift of Dr. H. Kung (Department of Biochemistry, Roche Institute of Molecular Biology) and was purified according to the procedure of Kung *et al.* (20). A unit of RNA polymerase activity is defined as 1 nmol of ATP incorporated into an acid-insoluble form in 20 min at 37° with a single-stranded HeLa DNA as template under the conditions described in the next section.

Preparation of RNA·DNA Hybrid for Uncoupled Reaction. HeLa cell DNA, at a concentration of 330 μg/ml in 15 mM NaCl, 1.5 mM sodium citrate, 10 mM Tris·HCl (pH 7.9), was denatured by heating at 100° for 10 min and then quickly chilled in ice water. After this treatment the DNA had a single-stranded molecular weight of approximately 4.5×10^6.

The nomenclature for HeLa cell DNA polymerases used in this paper is the following: *DNA polymerase α* is the high-molecular-weight (6–8S) DNA polymerase first detected in mammalian cells by Yoneda and Bollum (1) and studied in several laboratories (2); *DNA polymerase β* is the low-molecular-weight (3.5S) enzyme first described in HeLa cell nuclei by Weissbach *et al.* (3) and in calf thymus by Chang and Bollum (4); *DNA polymerase γ* is the synthetic RNA-dependent DNA polymerase described by Fridlender *et al.* (5) in HeLa cells and Maia *et al.* in chick cells (6). A separate and distinct DNA polymerase found in mitochondria (7) will not be discussed in this paper.

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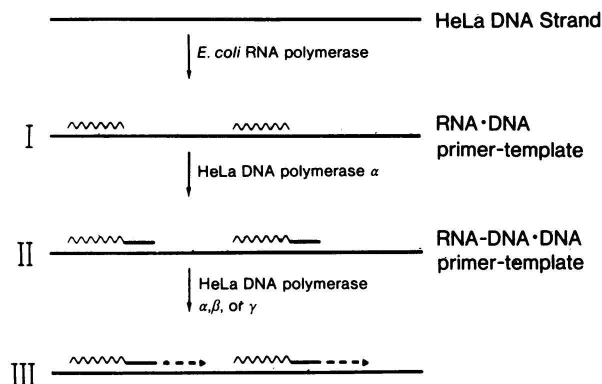

FIG. 1. Experimental scheme for DNA synthesis *in vitro* by HeLa cell DNA polymerases.

A partial DNA·RNA hybrid was prepared in 1 ml of a solution containing 50 mM Tris·HCl (pH 7.9), 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 80 μM EDTA, 200 μg of bovine serum albumin, 2 mM KPO_4 (pH 7.5), 10 μM rabbit liver soluble RNA, 0.1 mM ribonucleoside triphosphates, 120 μg of HeLa denatured DNA, and 3.4 units of DNA-dependent RNA polymerase (*E. coli*).

After incubation at 37° for 20 min, approximately 1–2% of the DNA was transcribed, as judged by [α-^{32}P]ATP incorporation in parallel experiments. The reaction mixture was then extracted with phenol–chloroform (1:1) saturated with 0.3 M Tris·HCl (pH 7.5). The phenol–chloroform was subsequently removed by extraction with ether. The DNA·RNA hybrid so isolated was then used in the DNA polymerase reaction (uncoupled reaction). Control experiments show that the ribonucleotides still remaining do not interfere with the subsequent reaction catalyzed by DNA polymerase. When labeled RNA was desired, [α-^{32}P]ATP was used in the reaction mixture and the incorporation of radioactivity was assayed as described. The RNA·DNA hybrid product banded at the density of single-stranded DNA in a CsCl gradient.

Coupled RNA-Primed DNA Synthesis. The complete system was the same described above for the uncoupled reaction except that it contained also the deoxyribonucleoside triphosphates at 40 μM each (specific activity as specified) and the designated DNA polymerase from HeLa cell, in addition to RNA polymerase.

^{32}P Transfer Experiments. The coupled reaction mixture was modified as follows: the four ribonucleoside triphosphates were present at a concentration of 1 mM; deoxynucleoside [α-^{32}P]-triphosphates (10–16 Ci/mmol) were used at 10 μM, and the concentration of DNA polymerase α was 25 units/ml. The reaction was carried out at 37° for 1 hr and then the transfer of [^{32}P]phosphate to ribonucleotides was studied as described by Pigiet *et al.* (17).

RESULTS

RNA-Primed DNA Synthesis In Vitro by HeLa Cell DNA Polymerases. The experimental scheme used in these studies is outlined in Fig. 1. HeLa DNA, either single- or double-stranded, was converted to a partial RNA·DNA hybrid with *E. coli* RNA polymerase (structure I). This structure was tested as a primer-template for the various HeLa cell DNA polymerases since it was known that HeLa cell DNA polymerases utilize pure single-stranded DNA poorly, if at all (3, 13). Table 1 shows the response of HeLa cell DNA polymerases α, β, and γ to a partial RNA·DNA hybrid (structure I) prepared in the coupled reaction. When the enzymes are added to a template that contains small RNA pieces synthesized by the RNA polymerase *in situ* on a single-stranded DNA, DNA polymerases β and γ show little copying of the DNA and are not significantly stimulated by RNA synthesis. However, DNA polymerase α was active in this system, and inhibition of RNA synthesis by omission of either one or all four ribonucleotides or by omission of RNA polymerase reduced DNA synthesis by DNA polymerase α by up to 20-fold. This would indicate that the stimulation of DNA synthesis required RNA synthesis. As expected, all four deoxyribonucleoside triphosphates were required for DNA synthesis and neither RNA nor DNA was made in the absence of a DNA template.

TABLE 1. *Response of HeLa cell DNA polymerases α, β, and γ to an RNA-primed DNA template formed in situ*

	[^{3}H]dNTP incorporated (pmol) by		
	DNA polymerase α	DNA polymerase β	DNA polymerase γ
Complete system	57.4	14.2	5.1
− rATP, rCTP, rGTP, rUTP	3.2	10.8	4.3
− rATP	10.0	—	—
− RNA polymerase	1.8	11.9	4.5
− DNA polymerase	0.9	—	—
− dTTP, dCTP, dGTP	0.6	—	—

The complete system (50 μl volume) is the one described for the coupled reaction in *Materials and Methods.* Deoxyribonucleotides were present at 40 μM with a specific activity of 156 cpm/pmol. Incubations were carried out at 37° for 30 min with 0.62, 0.93, and 0.25 unit of DNA polymerases α, β, and γ, respectively. [^{3}H]dNTP refers to a mixture of all four deoxynucleoside [^{3}H]-triphosphates. The template was single-stranded HeLa DNA (molecular weight 4.5×10^6), 120 μg/ml. —, not done.

In uncoupled reactions, where RNA·DNA hybrid (structure I) was synthesized independently and then incubated with the DNA polymerases, the same results were obtained. DNA polymerase α was markedly stimulated by the RNA·DNA hybrid when compared to a single-stranded DNA template, while DNA polymerases β and γ showed little response to either template (Table 2a). Similar results were also obtained using HeLa native DNA as template in the uncoupled reaction (Table 2b), but the extent of DNA synthesis was 50% less than that found with single-stranded DNA template.

Fig. 2 shows that, under the conditions of the coupled reaction, where RNA polymerase, DNA polymerase, and the appropriate substrates were added together, RNA synthesis was linear for approximately 15–20 min and then leveled off. However, the synthesis of DNA by DNA polymerase α started after a lag period of 5 min and then proceeded at a constant rate for approximately 40–50 min. If a preformed DNA·RNA hybrid (structure I) from the uncoupled reaction is used as a template, DNA synthesis by DNA polymerase α starts immediately and is linear for approximately 30 min. These results suggest that an RNA of a certain length is

TABLE 2. *Response of HeLa cell DNA polymerases to RNA- and DNA-primed templates*

Primer-template	$[^3H]$dNTP incorporation (pmol) by DNA polymerase α	DNA polymerase β	DNA polymerase γ
(a) RNA-primed single-stranded HeLa DNA template (structure I)	76.0	24.3	7.2
Without ribonucleoside triphosphates	6.9	24.0	7.1
(b) RNA-primed double-stranded HeLa DNA template	35.5	21.0	3.3
Without ribonucleoside triphosphates	5.28	26.0	3.0
(c) DNA-primed single-stranded HeLa DNA template (structure II)	108.0	84.0	18.9
Without ribonucleoside triphosphates	6.9	24.0	7.1
Without deoxyribonucleoside triphosphates (d)	—	23.0	6.8

(a) Fifty-microliter reaction mixtures contained 100 μg/ml of the DNA·RNA hybrid (structure I) prepared as described in *Materials and Methods* (uncoupled reaction) and 0.62, 0.93, and 0.25 unit of DNA polymerases α, β, and γ, respectively. The incubations were carried out at 37° for 30 min with 40 μM $[^3H]$-deoxyribonucleotides (specific activity, 62 cpm/pmol). The single-stranded DNA used in these studies (a, b, and c) had a molecular weight of 700,000.

(b) Fifty-microliter reaction mixtures contained 100 μg/ml of the DNA·RNA hybrid prepared as described in *Materials and Methods* for the uncoupled reaction except that HeLa native DNA was used.

(c) Fifty-microliter reaction mixtures contained 100 μg/ml of the DNA·RNA hybrid (structure I) formed as in (a) and 0.62 unit of DNA polymerase α, which converts structure I into structure II. The polymerization reaction was carried out at 37° for 30 min in the presence of 40 μM unlabeled deoxyribonucleotides and DNA polymerase α was then inactivated by heating 7 min at 60°. To each reaction mixture was added $[^3H]$dATP (final specific activity 62 cpm/pmol average) and 0.62, 0.93, and 0.25 unit of DNA polymerases α, β, and γ, respectively, to permit synthesis of structure III. After 30 min at 37° the acid-precipitable radioactivity was analyzed as described in *Materials and Methods*. The control reaction, lacking ribonucleoside triphosphates to prevent the initial formation of structure I, was carried out in exactly the same manner.

(d) In this control reaction, an RNA·DNA hybrid was incubated with DNA polymerase α in the absence of deoxynucleoside triphosphates. After heat-inactivation of the enzyme, the desired DNA polymerase and $[^3H]$deoxynucleoside triphosphates were added and the mixture was incubated at 37° for 30 min.

required to prime DNA synthesis, although the minimum length of the RNA primer has not been determined.

Covalent Attachment of the RNA Primer to the DNA Synthesized by DNA Polymerase α. The RNA-mediated stimulation of DNA synthesis by DNA polymerase α suggests that RNA chains complementary to the HeLa DNA template are serving as primers for the covalent extension by DNA polymerase, yielding RNA-DNA tandemly linked chains having RNA at their 5′-end and DNA at their 3′-end (structure II of Fig. 1). Direct evidence for the presence of linked RNA-DNA molecules was obtained by cesium chloride–cesium sulfate equilibrium centrifugation studies and by ^{32}P transfer experiments.

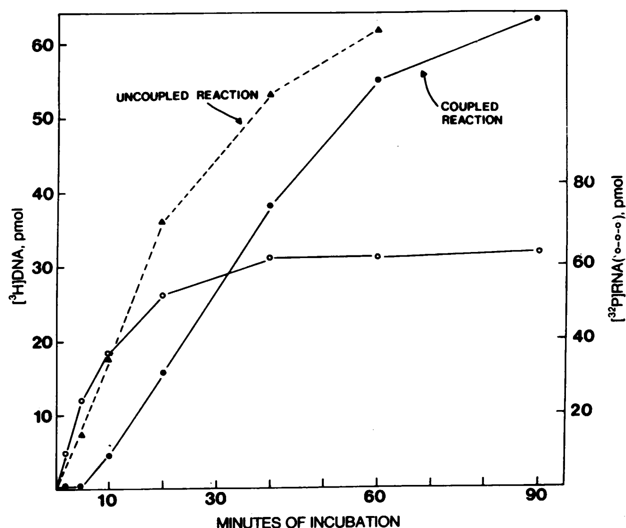

FIG. 2. Kinetics of DNA synthesis by HeLa cell DNA polymerase α in the coupled and uncoupled reaction. The coupled reaction described in *Materials and Methods* (50 μl volume) contained the four ^{32}P-labeled ribonucleoside triphosphates at 60 μM each (specific activity 43 cpm/pmol) and the four ^3H-labeled deoxyribonucleoside triphosphates at 40 μM each (specific activity 93 cpm/pmol). Incubation was at 37° with 0.17 unit of RNA polymerase and 0.62 unit of DNA polymerase. $[^{32}P]$RNA, O; $[^3H]$DNA, ●. The uncoupled reaction (50-μl volume) contained 2.5 μg of preformed RNA·DNA hybrid, 20 μg of bovine serum albumin, 50 mM Tris·HCl (pH 8.5), 7.5 mM $MgCl_2$, 0.5 mM dithiothreitol, and the four ^3H-labeled deoxyribonucleoside triphosphates at 40 μM each (specific activity 93 cpm/pmol). Incubation was at 37° with 0.62 unit of DNA polymerase. $[^3H]$-DNA, ▲. The results shown represent acid-precipitable radioactivity.

For the equilibrium centrifugation experiment, an uncoupled reaction in which DNA polymerase α utilized a preformed RNA·DNA hybrid (structure I) as a template was carried out. The RNA·DNA primer-template was labeled with ^{32}P in the RNA moiety and the synthesis of new DNA, catalyzed by DNA polymerase α, was carried out in the presence of $[^3H]$deoxynucleoside triphosphates. Thus, a tandemly linked RNA-DNA chain (structure II) duplexed to the HeLa DNA template would contain ^{32}P in the RNA segment and ^3H in the newly synthesized DNA portion.

The results of the CsCl–Cs_2SO_4 isopycnic centrifugation of the products of such a reaction are presented in Fig. 3. The $[^{32}P]$RNA and newly synthesized $[^3H]$DNA, which band at densities intermediate between the RNA and DNA markers, probably represent a covalent association between RNA and DNA since the denaturing conditions used with formaldehyde should prevent any reassociation between RNA and DNA. If these molecules banding at intermediate densities represent covalently linked RNA-DNA chains, then treatment with alkali to destroy the RNA should result in a shift of the $[^3H]$-DNA from the hybrid density to the density of known DNA. This was observed, as shown in Fig. 3. The broadness of the

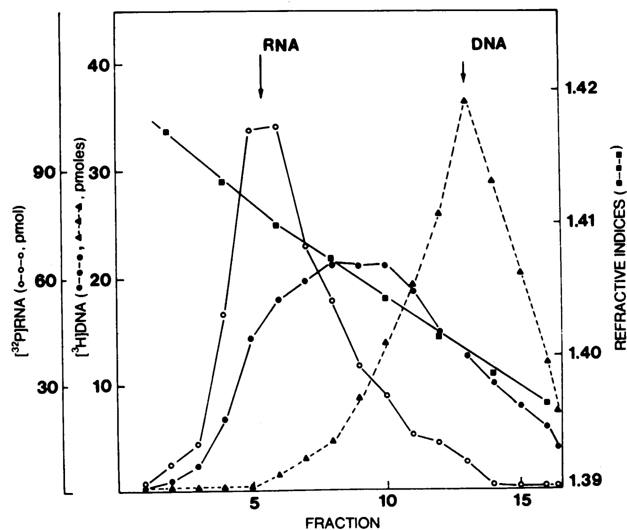

FIG. 3. Formaldehyde–CsCl–Cs_2SO_4 gradient centrifugation of the RNA-primed reaction product. RNA–DNA covalently linked molecules (structure II) were synthesized in a 1.5-ml uncoupled reaction mixture that contained 45 μg/ml of preformed RNA·DNA hybrid (structure I) (the RNA was labeled with [α-^{32}P]ATP), the four [^{3}H]deoxyribonucleoside triphosphates at 40 μM (specific activity 170 cpm/pmol), and the reagents required for the DNA polymerase reaction (as described in *Materials and Methods*). Incubation was at 37° for 30 min with 12.4 units/ml of DNA polymerase α. The polymerization reaction was halted by heating the mixture for 5 min at 60°, which inactivates DNA polymerase α.

To 0.2 ml of this reaction mixture were added 0.02 ml of 0.4 M EDTA, 0.05 ml of 1 M KPO_4 (pH 7.5), 0.002 ml of 10% Sarkosyl, and 0.075 ml of 10 M HCHO, and the final volume was adjusted to 0.75 ml with water. The mixture was then heated at 90° for 7 min, cooled, and diluted to 2 ml. Saturated CsCl (8 ml) and 1 ml of saturated Cs_2SO_4, both containing 0.3 M HCHO, 0.05 M KPO_4 (pH 7.5), 5 mM EDTA, were then added and the mixture was centrifuged in a 50 Ti rotor at 45,000 rpm at 25° for 44 hr. After centrifugation, fractions were collected, refractive indices were measured, and acid-insoluble radioactivity was determined. [^{32}P]RNA, O; [^{3}H]DNA, ●. Another 0.2-ml aliquot of the reaction mixture was first incubated at 37° overnight in 0.1 M NaOH to destroy RNA, then neutralized and treated as the sample described above before centrifugation. [^{3}H]DNA, ▲. No ^{32}P-labeled acid-precipitable radioactivity was present in this gradient. *Bombyx mori* ribosomal [^{3}H]RNA and HeLa [^{14}C]DNA, denatured under the same conditions, were run in a parallel tube as markers and are indicated by *arrows* in the figure.

peak is probably due to the low molecular weight of the DNA chains (see sucrose gradient centrifugation).

The presence of ribonucleotides covalently attached by a phosphodiester link from their 3′-OH group to the 5′-OH of the deoxyribonucleotides was confirmed by ^{32}P transfer experiments. RNA-DNA linked chains (structure II) were synthesized in a coupled reaction containing single-stranded HeLa DNA, unlabeled ribonucleotide triphosphates, RNA polymerase, and DNA polymerase α in the presence of deoxyribonucleoside triphosphates labeled with ^{32}P at the α-position. The RNA-DNA linked chains synthesized were isolated by gel filtration as described in *Materials and Methods* and then hydrolyzed with alkali. The distribution of ^{32}P in the four ribonucleotides was examined by the technique recently described by Pigiet *et al.* (17) and is shown in Table 3. A nonrandom distribution of ribonucleotide sequences occurred at the

TABLE 3. *^{32}P transfer experiments with the products of RNA-primed DNA synthesis catalyzed by HeLa cell DNA polymerase α*

[α-^{32}P]dNTP substrate	Percentage ^{32}P transfer to ribonucleotides			
	CMP	AMP	GMP	UMP
dTTP	4.61	27.89	36.26	31.23
dGTP	1.86	14.93	32.78	50.55
dCTP	18.04	18.61	46.73	16.65
dATP	9.88	12.74	46.66	30.84
All	6.60	16.35	48.72	28.27

With each substrate, RNA–DNA chains (structure II) were synthesized in 4 ml of the coupled reaction mixture described in *Materials and Methods.* [α-^{32}P]Deoxynucleoside triphosphates (New England Nuclear Corp.) were present at 10 μM (specific activities 2500 cpm/pmol when each labeled deoxyribonucleotide was present and 1000 cpm/pmol when all four were present). Total radioactivity in ribonucleotides after Dowex-1 chromatography was: 2.4×10^5, 1.3×10^5, 9.8×10^4, 1.5×10^5, and 2.5×10^5 cpm, respectively, after incubation with dTTP, dATP, dCTP, dGTP, and all four nucleotides.

RNA-DNA linkage. CMP was clearly present at a lower frequency than any of the other ribonucleotides, in particular when adjacent to dGMP or DTMP. GMP was present at higher frequency when adjacent to dCMP or dAMP, and UMP was more frequently found adjacent to dGMP. Nonetheless, it is clear that all four ribonucleotides do occur next to all deoxyribonucleotides.

The amount of ^{32}P transferred to ribonucleotides was 1–1.5% of the total acid-precipitable counts. Under the conditions used, no detectable radioactivity was incorporated into RNA, establishing that our procedure did not label internucleotide linkages in RNA.

Size of Products Synthesized. In order to determine the length of the DNA molecules formed, the products obtained in the uncoupled reaction with DNA polymerase α and a preformed RNA·DNA hybrid (structure I) were examined in HCHO–sucrose gradients. The structure II products were synthesized on a HeLa DNA template strand as described in the legend to Fig. 3. The linked [^{32}P]RNA and [^{3}H]DNA molecules so formed cosedimented, after heat denaturation in the presence of formaldehyde, at about 5 S. After additional alkali treatment to destroy RNA, the remaining [^{3}H]DNA sedimented at 2.8 S, which indicated an apparent molecular weight of approximately 30,000, a size much smaller than that of the original DNA template (4.5×10^6). Parallel experiments showed that the [^{32}P]RNA primer, initially formed by RNA polymerase in the uncoupled reaction, was heterogeneous in size with a main peak sedimenting at 3 S. These combined data indicate that [^{32}P]RNA and the [^{3}H]DNA were covalently attached in the uncoupled reaction, forming a polymer containing approximately 100 deoxynucleotides attached to an RNA chain of about the same size.

DNA-Primed DNA Synthesis In Vitro by HeLa DNA Polymerases. The previous experiments have shown that DNA polymerase α can utilize the RNA portion of an RNA·DNA hybrid duplex (structure I) as a primer to form an RNA-DNA covalently linked chain presumably held in a duplex configuration with the corresponding HeLa DNA template strand (structure II). Molecules of this type, synthesized as de-

scribed in the footnote (c) to Table 2 (and after heat-inactivation of DNA polymerase α), were again incubated with DNA polymerase α or β or γ. Table 2c indicates that both DNA polymerase β or γ can now utilize such a template to form structure III even though these enzymes could not utilize the original RNA·DNA hybrid structure I. As expected, DNA polymerase α can also utilize structure II.

The DNA synthesis observed in Table 2c is not due to an alteration of the template by a contaminant in the DNA polymerase α preparation. The control experiment in which DNA polymerase α is preincubated with a hybrid RNA·DNA template in the absence of deoxynucleoside triphosphates showed no increase in template ability when subsequently incubated with DNA polymerase β or γ (Table 2c).

Direct proof that the initial [^{3}H]DNA chain, made by DNA polymerase α by extension of the RNA primer to form structure II, can be further extended in size by DNA polymerase β (or α) to give structure III was shown by analyzing the products formed in a formaldehyde–sucrose gradient. The size of the newly synthesized [^{3}H]DNA molecules was examined in this gradient before and after reincubation of structure II molecules with DNA polymerase α or β for 30 min at 37°. Both enzymes lengthened the [^{3}H]DNA segment from 2.8 S to 4–5 S. Though the data again are not shown, DNA polymerase γ can also extend, in the same manner, the RNA-DNA chains. Thus, RNA-DNA linked molecules hybridized to the proper DNA template (structure II) can act as effective primers for these three HeLa DNA polymerases and permit synthesis of longer DNA chains.

DISCUSSION

Previous work (9, 10) has indicated that a synthetic oligoribonucleotide [oligo(A)] could be used as a primer for the three mammalian DNA polymerases (α, β, and γ). However, it was not known if a "natural" RNA could also function as a primer for these three enzymes. The main objective in this study was to analyze and compare the responses of three known HeLa cell DNA polymerases (α, β, and γ) to RNA- and DNA-initiated DNA templates. We have used, therefore, a single-stranded HeLa DNA template containing an RNA primer synthesized by *E. coli* RNA polymerase. The major conclusion from our data is that only DNA polymerase α is able to use such a primer. Proof for the covalent linkage of the priming RNA to the newly synthesized DNA via a $3' \rightarrow 5'$ phosphodiester bond was provided by ^{32}P transfer experiments. These last studies make it possible to identify the nucleotides at the RNA-DNA junction. According to our results, the switch from RNA to DNA synthesis *in vitro* is not caused by a unique sequence-determined signal. All four ribonucleotides are represented in the RNA-DNA linkages, but the linkage does not appear to be a completely random process. Similar results have been reported in studies of the synthesis of polyoma DNA in isolated nuclei (17). Our studies do not exclude the possibility that a more specific sequence at the RNA-DNA link may exist *in vivo* or within the RNA preceding RNA-DNA junction by one or more nucleotides.

A last point to mention is the ability by all three major HeLa polymerases (α, β, and γ) to extend the newly synthesized deoxyribonucleotide chains. The RNA-DNA linked molecules on a HeLa DNA template strand (structure II) represent putative "natural" *in vitro* primer templates for DNA polymerase with initiation sites on one single strand that can be extended by all three DNA polymerases (α, β, or γ). This would indicate that with a DNA template, DNA polymerases β and γ are capable of DNA-primed but not RNA-primed DNA synthesis, while DNA polymerase α is capable of both RNA-primed and DNA-primed synthesis. The fact that DNA polymerases β and γ can utilize a synthetic oligoribonucleotide but not a "natural" RNA primer represents an important distinction between the use of synthetic and natural primer-templates. One can speculate, therefore, that whereas all human DNA polymerases could function in DNA repair or DNA elongation processes, only DNA polymerase α could initiate new DNA synthesis with an RNA primer provided by RNA polymerase.

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