Proc. Natl. Acad. Sci. USA
Vol. 73, No. 10, pp. 3418–3422, October 1976
Biochemistry

Stepwise biosynthesis *in vitro* of globin genes from globin mRNA by DNA polymerase of avian myeloblastosis virus

(rabbit globin mRNA/complementary DNA/"self-priming"/DNA nucleotidyltransferase of *Escherichia coli*)

FRANÇOIS ROUGEON AND BERNARD MACH

Department of Pathology, University of Geneva, Geneva, Switzerland

Communicated by Jacques Monod, July 14, 1976*

ABSTRACT **Two approaches have been explored for the synthesis of double-stranded DNA from single-stranded DNA template complementary to rabbit 9S globin mRNA (cDNA). (*i*) cDNA was elongated with dCMP or dTMP homopolymeric tracts using terminal deoxynucleotidyltransferase (EC 2.7.7.31; nucleosidetriphosphate:DNA deoxynucleotidylexotransferase). cDNA-dC, in the presence of an oligo(dG)$_{10}$ primer, was an efficient template with either DNA polymerase of *Escherichia coli* (EC 2.7.7.7; deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase) or RNA-directed DNA polymerase of avian myeloblastosis virus. cDNA-dT [with an oligo(dA)$_{10}$ primer] functioned as template only with *E. coli* polymerase. (*ii*) cDNA, without homopolymeric tails, was also efficiently copied in the absence of oligonucleotide primer, by DNA polymerase of avian myeloblastosis virus or of *E. coli*. The product of the reaction consisted of long hairpin molecules which could be converted into DNA duplex (melting temperature, 93°) by digestion with single-strand nuclease S1. The data indicate that a loop structure on the 3′ end of cDNA allowed DNA synthesis to take place by a "self-priming" mechanism. Some of the double-stranded DNA synthesized corresponded to the entire sequence of the 9S mRNA template. The synthesis of full-length double-stranded DNA from mouse globin mRNA and immunoglobulin light chain mRNA is also discussed.**

The DNA polymerase found in RNA tumor viruses, such as avian myeloblastosis virus (AMV), copies RNA or DNA templates by the elongation of a hydrogen-bonded "primer-initiator" molecule (1–5). *In vitro*, the product of the endogenous reaction is composed of single- and double-stranded DNA molecules (review in ref. 6). In the presence of an exogenous RNA template, such as mRNA from animal cells, the transcription production of AMV DNA polymerase is primarily single-stranded DNA, even in the absence of actinomycin D (7), although the synthesis of a small amount of double-stranded DNA of short size has been reported (8, 9). We have investigated the possibility of obtaining a double-stranded DNA molecule from a given mRNA template, using two different approaches.

(*i*) A complete cDNA transcript of globin 9S mRNA was elongated with a homopolymeric tract (with terminal transferase) and was then replicated in the presence of a complementary oligonucleotide primer with *Escherichia coli* or AMV DNA polymerase. This technique has been used in this laboratory to insert a mammalian gene sequence into a bacterial plasmid (10).

(*ii*) The cDNA alone was used as primer-template and copied with AMV or *E. coli* DNA polymerase ("self-priming"). As mentioned briefly earlier (10), the results indicate that AMV and *E. coli* DNA polymerases can convert single-stranded cDNA, such as cDNA from rabbit globin mRNA, into a full-length, well-matched DNA duplex. This simpler approach has made possible the construction of new bacterial plasmids carrying almost the entire sequence length of mRNA from rabbit hemoglobin, mouse hemoglobin, and mouse immunoglobulin light chain (in preparation).

Abbreviations: AMV, avian myeloblastosis virus; cDNA, complementary DNA.

* *Deceased.*

MATERIALS AND METHODS

RNA, DNA, and Enzymes. 9S rabbit globin mRNA was purified as described (11). mRNA for α and β globin chains was fractionated by gel electrophoresis and recovered as described (10, 11). [^{3}H]DNA of simian virus 40, digested with *Hin*(II + III) restriction endonuclease (12), was a gift of Dr. G. Bernardi and Dr. P. Chambon. AMV DNA polymerase was a generous gift from Dr. J. Beard. *E. coli* DNA polymerase (EC 2.7.7.7; deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase) ("Klenow fraction") was from Boehringer. Calf thymus terminal deoxynucleotidyltransferase (EC 2.7.7.31; nucleosidetriphosphate:DNA deoxynucleotidylexotransferase) and S1 nuclease were prepared as described (10).

Synthesis of Complementary DNA (cDNA). Unlabeled cDNA was synthesized in a 1-ml mixture containing 50 mM Tris·HCl (pH 8.2), 50 mM KCl, 7.5 mM $MgCl_2$, 1 mM dithiothreitol, 0.2 mM each dNTP, 10 μg of dT_{12-18} (P-L Biochemicals), 50 μg/ml of actinomycin D, 150 units of AMV DNA polymerase, and 50 μg of 9S globin mRNA. After 1 hr at 37°, EDTA was added to 10 mM and NaOH to 0.3 M, and the sample was incubated for 20 min at 80°. After neutralization and gel filtration (Sephadex G-75), the excluded volume was precipitated with ethanol and fractionated on an alkaline sucrose gradient (10). The absorbance tracing (A_{260}) was recorded and all cDNA corresponding to a chain length of more than 250 nucleotides was precipitated. [^{3}H]cDNA was synthesized as reported (10) with [^{3}H]dCTP (specific activity 10^3 or 10^4 cpm/pmol), from 9S globin mRNA or from fractionated α or β globin mRNA.

Elongation of cDNA. Unlabeled cDNA (20 μg/ml) was incubated at 37° in a 100-μl reaction mixture containing 100 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), pH 7.2, 8 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.5 mM dTTP or dCTP, and terminal transferase. The elongation reaction was monitored in parallel samples with either [^{3}H]dTTP or [^{3}H]dCTP (500 cpm/pmol); trichloroacetic acid-precipitable radioactivity was measured. Conditions were chosen where an average of 40 dTMP residues or 20 dCMP residues had been added. The mixture was made 10 mM EDTA, extracted with chloroform–isoamylalcohol (20:1), and filtered on Sephadex G-75. The excluded fraction was lyophilized.

Synthesis of Double-Stranded DNA. With AMV DNA polymerase, the reaction was as described above for the synthesis of cDNA except that actinomycin D was omitted and mRNA was replaced by cDNA as template. With *E. coli* DNA polymerase, the mixture was the same except that Tris·HCl was pH 7.6, $MgCl_2$ was 10 mM, and 20 μg/ml of bovine serum al-

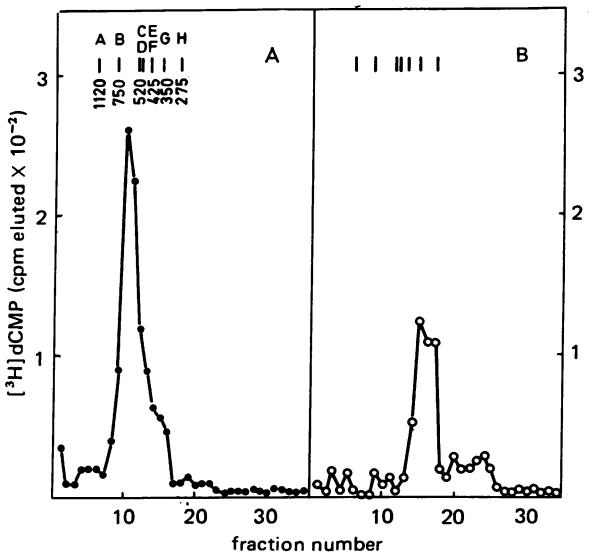

FIG. 1. Acrylamide gel electrophoresis of DNA in 98% formamide. (A) [^{3}H]cDNA was fractionated on an alkaline sucrose gradient, and an aliquot (4000 cpm) of the heaviest fractions was analyzed by gel electrophoresis (11). 1-mm slices were crushed and radioactivity was determined. Vertical lines indicate the position of *Hin*(II + III) fragments of ^{3}H-labeled simian virus 40 run in a parallel gel. Chain lengths are estimated from the values of ref. 12 and assuming a size of 5000 base pairs for simian virus 40. (B) The product of a reaction with cDNA-dC-dG_{10} and AMV DNA polymerase (see Fig. 2A) was treated with S1 nuclease and analyzed as indicated for (A) above. As indicated in *Materials and Methods*, the labeled DNA template was heterogeneous in size, ranging from 200 to 650 nucleotides.

bumin were added. Incubation time, and the concentration of enzyme, template, primer, and triphosphates are given in the legends. Trichloroacetic acid-precipitable radioactivity was measured on an aliquot of the reaction. Alternatively, when [^{3}H]cDNA was used as template, synthesis of the second DNA strand was assayed by measuring the resistance of the [^{3}H] cDNA template to single-strand nuclease S1. The reaction product was treated with an excess of S1 nuclease in a 0.1-ml mixture as described (10). The size of the reaction product was determined by sedimentation in an alkaline sucrose gradient (10) using a SW 50.1 rotor for 12 hr at 20° and 45,000 rpm, with appropriate markers whose sizes had been determined by gel electrophoresis (Fig. 1).

RESULTS

cDNA Synthesis in Presence and Absence of Actinomycin D. To determine if AMV DNA polymerase could synthesize double-stranded DNA in one step starting from globin 9S mRNA, the reaction was studied in the presence and absence of actinomycin D. Analysis of the product size and of the degree of double-strandedness (resistance to S1 nuclease) with and without denaturation, indicated that most of the cDNA synthesized in the presence and absence of actinomycin D consisted of single-stranded DNA (data not shown). Similar conclusions have been reached by others (7).

In the presence of actinomycin D, the yield of cDNA synthesized corresponded to 50–60% of the input template. Analysis of the cDNA size has shown that the longest cDNA molecules (600–650 base pairs) correspond in length to complete transcripts of the globin mRNA template (Fig. 1). The size was estimated electrophoretically with simian virus 40 fragments that had been digested with *Hin*(II + III) as standards (12). When saturating amounts of polymerase were used, the size of the cDNA product did not show a clear-cut dependency upon the concentration of triphosphates (7, 13).

Table 1. Synthesis of double-stranded DNA from cDNA templates

Template	Primer	pmol of dNTPs polymerized: *E. coli* DNA polymerase I	pmol of dNTPs polymerized: AMV DNA polymerase
cDNA-dT	None	16.7	3
	dA_{10}	55.3	7.2
cDNA-dC	None	56	22.8
	dA_{10}	—	26.3
	dC_{10}	—	30
	dG_{10}	62.7	71.2
	dG_{10} (minus dATP)	—	8.3
No cDNA		0.5	0.4
	None	57.6	61.6
cDNA	dC_{10}	59	74.4

Incubation mixtures contained, in 25 μl, 120 pmol of cDNA nucleotides and, when indicated, 25 pmol of oligonucleotide primer. Reactions were for 1 hr at 30° with 1 unit of *E. coli* DNA polymerase I and at 37° with 1 unit of AMV DNA polymerase. Incorporation of [^{3}H]dCMP into trichloroacetic acid-precipitable material was measured. Results are expressed as total picomoles incorporated (assuming an equal amount of the four deoxynucleoside triphosphates).

cDNA Elongated with Homopolymeric Tracts Used as Template in Presence of Complementary Primer. Globin 9S cDNA (prepared in unlabeled form for these experiments) functioned as an active primer for the polymerization of dCMP or dTTP with terminal transferase. Under standard conditions (*Materials and Methods*), an average of 100 dCMP or dTTP nucleotides were added to each cDNA molecule. In the case of dGMP, the polymerization reactions stopped sooner, and 10–15 residues were added on the average.

Elongated cDNA (cDNA-dT, cDNA-dC, and cDNA-dG) was tested for its template activity in the presence of the corresponding complementary primers (initiators), with either *E. coli* DNA polymerase I (Klenow enzyme, ref. 14) or AMV DNA polymerase. Different permutations of primers were used and, as expected, only the primers capable of annealing to the elongated 3′-OH end of the cDNA stimulated DNA synthesis significantly. The results are summarized in Table 1 and Fig. 2A. cDNA-dC was an efficient template for both AMV and *E. coli* polymerases, with a strong dependency on the presence of primer in the case of the viral enzyme. cDNA-dT, in the presence of oligo(dA) primer, was copied much more efficiently with *E. coli* polymerase than with AMV polymerase. In all cases, DNA synthesis was totally inhibited by the presence of actinomycin D or by the absence of one of the four nucleotides. The size of the complementary DNA strand synthesized from the elongated cDNA template was analyzed after treatment of the reaction product with nuclease S1. Fig. 1B shows the profile of a gel electrophoresis in 98% formamide, which indicates an average length of about 350 nucleotides. The unlabeled cDNA template used was heterogeneous in size, ranging from 200 to 650 nucleotides.

Synthesis of Double-Stranded DNA in Absence of Added Primer. A reproducible observation with elongated cDNA

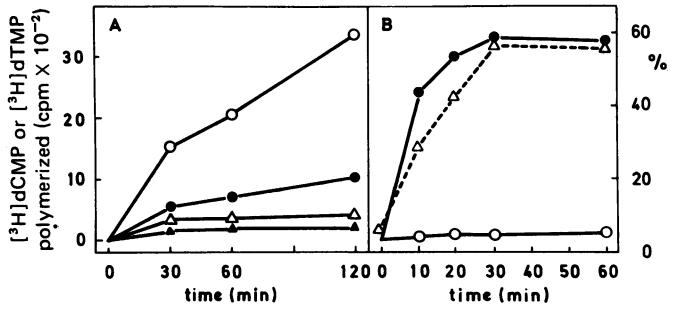

FIG. 2. Time course of the conversion of cDNA into double-stranded DNA with AMV DNA polymerase. (A) cDNA-dC_{20} and cDNA-dT_{40} as template. Reaction mixtures (100 μl) contained 280 pmol of cDNA, 3 units of AMV DNA polymerase, and 0.2 mM [^{3}H]dCTP or [^{3}H]dTTP (500 cpm/pmol). Acid-precipitable radioactivity was measured on 10-μl aliquots. (●) cDNA-dC_{20} and (○) cDNA-dC_{20}-dG_{10}, both with [^{3}H]dTTP. (▲) cDNA-dT_{40} and (△) cDNA-dT_{40}-dA_{10}, both with [^{3}H]dCTP. (B) cDNA template without homopolymeric ends. Reaction mixtures (50 μl) contained 240 pmol of cDNA, 1 unit of AMV DNA polymerase, and 0.2 mM [^{3}H]dCTP (500 cpm/pmol) without (●) or with (○) 50 μg/ml of actinomycin D. Acid-precipitable radioactivity was measured on 10-μl aliquots. In another incubation (△) the unlabeled cDNA template was replaced by ^{3}H-labeled cDNA template (200 pmol, labeled with [^{3}H]dCTP, 500 cpm/pmol) and aliquots were assayed for the resistance to nuclease S1 digestion, indicated in %.

templates was that considerable DNA synthesis was taking place with *E. coli* and AMV polymerase, even in the absence of added primer. We therefore explored the possibility of using as template the unelongated single-stranded cDNA. The reaction was efficient in the case of both DNA polymerases (Table 1). This primer-independent DNA synthesis was explored in greater detail with AMV DNA polymerase. Unlabeled cDNA was used as template (−strand), and the incorporation of radioactive nucleotides into the complementary (+)strand was followed (Fig. 2B and Table 2). In other experiments, ^{3}H-labeled cDNA, corresponding in length to the entire transcript of 9S globin mRNA (600–650 nucleotides), was used as template. The reaction was followed by measuring the acquisition of resistance to nuclease S1 of the [^{3}H]cDNA template (Figs. 2B and 3).

When unlabeled cDNA (−strand) was used as template, the newly synthesized DNA (+ strand) in the native reaction product was found to exist mainly in double-stranded form, as judged from its resistance to nuclease S1 (97%, Table 2). The reaction product, either as such or after treatment with nuclease S1, was denatured and allowed to reanneal, and the extent of double-strandedness was measured (Table 2). After denaturation and rapid cooling (0 time), the intact molecule exhibited considerable S1 resistance (65.2%), indicating intramolecular duplex formation ("snap-back"). In contrast, when the reaction product had first been exposed to nuclease S1, denaturation resulted almost exclusively in single-stranded material (see *Discussion*). When allowed to reanneal, the same reaction product became more than 77% S1-resistant. The results also show (Table 2) that globin mRNA competed with the labeled DNA strand (+) in the reassociation reaction, whereas cDNA (−strand) provided complete protection.

The DNA synthesized in this primer-independent reaction was also characterized with respect to size. Analyses were done by alkaline sucrose gradient sedimentation in the presence of [^{3}H]DNA markers whose sizes had been determined by gel electrophoresis. Fig. 3 shows the sedimentation profile of the globin [^{3}H]cDNA template (Fig. 3B) and of the same molecule after the reaction with AMV DNA polymerase (Fig. 3A). The size of the [^{3}H]cDNA template is now shifted to a higher molecular weight, and the largest molecules correspond in length to 1100–1200 nucleotides. This represents about twice the length of the original cDNA template. To measure the sequence length of the [^{3}H]cDNA template that had actually been copied into DNA, we first digested the reaction product with nuclease S1 and analyzed the S1-resistant DNA in an alkaline sucrose gradient. From the distribution of the nuclease-resistant ra-

Table 2. Secondary structure of [^{3}H]DNA synthesized by AMV DNA polymerase with unlabeled cDNA as template-primer

DNA	As such		After exposure to nuclease S1	
	cpm (− and + S1)	% [^{3}H]DNA in duplex form	cpm (− and + S1)	%[^{3}H]DNA in duplex form
Native	1080	97.2	646	98.6
	1032		633	
Denatured (zero time)	870	65.2	714	5.3
	568		38	
Reannealing (6 hr)	882	75.2	783	77.4
	664		606	
Reannealing (6 hr) + mRNA (0.5 μg/ml)	910	55.16	801	22.4
	502		180	
Reannealing (6 hr) + unlabeled cDNA (0.4 μg/ml)	978	82.8	700	98.2
	810		688	

Unlabeled cDNA (see *Materials and Methods*) was used as template in the absence of primer with AMV DNA polymerase ([^{3}H]dCTP, 500 cpm/pmol). The product (either as such or after digestion with nuclease S1) was assayed for its secondary structure under the different conditions indicated. Denaturation was at 98° for 5 min in H_2O and reannealing was done in 10 mM Tris·HCl (pH 7.6), 0.4 M NaCl, 1 mM EDTA at 65°, to a C_0t value of 5×10^{-3}. All samples were incubated for 1 hr at 45° with and without S1 nuclease, and acid-insoluble radioactivity was measured (10). [^{3}H]cDNA gave 4% S1-resistant radioactivity.

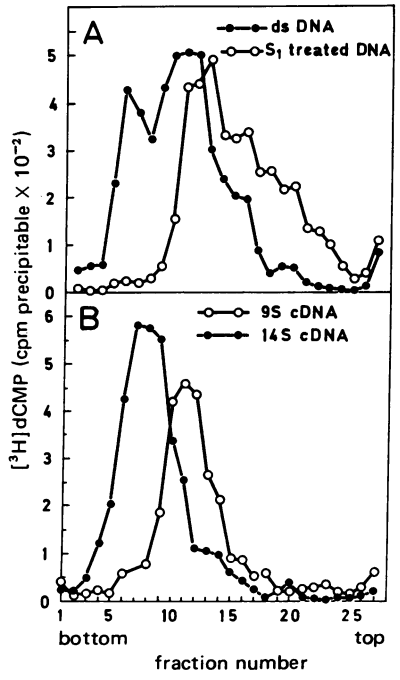

FIG. 3. Alkaline sucrose sedimentation of [^{3}H]cDNA template before and after its conversion into double-stranded DNA by AMV DNA polymerase. (A) [^{3}H]cDNA (125 cpm/pmol) was used as template with AMV DNA polymerase as in Fig. 2B. The replication reaction was assayed by following the resistance to nuclease S1 (Fig. 2). The replication reaction product (double-stranded DNA) was analyzed in alkaline gradients either as such (●) or after digestion with nuclease S1 (○). (B) [^{3}H]cDNAs as markers: an aliquot of the [^{3}H]cDNA used as template, and whose size had been determined by gas electrophoresis (see Fig. 1) (○); a [^{3}H]cDNA fraction synthesized from mouse immunoglobulin light chain mRNA (11), independently shown by acrylamide gel electrophoresis in 98% formamide to have an average chain length of 1100 bases (●).

dioactivity (Fig. 3A) it is evident that a main fraction corresponds in length to the original [^{3}H]cDNA template (full-length copies) while other molecules are smaller (partial copies).

The melting temperature (T_m) of the double-stranded DNA synthesized was determined by following the thermal elution of S1-treated reaction product from hydroxyapatite (Fig. 4). The observed curve indicated well-matched duplexes with a T_m of 93°.

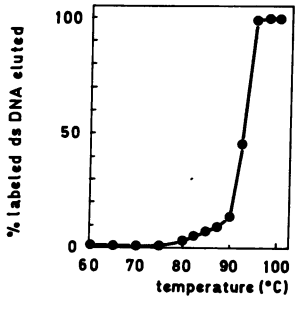

FIG. 4. Thermal melting curve of the double-stranded DNA (dsDNA) synthesized from cDNA by AMV DNA polymerase. 6000 cpm (24 pmol) of product of the DNA synthesis reaction with AMV DNA polymerase (Fig. 2A) was treated with nuclease S1 (10) and applied to a 0.5-ml column of hydroxyapatite in 40 mM sodium phosphate buffer (pH 6.8). The column was washed with 0.16 M sodium phosphate at the indicated temperatures, and the eluted acid-precipitable radioactivity was measured. It is expressed here as percent of the total radioactivity eluted from 60° to 98°.

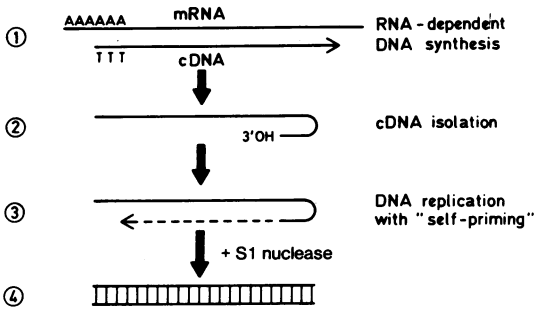

FIG. 5. Schematic representation of double-stranded DNA synthesis by "self-priming" (see *Discussion*).

cDNA from mRNA of both α and β globin functioned as template in the absence of primer and with the same efficiency. In addition, [^{3}H]cDNA of different length were tested for their primer-independent template activity. The results indicate (Table 3) that the cDNA fraction containing full-length transcripts of β globin mRNA is clearly the most active. Shorter cDNA molecules can be copied, but less efficiently.

Another specific single-stranded DNA (cDNA made from mouse globin mRNA) could also be copied in the absence of added primer (data not shown), with AMV DNA polymerase as well as with *E. coli* DNA polymerase. In another situation (cDNA made from mRNA of mouse immunoglobulin light chain), synthesis of double-stranded DNA in the absence of added primer proceeded well with *E. coli* DNA polymerase but very poorly with the AMV enzyme (data not shown).

DISCUSSION

The initial object of this study was the synthesis of a well-matched, double-stranded DNA sequence specified by a given mRNA, for its insertion into a bacterial plasmid. In this paper we describe the synthesis of full-length double-stranded DNA transcripts of rabbit globin 9S mRNA. Two main conclusions can be drawn from these studies. (*i*) AMV DNA polymerase, as well as *E. coli* DNA polymerase, can convert single-stranded cDNA into double-stranded structures. (*ii*) Long globin cDNA fractions, which include full-length transcripts, can be converted efficiently into double-stranded DNA without the addition of primer (initiator). This reaction must result from the

Table 3. Template-primer efficiency of β globin cDNA of different size

cDNA template	Incubation time (min)	cpm	% S1 resistance
β Globin cDNA, 550–650 nucleotides	0	1690 105	6.2
	30	2542 1244	48.9
β Globin cDNA, 100–250 nucleotides	0	1643 105	6.3
	30	4258 558	13.1

cDNA nucleotides (120 pmol), labeled with [^{3}H]dCTP (500 cpm/pmol), were incubated in a 50-μl reaction mixture (see *Materials and Methods*). The synthesis of double-stranded DNA was measured by following the conversion of the single-stranded [^{3}H]cDNA template into S1-resistant duplex. The sequence lengths are estimated as in the legend of Fig. 3.

existence of a well matched 3'-OH terminal loop in the template cDNA that is used as primer.

Since DNA polymerases cannot initiate the synthesis (or the replication) of DNA strands, the first approach was to provide a specific initiator-primer oligonucleotide capable of hydrogen-bonding to cDNA. For this, cDNA was elongated with monopolymeric tails and the complementary primer was used for replication. This procedure worked for globin cDNA, and the efficiency of both AMV and *E. coli* polymerases with the various primer molecules was analyzed. The use of oligo(dA) primer with cDNA-dT and *E. coli* polymerase, for instance, has allowed the synthesis of double-stranded globin DNA, which in turn could be inserted into a bacterial plasmid (10). The procedure should be generally applicable to any single-stranded DNA independent of its length and of its secondary structure. DNA polymerase from AMV was able to convert cDNA-dC, in the presence of a dG_{10} primer, into double-stranded DNA. The rather short length of the product was probably a consequence of the size distribution of the template cDNA. The relative efficiencies of cDNA-dT·dA_{10} and cDNA-dC·dG_{10} are similar to the relative efficiencies of the homopolymers dA_n·dT_{10} and dC_n·dG_{10} with either AMV DNA polymerase or the enzyme from *E. coli* (1, 15, 16). The efficiency of cDNA-dC alone could also be related to the ability of poly(dC) homopolymers to assume some secondary structure and to act as primer-template (15–17).

The data show that rabbit globin cDNA once reisolated, and without elongation, functioned as a template and a primer for its own copying, with DNA polymerase from either AMV or *E. coli*. Preliminary observations on such a reaction had been reported (9). The results presented here show that the product of the reaction with AMV polymerase consisted of DNA molecules about twice as long as the cDNA template and that the newly synthesized DNA was a sequence complementary to the template cDNA. After denaturation, the reaction product instantaneously assumed considerable base pairing ("snap-back"), indicating an intramolecular duplex. If, however, the reaction product had been first treated with nuclease S1, the template and the product DNA strands behaved as two distinct molecules, with similar length and showing perfect complementarity. From these results one can conclude that AMV DNA polymerase converted the long globin cDNA molecules into double-stranded DNA by the elongation of a 3'-OH terminus loop structure that functioned as a primer-initiator ("self-priming"). After synthesis of the second DNA strand, the loop is accessible to cleavage by nuclease S1 (see Fig. 5).

The copying of a single-stranded DNA without the addition of primer has been reported already with the DNA polymerase of phage T4 (18). In that case, DNA synthesis was shown to require first an initial exonucleolytic digestion of unpaired nucleotides on the 3'-OH terminus of a partly mismatched loop until a well-matched "primer" sequence became available. The same enzyme was then able to catalyze DNA synthesis by elongation. In the case described in this paper, since AMV DNA polymerase does not possess an associated 3' → 5' exonuclease activity, we must conclude that the structure that allowed the "self-priming" reaction to take place is a well-matched 3'-OH terminal loop in globin cDNA. The fact that short globin cDNA molecules are much less efficient primer-templates suggests that such loop structures are characteristic of the 5' end of the globin mRNA sequence.

The behavior of cDNA of mouse globin, which also functioned as primer-template with AMV polymerase, would suggest a perhaps more general distribution of the 3'-OH end loop. It was therefore of interest to observe that cDNA from mouse immunoglobulin light chains could not be copied efficiently in the absence of added primer by AMV DNA polymerase, although the "self-priming" reaction was active with *E. coli* DNA polymerase. This different behavior with the two enzymes could result from the existence, in this case, of a loop with an unpaired 3'-OH terminus sequence (which must first be digested). Alternatively the observed difference in primer-template efficiency could be related to the greater length of the light chain template cDNA (2) or to other structural differences.

Using a selection step for long double-stranded DNA molecules (after S1 nuclease digestion), we have constructed a series of *E. coli* plasmids carrying almost the full sequence length of α and β globin of rabbit and mouse and of mouse immunoglobulin light chain (in preparation). In some of these plasmids, the inserted globin sequence was 620 base pairs long, indicating therefore that the entire globin gene had been synthesized in the reaction *in vitro*.

Note Added in Proof. Since submission of this manuscript, another report has also described the synthesis of double-stranded globin DNA [Efstratiadis, A., Kafatos, F. C., Maxam, A. M. & Maniatis, T. (1976) *Cell* 7, 279–288].

We thank M. Olgiati aand P-A. Briand, who provided excellent technical assistance, P. Vassalli for advice and encouragement, G. Bernardi and P. Chambon for gifts of simian virus 40 fragments, and the Viral Cancer Program, National Cancer Institute for providing AMV DNA polymerase. F.R. was an E. Roosevelt Fellow. This work was supported by the Fonds National Suisse.

1. Baltimore, D. & Smoler, D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 1507–1511.
2. Hurwitz, J. & Leis, J. P. (1972) *J. Virol.* **9**, 116–129.
3. Leis, J. P. & Hurwitz, K. (1972) *J. Virol.* **9**, 130–142.
4. Ross, J., Aviv, H., Scolnick, E. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 264–268.
5. Verma, J. M., Temple, G. F., Fan, H. & Baltimore, D. (1972) *Nature New Biol.* **235**, 163.
6. Junghans, R. P., Duesberg, P. H. & Knight, C. A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4895–4899.
7. Efstratiadis, A., Maniatis, T., Kafatos, F. C., Jeffrey, A. & Vournakis, J. N. (1975) *Cell* **4**, 367–378.
8. Young, B. D., Harrison, P. R., Gilmour, R. S., Birnie, G. D., Hell, A., Humphries, S. & Paul, J. (1974) *J. Mol. Biol.* **84**, 555–568.
9. Verma, I. M., Temple, G. F., Fan, H. & Baltimore, D. (1973) *Fed. Proc.* **32**, 530.
10. Rougeon, F., Kourilsky, P. & Mach, B. (1975) *Nucleic Acids Res.* **2**, 2365–2378.
11. Farace, M.-G., Aellen, M.-F., Briand, P.-A., Faust, C. H., Vassalli, P. & Mach, B. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 727–731.
12. Dauna, K. & Nathans, D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2913–2917.
13. Diggelmann, H., Faust, C. H. & Mach, B. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 693–696.
14. Klenow, H., Overgaard-Hansen, K. & Patkars, A. (1971) *Eur. J. Biochem.* **22**, 371–387.
15. Wells, R. D., Flügel, R. M., Larson, J. E., Shendel, P. F. & Sweet, R. W. (1972) *Biochemistry* **11**, 621–629.
16. Rougeon, F., Brun, G. & Chapeville, F. (1974) *Eur. J. Biochem.* **41**, 253–261.
17. Inman, R. B. (1964) *J. Mol. Biol.* **9**, 624–637.
18. Englund, P. T. (1971) *J. Biol. Chem.* **246**, 5684–5687.