Oligonucleotide-Directed Switching of DNA Polymerases to a Dead-End Track†

Anne-Laure Guieysse,‡ Danièle Praseuth,‡ Mikkail Grigoriev,§ Annick Harel-Bellan,§ and Claude Hélène*,‡

Laboratoire de Biophysique, INSERM U.201−CNRS URA 481, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75005 Paris, France, and Laboratoire d'Immunologie, URA CNRS 1156, Institut Gustave Roussy, 94805 Villejuif, France

Received March 9, 1995; Revised Manuscript Received May 8, 1995⊗

ABSTRACT: During DNA replication, the presence of oligonucleotides with partial homology to the template strand was shown to induce a switch of the polymerase from the normal template to the oligonucleotide. The latter acted as a dead-end template and led to abortive replication. The only prerequisite was that the oligonucleotide could form 7−9 base pairs with the newly synthesized DNA strand in order to switch templates. The switch occurred when base pairing of the oligonucleotide could take place with the 3′-end of the newly synthesized strand. These results show that oligonucleotides used in antisense or antigene strategies could have unexpected effects on replication. In addition, oligonucleotide-directed abortive replication might play an inhibitory role during PCR experiments on long DNA templates and lead to the amplification of truncated fragments.

Oligonucleotides can be used to selectively control gene expression according to several strategies (Hélène & Toulmé, 1990). Antisense oligonucleotides are targeted either to messenger RNAs, thereby inhibiting mRNA translation, or to viral RNAs where they block reverse transcription or viral RNA replication. Antigene oligonucleotides are targeted to double-helical DNA and inhibit transcription via triple-helix formation (Thuong & Hélène, 1993). Sense oligonucleotides can be used to trap cellular transcription factors or transactivating proteins of viral origin (Clusel et al., 1993). Ribozymes bind to messenger or viral RNAs in a sequence-specific way, but in addition to their antisense activity, they are engineered to induce catalytic cleavage of their target RNA (Rossi & Sarver, 1990). In addition, RNA or DNA oligonucleotides can be selected for binding to proteins or small molecules, the normal biological function of which does not involve any interaction with nucleic acids (Szostak, 1992). Here we report that oligonucleotides can inhibit DNA replication by inducing a switch of DNA polymerases from the normal template to the oligonucleotide acting as a dead-end template.

MATERIALS AND METHODS

Plasmid and Oligonucleotides. The plasmid constructs containing wild-type or mutant IL-2Rα promoter regions (positions −352, +110) were described previously (Grigoriev et al., 1992, 1993a) (see Figure 1). Unmodified oligonucleotides were purchased from Eurogentec (Belgium). They were purified on denaturing polyacrylamide gel. Acridine derivatives of oligonucleotides were obtained from Dr. N. T. Thuong (Orléans, France).

Replication Experiments Using T7 DNA Polymerase

Plasmid Template. The polymerization conditions with T7 DNA polymerase were as follows: the 5′-^{32}P-labeled primer 21-py or 20-py (20 nM) (see sequences in Figure 1) was incubated for 15 min at 30 °C with 1 μg of plasmid digested with *Hph*I or *Pst*I, alkali-denatured, and neutralized according to standard methods, in 20 mM Tris-HCl (pH 7.5), 25 mM NaCl, and 10 mM $MgCl_2$ in a final volume of 10 μL and in the presence of oligonucleotide when indicated (see Figure 1 for the location of primers and restriction sites on the template). The polymerization was initiated by adding 225 μM each dNTP and 0.6 unit of Sequenase (US Biochemicals). The reaction was stopped after 30 min at 30 °C, and products were analyzed on an 8% denaturing polyacrylamide gel. Data were quantitated on a Molecular Dynamics phosphorimager.

Oligonucleotide Template. A 47-base pair duplex (5′-TTGCTCACCCTACCTTCAACGGCAGGGGAATCTCCCTCTCCTTTTAT-3′) was 5′-^{32}P-labeled on the pyrimidine-rich strand and incubated for 15 min at 30 °C in the presence or absence of 15-mer oligonucleotides in 20 mM Tris-HCl (pH 7.5), 25 mM NaCl, and 10 mM $MgCl_2$. The polymerization was initiated by adding 225 μM each dNTP and 0.6 unit of Sequenase (US Biochemicals). The final volume was 10 μL. The reaction was stopped after 30 min at 30 °C, and products were analyzed on a 10% denaturing polyacrylamide gel.

Replication Experiments Using Taq Polymerase

The polymerization conditions with Taq polymerase were as follows: the reaction volume was 25 μL, containing 8 nM 5′-^{32}P-labeled primer 21-py, 1 μg of plasmid digested with *Hph*I, 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.01% Tween 20, 1 mM each dNTP, 2.5 units of Taq polymerase (Eurobio), and 10 μM oligonucleotide when indicated. Ten cycles were performed (2 min at 94 °C; 2 min at 46 °C; 2 min at 72 °C), and the products of polymerization were analyzed on an 8% denaturing polyacrylamide gel. Data were quantitated by a Molecular Dynamics phosphorimager.

Sequencing of Polymerization Bands

The band obtained after elongation of the 20-py primer with Sequenase (US Biochemicals) was purified from the

† This work was supported in part by grants from Rhône-Poulenc-Rorer. A.L.G. was the recipient of a fellowship from the *Ligue Nationale Française contre le Cancer.*

‡ Muséum National d'Histoire Naturelle.

§ Institut Gustave Roussy.

⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1995.

0006-2960/95/0434-9193$09.00/0

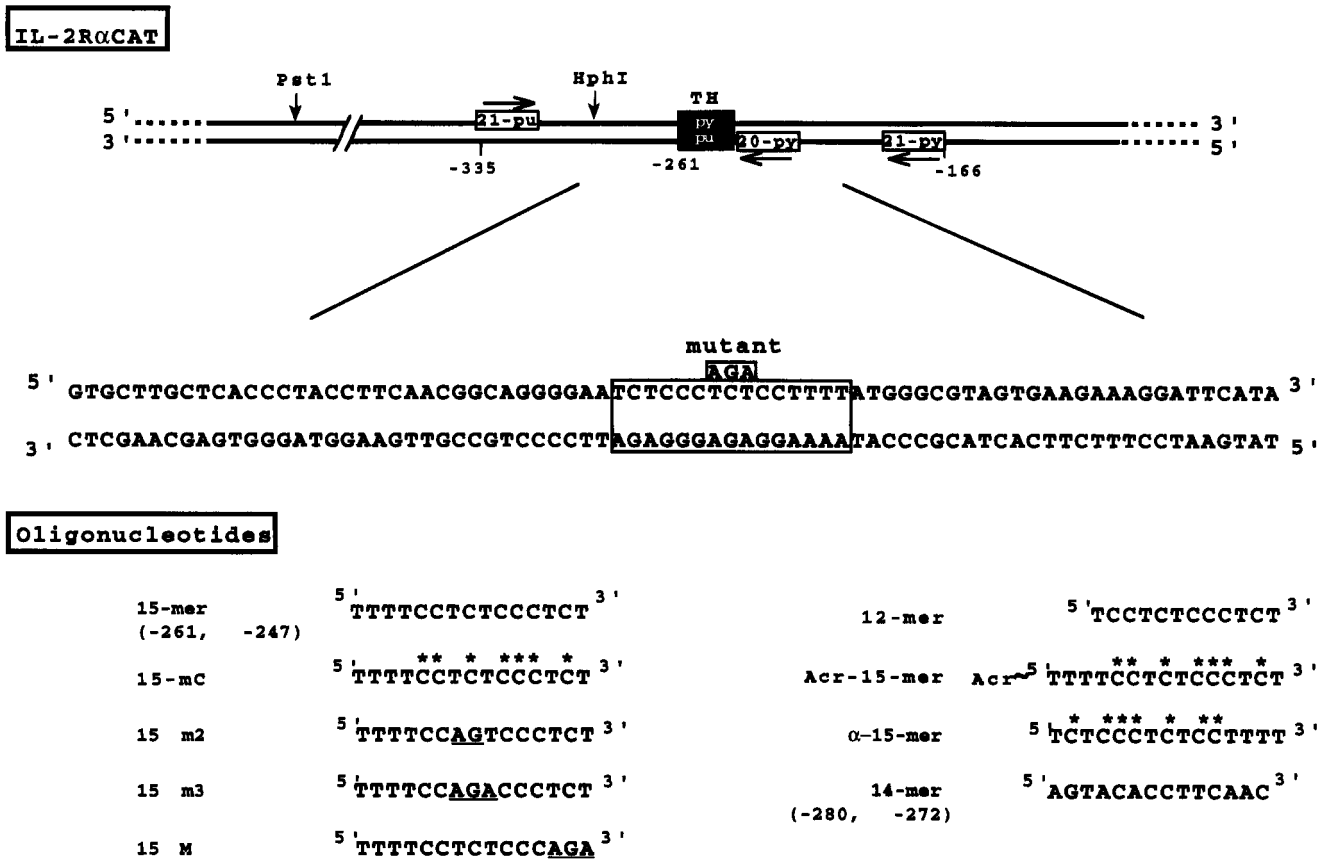

FIGURE 1: Top: Plasmid construct containing the IL-2Rα promoter region (−352 to +110) (Grigoriev et al., 1993a,b). TH indicates the position of the polypurine-polypyrimidine 15-bp sequence. The primers used for replication experiments are represented by open boxes, and horizontal arrows indicate the direction of polymerization (py indicates that they are complementary to the pyrimidine-rich strand and pu to the purine-rich strand). The mutant sequence with AGA replacing TCT in the upper strand is also indicated. The sequences of the primers indicated in the figure are as follows: 21-py, 5′-AGTTCAATTGCTGGAGGTGTG-3′, 20-py, 5′-CCTTTCTTCACTACGCCCAT-3′, 21-pu, 5′-ACTCAGCTTATGAAGTGCTGG-3′. Bottom: Oligonucleotides used in this study. C* represents methylated cytosines, and Acr represents an intercalating acridine derivative tethered to the 5′-phosphate of the oligonucleotide (Thuong & Hélène, 1993).

acrylamide gel and sequenced according to Maxam and Gilbert (1980) (G+A and G reactions). Products were analyzed on a 20% denaturing polyacrylamide gel.

RESULTS

Oligonucleotide-Induced Abortive Replication. We recently reported that an oligonucleotide−intercalator conjugate targeted to a 15 base pair polypurine-polypyrimidine sequence in the promoter region of the gene for the α-subunit of the interleukin-2 receptor (IL-2Rα) could inhibit transcription from this promoter in a sequence-specific way (Grigoriev et al., 1992, 1993a,b) (Figure 1). This inhibition was due to triple-helix formation at the promoter site, as shown by using a mutant of the target sequence (Grigoriev et al., 1993a,b). Primer extension experiments with T7 DNA polymerase (Sequenase) or Taq polymerase were performed in the presence of the triple-helix-forming 15-mer oligonucleotide. An arrest of T7 DNA polymerase was observed six nucleotides downstream from the triple-helix-forming site when the polypyrimidine-containing strand (top strand in Figure 1) was used as a template, with 21-py oligonucleotide as a primer (Figure 2). No truncated product was observed in the absence of the 15-mer oligonucleotide.

Experiments using T7 DNA polymerase performed with a radiolabeled template did not reveal any cleavage of the template strand in the presence (or absence) of the 15-mer oligonucleotide, as analyzed by gel electrophoresis. These results ruled out the possibility that nuclease activity could account for the stop band in the pattern of elongation (Lyamichev et al., 1993). Kinetic studies of the appearance of the stop band revealed that the arrest occurred with the same efficiency even at short times of incubation (30 s), and we did not observe any pause site of the polymerase (data not shown).

Triplex Structure Is Not Involved in the Arrest of DNA Polymerase. During replication of the single-stranded template (top strand in Figure 1) the triple-helix site was formed only after the newly synthesized strand contained the complementary polypurine sequence. Therefore, if triple-helix formation by the 15-mer oligonucleotide at the polypurine-polypyrimidine sequence were responsible for the observed arrest of DNA polymerase 6 base pairs downstream, the triple helix should form more rapidly than the extension of the newly synthesized strand, and this would require that the triple-helix site is made accessible despite the presence of DNA polymerase. Even though this seemed very unlikely, especially under the experimental conditions used for replication, which are unfavorable to triple-helix formation, we designed an experiment to eliminate this possibility. DNA was replicated in the presence of 7-deaza-dGTP. When the polypurine sequence was synthesized it could no longer engage in Hoogsteen hydrogen-bonding interactions with the 15-mer oligonucleotide, and therefore no triple helix could form. Nevertheless, the abortive product of replication was

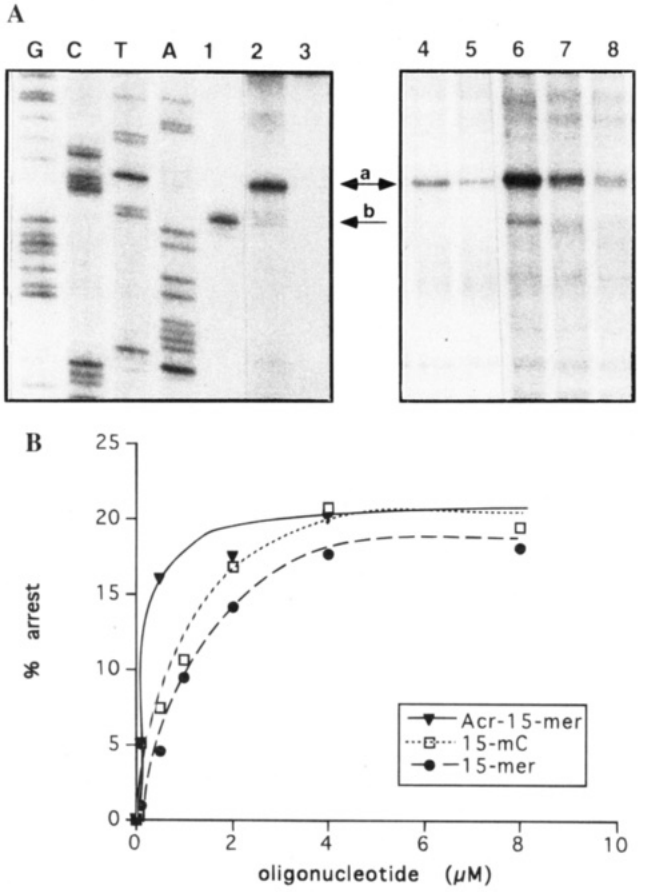

FIGURE 2: Replication experiments using T7 DNA polymerase and primer 21-py (Figure 1) on the plasmid IL2Rα-CAT linearized by *Pst*I or *Hph*I. The pyrimidine-rich strand is replicated. (A) Stop of elongation induced by 10 μM α-15-mer (lane 1) [the same arrest band was obtained with an α-15-mer substituted at the 3′ or the 5′-end by a psoralen derivative (Thuong & Hélène, 1993)], 10 μM 15-mer (lane 2), 1 μM Acr−15-mer (lane 4), 1 μM 15-mC (lane 5); 10 μM 15-mC (lane 6), 10 μM 15 m2 (lane 7), and 15m3 (lane 8). Lane 3: without oligonucleotide. A, T, C, G: Sanger sequence of the synthesized strand. The position of arrest due to the β-oligonucleotides is shown by arrow a, and arrest due to the α-15-mer is shown by arrow b. The experiments in lanes 1−3 and 4−8 are two separate experiments. The 5′-^{32}P-labeled primer 21-py (20 nM) was incubated for 15 min at 30 °C with 1 μg of plasmid linearized with *Pst*I, and the polymerization was performed as described in Materials and Methods. Products were analyzed on an 8% denaturing polyacrylamide gel. The full-length replication product (4900 nt) is not shown on the gels. (B) Effect of increasing concentrations of 15-mer oligonucleotides (15-mer, 15-mC, and Acr−15-mer) on the fraction of replication products arrested at the site indicated by arrow a in part A. The plasmid was linearized with *Hph*I, and the polymerization conditions were the same as in Figure 1. The abortive product (101 nt) was compared to the full-length product (149 nt) after gel electrophoresis (8% acrylamide) and quantitative analysis with a phosphorimager (see Figure 4).

still observed. This experiment ruled out triple-helix formation as a cause of replication arrest.

The Purine-Rich Strand Is Not Involved in Abortive Replication. In the experiments reported earlier, polymerization was carried out on an alkali-denatured plasmidic template, the complementary purine-rich strand (bottom strand in Figure 1) being present during the elongation. In order to exclude any participation of this complementary strand in the replication arrest, elongations were performed on a single-stranded pyrimidine template (460 nt) obtained by 30 successive cycles of linear primer extension using 21-pu as primer (Figure 1) on the template linearized by *Pst*1 digestion. To avoid any interference by the 21-pu primer that was not annealed to the template, the latter was removed on a Sephadex G25 column (Boehringer). In the presence of the 15-mer oligonucleotide, the stop band appeared with equal intensity. This showed that the purine strand of the template was not involved in the mechanism of arrest.

To determine whether the effect was strand-specific, elongation was performed using the purine-rich strand as template and the primer 21-pu. No replication-arrested band was detected in the presence of the 15-mer oligonucleotide, indicating that the process was asymmetric.

Analysis of the Product of Replication Arrest. In order to study the mechanism responsible for the production of the stop band, the DNA product resulting from the premature termination of DNA replication in the presence of the 15-mer oligonucleotide was purified from the acrylamide gel and sequenced according to Maxam and Gilbert (1980). As shown in Figure 3, the 3′-end of the DNA fragment was not complementary to the DNA template, but was instead complementary to the 5′-end of the 15-mer oligonucleotide over six nucleotides. The expected sequence of the last six nucleotides should be 5′-TTCCCC-3′ if the polymerase used the plasmidic DNA as template. We found that the nucleotides incorporated were 5′-GGAAAA-3′. This result indicated that the six terminal nucleotides of the truncated product resulted from replication by the polymerase of the 15-mer oligonucleotide sequence instead of the normal template. The sequence of the 15-mer oligopyrimidine includes a symmetric 9-mer sequence TCTCCCTCT, which could pair over nine nucleotides with the newly replicated strand. Therefore, the simplest explanation was that the 15-mer was hybridizing to the 3′-end of the newly synthesized DNA when it reached the end of the polypurine sequence and that a switch of template occurred from the single-stranded DNA to the 15-mer oligonucleotide. The 5′-end of the oligonucleotide determined the position of the arrest of the polymerase.

In order to confirm this mechanism, several additional experiments were performed: (i) Elongation with T7 DNA polymerase was performed with increasing concentrations of 15-mer oligonucleotide. The results presented in Figure 2B show that the arrest of replication was dependent on the oligonucleotide concentration. A plateau was reached above 4 μM 15-mer. This plateau corresponded to about 20% of aborted replication when T7 DNA polymerase was used.

(ii) We next studied the effect of length and base composition of the oligonucleotide on the truncated product. Various oligonucleotides were tested for their ability to induce an arrest of elongation. When the oligonucleotide was shortened from 15 to 12 nucleotides by removing 3 nucleotides from the 5′-end, the abortive DNA product was reduced in length by 3 nucleotides (12-mer in Figure 1) (results not shown). A 15-mer α-oligonucleotide was synthesized with α-anomers of the nucleotide units. It had the same sequence as the original 15-mer (synthesized with natural β-anomers) but in the reverse orientation to account for the *parallel* orientation of the two strands in α−β hybrids (Praseuth et al., 1987). A truncated DNA fragment was still obtained in the presence of this α-15-mer, but it was six nucleotides shorter than the fragment observed with the (β) 15-mer (Figure 2A). This result was in agreement with hybridization of the α-15-mer over nine bases with the newly

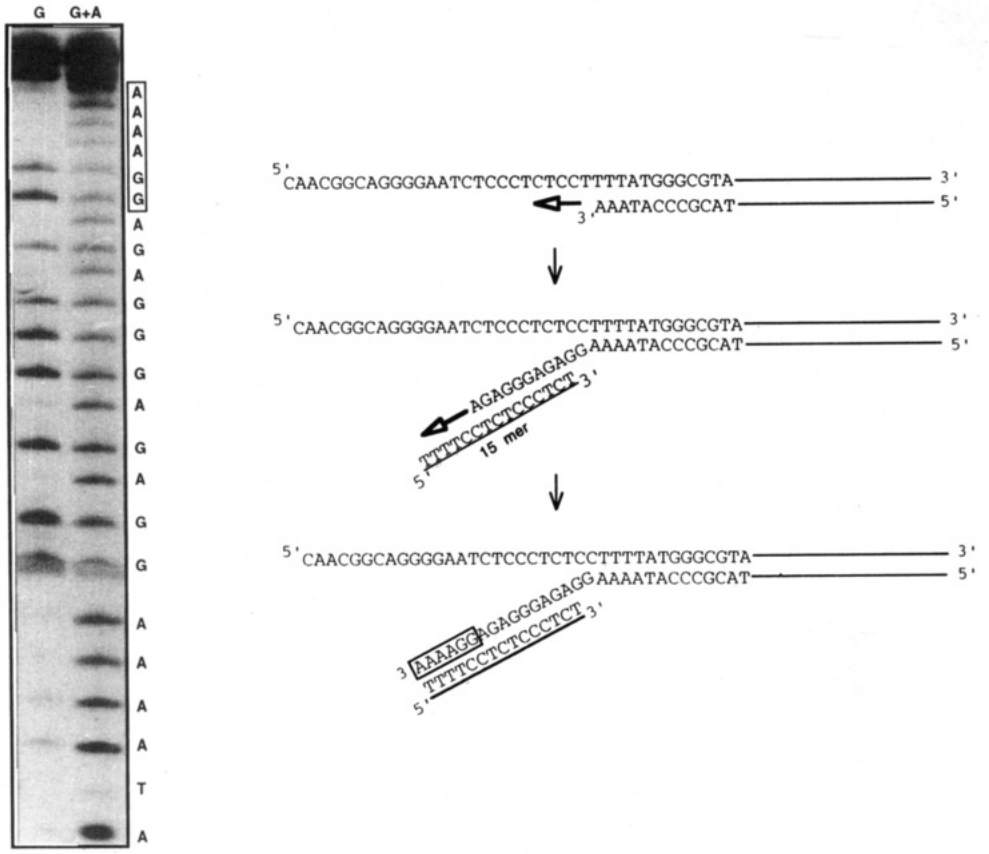

FIGURE 3: Left: Chemical sequence of the truncated product. Polymerization of the 5′-^{32}P labeled primer 20-py (see position in Figure 1) was performed on plasmid linearized with PstI in the presence of 10 μM of 15-mC (see Materials and Methods). The band corresponding to replication arrest (band a in Figure 2) was extracted from the gel, sequenced according to Maxam and Gilbert (1980) (G+A and G reactions), and analyzed on a 20% denaturing polyacrylamide gel. Right: Proposed mechanism of template switching.

synthesized DNA and the incapacity of DNA polymerase to use an α-oligonucleotide as a template.

(iii) Two or three mutations were introduced in the oligonucleotide (15m2, 15m3 and 15M in Figure 1), so that the newly synthesized strand could form only seven (15m2) or six (15m3 and 15M) base pairs with the 15-mer oligonucleotide. Only a weak arrest was observed with 15m2, and nearly no arrest was observed with 15m3 (Figure 2A). These results showed that the minimal length of double-helix formation between the oligonucleotide and the newly synthesized strand involved about seven base pairs. When the template strand was also mutated (see Figure 1) to compensate for the mutations in oligonucleotide 15M and restore the formation of nine base pairs, a truncated product elongated by six nucleotides was observed (results not shown).

Arrest Is Independent of the Location of the Oligonucleotide Hybridization Site on the Replicated Strand. To determine whether the mechanism of strand switching was peculiar to the 15-mer oligonucleotide or specific for polypyrimidine sequences, a 14-mer oligonucleotide (Figure 1) complementary over nine base pairs in its 3′ part to a distinct sequence (positions −280 to −272) of the newly synthesized strand was used in polymerization reactions. An abortive DNA fragment terminating five bases after the last hybridized base was detected (Figure 4). This result showed that the observation made with the 15-mer oligopyrimidine was not related to a special property of this sequence. We also showed that a similar stop of DNA polymerase replication was observed when the other strand of the IL-2Rα promoter fragment (containing the 15-mer polypurine sequence, bottom strand of Figure 1) was used as a template in the presence of an oligonucleotide able to hybridize over nine base pairs to the newly synthesized strand (results not shown).

Experiments Using Other DNA Polymerases. With Taq polymerase, we observed the same truncated product in the presence of the 15-mer oligonucleotide, and a succession of cycles (denaturation, hybridization, polymerization) allowed us to amplify the fragment due to strand switching (Figure 4). When the Taq polymerase switched template, the 3′-end of the newly synthesized fragment was complementary to the 15-mer oligonucleotide. After a denaturation step, this oligonucleotide could then be used as a primer, and polymerization could occur on the DNA product resulting from the switch of template. Then, a succession of cycles gave rise to exponential amplification of this product when the original DNA template was still linearly replicated.

Since sequenase and Taq DNA polymerase lack 3′→5′ exonuclease activity, the same experiments were performed with native *Escherichia coli* DNA polymerase I (Biolab's). This polymerase exhibits both 5′→3′ and 3′→5′ exonuclease activity. The same truncated product was observed with the same efficacy as the one obtained with the other polymerases. The Klenow fragment of *E. coli* DNA polymerase I, which lacks the 5′→3′ exonuclease activity, also gave the same product.

All of these experiments confirmed the hypothesis presented earlier, which assumed that DNA polymerases were able to switch templates when an oligonucleotide was present in the replication mixture, provided that it could form about 7−9 base pairs with the newly synthesized DNA fragment.

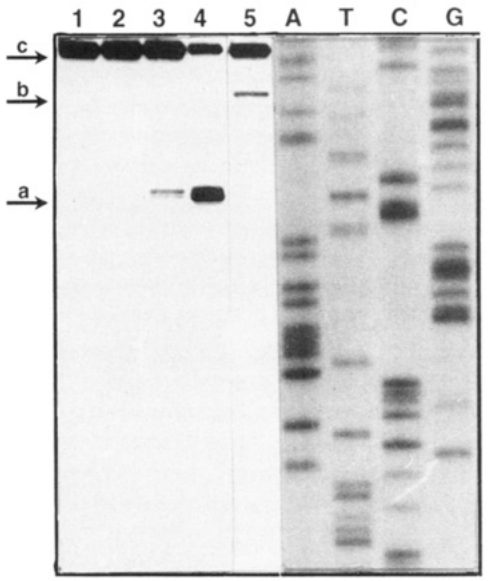

FIGURE 4: Replication experiments using Taq polymerase and the primer 21-py on the plasmid IL2Rα-CAT linearized by *Hph*I. Stop in elongation induced by 10 μM 15m3 (lane 2), 15-mC (lane 3), Acr−15-mer (lane 4), or 14-mer (lane 5). Lane 1: without oligonucleotide. Lanes labeled A, T, C, and G correspond to sequences of the synthesized strand obtained with the Sanger technique. The 5′-^{32}P-labeled primer 21-py (8 nM) was incubated with 1 μg of plasmid digested with *Hph*I and 10 μM oligonucleotide. Ten cycles of polymerization using Taq polymerase were performed (see Materials and Methods). The products of polymerization were analyzed on an 8% denaturing polyacrylamide gel. The position of the arrest due to the 15-mers is shown by arrow a, the arrest due to the 14-mer is indicated by arrow b, and the full-length replication product terminating at the cleavage site of *Hph*I is shown by arrow c. See Figure 1 for the sequence of the 14-mer.

With T7 DNA polymerase (Sequenase) at 30 °C we did not observe any increase in the intensity of the stop band when the number of base pairs increased to 10 or above.

Experiments with Modified Oligonucleotides. We then examined the effect of intercalator substitution at the 5′-end of the 15-mer oligonucleotide on the extent of DNA replication arrest. Acridine-substituted oligonucleotides have previously been used to inhibit gene expression in both the antisense and antigene approaches (Hélène & Toulmé, 1990; Thuong & Hélène, 1993). At low concentrations, the 5′-substituted Acr−15-mer was more efficient to arrest replication with T7 and Taq DNA polymerases than the unsubstituted 15-mer (Figures 2 and 4). However, the acridine could not be involved in stabilizing the nine base pairs formed by the 15-mer with the newly synthesized strand since it was attached to the nonhybridized 5′-end of the 15-mer. The switch of template might involve an oligonucleotide bound to the polymerase rather than a free oligonucleotide exchanging with the original template. The acridine−oligonucleotide conjugate could bind more tightly to the polymerase than the unsubstituted oligomer, as previously observed with RNA polymerase (Toulmé et al., 1986). This could explain why Acr−15-mer was more efficient at low concentrations than 15-mer due to tighter binding to DNA polymerases. The experiments described in the following show that oligonucleotides can bind to DNA polymerase−DNA complexes. It should be noted that, once strand switching has taken place, the Acr−15-mer conjugate can bind more efficiently to the abortive product and act as a more efficient primer for Taq polymerase during further elongation steps.

Elongation of a Double-Stranded Blunt-End DNA by Using an Oligonucleotide as Template. During the experiments reported earlier, we also observed that DNA fragments longer than the original template were synthesized in the presence of the 15-mer oligonucleotide. The length difference between the successive elongation products corresponded to the length of the oligonucleotide. To study the products of polymerization, experiments were performed with a blunt-ended 47-base pair double-stranded oligonucleotide with one ^{32}P-labeled strand (see Materials and Methods for the sequence). This 47-base pair DNA fragment was incubated with the 15-mer oligonucleotide in the presence of the four dNTPs. The ^{32}P-labeled strand was elongated, and bands corresponding to $(47 + 15n)$ nucleotides ($n = 1, 2, 3, ...$) appeared on a denaturing gel (Figure 5). Sequencing studies showed that these newly synthesized DNA fragments were elongated by multiples of 15 nucleotides, with sequences complementary to the 15-mer. When Acr−15-mer was used instead of the 15-mer, only one elongated fragment (+15 nt) was observed (Figure 5). The same results were obtained when the other strand of the 47-base pair duplex was 5′-^{32}P-labeled. The same phenomenon was observed with oligonucleotides of sequences and lengths different from those of the 15-mer. Elongation products corresponding to multiples of the oligonucleotide length were obtained (results not shown). These results are consistent with DNA polymerase using the 15-mer oligonucleotide as a template when it binds to a blunt-ended DNA fragment. When the 15-mer was 5′-substituted by an acridine, elongation could not occur after a first replication of the 15-mer sequence because the 3′-end of the newly synthesized strand was then facing an acridine derivative. These experiments showed that the 15-mer and Acr−15-mer could bind to DNA polymerase, at least when the polymerase was bound to double-helical DNA with blunt ends. It should be noted that a similar situation is encountered when DNA polymerases reach the end of a primed single-stranded template.

DISCUSSION

The results presented in this paper show that oligonucleotides used in antisense, sense, or antigene approaches could have unexpected effects on another biologically important function, namely, replication. This effect occurs via a switch of the template of DNA polymerase to the added oligonucleotide, provided that it can form about 7−9 base pairs with the newly synthesized strand. The oligonucleotide then acts as a dead-end track for the polymerase, thereby leading to abortive replication. This shift of template was made possible with the system described in this study, because nine consecutive base pairs could be formed between the 15-mer oligonucleotide and the neosynthesized purine-rich strand. This was due to the partial symmetry of the oligonucleotide sequence represented by the motif TCTC-CCTCT. When oligonucleotides having two or three mutations in the symmetrical part of the sequence (15m2 and 15m3 in Figure 1) were added during the polymerization instead of the 15-mer, the stop decreased or disappeared (Figure 2A). This result showed that six consecutive base pairs between the oligonucleotide and the neosynthesized strand were not stable enough to allow the oligonucleotide to compete with the template. Experiments performed in the presence of different oligonucleotides showed that this phenomenon was not dependent on the sequence of the

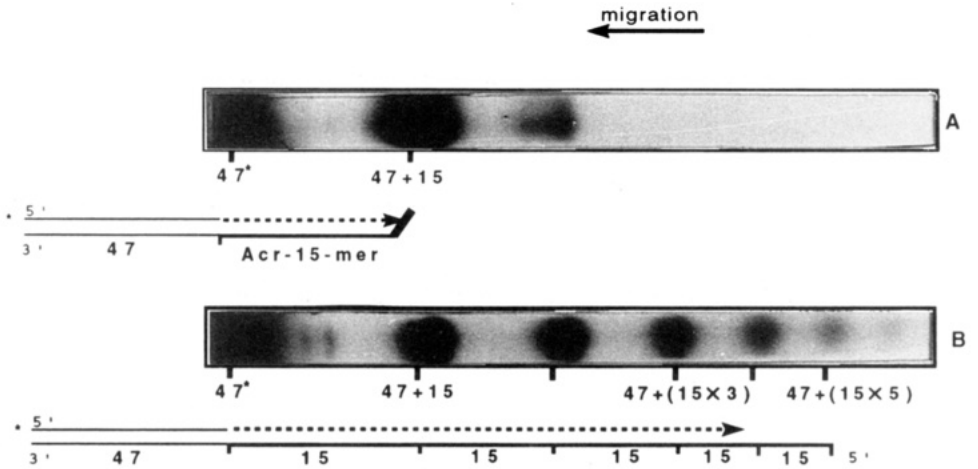

FIGURE 5: Elongation of a double-stranded 47-bp template by T7 DNA polymerase (Sequenase) in the presence of 10 μM Acr−15-mer (lane A) or 10 μM unsubstituted 15-mer (lane B). The 47-bp template was 5′-^{32}P-labeled on one strand and annealed to the complementary strand. The 47-base pair double-stranded fragment was incubated in the polymerization mix in a final volume of 10 μL in the presence or absence of 15-mer oligonucleotides, and the polymerization reaction using Sequenase was performed as described in Materials and Methods. Products were analyzed on a 10% denaturing polyacrylamide gel. The broken line represents the neosynthesized strand. The second (weak) band corresponding to 2 × 15 nt elongation (lane A) is due to contamination of the Acr−15-mer by a small fraction of unsubstituted 15-mer.

oligonucleotide, provided that it could form 7−9 base pairs with the neosynthesized strand.

We propose a model for the *inter*strand switching mechanism whereby the oligonucleotide present in the reaction mixture hybridizes to the newly synthezised strand during the replication process. The polymerase switches from its template to the oligonucleotide. This leads to an arrest of replication when the polymerase reaches the 5′-end of the oligonucleotide. This mechanism assumes that the oligonucleotide can hybridize to the neosynthezised strand during polymerization. The oligonucleotide probably interacts with the polymerase, facilitating the formation of a new double-stranded template when the oligonucleotide can hybridize over 7−9 nucleotides with the growing strand. Several experiments are in agreement with this hypothesis. Consistent with this interaction, the acridine−15-mer conjugate was found to be more efficient to induce template switching at lower concentrations than the unmodified 15-mer (Figures 2 and 4). The presence of an acridine could enhance the binding of the oligonucleotide to the polymerase. We have indeed observed earlier that acridine−oligonucleotide conjugates can bind to *E. coli* RNA polymerase and inhibit transcription in a non-sequence-specific way (Toulmé et al., 1986). Furthermore, DNA polymerases are able to elongate a double-stranded DNA with blunt ends by using an oligonucleotide as template (Figure 5). This phenomenon can be explained if the polymerase, when it is bound to the double-stranded DNA, also interacts with the oligonucleotide.

The fraction of abortive products increased with 15-mer oligonucleotide concentration until a plateau was reached at about 20% above 4 μM. This plateau was unchanged when the oligonucleotide length was increased from 12 to 25 nucleotides, provided that 9 base pairs could form with the newly synthesized strand (results not shown). The plateau might correspond to the probability that the polymerase switches from the donor to the acceptor oligonucleotide template once the latter is bound to polymerase, as compared with the elongation of the initial template. The acridine-substituted 15-mer had a higher activity at lower concentrations than the unsubstituted oligomer, suggesting again that acridine substitution increases the affinity for the DNA polymerase. It is not possible to exclude a mechanism involving dissociation of the polymerase from the newly synthesized strand, followed by strand invasion by the oligonucleotide and rebinding of the polymerase to the new substrate. However, this mechanism is very unlikely with T7 DNA polymerase (Sequenase), which is a highly processive enzyme. As a matter of fact, we did not observe any pause site with this enzyme even at short times of incubation.

We have shown that strand switching is also observed with Taq DNA polymerase and leads to the amplification of a truncated fragment, using the switch oligonucleotide as a primer. The loss of efficacy of PCR amplification when long DNA fragments are used (Barnes, 1994) might involve such strand-switching reactions to the primer oligonucleotides used in excess when they can form 7−9 base pairs with any of the two DNA strands. Therefore, the choice of oligonucleotide primers in PCR should also take into account the possibility that these primers may form a limited number of contiguous base pairs with any other sequence in the DNA fragment to be amplified. Such partial hybridization should be minimized to avoid unexpected amplification reactions.

Strand transfer reactions have been previously observed with several polymerases. *Intramolecular* template switching can occur *within the same strand* when the template strand contains stable secondary structures, such as hairpins. The polymerase may jump across the base of the hairpin, resulting in a replicated fragment with a deletion (Cariello et al., 1991). Strand switching from the initial template strand *to the complementary strand* during strand displacement can also occur, provided inverted repeats exist that allow base pairing between the neosynthesized and the displaced strands. Lechner et al. (1983) previously reported such a process, with T7 DNA polymerase elongating by strand displacement from a nick on a circular template. Strand switching occurred within the same molecular complex since the displaced strand remained linked to the template−polymerase complex. It was not observed when the polymerase harbored 3′→5′ exonuclease activity, in contrast to our present

observations with oligonucleotides where the 3′→5′ exonuclease did not play any role. Both *intermolecular and intramolecular* template switchings are an essential mechanism of viral reverse transcriptases (RT) and play a role in the genetic variations displayed by the virus (Peliska & Benkovic, 1992, 1994). A minimal region of homology is required between both templates (Ouhammouch & Brody, 1993). The RNaseH activity is essential for RT-mediated strand transfer. Degradation of the RNA template occurs concomitantly with polymerization, thereby exposing part of the neosynthesized DNA to hybridization with the acceptor template. As stated by several authors (Destefano et al., 1994; Peliska & Benkovic, 1992), an active role of the acceptor template via strand invasion is probably made possible by the existence of a three-stranded intermediate in association with the enzyme.

In the present work, two types of template-switching reactions involving DNA polymerases were observed with oligonucleotides: (i) transfer occurring at internal sequences on the template; (ii) transfer occurring at the end of a blunt-ended DNA fragment. We propose that a direct interaction between DNA polymerase and oligonucleotide greatly favors the first mechanism and is a prerequisite for the second one. Our results suggest that oligonucleotides might have unexpected effects by inducing an abortive oligonucleotide-directed template-switching reaction. Prokaryotic systems have been used previously to assess the role of oligonucleotides as transcription or replication inhibitors (Duval-Valentin et al., 1992; Maher, 1992; Hacia et al., 1994; Samadashwily & Mirkin, 1994). Some of the unexpected abortive products observed by Hacia et al. (1994) and by Samadashwily and Mirkin (1994) during DNA replication might be explained by the strand-switching reaction described in the present paper. Further experiments should indicate whether strand-switching reactions might also occur in eukaryotic systems during replication in the presence of oligonucleotides.

REFERENCES

Barnes W. D. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 2216−2220.

Buiser, R. G., Destefano, J. J., Mallaber, L. M., Fay, P. J., & Bambara, R. A. (1991) *J. Biol. Chem. 20*, 13103−13109.

Cariello, N. F., Thilly, W. G., Swenberg, J. A., & Skopok, T. R. (1991) *Gene 99*, 105−108.

Clusel, C., Ugarte, E., Enjolras, N., Vasseur, M., & Blumenfeld, M. (1993) *Nucleic Acid Res.* 15, 3405−3411.

Destefano, J. J., Bambara, R. A., & Fay, P. J. (1994) *J. Biol. Chem. 269*, 161−168.

Duval-Valentin, G., Thuong, N. T., & Hélène, C. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 504−508

Engler, M. J., Lechner, R. L., & Richardson, C. C. (1983) *J. Biol. Chem. 258*, 11165−11173.

Grigoriev, M., Praseuth, D., Robin, P., Hémar, A., Saison-Behmoaras, T., Dautry-Varsat, A., Thuong, N. T., Hélène, C., & Harel-Bellan, A. (1992) *J. Biol. Chem 267*, 3389−3395.

Grigoriev, M., Praseuth, D., Guieysse, A. L., Robin, P., Thuong, N. T., Hélène, C., & Harel-Bellan, A. (1993a) *Proc. Natl. Acad. Sci. U.S.A. 90*, 3501−3505.

Grigoriev, M., Praseuth, D., Guieysse, A. L., Robin, P., Thuong, N. T., Hélène, C., & Harel-Bellan, A. (1993b) *C.R. Acad. Sci. Paris, Life Sci. 316*, 492−495.

Hacia, J. G., Dervan, P. B., & Wold, B. J. (1994) *Biochemistry 33*, 6192−6200.

Hélène, C., & Toulmé, J. J. (1990)*Biochim. Biophys. Acta 17*, 99−125.

Lechner, R. L., Engler, M. J., & Richardson, C. C. (1983) *J. Biol. Chem. 258*, 11174−11184.

Lyamichev, V., Brow, M. A. D., & Dahlberg, J. E. (1993) *Science 260*, 778−783

Maher, L. J., III (1992) *Biochemistry 31*, 7587−7594.

Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 497−559.

Ouhanmouch, M., & Brody, E. N. (1992) *Nucleic Acids Res. 20*, 5443−5450.

Peliska, J. A., & Benkovic, S. J. (1992) *Science 258*, 1112−1118.

Peliska, J. A., & Benkovic, S. J. (1994) *Biochemistry 33*, 3890−3895.

Praseuth, D., Chassignol, M., Takasugi, M., Le Doan, T., Thuong, N. T., & Hélène, C. (1987) *J. Mol. Biol. 196*, 939−942.

Rossi, J. J., & Sarver, N. (1990) *Trends Biotechnol. 8*, 179−183.

Samadashwily, G. M., & Mirkin, S. M. (1994) *Gene 149*, 127−136.

Szostak, J. W. (1992) *Trends Biotechnol. 17*, 89−93.

Thuong, N. T., & Hélène, C. (1993) *Angew. Chem., Int. Ed. Engl. 32*, 666−690.

Toulmé, J.-J., Kirsch, H. M., Loreau, N., Thuong, N. T., & Hélène, C. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1227−1231.

BI950544R