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Specific inhibition of transcription by triple helix-forming oligonucleotides

(triplex/*in vitro* transcription/β-lactamase/psoralen)

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ABSTRACT **Homopyrimidine oligonucleotides bind to the major groove of a complementary homopyrimidine·homopurine stretch by triple helix formation. The *bla* gene from transposon Tn3 contains a homopyrimidine·homopurine sequence of 13 base pairs located just downstream of the RNA polymerase binding site. A 13-mer homopyrimidine oligonucleotide targeted to this sequence was tested for its effect on transcription of the *bla* gene *in vitro*. We show that the consequence of triple helix formation in front of the *Escherichia coli* RNA polymerase–promoter complex is to block the holoenzyme at its start site during a period that is dependent on temperature. The temperature dependence of transcription inhibition shows a direct correlation between this effect and the stabilization of the triple helix. Substitution of 5-methylcytosine to cytosine in the 13-mer oligonucleotide enhances triplex stability and transcription inhibition. Transcription inhibition by this synthetic repressor was also confirmed by footprinting studies demonstrating its specificity of action. The 13-mer oligonucleotide containing a psoralen derivative covalently linked to its 5′ end shows an irreversible and specific inhibition of transcription initiation after exposure to light of wavelength >310 nm.**

Artificial control of gene expression at the transcriptional level is an essential strategy toward development of a genetic therapy. With this goal in mind, extensive investigations have been performed using anticancer drugs able to bind DNA, such as intercalators or minor groove ligands (1–3). A crucial question with these chemical reagents concerns the stability of their complexes (i.e., residence time) with the nucleic acid target (4); this parameter determines the efficiency of inhibition of biological processes such as transcription or replication that involve proteins moving along nucleic acids. Another major problem is the sequence specificity of a particular drug with respect to a nucleic acid target, intercalators and minor groove binders having a limited sequence specificity.

For these reasons, oligonucleotides directed to a precise DNA sequence provide an interesting approach to artificially control the transcriptional process. Homopyrimidine oligonucleotides bind to complementary homopyrimidine·homopurine stretches by triple helix formation (5–9). We have studied the ability of an oligonucleotide–DNA complex to inhibit transcription by arresting *Escherichia coli* RNA polymerase (RNAP) *in vitro*. We have developed a transcription and footprinting assay on a DNA fragment carrying the *bla* promoter of *E. coli*, which enabled oligonucleotide–DNA complex formation under conditions in which the DNA was actively transcribed.

Here, we present the results obtained with a 13-mer oligonucleotide directed to the *E. coli* β-lactamase gene under the control of the *bla* promoter. This promoter was used in our experiments because it contains a homopurine·homopyrimidine sequence located just downstream of the promoter site from +22 to +34 relative to the transcriptional start site (Fig. 1). This potential triple helix site is close to the downstream limit of the region protected by the RNAP, which is specifically bound to the promoter (11). The *bla* promoter that we studied previously (11–13) presents a type of regulation that we termed "constitutive regulation." We showed earlier that, *in vitro* and *in vivo*, transcription from this promoter is inhibited by excess RNAP due to the formation of an inactive specific complex of two RNAPs per promoter above a critical RNAP/promoter ratio. This particular behavior did not interfere with our experiments under the conditions used here (12, 13). In this study, we performed footprinting experiments to optimize the conditions of saturation of the target by the oligonucleotide in the presence of RNAP. We show also by *in vitro* transcription experiments that *E. coli* RNAP can be inhibited by an oligonucleotide engaged in a triple helix. Moreover, by using the 13-mer linked to a photoreactive psoralen group at the 5′ end, the progression of the RNAP during transcription is irreversibly blocked after exposure to UV-A light (>310 nm). To our knowledge, this is the first example of how progression of RNAP on a precise gene can be artificially and specifically blocked by triple helix formation downstream of the transcription initiation site.

MATERIALS AND METHODS

Promoter Fragment. A 267-base-pair (bp) *Hph* I/*Eco*RI fragment, carrying the *bla* promoter, was isolated from the plasmid pBR322 and labeled with α-^{32}P at the *Eco*RI 3′ end for the footprinting experiments.

RNAP. *E. coli* RNAP was prepared according to Burgess and Jendrisak (14). Our preparation had a minimum of 90% σ subunit. From the titration experiments using the abortive initiation assay, we found that our enzyme preparation contained a minimum of 45% active molecules (10). A similar result was found by the nitrocellulose binding assay (15) on the *tetR* promoter.

Oligonucleotides. The oligopyrimidines used in this work were synthesized by the phosphoramidite method and purified on a 20% polyacrylamide gel. The sequence of the specific 13-mer is 5′-d(CTTTTTCCTTCTC)-3′ and is targeted to the β-lactamase gene at positions +22 to +34 relative to the start site located at +1. Psoralen-derivatized oligonucleotides were obtained on an oligonucleotide automatic

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Abbreviations: RNAP, RNA polymerase; 13-mer (^{m}C), 13-mer methylated at C-5 position of cytosine; pso-13-mer, psoralen-substituted 13-mer.

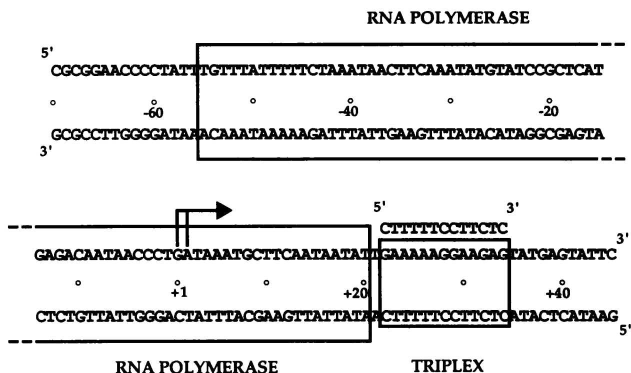

FIG. 1. Sequence of the β-lactamase promoter from *E. coli*. The β-lactamase transcription begins at positions +1 and +2 (10). Under the experimental conditions described in *Materials and Methods*, the major transcription start site is at G (+1). Boxes designate the regions protected by the RNAP (−55 to +20) and by the 13-mer oligonucleotide (+22 to +34) against DNAse I digestion.

synthesizer (Pharmacia) by using the phosphoramidite derivative of 5-(ω-hydroxyhexyloxy)psoralen, which was coupled to the 5′ end of the oligonucleotide attached to the solid support. The sequence of the nonspecific oligonucleotides used as control were 5′-d(CACACCGAGGGCG)-3′, and 5′-pso-d(TTTTCTTTTCCCCCCT)-3′, a 16-mer containing a psoralen derivative at its 5′ end. Fig. 1 shows the *bla* promoter sequence with the location of the triple helix and RNAP.

Protection Against DNase I Attack (Footprinting). The oligonucleotide and DNA fragment concentrations in all experiments are referred to as strand concentration. Experiments were performed as described in ref. 11, including the following modifications: the 267-bp fragment (5 nM; 10^5 cpm) was incubated with RNAP (30 nM, active molecules) at 37°C in a 120-μl solution containing 10 mM Tris·HCl (pH 7.8), 5 mM $MgCl_2$, 100 mM NaCl, 5 mM $CaCl_2$, 0.1 mM spermine, and 1 mM dithiothreitol. After 45 min of incubation, aliquots of 10 μl were withdrawn and put into 12 tubes containing heparin at 150 μg/ml (final concentration) in order to remove all nonspecific complexes located outside of the promoter and to trap all free RNAP. After the heparin challenge (performed for 1 min), the incubation was continued at different temperatures, after a rapid temperature shift down as indicated in the figure legends. The oligonucleotide was then added at 100 μM and the mixture was incubated for 10 min. DNase I was added (final concentration, 0.2 μg/ml) and the digestion was stopped after 10 sec (25°C–30°C) or 15 sec (15°C–20°C) by adding 4 μl of a solution containing 50 mM EDTA, 2% SDS, and 20 μg of tRNA. After two ethanol precipitations, the digests were analyzed on a denaturing polyacrylamide gel. For the footprinting experiments in the presence of the four XTPs, the nucleotides were added (500 μM each) for different periods of time before DNase I digestion.

***In Vitro* Transcription Directed by the 267-bp Fragment.** Transcription assays were performed in a 10-μl solution containing 10 mM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mM spermine, and 1 mM dithiothreitol, with 267-bp fragment (5 nM) and active RNAP (30 nM). The final nucleotide concentrations were 500 μM for ATP, GTP, and CTP, and 10 μM for UTP, including [α-^{32}P]UTP at 20 Ci/mmol (1 Ci = 37 GBq). Experiments were carried out as described in the footprinting section except that after incubations with RNAP and different oligonucleotides, the mixture of the four ribonucleoside triphosphates was added and the incubation was continued for different periods of time as given in the figure legends. Reactions were quenched as before, and after two ethanol precipitations the samples were heated and loaded on a 20% denaturing polyacrylamide gel.

Irradiation Experiments. Experiments were performed under the same conditions as in transcription experiments, except that after preincubation of RNAP with the 267-bp fragment bearing the *bla* promoter, heparin was added followed by the psoralen-modified 13-mer at 50 μM (final concentration) in a 120-μl solution at 30°C. After 10 min of incubation in darkness the solution was then exposed at 30°C in a glass tube to UV light from a 200-W xenon lamp filtered by 5-mm-thick Pyrex glass to eliminate radiation below 310 nm. Ten-microliter aliquots were withdrawn after increasing times of irradiation and transcription assays were then performed for 10 min (see Fig. 5). Transcription kinetics were also performed as described in the Fig. 5 legend. As a control, the same procedure was applied without irradiation in the presence of the 13-mer–psoralen conjugate. Other control experiments were performed in the presence of the 13-mer without psoralen followed by irradiation under the same conditions and using the psoralen-modified 13-mer in the presence of another promoter (*tetR* of plasmid pSC101) with UV-A exposure. A psoralen–16-mer conjugate was used as a control (see Fig. 5*b*).

RESULTS

Footprinting Study of the 267-bp DNA Fragment in the Presence of the 13-mer and the RNAP. The 13-mer is bound to the major groove of the homopurine·homopyrimidine sequence from positions +22 to +34. In the pH 7.8 buffer used in our experiments, complete protection was reached at ≈70 μM oligonucleotide for 5 nM double-stranded DNA at 30°C (Fig. 2). Above 30°C, no footprint could be detected as a result of complex instability at this temperature under our

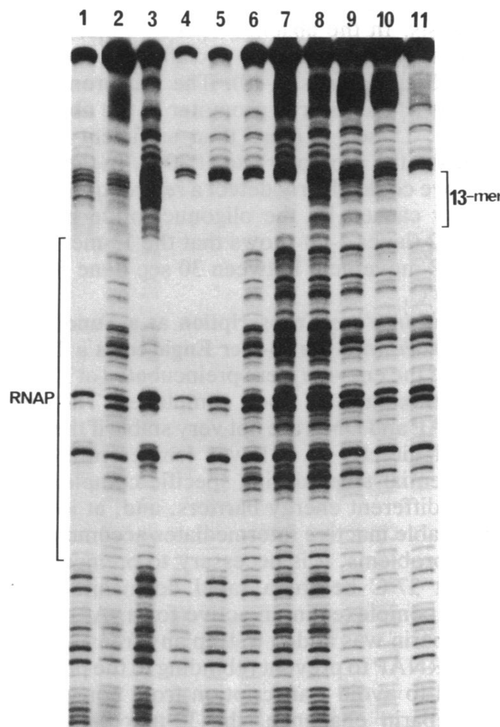

FIG. 2. DNase I footprinting of RNAP and the 13-mer with or without XTPs at 30°C. Lanes: 1, with RNAP after 15 min of heparin challenge; 2, DNA alone; 3, with RNAP after 1 hr of incubation with heparin; 4, with RNAP and 13-mer; 5–10, with RNAP and XTPs in the presence (lanes 5–7) or absence (lanes 8–10) of specific 13-mer—times of incubation were 30 sec (lanes 5 and 8), 2 min (lanes 6 and 9), and 15 min (lanes 7 and 10); 11, 13-mer alone.

conditions. At the pH of the experiments (pH 7.8), the triplex is dissociated above 30°C. The experiments in the presence of the 13-mer alone (Fig. 2, lane 11) showed one site hypersensitive to nuclease attack located at position +35. Taken together with the fact that DNase I can be considered as a conformational "reporter" (16–18), this result indicates that the oligonucleotide specifically bound induces a distortion of the DNA on the 3′ side of the 13-mer.

The next question was to determine whether it was possible to bind both RNAP and the oligonucleotide to the same DNA fragment. We have previously shown that the RNAP protects the promoter from −55 to +20 against DNase I attack (ref. 11; Fig. 2, lane 1). Under conditions in which the RNAP is in excess when compared to the DNA fragment, some nonspecific complexes appear just after preincubation, and they could compete with the oligonucleotide. For this reason, we performed a challenge with heparin before addition of the 13-mer. Fig. 2 (lane 4) shows an experiment performed at 30°C, where the regions protected by the protein and the oligonucleotide are in contact, showing that the two sites are not exclusive in spite of their proximity. All the control experiments were performed in the presence of a noncomplementary 13-mer to determine the effect of the presence of a short polyanion on RNAP complex stability. No difference was detected in the presence or absence of this control 13-mer.

Interaction of RNAP with the Promoter During mRNA Initiation in the Presence of the 13-mer Probed by DNase I Attack. DNase I cleavage was performed on polymerase–promoter complexes at different times after the addition of all four XTPs. The DNase I cleavage reaction requires ≈10 sec at 30°C. Therefore, it was possible to follow the clearing of the RNAP binding site on the promoter during the initiation of transcription. After transcription is initiated, no footprint is detected because of the positional heterogeneity of the different complexes, which are not synchronized during RNA synthesis. In the absence of the 13-mer, the enzyme footprint disappeared within 30 sec after the addition of the four XTPs (Fig. 2, lanes 8–10). The *bla* promoter can be considered as a "fast start" promoter in the absence of any obstacle (13); 15 sec is more than sufficient time for the RNAP to leave the promoter site at 30°C. In the presence of the 13-mer, we could clearly detect a retardation of RNAP on the promoter caused by the oligonucleotide bound downstream. Fig. 2 (lanes 5–7) shows that the 13-mer was able to inhibit RNAP movement between 30 sec (lane 5) and 2 min (lane 6) at 30°C.

Efficiency of *in Vitro* Transcription as a Function of Time and Temperature with the 13-mer Engaged in a Triple Helix. The DNA and the enzyme were preincubated at 37°C in order to obtain a maximum of open complexes. The complexes between RNAP and DNA are not very stable if the incubation is performed directly at a lower temperature. There are several sequential steps during specific complex formation that involve different energy barriers, and, at low temperature, some stable inactive intermediates accumulate (15). To avoid these problems, it is necessary to preincubate RNAP and DNA at 37°C before a rapid downshift in order to maintain the complexes in an active form. After this preincubation, heparin was added to trap any free and nonspecifically bound RNAP to prevent rebinding to the promoter after initiation and to avoid transcription from nonspecific sites. After the heparin challenge, the 13-mer was added, the temperature was downshifted to stabilize the triplex, and incubation continued for 10 min. Transcription was initiated by addition of the four ribonucleoside triphosphates and the elongation process was terminated after increasing periods of time. When elongation was terminated after a long incubation (10–40 min depending on temperature), full-length transcripts of 95 nucleotides accumulated, with some intermediate RNA

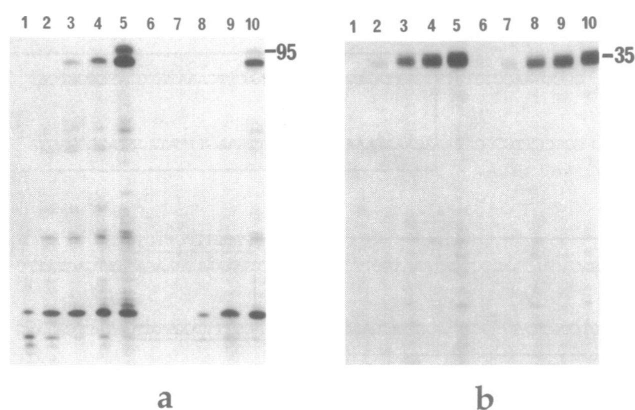

FIG. 3. (*a*) *In vitro* elongation kinetics of *bla*–RNAP complexes without (lanes 1–5) or with (lanes 6–10) triple helix at 25°C. RNAP and the 267-bp fragment bearing the *bla* promoter were preincubated for 45 min at 37°C. The oligonucleotide (100 μM) was added at 25°C for 10 min after heparin challenge. Transcription was assayed by adding the four XTPs and was quenched after increasing periods of time. Times of incubation in the presence of the four ribonucleotides were as follows: lanes 1 and 6, 30 sec; lanes 2 and 7, 1 min; lanes 3 and 8, 2 min; lanes 4 and 9, 4 min; lanes 5 and 10, 10 min. Note that there are several pause sites that yield transcripts of intermediate sizes. A strong pause site exists a few nucleotides before the end of the DNA fragment; it gives a strong band below the full-length transcripts of 95 nucleotides. (*b*) *In vitro* transcription kinetics of *tetR*–RNAP complexes without (lanes 1–5) or with (lanes 6–10) 13-mer as described in *a* at 25°C (final temperature). Times of incubation were as follows: lanes 1 and 6, 30 sec; lanes 2 and 7, 1 min; lanes 3 and 8, 3 min; lanes 4 and 9, 6 min; lanes 5 and 10, 15 min.

species corresponding to natural pause sites, most of them being abortive (19). Transcription assays revealed a clear inhibitory effect of the 13-mer on transcription between 20°C and 30°C (Figs. 3*a* and 4), which was detected by the retarded synthesis of both intermediates (pauses) and full-length transcripts during the elongation process in the presence of the 13-mer. Control experiments with nonspecific oligonucleotides revealed that there was no effect on transcription by RNAP. The synthesized RNAs enabled us to approximately measure the rate of progression of the polymerase along the DNA. Fig. 4 shows the concentration of the full-length transcripts (measured from radioactivity on the gels) in the presence of the 13-mer relative to that in the absence of the

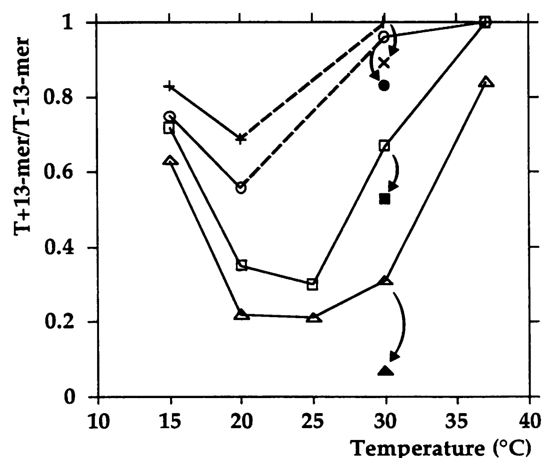

FIG. 4. Quantification of the 95-mer mRNA in the presence (T + 13-mer) and absence (T − 13-mer) of triple helix-forming oligonucleotide. The ratio of transcript amounts was determined at different temperatures after 4 (△), 10 (□), 15 (○), and 20 (+) min of elongation. Arrows show the shift of the ratio obtained with the 13-mer methylated at the C-5 of cytosine (at 30°C only). Note that there are no data points at 25°C for 15 and 20 min of elongation.

13-mer as a function of temperature. This ratio reflects the rate of dissociation of the 13-mer from its target sequence. When the oligonucleotide dissociates, RNAP can move along the DNA fragment and synthesize intermediates and full-length transcripts. The experimental conditions do not allow for reinitiation. Therefore, the concentration of full-length transcripts reaches a plateau value when all RNAP molecules have moved away from the promoter toward the end of the DNA fragment. Even in the presence of the oligonucleotide, the same amount of transcripts are synthesized if one waits long enough for all DNA–oligonucleotide complexes to have dissociated once. Rebinding of the oligonucleotide after RNAP has moved will not interfere any more with transcription. The results presented in Fig. 4 show that, at 20°C and in the presence of the 13-mer, it takes ≈15 min to reach 50% of the transcripts synthesized in the absence of oligonucleotide. When the transcription experiment was performed at 37°C, no difference was detected with or without the oligonucleotide. This was consistent with the fact that at this temperature no triple helix was detected in footprinting experiments. Very little triple helix effect was detected at 15°C (Fig. 4). Indeed, we showed in our previous work (11) that at this temperature the footprint of the RNAP on the *bla* promoter extended to +30, thus covering a major part of the oligonucleotide binding site. A consequence of this is the prevention of triple helix formation, which in turn prevents the inhibition process.

Sequence Specificity of Transcription Inhibition. The same experiments were performed with the 13-mer methylated at the C-5 position of cytosine [13-mer (^{m}C)] in order to stabilize the triple helix (20, 21). Fig. 4 shows that, at 30°C, a more efficient inhibition of RNAP sliding can be achieved with the modified 13-mer as compared to the unmodified one. Several control experiments were performed with a 13-mer that was not able to form a triple helix. No effect was detected on transcription. However, we could not exclude a sequence-dependent action due to the specific 13-mer oligonucleotide binding to the RNAP itself. The experiments performed at 15°C, 30°C, and 37°C with the nonmodified 13-mer indicated that this is probably not the case. Nevertheless, to add another control to the specificity of action of the oligonucleotide, we investigated the effect of the *bla*-specific 13-mer on transcription from the *tetR* promoter (from plasmid pSC101). Fig. 3*b* shows the results of transcription kinetics at 25°C in the absence (lanes 1–5) and in the presence (lanes 6–10) of the 13-mer. No difference was detected and RNAP progression was not perturbed, whereas a strong inhibitory effect was seen when the β-lactamase gene was used (Fig. 3*a*).

Irreversible Inhibition of Transcription by an Oligonucleotide–Psoralen Conjugate. Psoralen-substituted oligonucleotides can be cross-linked to a complementary sequence upon irradiation at wavelengths >310 nm (22–24). The sequence of the homopurine sequence on the β-lactamase gene is preceded by two thymines (Fig. 1). Therefore, irradiation of the triple helical complex was expected to induce a cross-linking of the psoralen-substituted 13-mer (pso-13-mer) to the promoter sequence. Transcription assays performed at 30°C in the presence of the psoralen-modified 13-mer containing 5-methylcytosine [pso-13-mer (^{m}C)] revealed a strong enhancement of inhibition after irradiation as compared to the effects observed with the unsubstituted oligonucleotide or the substituted 16-mer control. To determine the best conditions of irradiation, light exposure was performed for increasing periods of time after preincubation of the components, followed by addition of the four XTPs. Fig. 5*b* presents the results in the presence of psoralen-substituted or unsubstituted 13-mer oligonucleotides and a substituted nonspecific 16-mer. A strong irreversible inhibition of transcription (>50%) was observed during the first 5 min of irradiation in the presence of pso-13-mer (^{m}C). At longer irradiation times, the inhibitory effects leveled off. This probably reflects the light-induced destruction of the psoralen ring as observed by others (23). It should be noted that a nonspecific psoralen-substituted 16-mer had no marked effect on transcription. The slight inhibition observed upon irradiation was similar to that observed upon irradiation in the presence of the non-substituted 13-mer.

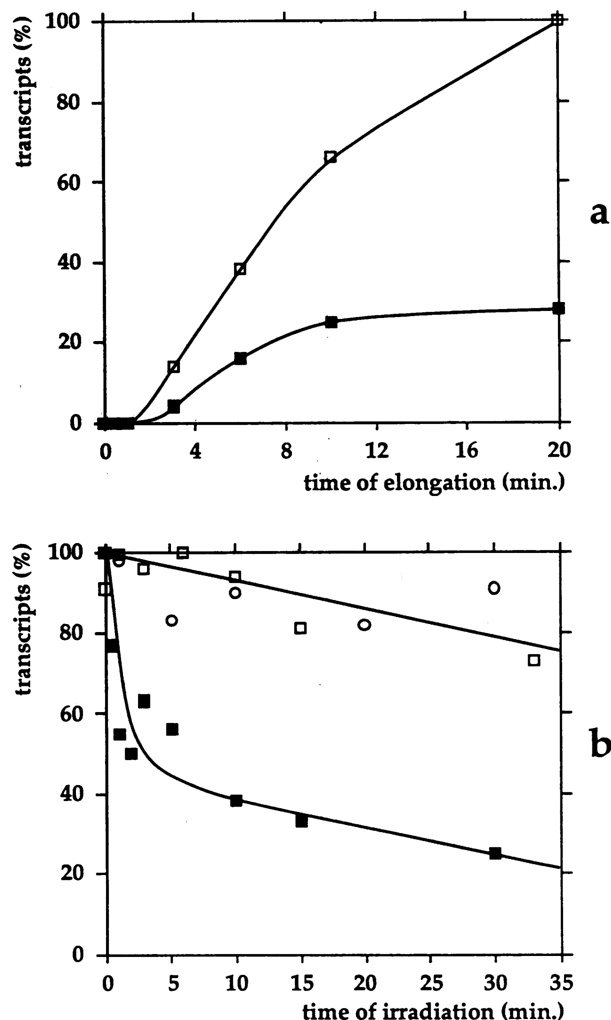

FIG. 5. (*a*) Quantification of the two longer transcripts as a function of elongation time at 30°C after 0 (□) and 30 (■) min of UV-A exposure (wavelength >310 nm) using the pso-13-mer (^{m}C). The RNAP–promoter complex formed at 37°C was incubated with the oligonucleotide for 10 min at 30°C before exposure to light. (*b*) Quantification of the 95-mer mRNA as a function of the time of irradiation at 30°C using the pso-13-mer (^{m}C) (■), the 13-mer (^{m}C) (□), and the nonspecific pso-16-mer (^{m}C) (○). Incubation of the RNAP–promoter complex and oligonucleotide was for 10 min at 30°C before exposure to light followed by 10 min of elongation.

Kinetics of transcription were analyzed after 0 or 30 min of irradiation. Unlike the retardation effect of the unsubstituted 13-mer, the inhibition observed after irradiation in the presence of pso-13-mer reflected irreversible blockage of the RNAP as shown by the plateau obtained after ≈10 min of elongation at 30°C. Photoinduced cross-linking of pso-13-mer induces permanent blockage of RNAP on its promoter.

DISCUSSION

We have shown in this study that a 13-mer oligonucleotide targeted to a sequence located downstream of the *bla* promoter is able to prevent initiation of transcription by *E. coli*

RNAP *in vitro*. This work demonstrates that a triple helix-forming oligonucleotide can act as a specific transcriptional repressor when bound downstream of RNAP. It was earlier demonstrated that triple-stranded polynucleotides formed by poly(dA)·poly(dT) with poly(rU) and by poly[d(AG)]·poly-[d(CT)] with poly[r(CU)] were not substrates for RNAP (25). A correlation between oligonucleotide-directed triple helix formation and repression of c-*myc* gene transcription was reported by Cooney *et al.* (26). The oligonucleotide was targeted to a transcription factor binding site located upstream of the RNAP, thereby decreasing transcription activation.

In vitro transcription experiments on the DNA fragment carrying the *bla* promoter show that the consequence of the presence of a triple helix immediately downstream of the RNAP–promoter complex is to block the holoenzyme at its start site. The 5′ end of the oligonucleotide, located at position +21, is contiguous to the RNAP footprint. The absence of accumulation of short (<20) transcripts seems to indicate that the triple helix acts at the boundary of the RNAP–DNA complex and prevents any movement of RNAP along the DNA. We observed that after a long incubation time (dependent on temperature), the final amount of the run-off transcripts was approximately the same in the presence and in the absence of triple helix. This demonstrates that RNAP is transiently blocked at the promoter but not dissociated by the oligonucleotide. For this reason, it was necessary to perform kinetic transcription experiments to detect the retardation of the RNAP start caused by the triple helix. The enzyme remains blocked on its promoter in the presence of the four ribonucleoside triphosphates and of the triple helix during a period that is dependent on temperature. This fact is related to stabilization of the oligonucleotide bound to the DNA target when the temperature is decreased. It is difficult to determine whether initiation of transcription occurs as a result of passive dissociation of the oligonucleotide or whether dissociation is activated by the RNAP. Long residence times of oligonucleotides on DNA have been described in triplex structures (27, 28). This is strongly dependent on temperature but also on pH because triplex formation requires protonation of cytosines in the triple helix-forming oligonucleotide. All experiments described in the present work have been carried out at pH 7.8, which is not favorable to triplex formation. In these conditions, the highest temperature at which it was possible to detect the triple helix was 30°C. Above this value, the 13-mer was not sufficiently stable and the equilibrium was displaced toward dissociation. Decreasing the temperature of transcription stabilized the 13-mer triplex and enhanced the inhibitory effect. The experiments at different temperatures show a correlation between the relative stabilities of complexed molecules and the retarded initiation of transcription. The stabilization of the oligonucleotide on its DNA target increases its blocking effect on RNAP. The experiments performed at 37°C (a temperature at which no triplex was detected) and those using another promoter at 25°C (*tetR* of the plasmid pSC101) as a control demonstrate the specificity of oligonucleotide action. The inhibition observed on the *bla* promoter with a 13-mer was more efficient when the oligonucleotide was modified by methylation of cytosines in agreement with the enhanced stability of triplexes (20, 21).

Having demonstrated the existence of a clear inhibitory effect of triple helix formation on transcription initiation, our objective was to make this specific inactivation irreversible. Toward this goal, we used a psoralen-modified 13-mer able to react covalently with the DNA target after a short exposure to light of wavelengths >310 nm. Upon UV irradiation, an intercalated psoralen is able to react with adjacent pyrimidine bases, mostly with thymines, to form monoadducts and bis adducts (at 5′-TpA-3′ sequences). Specific inhibition of transcription occurs on the *bla* gene after illumination in the presence of the triple helix-forming pso-13-mer. Preliminary experiments showed that pso-13-mer bound to double-stranded DNA in a triple-helical structure was able to cross-link to one of the two thymines that precede the homopurine sequence on the 5′ side of the sense strand. Initiation complexes are irreversibly arrested due to covalent linking of the psoralen–oligonucleotide conjugate. The inhibition of *in vitro* transcription under physiological conditions demonstrates that it is possible to design synthetic repressors. The recent development of nuclease-resistant α oligodeoxynucleotides, which are capable of forming triple helices (5, 7, 29), opens the possibility of using psoralen-derivatized α oligodeoxynucleotides *in vivo* to modulate gene expression at the level of transcription.

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