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PSF/p54^{nrb} Stimulates "Jumping" of DNA Topoisomerase I between Separate DNA Helices†

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ABSTRACT: We have previously shown [Straub et al. (1998) *J. Biol. Chem. 273*, 26261] that the pyrimidine tract binding protein associated splicing factor PSF/p54^{nrb} binds and stimulates DNA topoisomerase I. Here we show that cleavage and religation half-reactions of topoisomerase I are unaffected by PSF/p54^{nrb}, whereas the propensity of the enzyme to jump between separate DNA helices is stimulated. To demonstrate such an effect, topoisomerase I was first captured in suicidal cleavage of an oligonucleotide substrate. Subsequently, a cleavage/ligation equilibrium was established by adding a ligation donor under conditions allowing recleavage of the ligated substrate. Finally, a second oligonucleotide was added to the mixture, which also allowed suicidal cleavage by topoisomerase I, but did not accommodate the ligation donor of the first oligonucleotide. Thus, topoisomerase I was given the choice to engage in repeated cleavage/ligation cycles of the first oligonucleotide or to jump to the second suicide substrate and get trapped. PSF/p54^{nrb} enhanced the cleavage rate of the second oligonucleotide (11-fold), suggesting that it stimulates the dissociation of topoisomerase I after ligation. Thus, stimulation of topoisomerase I catalysis by PSF/p54^{nrb} seems to be affected by mobilization of the enzyme.

Recently, we reported (*1*) a direct interaction of topoisomerase I with PSF[1] and its smaller homologue p54^{nrb}. Interactions between these proteins, which copurified in a 1:1:1 ratio from nuclear extracts, could be demonstrated by several biochemical procedures. A physiological role of the interaction seemed feasible, since topoisomerase I and PSF showed a patched colocalization in cell nuclei, which varied with cell cycle. We also demonstrated a functional effect: the DNA relaxation activity of topoisomerase I was 16-fold higher when the enzyme was complexed with PSF/p54^{nrb}. The DNA relaxation activity of pure recombinant topoisomerase I could be stimulated up to 12-fold by addition of recombinant PSF or PSF/p54^{nrb}. Since the RNA splicing factors did not have an endogenous DNA relaxation activity, we concluded that topoisomerase I gets stimulated by the interaction with the PSF/p54^{nrb} heterodimer.

However, the biological implications of the interaction could not be judged from our previous data, because the DNA relaxation activity summarizes several distinct steps comprising the catalytic cycle of topoisomerase I. These include association with the DNA duplex, cleavage of one strand, passage of the other strand, religation of the cleaved strand, and dissociation from the resealed DNS duplex. The stimulatory effect of PSF/p54^{nrb} could be exerted at several positions of the cycle. In each case, the DNA relaxation activity would be increased, whereas the biological properties of the enzyme might be affected quite differently, depending on which step is modulated: Increases in cleavage and/or ligation rates would affect the cellular levels of covalently DNA-linked catalytic intermediates, thus altering the potential of the enzyme for disturbing the integrity of the genome (*2*). Changes in DNA avidity, on the other hand, would affect the way in which topoisomerase I accesses DNA sites for processing. To gain more insight into the precise mechanism and the putative physiological consequences of the stimulatory interaction, we have here carried out a detailed investigation of the influence of PSF/p54^{nrb} on defined segments of the catalytic cycle of topoisomerase I.

EXPERIMENTAL PROCEDURES

Proteins. Recombinant human topoisomerase I was expressed in *S. cerevisiae* and purified as decribed in (*3*, *4*). A typical example is shown in Figure 1a, lane 3. The PSF/p54^{nrb} heterodimer was copurified with DNA topoisomerase I from human A431 epidermoid cells (ATCC 1555) as described previously (*1*). Briefly, nuclear extracts were adsorbed to Ni-NTA-agarose (Qiagen, Hilden, Germany), eluted with 200 mM imidazole, and passed through a Source-15Q column (Pharmacia, Uppsala, Sweden). A typical preparation of the trimeric protein complex is shown in

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[1] Abbreviations: BisTrisPropane, 1,3-bis[tris(hydroxymethyl)methylamino]propane; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NTA, nitrilotriacetic acid; PBS, phosphate-buffered saline solution; PMSF, phenylmethylsulfonyl fluoride; PSF, pyrimidine tract binding protein associated splicing factor; p54^{nrb}, nuclear RNA-binding protein of 54 kDa; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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Figure 1a, lane 1. Subsequently, the PSF/p54^{nrb} heterodimer was precipitated with 1 M ammonium sulfate, whereas topoisomerase I remained soluble. Precipitated PSF/p54^{nrb} was renatured and further purified by gel permeation chromatography (Superdex 200 HR30/10, Pharmacia, Uppsala, Sweden). Peak fractions were dialyzed against 15 mM HEPES, pH 7.5, 300 mM NaCl, 1 mM PMSF, 10% glycerol, and stored at −80 °C. Figure 1a, lane 2 shows a representative example of the purified PSF/p54^{nrb} heterodimer. The trimeric complex was reconstituted by incubating a 10-fold molar excess of isolated PSF/p54^{nrb} with pure recombinant topoisomerase I at 37 °C for 15 min in assay buffer (10 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.3 μg/μL BSA, and 0.5 mM DTT). The overall DNA relaxation activity of various preparations was characterized by relaxation of supercoiled pUC 18 plasmid DNA, as described in (*5*). Figure 1b shows representative examples of pUC 18 relaxation time courses obtained with 5 ng of topoisomerase I (top), 5 ng of topoisomerase I preincubated with a 10-fold molar excess of PSF/p54^{nrb} (middle), or an equivalent amount of the PSF/p54^{nrb} dimer alone (bottom). Quantitative data derived from multiple determinations of relaxation kinetics are summarized in Figure 1c.

Synthetic DNA Substrates. Oligonucleotides were supplied by Carl Roth (Karlsruhe, Germany). Scissile DNA strands were 5′-radiolabeled with ^{32}P, whereas all other strands were cold-phosphorylated using T4 polynucleotide kinase (Amersham, Little Chelford, U.K.). Scissile and noncleaved strands were mixed at a ratio of 1:1.5. Duplex-forming strands of the substrates shown in Figure 2 b+c were added in 2-fold excess. The mixtures were heated to 92 °C and then slowly cooled to 20 °C. Hybridized substrates were purified by nondenaturing gel electrophoresis.

Kinetic analysis of cleavage and religation half-reactions followed published procedures (*4*−*6*). Cleavage reactions were carried out in assay buffer at 15 °C. They were started by adding 1 pmol of topoisomerase I to 10 fmol of oligonucleotide substrate and were stopped after various incubation periods by adding 500 mM NaCl and 75% ethanol. Alternatively, cleavage reactions were followed by ligation reactions. In this case, 50 pmol of AG dinucleotide (as ligation donor) and 300 mM NaCl (to prevent recleavage of the ligated product) were added and incubations continued at 20 °C for various periods of time before stopping with 75% ethanol. Ethanol precipitates were sedimented, dried, and digested with 1 mg/mL trypsin in 10 mM Tris-HCl, 0.1 mM EDTA at 37 °C for 30 min. Samples were finally mixed with 1 volume of loading buffer (80% formamide, 50 mM Tris−borate, pH 8.3, 1 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene xyanol) and subjected to denaturing polyacrylamide gel electrophoresis. Reaction products were visualized by autoradiography.

Analysis of Enzyme "Jumping" between Separate DNA Helices. Two picomoles of unlabeled *substrate 1* (Figure 2b) was incubated with 0.5 pmol of topoisomerase I (optionally preincubated with PSF/p54^{nrb}) in assay buffer at 25 °C for 30 min. Under these conditions, all of the enzyme became trapped in suicidal cleavage, as demonstrated by the complete absence of residual DNA relaxation activity after the incubation. Subsequently, 10 fmol of labeled *substrate 2* (Figure 2c) was added, and incubation was continued for another 15 min. Topoisomerase I was then released from suicidal cleavage of *substrate 1* by addition of 50 pmol of AG. Reactions continued at 37 °C for various time periods and were finally stopped by ethanol precipitation. Precipitates were trypsinized and subjected to denaturing polyacrylamide gel electrophoresis. Reaction products were visualized by autoradiography.

Retrieval of Quantitative Data and Statistics. For the data shown in Figure 3b+d and Figure 4c−e, X-ray films were scanned with a transilluminating flatbed scanner linked to a Macintosh G3 PowerPC. Densitograms were analyzed using the software NIH Image. For the data shown in Figure 5c, radioactive bands were cut out of dried gels, equilibrated for 12 h with scintillation fluid, and measured in a scintillation counter. Percentages of cleavage or ligation were determined by comparing within one lane the intensities of radioactive bands corresponding to cleaved and uncleaved or cleaved and religated reaction products, respectively. Data of 3 (Figure 5c) or 5 (Figure 3b+d; Figure 4c−e) individual experiments with similar outcome were pooled for statistical analysis. Apparent rate constants derived from the data by nonlinear regression analysis as previously described (*6*) are stated as mean values ± standard error. The significance of differences was tested by Wilcoxon's signed rank test for unpaired samples.

RESULTS

PSF/p54^{nrb} Stimulates the DNA-Relaxation Activity of Topoisomerase I. We purified DNA topoisomerase I from nuclear extracts of human A431 cells in a form complexed with the RNA splicing factors PSF (115 kDa) and p54^{nrb} (54 kDa). A typical example of the trimeric 1:1:1 complex is shown in Figure 1a, lane 1. The PSF/p54^{nrb} heterodimer could be separated from topoisomerase I by precipitation with 1 M ammonium sulfate (Figure 1a, lane 2). Figure 1b shows representative examples of DNA relaxation kinetics obtained with topoisomerase I alone (top) or after preincubation with PSF/p54^{nrb} (middle). The bottom panel of Figure 1b attests to the fact that equivalent amounts of PSF/p54^{nrb} alone did not relax the plasmid under similar conditions. Figure 1c summarizes quantitative data derived form five similar experiments carried out with independent protein preparations. The specific DNA relaxation activity of the trimeric complex was about 16 times higher than that of pure recombinant human topoisomerase I (Figure 1c, compare columns 1 and 3). The activity of the recombinant enzyme could be increased to a similar extent (12-fold) by preincubation with isolated PSF/p54^{nrb} (Figure 1c, compare columns 3 and "2+3"). None of the PSF/p54^{nrb} preparations used in this study had an endogenous DNA relaxation activity after ammonium sulfate precipitation (Figure 1c, column 2). Therefore, ruling out additive effects, the 12-fold increase in DNA relaxation activity seen upon preincubation of topoisomerase I with PSF/p54^{nrb} seems to be due to activation of the enzyme by the RNA splicing factor.

PSF/p54^{nrb} Does Not Alter the Cleavage and Religation Rates of Topoisomerase I. From our previous work (*1*) and the data shown in Figure 1b+c, we could not conclude which step of the catalytic cycle of topoisomerase I was actually modulated by PSF/p54^{nrb}. To address this question, we dissected the catalytic cycle of topoisomerase I into cleavage and religation half-reactions (*7*), using a partially double-

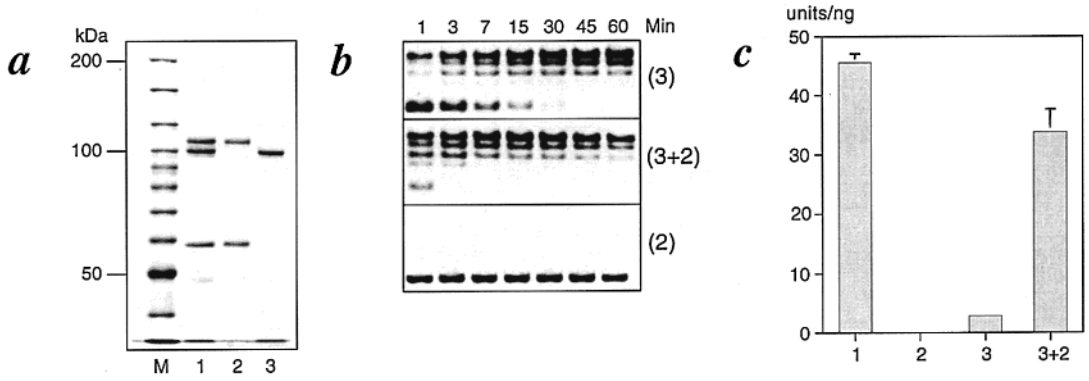

FIGURE 1: (a) Representative examples of the protein preparations used: The SDS−polyacrylamide gel stained with Coomassie blue shows marker proteins (M), the heterotrimeric complex of topoisomerase I, PSF, and p54^{nrb} copurified from A431 cells (lane 1), the PSF/p54^{nrb} heterodimer isolated from the trimeric complex by ammonium sulfate precipitation (lane 2), and recombinant human topoisomerase I produced in yeast (lane 3). (b) Representative time courses of DNA relaxation: 300 ng of completely supercoiled pUC18 plasmid DNA was incubated with 5 ng of pure topoisomerase I (top) or 5 ng of enzyme preincubated with a 10-fold molar excess of PSF/p54^{nrb} (middle), or an equivalent amount of PSF/p54^{nrb} alone (bottom). Panel numbers on the right margin correspond to lane numbers in (a). (c) Quantitative analysis of DNA relaxation activity determined, as shown in (b). One enzymatic unit was defined as the amount of enzyme completely relaxing 300 ng of supercoiled pUC18 plasmid DNA at 37 °C in 30 min. Column numbers correspond to lane numbers in (a). Column "3+2" shows the activity of pure recombinant topoisomerase I reconstituted with a 10-fold molar excess of PSF/p54^{nrb} heterodimer. Enzymatic units in columns 1, 3, and "3+2" are related to the protein amount of topoisomerase I present in these fractions. In lane 2, the amounts of isolated PSF/p54^{nrb} heterodimer tested were equivalent to those in column 1. Columns represent mean values determined with 5 independent sets of protein preparations. Error bars indicate standard errors of the mean. For pure recombiant topoisomerase I (lane 3), the error was less than 5% and is not indicated. For the isolated PSF/p54^{nrb} heterodimer (lane 2), a statistic analysis was not meaningful, because endogenous DNA relaxation activity was not detectable in any of the preparations tested.

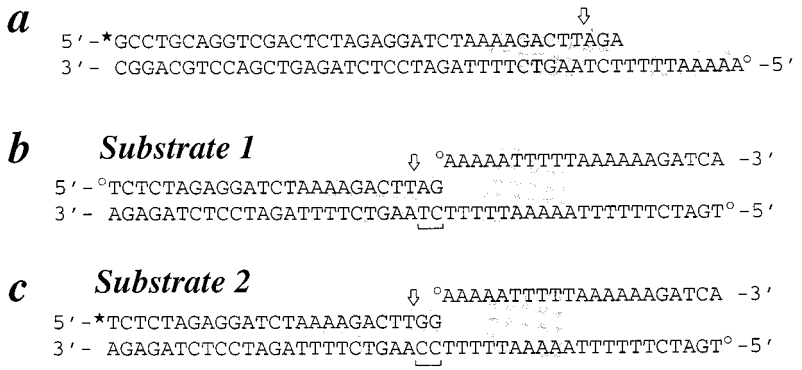

FIGURE 2: Oligonucleotide substrates. (a) Partially double-stranded oligonucleotide used for kinetic analysis of topoisomerase I cleavage and ligation half-reactions (see Figure 3). (b and c) Fully double-stranded oligonucleotides used for measuring the interhelix mobility of topoisomerase I. (b) was the unlabeled substrate used for capturing and releasing topoisomerase I. It corresponds to the open bars in Figure 4a and is referred to in the text as *substrate 1*. (c) was the labeled substrate used to trap topoisomerase I in suicidal cleavage after dissociation from *substrate 1*. It corresponds to the shaded bars in Figure 4a and is referred to in the text as *substrate 2*. The positions of radiolabel are indicated by asterisks (*); sites of cold phosphorylation are marked by open superscript circles (^{o}). Cleavage sites are indicated by downward open arrows.

stranded oligonucleotide (Figure 2a) as substrate. As previously described (*8*), such substrates are preferentially cleaved by topoisomerase I at the position indicated (Figure 2a, arrow), and the AGA trinucleotide that is cleaved off diffuses away from the reaction site. Thus, the catalytic cycle cannot progress beyond the stage of the covalent DNA intermediate of topoisomerase I. It should be noted that with this type of substrate cleavage efficiency is usually less than 60%, because the substrate dimerizes via the palindromic sequence of the protruding end of the bottom strand (*8*). However, the annealing temperature of that sequence is not higher than 20 °C. Therefore, the DNA duplex downstream of the cleavage site known to be crucial for the cleavage reaction (*9*) is comparatively unstable. This causes the cleavage reactions to be comparatively slow, which is favorable for a detailed kinetic analysis. Upon comparing representative autoradiograms of cleavage time courses, it became obvious that cleavage by topoisomerase I reconstituted with PSF/p54^{nrb} was not notably faster than cleavage by an equivalent amount of the pure enzyme (Figure 3a, compare lanes 2−8 with lanes 9−15). This notion was further substantiated by the quantitative analysis shown in Figure 3b, which summarizes the experiment shown in Figure 3a and four more similar ones. Kinetics of suicidal cleavage were pseudo-first-order, which is in good agreement with previous observations (*6*). Apparent time constants of initial cleavage rates derived from the data by nonlinear regression were 0.35 ± 0.08 min^{-1} for topoisomerase I alone and 0.43 ± 0.07 min^{-1} for topoisomerase I complexed with PSF/p54^{nrb}. These values did not differ significantly from each other.

Next, we investigated the kinetics of the ligation half-reaction. In this case, the partially double-stranded substrate shown in Figure 2a was precleaved with topoisomerase I (alone or preincubated with PSF/p54^{nrb}). Ligation was subsequently started by adding an excess of AG dinucleotide (as ligation donor) together with 300 mM NaCl, which

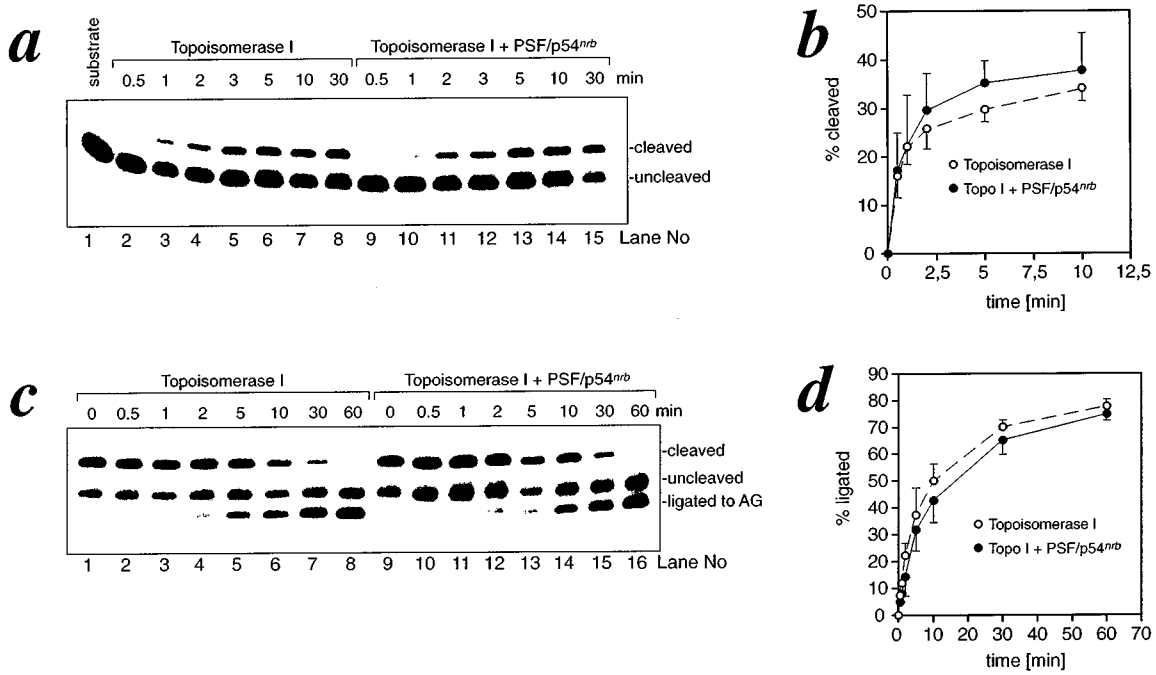

FIGURE 3: (a) Cleavage kinetics, representative examples: 10 fmol of the oligonucleotide substrate shown in Figure 2a (lane 1) and 1 pmol of topoisomerase I alone (lanes 2−8) or enzyme preincubated with a 10-fold molar excess of PSF/p54^{nrb} (lanes 9−15) were incubated at 15 °C. Reactions were stopped after the incubation periods indicated at the top. Reaction products were separated by denaturing gel electrophoresis and visualized by autoradiography. Cleaved bands are shifted upward, because a trypsin-resistant peptide of topoisomerase I remains attached. (b) Quantitative analysis of cleavage kinetics was done by densitometric scanning of the autoradiograms shown in (a) and four more similar sets. Mean values obtained with topoisomerase I alone (○) or preincubated with PSF/p54^{nrb} (●) and corresponding standard errors of the mean (bars) are shown. Initial rate constants derived by nonlinear regression were 0.35 ± 0.06 min^{-1} for topoisomerase I alone and 0.43 ± 0.07 min^{-1} for topoisomerase I complexed with PSF/p54^{nrb}. Rate constants were not significantly different from each other. (c) Ligation kinetics, representative example: 10 fmol of oligonucleotide (Figure 2a) was reacted with 1 pmol of topoisomerase I alone (lanes 1−8) or preincubated with PSF/p54^{nrb} (lanes 9−16). After 1 h, cleavage reactions were stopped with 300 mM NaCl, and ligation was started by adding 50 pmol of AG. Ligation reactions were carried out at 25 °C for the time periods indicated at the top. Samples were then subjected to electrophoresis and autoradiography. (d) Quantitative analysis of ligation kinetics was done by densitometric scanning of the autoradiograms shown in (c) and four more similar sets. Mean values obtained with 5 independent preparations of topoisomerase I alone (○) or preincubated with PSF/p54^{nrb} (●) and corresponding standard errors of the mean (bars) are shown. Initial rate constants derived by nonlinear regression were 0.066 ± 0.011 min^{-1} for topoisomerase I alone and 0.050 ± 0.006 min^{-1} for topoisomerase I complexed with PSF/P54^{nrb}. These rate constants differed insignificantly from each other.

precludes recleavage of the ligated product (*7*). As shown in Figure 3c, ligation time courses were very similar with and without PSF/p54^{nrb} (Figure 3c, compare lanes 1−8 with lanes 9−16). A quantitative analysis of five such experiments is summarized in Figure 3d. Ligation kinetics were pseudo-first-order. Apparent initial rate constants derived by nonlinear regression of the data were 0.066 ± 0.011 min^{-1} for topoisomerase I alone and 0.050 ± 0.006 min^{-1} for topoisomerase I complexed with PSF/p54^{nrb}. These values did not differ significantly from each other. Taken together, the experiments shown in Figure 3 suggest that the stimulatory effect of PSF/p54^{nrb} on topoisomerase I catalysis (demonstrated in Figure 1b+c) is not exerted on the rates of DNA cleavage or DNA ligation. In light of these data, DNA association could also be ruled out as a step possibly modulated by PSF/p54^{nrb}, because it is a determinant of the cleavage reaction and changes would consequently become apparent in the cleavage kinetics (Figure 3a,b). Thus, DNA dissociation seemed to be the only step of the catalytic cycle that could possibly be influenced by PSF/p54^{nrb}. Since we were unable to think of a way to measure DNA dissociation directly, we approached the problem in an indirect manner, studying the propensity of topoisomerase I to "jump" between separate DNA helices.

PSF/p54^{nrb} Stimulates "Jumping" of Topoisomerase I between Separate DNA Helices. (A) Rationale. Our approach is schematized in Figure 4a. First, we trapped topoisomerase I in suicidal cleavage of an oligonucleotide substrate (termed *substrate 1*). Then we established a cleavage/ligation equilibrium by adding an excess of AG dinucleotide as appropriate donor for the ligation of *substrate 1*. In contrast to the experiments shown in Figure 3c, the immediate recleavage of the ligated product was in this case not prevented by increasing the salt concentration upon addition of the ligation donor. Finally, we added a second oligonucleotide (termed *substrate 2*), which could also be cleaved by topoisomerase I in a suicidal manner, but did not accommodate the ligation donor of *substrate 1* (i.e., the AG dinucleotide). In this situation, topoisomerase I molecules dissociating from *substrate 1* after ligation would have a chance to be trapped in suicidal cleavage of *substrate 2*, whereas those engaging in multiple cleavage/ligation cycles of *substrate 1* would not. Thus, the cleavage rate of *substrate 2* (monitored by a 5′-label on the scissile strand) could be used as an indication of topoisomerase I "jumping" between separate DNA helices.

(B) Setup and Characterization. To allow at the same time suicidal cleavage and multiple cycles of cleavage/ligation on two separate pieces of double-stranded DNA, we designed

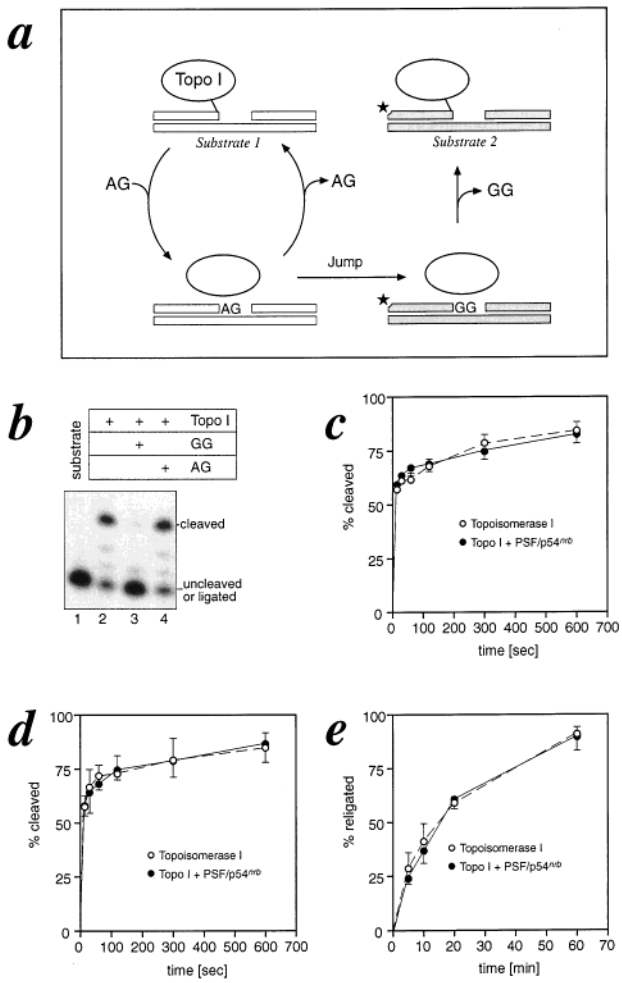

FIGURE 4: Experimental setup and control experiments for the determination of enzyme mobility between separate DNA helices. (a) Setup: Topoisomerase I (open ellipsoid) is covalently trapped in suicidal cleavage on *substrate 1* (open bars, corresponding to Figure 2b). Subsequently a cleavage/ligation equilibrium is initiated by adding AG as appropriate ligation donor. At the same time, *substrate 2* (shaded bars, corresponding to Figure 2c) is added, which also allows suicidal cleavage by topoisomerase I, but does not accommodate AG as ligation donor. Thus, *substrate 2* gets irreversibly cleaved by topoisomerase I dissociating from *substrate 1* after religation. Cleavage of *substrate 2* is monitored by the radioactive 5′-label of the scissile strand (*). (b) Challenge of cleaved *substrate 2* with an excess of AG or GG. 10 fmol of *substrate 2* was 5′-labeled on the scissile strand (lane 1) and reacted with 1 pmol of topoisomerase I (lanes 3−4). After 1 h, the cleavage reaction was stopped with 300 mM NaCl, and 50 pmol of AG (lane 4) or GG (lane 3) was added. Reactions continued for another 45 min, and samples were finally subjected to electrophoresis and autoradiography. (c−e) Quantitative analyses of cleavage and ligation kinetics of *substrate 1* and *substrate 2* were carried out as described in Figure 3. Mean values obtained with three independent preparations of topoisomerase I alone (○) or preincubated with PSF/p54^{nrb} (●) and corresponding standard errors of the mean (bars) are shown. (c) Cleavage kinetics of *substrate 1*; (d) cleavage kinetics of *substrate 2*; (e) ligation kinetics of *substrate 1* using an excess of AG as ligation donor. Initial rate constants derived by nonlinear regression of the curves in (e) were 0.060 ± 0.008 min^{-1} for topoisomerase I alone and 0.049 ± 0.0006 min^{-1} for topoisomerase I complexed with PSF/P54^{nrb}. These values differed insignificantly from each other.

two fully double-stranded oligonucleotides, which are almost identical (Figure 2b,c). Both are cleaved preferentially at the position indicated by arrows. Due to a nick between positions −2 and −3 relative to the cleavage position, the cleavage reaction releases a dinucleotide, while subsequent ligation to the adjacent 5′-end is precluded by cold phosphorylation. The two substrates differ at the −1 position relative to the cleavage site, which is an A/T in *substrate 1* and a G/C in *substrate 2* (Figure 2 b+c, brackets). Therefore, suicidal cleavage by topoisomerase I releases AG from *substrate 1*, but GG from *substrate 2*. Consequently, the gap of two bases created by suicidal cleavage of *substrate 1* will accommodate AG as ligation donor, whereas *substrate 2* would require GG. The control experiment in Figure 4b confirms that an excess of AG did not induce religation of *substrate 2* (whereas an excess of GG did). Thus, suicidal cleavage of *substrate 2* could occur in the presence of a cleavage/ligation equilibrium of *substrate 1* established by addition of AG. To use the system for comparing properties of topoisomerase I with and without PSF/p54^{nrb}, we had to ascertain that cleavage rates of *substrate 1* and *substrate 2* were the same for both enzyme conditions. It should be noted that these oligonucleotides were cleaved much more rapidly by topoisomerase I than the one used for the experiments in Figure 3, because they provide a longer and more stable DNA duplex downstream of the cleavage site. Therefore, we could not determine cleavage rates precisely. However, it can be clearly seen that the cleavage time courses of *substrate 1* (Figure 4c) and *substrate 2* (Figure 4d) were virtually the same with (●) or without (○) PSF/p54^{nrb}. Moreover, the cleavage time courses of *substrate 1* and *substrate 2* were only marginally different from each other (Figure 4, compare panel c to panel d), indicating that topoisomerase I had a similar cleavage avididity for both of them. Similar data, as shown in Figure 4d, were also obtained when the cleavage kinetics of *substrate 2* were determined in the presence of an excess of AG (not shown), confirming that cleavage of *substrate 2* was not reversed by religation when AG was present (which is also corroborated by the data shown in Figure 4b). Finally, we ascertained that the religation kinetics of *substrate 1* were the same with (●) and without (○) PSF/p54^{nrb} (Figure 4e). The initial rate constants derived from ligation time courses were 0.06 ± 0.008 min^{-1} for topoisomerase I alone and 0.049 ± 0.006 min^{-1} for topoisomerase I complexed with PSF/p54^{nrb} and did not differ significantly from each other, or from the values obtained with the partially single-stranded substrate (Figure 3d). Thus, differences in the religation rate of *substrate 1* could be ruled out as a putative cause of differences in the cleavage rate of *substrate 2*. On the basis of all the data shown in Figure 4, we concluded that the experimental setup was suitable for determining the mobility of topoisomerase I between two separate DNA helices.

(C) Data. The actual experiments addressing the effect of PSF/p54^{nrb} on the interhelix mobility of topoisomerase I are summarized in Figure 5. Figure 5a shows a representative result: An equal amount of topoisomerase I alone (lanes 2−8) or preincubated with PSF/p54^{nrb} (lanes 9−15) was trapped on *substrate 1* and then incubated with *substrate 2*. Figure 5a, lane 1 shows *substrate 1* alone (5′-labeled on the scissile strand). Lanes 2 and 9 show that in the absence of AG *substrate 2* was not cleaved within the time frame of the experiment. Thus, trapping of topoisomerase I on *substrate 1* appeared to be complete under both enzyme conditions (i.e., free topoisomerase I was absent at the onset of the second reaction step). Upon addition of AG, *substrate*

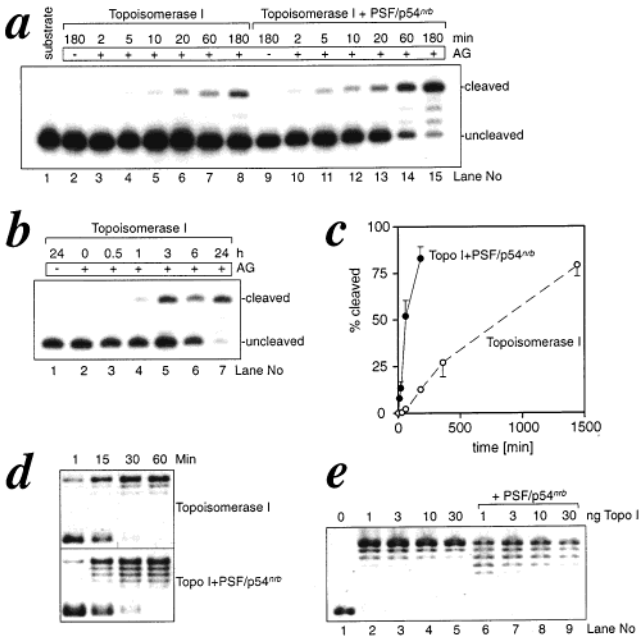

FIGURE 5: Jumping of topoisomerase I between separate substrate molecules. (a) 2 pmol of unlabeled *substrate 1* (Figure 2b) was reacted with 0.5 pmol of topoisomerase I for 1 h. The enzyme was optionally preincubated with PSF/p54^{nrb}. Subsequently, 50 pmol of AG was added together with 30 fmol of labeled *substrate 2* (Figure 2c), and incubations were continued for various time periods. Finally, the samples were subjected to gel electrophoresis and autoradiography. Control reactions (lanes 2 and 9) did not contain AG. Lane 1 shows *substrate 2* alone. (b) Extended reaction periods carried out with topoisomerase I alone. Lane 1 shows the control without addition of AG. (c) Quantitative analysis of the data by liquid scintillation counting of the radioactive bands. Percentages of cleavage were calculated from the relative amounts of cleaved and uncleaved reaction products within each lane. Mean values of cleavage obtained with topoisomerase I alone (○) or preincubated with PSF/p54^{nrb} (●) and corresponding standard errors (bars) summarize the results of three similar experiments. Initial rate constants of cleavage derived from the data by nonlinear regression were 0.0011 ± 0.0001 min^{-1} for topoisomerase I alone and 0.012 ± 0.002 min^{-1} for topoisomerase I complexed with PSF/P54^{nrb}. The 11-fold difference was significant on the $p < 0.0001$ level. (d) Comparison of relaxation time courses between equivalent activities of topoisomerase I alone and bound to PSF/p54^{nrb}: 300 ng of pUC18 plasmid DNA was incubated with 1 unit of topoisomerase I alone (top), or 1 unit of topoisomerase I was preincubated with PSF/p54^{nrb} (bottom). Determination of activity units and all other assay conditions were the same as in Figure 1. (e) Relaxation endpoints obtained at different enzyme-to-plasmid ratios: Various amounts of topoisomerase I protein were incubated with 300 ng of pUC18 plasmid DNA for 60 min. All other assay conditions were the same as in Figure 1. Lane 1 shows plasmid alone. Lanes 2−5 show results obtained with various amounts of topoisomerase I alone. Lanes 6−9 show corresponding results obtained with PSF/p54^{nrb}-complexed enzyme.

2 was cleaved in a time-dependent manner. However, cleavage was more rapid in the presence of PSF/p54^{nrb} (lanes 10−15) than with topoisomerase I alone (lane 3−8). In the presence of PSF/p54^{nrb}, cleavage of *substrate 2* was almost complete after 180 min, whereas with topoisomerase I alone a similar extent of cleavage took up to 24 h (Figure 5b). It should be noted that due to an unquenchable phosphatase activity present in our PSF/p54^{nrb} preparations, some of the 5′-label on the scissile strand of *substrate 2* was lost during longer periods of incubation. However, we could ascertain that 5′-dephosphorylation was the same for cleaved and uncleaved DNA strands (not shown) and thus did not affect the relative proportions of the reaction products used for quantitative analysis. Figure 5c summarizes in quantitative terms the experimental data of three similar experiments as shown in Figure 5a+b. In the presence of PSF/p54^{nrb}, cleavage of *substrate 2* started within 2 min after addition of AG and progressed in a steep (initially linear) fashion, whereas with topoisomerase I alone cleavage had a much lower initial rate and was preceded by a lag period of 5−10 min. Initial rate constants derived from the data in Figure 5c by nonlinear regression were 0.0011 ± 0.0001 min^{-1} for topoisomerase I alone and 0.012 ± 0.001 min^{-1} for the topoisomerase I−PSF/p54^{nrb} complex. The 11-fold difference was significant at the $p < 0.0001$ level.

PSF/p54^{nrb} Makes Topoisomerase I More Processive at Low Enzyme Concentrations. The experimental results in Figure 5a−c suggest that PSF/p54^{nrb} stimulates the dissociation of topoisomerase I from the DNA substrate after religation has been completed. A prediction of these results is that PSF/p54^{nrb} should make topoisomerase I more distributive and should give rise to a wider range of DNA topoisomers in plasmid relaxation assays. This is actually the case in the early time points (1 and 3 min) of the relaxation time courses shown in Figure 1b, whereas it is less apparent later on. Since these assays were normalized to topoisomerase I protein, we suspected that the distributive relaxation mode of the PSF/p54^{nrb}-bound enzyme might be masked by its 12-fold higher specific activity. Therefore, we repeated these experiments—this time normalizing to topoisomerase I activities. Figure 5d shows a comparison of the plasmid relaxation kinetics obtained with 1 unit of topoisomerase I alone (top) and 1 unit of topoisomerase I complexed with PSF/p54^{nrb}. Under these conditions, it became clear that PSF/p54^{nrb}-complexed topoisomerase I had a more distributive relaxation mode than topoisomerase I alone. At the reaction equilibrium (after 30 min), the PSF/p54^{nrb} -bound enzyme created a ladder of at least four partially relaxed topoisomers in addition to the completely relaxed plasmid form. In contrast, the pure enzyme produced completely relaxed plasmid as the major reaction product and only traces of not more than two more partially relaxed topoisomers. To corroborate our notion that the more distributive relaxation mode of PSF/p54^{nrb}-complexed topoisomerase I might actually be masked at high enzyme-to-plasmid ratios, we compared relaxation endpoints obtained with various amounts of topoisomerase I ± PSF/p54^{nrb}. A typical result is shown in Figure 5e. It demonstrates that at low enzyme concentrations topoisomerase I was clearly more distributive, when bound to PSF/p54^{nrb} (compare lanes 2 and 3 with lanes 6 and 7), whereas such an effect became less apparent with increasing enzyme concentrations (compare lane 5 to lane 9). These results are in good agreement with the other data presented in this paper showing that PSF/p54^{nrb} stimulates DNA dissociation of topoisomerase I after ligation, but does not influence DNA association before cleavage. Thus, it favors distributive relaxation only at molar excess of plasmid over enzyme, where each topoisomerase I molecule has to interact with many plasmid molecules.

DISCUSSION

We have previously reported (*1*) that topoisomerase I interacts with the heterodimeric RNA splicing factor PSF/p54^{nrb} and gets stimulated by the interaction. Here we

performed a closer investigation of the precise mechanism of stimulation. We show that the increase in DNA relaxation activity is not due to changes in the rates of DNA cleavage or DNA ligation. It seems to be affected by an increase in the rate by which the enzyme moves between separate DNA helices. The most plausible interpretation of our data is that PSF/p54^{nrb} mobilizes topoisomerase I after religation has been completed, which points to an increase in the DNA dissociation rate, although we do not have direct evidence proving this. However, we show clearly that topoisomerase I alone has a pronounced inclination to subject a chosen substrate molecule to multiple cycles of cleavage/ligation. Relaxation activity is usually judged in quantitative terms by the disappearance of supercoiled plasmid molecules. To achieve complete relaxation under catalytic conditions (i.e., molar excess of plasmid over enzyme), each enzyme molecule has to interact with several plasmid molecules. It is easily conceivable that in this situation the pronounced disinclination of the pure enzyme to detach from a given DNA molecule will limit its overall relaxation activity in a more decisive manner than the DNA turnover rate itself. Therefore, it is reasonable that PSF/p54^{nrb} increases the overall DNA relaxation activity of topoisomerase I about 12-fold, as it increases the exchange rate of the substrate by a similar order of magnitude (11-fold). Our data might even suggest that topoisomerase I requires interacting proteins such as PSF/p54^{nrb} in order to be converted from a mere swivel to a true catalysator capable of processing multiple separate DNA segments.

The way in which PSF/p54^{nrb} seems to modulate the function of topoisomerase I is also conceivable from a teleological point of view. On one hand, PSF/p54^{nrb} does not change the cleavage/ligation equilibrium of topoisomerase I. Thus, it does not hold a potential of converting topoisomerase I into a cell poison (*10*). On the other hand, certain biological processes are known to require the coordinated interaction of topoisomerase I with separate DNA segments, between which the enzyme cannot move just by sliding along the DNA duplex. One such example is the control of DNA supercoils generated in front of and behind transcription complexes. The degree of supercoiling at these positions has been shown to control the transcription process to some extent (*11*). PSF/p54^{nrb} is known to coordinate the assembly of the spliceosome, which is also a transcription linked process (*12*, *13*). Thus, PSF/p54^{nrb} might regulate the activity and spatial distribution of topoisomerase I in the vicinity of transcription complexes in the fashion outlined in this study.

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