The Transducer Domain Is Important for Clamp Operation in Human DNA Topoisomerase IIα*

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DNA topoisomerase II is a multidomain homodimeric enzyme that changes DNA topology by coupling ATP hydrolysis to the transport of one DNA helix through a transient double-stranded break in another. The process requires dramatic conformational changes including closure of an ATP-operated clamp, which is comprised of two N-terminal domains from each protomer. The most N-terminal domain contains the ATP-binding site and is directly involved in clamp closure, undergoing dimerization upon ATP binding. The second domain, the transducer domain, forms the walls of the N-terminal clamp and connects the clamp to the enzyme core. Although structurally conserved, it is unclear whether the transducer domain is involved in clamp mechanism. We have purified and characterized a human topoisomerase IIα enzyme with a two-amino acid insertion at position 408 in the transducer domain. The enzyme retains both ATPase and DNA cleavage activities. However, the insertion, which is situated far from the N-terminal dimerization area, severely disrupts the function of the N-terminal clamp. The clamp-deficient enzyme is catalytically inactive and lacks most aspects of interdomain communication. Surprisingly, it seems to have retained the intersubunit communication, allowing it to bind ATP cooperatively in the presence of DNA. The results show that even distal parts of the transducer domain are important for the dynamics of the N-terminal clamp and furthermore indicate that stable clamp closure is not required for cooperative binding of ATP.

Eukaryotic topoisomerase II is a homodimeric enzyme consisting of three distinct regions. The N-terminal and core regions, containing the two catalytic entities, are highly conserved among eukaryotic organisms and also share homology with DNA gyrase, which represents the bacterial DNA topoisomerase II counterpart. The active site for ATP hydrolysis is encompassed in the N-terminal region, whereas that of DNA cleavage and ligation is located in the central region. The C-terminal part shows no sequence conservation and is dispensable for catalytic activity in vitro (9–11).

The N-terminal region of topoisomerase II forms an ATP-operated clamp that closes upon ATP binding, allowing trapping of the T segment (6, 8, 12). The crystal structures of the yeast and bacterial N-terminal topoisomerase II fragments reveal that the dimeric N-terminal clamp contains two domains in each protomer (8, 12). The most N-terminal domain holds the ATP-binding site that dimerizes upon nucleotide binding (8, 12, 13). This domain shares homology with the GHKL-type ATPases including topoVI-B (14) and MutL (15), for which a similar clamp operation has been observed. The second domain, called the transducer domain, bridges the N-terminal ATPase domain and the core region. Based on the yeast topoisomerase II structure, the transducer domain comprises the walls in a 6 Å-wide hole formed upon clamp closure, and it has been suggested to push the T segment through the DNA gate (12). The domain furthermore contains a loop that extends into the ATP-binding pocket and harbors a highly conserved lysine that contacts the γ-phosphate of the bound nucleotide (8, 12, 13). Based on structural and biochemical analyses of the loop region, communication between the ATP-binding GHKL domain and the central domain of the enzyme responsible for DNA cleavage/ligation has been suggested to go through the transducer domain (13, 16). Upon nucleotide binding and closure of the N-terminal clamp, the transducer domain undergoes a large domain rotation, probably facilitating opening of the DNA gate in the G segment bound by the central domain (14). Recent studies surprisingly revealed that the transducer domain also bears structural homology to MutL and the archaean topoVI-B, and similar conformational effects of nucleotide binding have been reported for the transducer domain of these GHKL-ATPases, indicating that the structure and motion of this domain play a conserved role in the clamp mechanism (14).

According to the available structures of the yeast topoisomerase II core, it is a heart-shaped dimer comprised of two domains, A' and B', showing homology to the subunits of DNA gyrase, gyrase A and gyrase B, respectively (17). The A' domain contains the active site tyrosine covalently attached to the 5' end of the DNA during cleavage (18) and encompasses a large cavity holding the transported DNA after passage through the G segment (17). The B' domain constitutes the interface be-
between the transducer domain and the A' domain (17). It is essential for DNA cleavage (17, 19) and has a proposed role in the separation of the cleaved DNA ends moving the A' domain by undergoing large rearrangements in response to signals from the ATPase domain (5).

Minimization of energy usage in DNA topoisomerase II requires a tight coupling between ATP consumption and DNA strand passage (20). Several observations have indicated that this is obtained through an extensive interdomain and intersubunit communication facilitating an appropriate temporal order of catalytic steps and conformational changes. Thus, binding of ATP to the N-terminal domain stimulates cleavage of the G segment bound by the core region, and DNA binding increases the turnover rate of ATP hydrolysis (4, 7). Furthermore, ATP binding occurs cooperatively only in the presence of a DNA substrate, meaning that DNA is required for intersubunit communication between the two ATP-binding sites in topoisomerase II (20). Binding of ATP per se induces clamp closure and triggers enzyme catalytic activity (6, 21), whereas hydrolysis of ATP is believed to accelerate the DNA strand passage event as well as enzyme resetting (7, 22).

In an earlier study, we have demonstrated that deletion of amino acids 351–408 located in the C-terminal part of the transducer domain of human topoisomerase IIa disturbs interdomain communication (4). To further dissect the role of the transducer domain, we have characterized a human topoisomerase IIa enzyme with two amino acids, leucine and glutamic acid, inserted at position 408. The insertion leaves both catalytic domains active, but strand passage is abolished. The clamp function is severely disrupted, even though the insertion is situated far from the dimerization domain, demonstrating the importance of the transducer domain for the clamp mechanism. The clamp-deficient enzyme is disturbed in domain communication, because the ATPase activity of the mutant enzyme lacks the normal response to DNA, and the DNA cleavage activity is unaffected by nucleotide binding. However, to our surprise, the DNA-mediated cooperativity in ATP binding seems to persist in 408i, indicating that stable clamp closure is not a prerequisite for intersubunit communication.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids—**The Saccharomyces cerevisiae strains BJ201 (Mata ura3 trpl pep4::HIS3 prb1 can1 top2::TRP1) and JEL1Δtop1 (kindly provided by J. C. Wang) were used for complementation and overexpression of topoisomerase II constructs, respectively. Plasmid pBY105 contains the yeast TPI promoter inserted into the JEL1/H11032 domain (23). To test the ability of 408i to complement the lack of endogenous topoisomerase II in BJ201, the LEU2-based pH7408i construct was transformed into BJ201, and the cells were transferred to media plates containing 5-fluoro-orotic acid (1 mg/ml) to select against the URA3 plasmid carrying the Schizosaccharomyces pombe top2' gene (10). pH7500 was used as a positive control.

**Topoisomerase IIa Induction, Overexpression, and Purification—**The recombinant human topoisomerase II enzymes were overexpressed in yeast strain JEL1Δtop1 by the addition of galactose to glucose-free medium (18). Yeast cells were extracted with 2 volumes of extraction buffer (50 mM Tris-HCl, pH 7.8, 1 mM NaCl, 1 mM phenylmethylsulfonyl fluoride) and 1 volume of acid-washed glass beads (425–800 μm; Sigma). Further preparation of yeast extracts was done according to the procedure of Jensen et al. (10). The initial purification step using a 6-mL Ni²⁺-nitrilotriacetic acid-agarose column was as described previously by Biersack et al. (24). For further purification, the fractions pooled from the Ni²⁺ column were loaded onto a 1-mL heparin-Sepharose column (Amersham Biosciences), and elution was performed by a 15-mL linear NaCl gradient ranging from 400 to 1500 mM. The peak fractions were further applied to a phosphocellulose column (P11 cellulose phosphate; Whatman) for concentration of the enzyme. Elution was performed in 750 mM NaCl, 0.5 mM dithiothreitol, 0.1 mM EDTA, 40% glycerol, 50 mM Tris-HCl, pH 7.7. Fractions containing topoisomerase IIa enzyme were pooled and stored in liquid nitrogen for later use.

Homogeneity of the topoisomerase II preparations was determined by SDS-polyacrylamide gel analysis after staining with Coomassie Blue dye.

**Topoisomerase IIa-mediated DNA Relaxation—**DNA relaxation was performed by incubating 2 or 10 nm topoisomerase II and 6.5 nm supercoiled pUC19 DNA in 50 mM Tris-HCl, pH 8, 140 mM KC1, 1 mM EDTA, 8 mM MgCl₂ supplemented with 1 mM ATP. The reactions were incubated at 37°C and stopped after 20 min by the addition of SDS and EDTA to a final concentration of 0.1% and 10 mM, respectively. The samples were subjected to electrophoresis in 1% agarose gels in TBE buffer (100 mM Tris borate, pH 8.3, 2 mM EDTA). DNA was stained with 1 μg/ml ethidium bromide and visualized by UV light.

**Clamp Closing Assay—**For clamp closing experiments, 100 nm topoisomerase IIa was preincubated with 6.5 nm negatively supercoiled pUC19 DNA for 5 min in a total volume of 20 μl of 50 mM Tris-HCl, pH 8, 140 mM KC1, 1 mM EDTA, 5 mM MgCl₂. After preincubation, AMPFPNP (Roche Applied Science) was added to a final concentration of 1 mM, and the reactions were incubated for an additional 5 min. The reactions were next stopped by the addition of either NaCl or SDS to final concentrations of 800 mM and 1%, respectively. The sample volume was increased to 70 μl by adding 50 μl of an 800 mM NaCl solution. To trap enzyme-DNA catenanes, phenol extraction was performed by adding 1 volume of phenol. The samples were vortexed and centrifuged at 13,000 rpm in an Eppendorf centrifuge for 10 min. The phenol phase was removed, ethanol-precipitated, and dissolved in 10 μl of TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA) for gel analysis. The combined phenol phase and phenol/water interphase was washed one time in high salt washing buffer (2 mM NaCl, 50 mM Tris-HCl, pH 7.9, and 5 mM MgCl₂) and one time in 0.6 M LiCl. Upon removal of the water phase after the last wash, the remaining material was ethanol-precipitated and dissolved in 10 μl of TE buffer containing 1 mg/ml proteinase K. The samples were next subjected to electrophoresis in a 1% agarose gel in TBE buffer containing 1 μg/ml ethidium bromide.

**Clamp Closing Assay Analyzed by Analytical CsCl Density Gradient Ultracentrifugation—**The technique was essentially as described previously by Morris et al. (25). Reaction mixtures of 30 μl containing 150 nm topoisomerase II, 250 nm relaxed pBR322 (topoGEN), or linearized pUC19 plasmid DNA in sample buffer (50 mM Tris-HCl, pH 8, 60 mM NaCl, 1 mM EDTA, 8 mM MgCl₂) were incubated for 5 min at 30°C. AMPFPNP was added to a final concentration of 1 mM, and incubation was continued for 10 min. The samples were then centrifuged at 130,000 rpm and 334 μl of saturated CsCl solution were added. The samples were run at 20°C and at 40,000 rpm in a Beckman XL-A analytical ultracentrifuge (Beckman Instruments). Scans were taken at 260 and 280 nm until equilibrium was reached (about 40 h).

**ATP Binding Assay—**100 nm topoisomerase IIa was incubated with 0.1 μM [γ-32P]ATP (600 Ci/mmole) for 20 min. In the presence or absence of 4.5 μM pUC19 plasmid DNA or 20 μM AMPFPNP in 50 mM Tris-HCl, pH 8, 140 mM KC1, 1 mM EDTA, 8 mM MgCl₂, the samples 1 The abbreviation used is: AMPFPNP, adenylylimidophosphate.
were incubated for 10 min at 37 °C and loaded onto a nitrocellulose filter using a slot blot apparatus (PR 648 Slot Blot Manifold; Amersham Biosciences). The filter was washed in a buffer containing 50 mM Tris-HCl, pH 7.9, and 800 mM KCl. Loading of samples was performed at a rate of 1 μl/min. A Molecular Imager (Bio-Rad) was used for quantification. For measurement of protein retention, a similar experiment was performed in the absence of [γ-32P]ATP, but in this case the filter was subjected to Western blot analysis with the pantethistidine antibody (Qiagen) recognizing the His-tagged topoisomerase II enzyme.

**Topoisomerase II-mediated Hydrolysis of ATP**—The ATPase assay was based on the method of Osheroff et al. (26). The reactions contained 100 nM topoisomerase II in the presence or absence of 20 nM negatively supercoiled pUC19 plasmid DNA. The reactions were carried out in 20 μl of 50 mM Tris-HCl, pH 8, 140 mM KCl, 1 mM EDTA, 8 mM MgCl2, containing a final concentration of 1 mM cold ATP and 2 μCi (0.033 μM) of [γ-32P]ATP (3000 Ci/mmol; Amersham Biosciences). The mixtures were incubated at 37 °C, and 2.5-μl aliquots were spotted onto thin layer cellulose plates impregnated with polyethyleneimine (Merck). Chromatography was performed using freshly made 0.4 M NH4HCO3. Levels of free phosphate were quantified using a Molecular Imager (Bio-Rad).

**Oligonucleotides**—DNA oligonucleotides were obtained from DNA Technology Corp. and purified by preparative polyacrylamide gel electrophoresis as described by Andersen et al. (27). The 28-mer used as the bottom strand in the suicide substrate was modified at the 3′ end by a phosphate group to inhibit ligation to this end.

**Topoisomerase II-mediated Cleavage of Suicide Substrates**—Hybridization and labeling of the synthetic oligonucleotides were done according to the procedures described by Andersen et al. (27). For topoisomerase II-mediated DNA cleavage, 20 nM topoisomerase II was incubated with 0.05 pmol of labeled substrate in 50 μl of 10 mM Tris-HCl, pH 7.0, 2.5 mM MgCl2, 2.5 mM CaCl2, 20 mM NaCl, and 0.1 mM EDTA at 37 °C, and the reactions were stopped by the addition of SDS to 1%. Covalent topoisomerase II-DNA cleavage complexes were recovered from a phenol/water interphase according to Gocke et al. (28). The complexes were subsequently ethanol-precipitated and treated with proteinase K (500 μg/ml) for 3 h at 42 °C. One volume of gel loading buffer (50% formamide, 0.05% bromphenol blue, 0.03% xylene cyanole, 5 mM EDTA) was added, and the material was subjected to electrophoresis in a 12% denaturing polyacrylamide gel.

**Topoisomerase II-mediated Ligation**—A topoisomerase II-mediated suicide cleavage reaction was performed as described above. After incubation at 37 °C for 45 min, the cleavage reaction was stopped by the addition of NaCl to 0.4 M, thereby preventing further cleavage during the ligation reaction. Ligation was initiated by the addition of 50 pmol of 45-mer ligation substrate. After 15 min of further incubation, the reaction was stopped by addition of SDS to 1%. The samples were ethanol-precipitated, proteinase K-digested, and analyzed by electrophoresis in a 12% denaturing polyacrylamide gel.

**Topoisomerase II-mediated Cleavage of Circular DNA**—Topoisomerase II-mediated DNA cleavage was performed by incubating 100 nM of topoisomerase II and 6.5 nM negatively supercoiled pUC19 DNA in a total volume of 20 μl in 50 mM Tris-HCl, pH 8, 140 mM KCl, 1 mM EDTA, 8 mM MgCl2. The samples were incubated at 37 °C for 7 min, and the cleavage products were trapped by the addition of SDS to 1%. After proteinase K digestion (0.8 mg/ml), the samples were subjected to electrophoresis in 1% agarose gels in TAE buffer (40 mM Tris acetate, pH 8.3, 2 mM EDTA). RNA was visualized by ethidium bromide staining (1 μg/ml). When reactions were carried out in the presence of ATP or AMPPNP, the concentration of the nucleotide was 1 mM.

**RESULTS**

**Human Topoisomerase IIα Containing a Two-amino Acid Acid Insertion at Position 408 Is Unable to Complement Growth in a Yeast top2 Deletion Strain**—To study the role of the transducer domain in DNA topoisomerase II, we have characterized a human topoisomerase IIα enzyme (408i) having an insertion of two amino acids at position 408 in the transducer domain (Fig. 1A, left panel). Based on alignment to the homologous part of yeast topoisomerase II, the insertion is located in the lower
part of the transducer domain, as indicated in the structure of the N-terminal yeast fragment (Fig. 1A, right panel). The insertion has been presented earlier in a linker scanning analysis of human topoisomerase IIα (10), where it was found to be among the few that abolished the ability of the human enzyme to sustain growth in an S. cerevisiae top2 deletion strain. This conclusion was confirmed by the complementation analysis shown in Fig. 1B. For studies of the in vitro capabilities of 408i, the enzyme fused to a hexahistidine tail at the C-terminal end was overexpressed in a top1 null strain and purified to homogeneity as seen from the Coomassie-stained gel in Fig. 1C.

Purified 408i Is Unable to Perform Relaxation of Supercoiled Plasmid DNA—To investigate whether the inability of 408i to complement is due to a defect in the DNA strand passage activity of the enzyme, a DNA relaxation assay was performed, and the catalytic activity of the mutant enzyme was compared with that of the wild-type enzyme. Although 10 nM of the wild-type enzyme relaxed all the supercoiled DNA within 20 min, the insertion mutant showed no sign of relaxation in these molar ranges (Fig. 2). Thus, consistent with the lack of in vivo complementation, these results reveal that insertion of two amino acids at position 408 is detrimental to the overall enzyme activity. Either the mutant enzyme fails to perform one or more of the steps involved in strand passage and catalytic turnover, or alternatively, the two-amin acid insertion disrupts correct folding of the protein.

Insertion at Position 408 Severely Disturbs the Function of the N-terminal Clamp—Preceding strand passage, topoisomerase II binds ATP and changes into the closed clamp conformation. To test whether the enzyme, bearing an insertion in the N-terminal transducer domain, has retained this important function of the N terminus, a clamp closing assay was performed (modified from Bjergbaek et al. (4)). The enzyme was preincubated with supercoiled plasmid DNA before the addition of the nonhydrolyzable ATP analog, AMPPNP. With the wild-type enzyme this leads to the formation of salt stable topoisomerase II-DNA interlinked complexes, which can be collected from a phenol-water interphase (Fig. 3A). The mutant enzyme, however, was unable to trap a detectable amount of supercoiled DNA under the present experimental conditions. Thus, insertion of two amino acids at position 408 disturbs stable clamp closure. None of the enzymes allowed trapping of DNA upon the addition of SDS, which disrupts the interlink between enzyme and DNA or in the absence of AMPPNP, where stable clamp closing does not occur.

To perform a more detailed investigation of the clamp function of 408i, the enzyme was subjected to analytical CsCl density ultracentrifugation. In this assay, the clamp closing reaction can be studied in the presence of higher concentrations of DNA and enzyme, and it is thus possible to
**FIG. 4.** 408i binds ATP. **Upper panel**, topoisomerase II was incubated with 0.3 nM $[^32P]ATP$ in the absence or presence of supercoiled plasmid DNA and cold AMPPNP as indicated. As a control, heat-inactivated enzyme (HI) was subjected to analysis as well. The samples were incubated for 5 min at 37 °C and loaded onto a nitrocellulose filter using a slot blot apparatus. **Lower panel**, histogram of the ATP binding experiment described in the upper panel. A Molecular Imager (Bio-Rad) was used for quantification of bound $[^32P]ATP$. Levels of ATP binding are relative to the amount of binding observed with the wild-type human topoisomerase II enzyme in the absence of DNA and AMPPNP. The results are the means ± S.D. of three independent experiments. 

**wt**, wild-type.

**Panel A**: Clamp Closure in Human DNA Topoisomerase IIα

> **408i Retains Normal ATP Binding Capabilities** — To investigate whether the clamp closing defect of 408i is caused by an AMPPNP- or DNA-mediated change in the retention efficiency of topoisomerase II on the nitrocellulose filter (data not shown).

> **408i Has ATPase Activity, but This Activity Is Not Stimulated by DNA**—To investigate whether the N-terminal ATPase domain has retained its intrinsic catalytic ability, we tested the ATPase activity of 408i. ATPase experiments were performed in the presence of 1 mM ATP, and thin layer chromatography followed by Molecular Imager analysis was used to measure the level of released $P_i$ (Fig. 5). The result shows that 408i is able to hydrolyze ATP, although its ATPase activity is lower than that of the wild-type enzyme (Fig. 5A). A comparison of 408i to that of an ATPase-deficient topoisomerase IIα enzyme (G164I) (7), which was purified in the same way, confirmed that the ATPase activity of 408i is not caused by contaminating phosphatases. ATPase activity is therefore an intrinsic capability of 408i, demonstrating that the N-terminal domain of the mutant enzyme folds into a catalytically active entity.

> **The 408i Enzyme Withholds both DNA Cleavage and Ligation Abilities**—To determine whether the insertion in 408i influences the activities of the central domain, we analyzed the ability of the enzyme to perform DNA cleavage and ligation. For this purpose, advantage was taken of the topoisomerase II suicide system, which allows a separation of the two half-reactions as schematically illustrated in Fig. 6A (upper panel). The suicide substrate consists of a 16-base-long 5’-recessed top strand with only three nucleotides 5’ to the cleavage position and a 28-base-long bottom strand. Use of the suicide substrate has been demonstrated to cause an uncoupling of the DNA cleavage and ligation half-reactions because of the release of the trinucleotide 5’ to the cleavage position on the top strand upon topoisomerase II-mediated cleavage (27). The substrate was labeled at the 3’ end of the recessed top strand and incubated with either the wild-type or the 408i enzyme. Cleavage complexes were isolated from a phenol/water interphase and analyzed by gel electrophoresis. As seen from the gel in Fig. 6A (lower panel), the mutant enzyme retains the ability to cleave the suicide DNA substrate with a cleavage level similar to that obtained with the wild-type enzyme.

The topoisomerase II cleavage complex generated upon cleavage of a suicide substrate is kinetically competent (27) and able to perform ligation if a suitable ligation substrate is added to the cleavage mixture as schematically illustrated in Fig. 6B (left panel). To investigate whether the central domain of 408i also withholds ligation activity, topoisomerase II-DNA cleav-
in the presence of DNA, the ATPase activity of 408i or wild-type enzyme was measured as in Experimental Procedures. The results are the means ± S.D. of three independent experiments.

The ATPase activity of 408i is not stimulated by DNA. A, the ATPase activity of either 408i (○), the wild-type enzyme (▲), or G164I (■) was measured by incubating enzyme with 1 mM ATP at 37 °C. The samples were removed at the indicated time points and spotted onto thin layer cellulose plates. The levels of free phosphate were quantified using a Molecular Imager (Bio-Rad). Hydrolysis is plotted relative to the amount of ATP hydrolyzed by 408i after 15 min. The results are the means ± S.D. of three independent experiments. B, the ATPase activity of 408i or wild-type enzyme was measured as in A in the presence (■) or absence (○) of DNA. The results are the means ± S.D. of three independent experiments. wt, wild-type.

To further verify that the cleavage/ligation domain of 408i retains a normal DNA interaction, the sequence specificity of the mutant and wild-type enzymes were compared using a 4330-bp pBR322 fragment labeled only at one 3' end as a DNA cleavage substrate. No difference was observed in the specificity of the two enzymes (data not shown), indicating that insertion at position 408 does not interfere with the normal DNA sequence preference of topoisomerase II. The result further substantiates that the central domain of 408i has retained its normal properties.

The Cleavage/Ligation Equilibrium of 408i Is Not Affected by ATP or AMPPNP—In addition to the stimulatory effect of DNA on topoisomerase II-catalyzed ATP binding and hydrolysis, the communication between the N-terminal and central regions is also manifested during topoisomerase II-mediated DNA cleavage, where ATP or an ATP analog shifts the DNA cleavage/ligation equilibrium toward cleavage (21, 29).

To further probe the communication abilities of the mutant enzyme, topoisomerase II-mediated cleavage of supercoiled plasmid DNA was performed in the absence or presence of ATP or the ATP analog, AMPPNP (Fig. 7, upper panel). Although more cleavage was obtained with the mutant enzyme relative to the wild-type enzyme in the absence of nucleotide (Fig. 7, lower panel), only the wild-type enzyme was stimulated by AMPPNP. This shows that 408i is unable to transmit the effect of nucleotide binding to the core region and thus indicates that the insertion directly or through inhibition of clamp closure abolishes the transmission event. In the presence of ATP, the wild-type enzyme relaxed the DNA. Even with these high enzyme concentrations, ATP only allowed 408i to cleave the DNA, in support of its inability to relax supercoiled DNA.

**DISCUSSION**

The ability of topoisomerase II to change the topological conformation of DNA is based on a highly controlled series of conformational changes that propagate from the N-terminal region throughout the entire enzyme. One large and very important switch occurs upon binding of ATP, which causes the N-terminal arms to dimerize and thereby closes the clamp. Because binding of a nonhydrolyzable ATP analog has been demonstrated to sustain one strand passage reaction (26, 30), ATP binding per se is sufficient to allow strand passage, whereas hydrolysis accelerates the reaction and resets the enzyme for a new catalytic cycle (22, 31).

In the present work, we have characterized a mutant human topoisomerase IIα enzyme that has a two-amino acid insertion at position 408 in the distal C-terminal part of the transducer domain. The enzyme has a severe defect in clamp closure but nevertheless can bind and hydrolyze ATP and is able to perform DNA cleavage and ligation. Our results show that the insertion causes a quite specific disturbance of the N-terminal clamp function. It results in an enzyme that fails to relax supercoiled DNA and therefore is unable to sustain mitotic growth in a yeast top2 null strain.

Taken together, our results strongly suggest that closure of the N-terminal clamp is the most important consequence of ATP binding and critical for the strand passage reaction. This is in agreement with results obtained from studies of a human topoisomerase IIα heterodimer, which was still able to relax DNA, although disabled in ATP binding in one subunit (7). The enzyme was disturbed in some aspects of domain communication but nevertheless was able to perform clamp closure, supportive of a strong correlation between clamp closure and DNA strand passage.

Surprisingly, we find that 408i, which is unable to make a
stable N-terminal dimerization, still seems to respond to DNA with cooperative ATP binding. This indicates that certain types of intersubunit communication can be mediated either via dimer contacts in the core region or through less stable interactions in the N-terminal dimerization domains, which might still occur in 408i, when DNA is bound to the enzyme.

The observations that DNA does not stimulate ATP hydrolysis in 408i but still seems to mediate cooperative ATP binding suggest that DNA normally influences the ATPase domain through two separate mechanisms, only one of which is retained in 408i. The stimulatory role of DNA on ATP hydrolysis, which is lacking in 408i, might thus rely on a fully coordinated clamp function and/or DNA strand passage. In support of two stimulatory mechanisms of DNA, studies of a cleavage-deficient topoisomerase II showed that this enzyme had a normal

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**Fig. 6.** 408i is able to cleave and ligate DNA. A, upper panel, schematic illustration of the topoisomerase II-mediated suicide cleavage reaction showing release of the trinucleotide 5' to the cleavage position on the top strand upon DNA cleavage. Arrows indicate the positions of topoisomerase II-mediated DNA cleavage. The asterisk indicates radioactive labeling of the substrate. Lower panel, for suicidal cleavage, the wild-type enzyme or 408i was incubated with supercoiled plasmid DNA at 37 °C in the absence or presence of ATP or the ATP analog, AMPPNP. The reactions were stopped after 7 min by the addition of SDS to 1%. After proteinase K digestion, the samples were subjected to electrophoresis in a 1% agarose gel. The enzyme and nucleotide used in the reactions are indicated above the lanes. C, DNA control. SC, supercoiled plasmid DNA. L, linear DNA. R/N, relaxed or nicked plasmid DNA. Cat, catenated plasmid DNA. Lower panel, histogram of the cleavage reactions of the wild-type enzyme and 408i in the absence of nucleotide. The results are the means ± S.D. of three independent experiments. wt, wild-type.

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**Fig. 7.** The cleavage/ligation equilibrium of 408i is not affected by ATP or AMPPNP. Upper panel, wild-type enzyme or 408i was incubated with supercoiled plasmid DNA at 37 °C in the absence or presence of ATP or the ATP analog, AMPPNP. The reactions were stopped after 7 min by the addition of SDS to 1%. After proteinase K digestion, the samples were subjected to electrophoresis in a 1% agarose gel. The enzyme and nucleotide used in the reactions are indicated above the lanes. C, DNA control. SC, supercoiled plasmid DNA. L, linear DNA. R/N, relaxed or nicked plasmid DNA. Cat, catenated plasmid DNA. Lower panel, histogram of the cleavage reactions of the wild-type enzyme and 408i in the absence of nucleotide. The results are the means ± S.D. of three independent experiments. wt, wild-type.
effect of DNA on ATP binding but a very reduced effect on ATP turnover (25).

In addition to the inability of 408i to increase its rate of ATP hydrolysis upon the addition of DNA, the clamp-disabled 408i enzyme does not show the normal increase in DNA cleavage when either ATP or an ATP analog is present. 408i thus has a disruption in the two-way communication, which normally exists between the N-terminal and central enzyme regions. Our findings correlate with the results obtained from studies of a topoisomerase IIo enzyme bearing a deletion of amino acids 351–408 (4). In addition to severe interdomain communication defects, the deletion enzyme had a malfunctioning N-terminal clamp. Our results confirm that the conformational changes involved in clamp closure are important for interdomain communication.

Sequence alignment with the recently crystallized yeast topoisomerase II fragment suggests that position 408 is situated at the outer surface in the distal end of the transducer domain and probably is in close contact with the B’ domain. It is thus located far from the ATP-binding site and the N-terminal dimerization area, both of which have well-established roles in the mechanism of clamp closure (8, 12). No obvious role for the transducer domain in the clamp mechanism has been suggested, yet comparison of several GHKL-ATPases surprisingly revealed that the structural similarity was not confined to the ATPase domain, but in some cases included the transducer domain as well (14, 15). The observed disturbance of clamp function by insertion of two amino acids at position 408 demonstrates an important role of the transducer domain for the dynamics of the N-terminal clamp consistent with the structural conservation of the domain.

Interdomain communication is critical for topoisomerase II to go through the conformational changes required for catalysis including clamp closure (4, 5, 8, 30). This complex process involves global rearrangements, and therefore it is difficult to identify residues critical for the process. However, the transducer domain harbors an important loop, in which a highly conserved lysine (Lys378) forms a hydrogen bond with the γ-phosphate of the bound nucleotide (8). Both structural and biochemical studies suggest that this loop is sensing the nucleotide-bound state of the enzyme and helps direct the motions of the ATP-binding domain relative to the transducer domain (13, 14, 16). According to the alignment, position 408 in human topoisomerase IIo is connected to this loop by a stretch of amino acids that directly traverses the transducer domain. The fact that position 408 is located at the interface of the B’ domain and the transducer domain thus indicates the existence of a structural communication pathway reaching from the ATPase domain to the DNA cleavage/ligation domain through the area around position 408. The observed clamp deficiency resulting from insertion at position 408 thus suggests that disturbance of communication between the transducer domain and the B’ domain is fatal for clamp function, maybe because of restrictions in the movements of the N-terminal arms that still remain in 408i after nucleotide binding.

The fact that 408i displays a significantly higher cleavage level on supercoiled plasmid DNA in the absence of nucleotide infers that the cleavage region in 408i does not fall into quite the same conformation as in the wild-type enzyme under these conditions. This observation leads to the hypothesis that the transducer domain in the absence of ATP normally induces a specific conformation of the B’ domain through the area around position 408, which results in an autoinhibition of the cleavage activity. Upon nucleotide binding, the inhibition is released, allowing a DNA gate to be formed. This interpretation is consistent with the crystal structure of the yeast core topoisomerase II that spontaneously falls into an open conformation in the absence of DNA and nucleotide (5, 32). In line with this, hypercleavage has also been reported for a catalytically active human topoisomerase IIo enzyme bearing a point mutation at position 437 (33), also located at the interface of the B’ domain and the transducer domain.

In conclusion, the insertion of two amino acids at position 408 in the transducer domain of human topoisomerase II severely disturbs the function of the N-terminal clamp. The clamp-deficient enzyme is catalytically inactive and lacks most aspects of interdomain communication. The results show that even distal parts of the transducer domain are important for the dynamics of the N-terminal clamp. Furthermore, the study strongly indicates that closure of the N-terminal clamp is a crucial prerequisite for strand passage.

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