1 **Crystal structure of *Sulfolobus solfataricus* topoisomerase III**

2 **reveals a novel carboxyl-terminal zinc finger domain essential**

3 **for decatenation activity**

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28 Running title: *Crystal structure of an archaeal DNA topoisomerase III*

29 **ABSTRACT**

30 DNA topoisomerases are essential enzymes for a variety of cellular processes
 31 involved in DNA transactions. Mechanistic insights into type IA DNA
 32 topoisomerases have come principally from studies on bacterial and
 33 eukaryotic enzymes. A structural understanding of type IA topoisomerases in
 34 Archaea is lacking. Here, we present a 2.1-angstrom crystal structure of
 35 full-length *Sulfolobus solfataricus* topoisomerase III (Sso topo III), an archaeal
 36 member of type IA topoisomerases. The structure shows that Sso topo III
 37 adopts a characteristic torus-like architecture consisting of a four-domain core
 38 region and a novel carboxyl-terminal zinc finger domain (domain V).
 39 Structure-based mutation analyses reveal that a novel zinc-binding motif in
 40 domain V is essential for the DNA decatenation activity of Sso topo III. Our
 41 data indicate that Sso topo III represents a subclass of Type IA
 42 topoisomerases capable of resolving DNA catenates using a domain
 43 V-dependent mechanism.

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58 **IMPORTANCE**

59 Type IA topoisomerases are omnipresent in all cellular life forms and serve

60 pivotal roles in cellular processes involved in DNA transactions. While
 61 considerable insights have been gained into Type IA topoisomerases from
 62 bacteria and eukaryotes, a structural understanding of type IA topoisomerases
 63 in Archaea remains elusive. we first determined the crystal structure of
 64 full-length *Sulfolobus solfataricus* topoisomerase III (Sso topo III), an archaeal
 65 member of type IA topoisomerases. Our structure provides the first molecular
 66 view of this archaeal topoisomerase, which removes negative supercoils and
 67 decatenates DNA catenane. Our findings manifest that Sso topo III may serve
 68 as an alternative prototype of type IA topoisomerases, whose decatenation
 69 mechanism differs from that of known bacterial and eukaryotic topoisomerases
 70 III such as *Escherichia coli* topoisomerase III (EcTOP3).

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79 **KEYWORDS**

80 Archaea, DNA topoisomerase, crystal structure, zinc finger, decatenation
 81 activity, enzyme mutation

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86 **INTRODUCTION**

87 The overall topological state of DNA in cells is altered by DNA

88 topoisomerases. In all three domains of life, conserved DNA topoisomerases
 89 are essential for a wide variety of cellular processes involved in DNA
 90 transactions (1-4). There are two types of topoisomerases (1). Type I
 91 topoisomerases cut only one strand of DNA, while type II topoisomerases
 92 cleave both strands of the DNA duplex. Type I enzymes can be further
 93 classified into three different families, types IA, IB and IC, on the basis of
 94 transiently formed covalent intermediates formed (5'- or 3'- covalent
 95 intermediate) (5). *Escherichia coli* topoisomerase I (EcTOP1) and
 96 topoisomerase III (EcTOP3) are the best-characterized bacterial type IA
 97 enzymes to date.

98 A conserved toroidal fold formed by four domains (I-IV) has been found in
 99 the structures of type IA enzymes including EcTOP1 (6), EcTOP3 (7),
 100 *Thermotoga maritima* topoisomerase I (TmTOP1) (8), *Mycobacterium*
 101 *tuberculosis* topoisomerase I (MtTOP1) (9), *Mycobacterium smegmatis*
 102 topoisomerase I (MsTOP1) (10), human topoisomerase III α (HsTop3 α) (11),
 103 human topoisomerase III β (HsTop3 β) (12) and a reverse gyrase (13) from
 104 *Archaeoglobus fulgidus*, a hyperthermophilic archaeon. Structural studies
 105 (14-16) as well as biochemical (17-20) and single-molecule (21-24)
 106 experimental data have provided abundant evidences in support of an
 107 enzyme-bridged strand passage model for type IA enzymes. According to this
 108 model, a type IA topoisomerase nicks one DNA single-strand, and remains
 109 attached to the two ends of the nick. That is, the enzyme is not only covalently

110 attached to one end of the nick, but also noncovalently bound to the other end
111 to generate a bridge through which another single-strand or the DNA duplex is
112 passed. The following step is the resealing of the nick and the release of the
113 enzyme. In this process, the OH group of the aromatic ring of the Tyr residue in
114 the enzyme nucleophilically attacks the scissile phosphodiester bond,
115 producing a transient 5' phosphotyrosine covalent bond.

116 Members of the type IA subfamily are further divided into two
117 mechanistically distinct subclasses based on their biochemical specificity for
118 substrates (2). EcTOP1 and EcTOP3 are the prototypes of these two
119 subclasses of type IA enzymes. EcTOP3 catalyzes the DNA catenates more
120 effectively than it relaxes negatively supercoiled DNA, whereas the converse is
121 true for EcTOP1 (23, 25-28). EcTOP3, but not EcTOP1, contains a unique
122 loop located close to the central hole (29). This positively charged loop, known
123 as the decatenation loop, is essential for the decatenation activity of EcTOP3
124 (24, 29).

125 Previous studies have characterized topoisomerase III (hereafter, Sso topo
126 III) from *Sulfolobus solfataricus*, a hyperthermophilic archaeon, as a member
127 of the type IA topoisomerase family (30, 31). A phylogenetic analysis of Sso
128 topo III, and the observation that Sso topo III inefficiently catalyzes the
129 relaxation of negatively supercoiled DNA, led to the suggestion that Sso topo
130 III is more similar to EcTOP3 than to EcTOP1 (30). More recently, Bizard et al.
131 provided convincing biochemical data in support of notion that Sso topo III is a

132 genuine decatenase that can efficiently unlink covalently closed catenanes
133 alone at temperatures up to 90°C (32). However, little is known about the
134 structure of type IA topoisomerases (except for reverse gyrase) from the
135 Archaea. The structural basis for the decatenase activity of Sso topo III is
136 unclear.

137 Here, we have determined the crystal structure of full-length Sso topo III.
138 Sso topo III adopts a classical elongated toroidal-like shape with four domains
139 (I-IV) forming the protein core and a novel carboxyl-terminal zinc finger domain
140 (domain V) tethered to the core against domain II. Furthermore, domain V is
141 conserved among DNA topoisomerases III from the TACK superphylum of the
142 Archaea. In addition, structure-based mutational analyses show that, whereas
143 the zinc-binding motif of domain V was required for efficient DNA relaxation by
144 Sso topo III, its depletion nearly abolished the DNA decatenation activity of the
145 enzyme. These findings indicate that Sso topo III may represent as a novel
146 type of topoisomerases III which employ a decatenation mechanism distinctly
147 different from that of known bacterial and eukaryotic equivalent.

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150 RESULTS

151 **Overall structure of intact Sso topo III** To obtain crystals of Sso
152 topo III, we overproduced and purified sufficient quantities of the mutant
153 enzyme (Y318F) in which the catalytic residue Tyr318 was replaced with Phe
154 (Fig. 1 A). The structure of the full-length enzyme was determined at 2.1 Å

155 resolution using molecular replacement (Table 1). The full-length protein
156 formed an asymmetric unit comprising four Sso topo III molecules. The four
157 molecules had essentially the same architecture. For convenience, one
158 molecule was chosen for the following structural analyses.

159 Our analysis showed that the overall structure of Sso topo III comprises four
160 domains (I–IV) (residues 1–593), forming the conserved core, and a novel
161 carboxyl-terminal domain V (residues 594–668) (Fig. 1A, 1B and S1). The
162 enzyme adopts a canonical toroidal-like elongated configuration with a large
163 hole (Fig. 1B and 1C), which is shared by all known type 1A topoisomerases
164 (33). The enzyme-bridged strand passage model proposes that the hole
165 accommodates single or double-stranded DNA (34).

166 Domain I (residues 1–219) exhibits a topoisomerase–primase fold (2), which
167 is found in type IA, IIA, and IIB topoisomerases, as well as in bacterial
168 primases. Within domain I, a disulphide bridge crosslinks Cys4 of a loop
169 (residues 1–10) and Cys34 of an α/β fold consisting of six parallel β strands
170 sandwiched with four α helices (Fig. S1). This connection results in tight
171 packing of domain I and stabilizes its architecture. In Domain II (residues
172 220–277, 408–474), the hinge region (residues 218–224, 471–480) is
173 composed of two loops linking domains I and II, and II and IV, respectively (Fig.
174 1B). The hinge is believed to serve as the principal pivot point for opening and
175 closing of the DNA gate in the strand passage reaction (6, 11, 35).

176 A long loop (residues 594–616) leads from helix α 18 of domain IV to
177 domain V (residues 594–668), thus linking the core of the enzyme to domain V
178 (Fig. 1B and S1). Domain V attaches to the top of the topoisomerase (domain
179 II) against the hinge region (Fig. 1B and 1C). Three antiparallel α -helices,
180 including helix α 19 (residues 616–636), helix α 20 (residues 640–649), and
181 helix α 21 (residues 655–666), are coiled around each other to constitute the
182 bulk of domain V (Fig. 1B). Interestingly, four residues (Cys602, Cys605,
183 Cys615 and His618) coordinate with a zinc atom to generate a novel
184 zinc-binding motif (Fig. 1D and S1). Among them, the three Cys residues come
185 from the long loop connecting domains IV/V and the His residue is from helix
186 α 19. The presence of the zinc-binding site was confirmed by an analysis of the
187 anomalous difference Fourier map (Fig. 1D). The recently characterized
188 mesophilic archaeal topoisomerase, *Methanosarcina acetivorans* topo III α
189 (*Mac* topo III α), also has one zinc-binding site (36). However, the zinc-binding
190 motif of *Sso* topo III is the Cys3His1 type, whereas that of *Mac* topo III α is
191 tetraCys type. For *Sso* topo III, the zinc finger motif may contribute to the
192 stabilization of domain V. As revealed by sequence alignment, the
193 carboxyl-terminal zinc finger motif of the Cys3His1 type is highly conserved
194 among topoisomerases from the Crenarchaeota (Fig. S2A), suggesting that
195 this motif plays an important role. Intriguingly, small proteins homologous to
196 domain V are also found in Thaumarchaeota and Bathyarchaeota, both of
197 which are phyla within the TACK superphylum where Crenarchaeota also

198 belong, as well as Euryarchaeota and Thorarchaeota (Fig. S3). Homologues of
 199 Sso topo III are present in Thaumarchaeota, Bathyarchaeota, Euryarchaeota
 200 and Thorarchaeota, but these enzymes almost lack the carboxyl-terminal
 201 domain similar to domain V in Sso topo III. It would be of interest to determine
 202 if the domain V-like small proteins physically and functionally interact with the
 203 type IA topoisomerases in these organisms. Notably, no structural elements
 204 similar to domain V of Sso topo III is found in the Protein Data Bank, as
 205 detected by DALI (37). These results suggest that the carboxyl-terminal fold of
 206 Sso topo III, also found in several archaeal lineages, is unique.

207 **Interface of domains II and V** A distinguishing feature of the overall
 208 structure of Sso topo III compared with that of other known available type IA
 209 topoisomerases is the presence of three helices in domain V located close to
 210 the hinge region of domain II (Fig. 1B). Two flexible loops, each about five
 211 residues long, link these three helices. This arrangement causes the three
 212 helices to pack against each other so that their movement is highly
 213 coordinated, but they maintain a degree of flexibility. Additionally, within the
 214 long linker loop (about 20 residues) connecting domains IV and V, there are
 215 three successive turns, which maintain free space between the two domains.
 216 The novel zinc-binding motif, located on the carboxyl-end of the long loop,
 217 contributes to the stabilization of the loop (Fig. 1D). By analogy to the other
 218 solved type IA topoisomerases (6), the interfaces between domain III and

domains IV and I in Sso topo III are conserved and far-ranging. However, the interface between domains II and V within the enzyme is unique.

The longest one of the three helices in domain V, helix $\alpha 19$, acts as the major binding platform for the interface between domains II and V. The interface is mostly, but not exclusively, hydrophobic (Fig. 1E). Extensive hydrophobic interactions mainly occur between the side chains of Leu219, Phe220, Ile221, Pro222, Leu223, Pro224 and Phe226 in the hinge region, which connects helix $\alpha 6$ of domain I with strand $\beta 9$ of domain II, and the side chains of Ala621, Leu625, Ala628, Leu631, Trp632 in helix $\alpha 19$ and Tyr656, Val657 in helix $\alpha 21$ of domain V (Fig. 1E). Moreover, the main chain amides of Gly654 and Lys655 in domain V form hydrophilic interactions with the backbone carbonyl groups of Phe220 and Asn218 in the hinge region, respectively, while the amide of Tyr656 makes hydrophilic interaction with the backbone carbonyl groups of Asn218 (Fig. 1F). In addition, the NH group on an indole group of Trp632 makes a hydrogen bond with the backbone carbonyl group of Pro222. These interactions further stabilize the attachment of domain V to domain II. Most importantly, the main chain carbonyl group and side chain amide of Arg635 in the carboxyl-end of helix $\alpha 19$ in domain V form hydrogen bonds with the main chain amide of Ile250 at the amino-end of helix $\alpha 7$ and the carbonyl group of Pro224 at the amino-end of strand $\beta 9$ in domain II, respectively (Fig. 1F). Domain V and the main part of domain II are connected by a single interaction, that is, double hydrogen bonds involving Arg635. This

241 may allow for independent and coordinated movements of domain V relative to
242 domain II at different stages during catalysis. Noticeably, Glu608 from domain
243 V forms salt bridge with Lys592 from domain IV on the other end of the
244 interface. Alignment of the sequences of type IA topoisomerases from the
245 Crenarchaeota shows that most of the above-mentioned residues contributing
246 to the interaction between domains II and V are highly conserved (Fig. S2B).
247 This implies that the interaction between domains II and V may be essential for
248 the functional role of domain V of the archaeal topoisomerases.

249 **Comparison of the interactions around the hinge region**
250 **among type IA topoisomerases** The studies on EcTOP3 (7, 14, 29)
251 indicated that the decatenation loop on domain IV is critical for efficient DNA
252 decatenation (Fig. 2A). A recent report on Sso topo III provides strong
253 biochemical evidence that the enzyme is a true decatenase (32). Surprisingly,
254 the structural equivalent of the decatenation loop is absent from Sso topo III
255 (Fig. 2A), suggesting that DNA decatenation by Sso topo III may differ
256 mechanistically from that by EcTOP3.

257 It is believed that the highly conserved hinge region joining domains II and IV
258 appears to be important for strand passage during catalysis (6). To understand
259 the decatenation mechanism of Sso topo III, we performed a structural
260 analysis of the potential interactions within the available type IA
261 topoisomerases between the hinge region and the carboxyl-terminal domain or
262 an additional subunit of the topoisomerase complex. No direct interactions

263 exist between the hinge regions and the C-terminus in TmTOP1 (8), MtTOP1
264 (9) and MsTOP1 (10). However, there are direct interactions between the
265 hinge region and parts of the carboxyl-terminal domain in several
266 topoisomerases or the other subunit of a topoisomerase complex (Fig. 2B).
267 The interactions may influence the opening state of their gates. The human
268 BLM helicase, topoisomerase III α (HsTop3 α) and RMI1 proteins form a
269 minimal protein complex, which catalyses the dissolution of double-Holliday
270 junctions (dHJ) to produce non-crossover products (38). A structural analysis
271 of the Top3 α –RMI1 complex showed that the hinge region makes intimate
272 contacts with the decatenation loop of the RMI1 subunit by hydrophobic
273 interaction, suggesting that this contact contributes to the modulation of the
274 opening-closing state of the gate in Top3 α (11) (Fig. 2B). Similar to the
275 Top3 α –RMI1 complex, Top3 β forms a complex with the TDRD3 subunit. A
276 structural analysis of the complex indicated that the hinge region of Top3 β is a
277 little bit apart from the insertion loop of TDRD3 (12) (Fig. 2B). In the case of
278 EcTOP1, the interaction between a unique α helix in domain VI of the
279 carboxyl-terminal domain and the hinge region mediates the major direct
280 contact between the carboxyl-terminal domain and the catalytic core (39) (Fig.
281 2B). The conformational changes of the carboxyl-terminal domain are
282 potentially transferred to the hinge region through the unique helix in domain
283 VI at some points in the catalytic cycle, thereby regulating the opening-closing
284 state of the gate. In *Sso* topo III, major contacts between the hinge region and

285 the longer helix $\alpha 19$ of domain V occur through stable and extensive
286 hydrophobic interactions (Fig. 2C), and several hydrogen bonds, located at the
287 two ends of the interface between domains II and V, are involved in the
288 interaction between domain V and the bulk of domain II and domain IV,
289 respectively (Fig. 1F). This network of interactions may allow domain V to
290 adhere to the hinge region mainly by the $\alpha 19$ helix during the strand passage
291 reaction, and possibly make the bulk of domain II and domain IV to move
292 relative to the hinge region at some points during the catalysis.

293 **Deletion of the zinc-binding motif reduces the relaxation**
294 **activity of Sso topo III** To explore the role of the carboxyl-terminal domain,
295 the zinc-binding motif in particular, in DNA relaxation by Sso topo III, a
296 carboxyl-terminally truncated mutant (597 Δ C), which lacked whole domain V,
297 and a quadruple mutant (CCCH/4A), in which the four residues (Cys602,
298 Cys605, Cys615 and His618) coordinating with the zinc atom were all replaced
299 with Ala, were generated by mutagenesis (Table S1). In the CCCH/4A, the
300 mutation of the zinc-binding motif (3Cys1His type) resulted in the elimination of
301 the zinc atom (Fig. 1D). These two mutant proteins were overproduced in *E.*
302 *coli* and purified to homogeneity. The two mutant proteins showed the same
303 optimal temperature as the wild-type enzyme (75°C) in DNA relaxation (Fig.
304 S4). 597 Δ C possessed about 50% of the wild-type activity (Fig. 3, compare top
305 and middle panel), whereas CCCH/4A retained about 15% of the wild-type

activity (Fig. 3, compare top and bottom panel). These results indicated that the zinc-binding motif is necessary for efficient relaxation by Sso topo III.

To compare DNA relaxation reactions catalysed by wild-type and mutants of Sso topo III, a time-course assay was performed (Fig. 4). The wild-type protein displayed a profile of topoisomers indicative of a distributive mechanism of relaxation (Fig. 4, top panel), as described recently (32). However, both 597 Δ C and CCCH/4A exhibit a relaxation profile differently from the wild-type Sso topo III (Fig. 4, compare top, middle, and bottom panel). The DNA relaxation reaction catalysed by 597 Δ C appeared to more distributive than that by the wild-type protein. Interestingly, the relaxation reaction becomes more distributive, when the unique zinc-binding motif was eliminated, than that as the carboxyl-terminal domain of Sso topo III was wholly deleted (Fig. 4).

Together, these results indicated that domain V, especially the zinc-binding motif, facilitates the relaxation of negatively supercoiled DNA by Sso topo III.

To probe the potential roles of domain V in SsoTopo III, we also determined ssDNA binding by the domain V-deletion mutant protein 594 Δ C (residues 1-594). The mutant protein showed a ~ 5-fold lower affinity than wild-type SsoTopo III for a 32-nt ssDNA containing the preferred cleavage site (designated C32) than the wild-type protein (Fig. S5). Therefore, it appeared that domain V contributes significantly to template binding by SsoTopo III.

The novel zinc-binding motif is essential for DNA

decatenation by Sso topo III To explore the role of the novel domain V

in Sso topo III-catalysed decatenation, wild-type and mutants of Sso topo III were subjected to a decatenation assay using kinetoplast DNA (kDNA) (40) as the substrate. Wild-type Sso topo III was optimally active in kDNA decatenation at 90°C, in consistent with the observation made previously (32) (Fig. S6A, B, C). The fast-migrating bands, corresponding to closed minicircles, were present when the reactions were performed at very high temperatures (90 °C) (Fig. 5A). These closed minicircles were released from the kDNA network by Sso topo III, because the enzyme apparently was unable to relax supercoiled DNA at 90 °C and above, but was still able to decatenate DNA catenanes (32). Therefore, the presence of closed minicircles at very high temperature was considered as a measure of the decatenation activity of Sso topo III. The bands corresponding to closed minicircles were undetectable in reactions containing 597ΔC (Fig. 5B), suggesting that the mutant protein had no detectable decatenation activity at very high temperature. Similarly, CCCH/4A was nearly inactive in decatenation (Fig. 5C). Taken together, we concluded that domain V, the zinc-binding motif in particular, is essential for the decatenation activity of Sso topo III.

DISCUSSION

Type IA topoisomerases are found in all three domains of life. Much of current knowledge about the enzymes has come from structural and biochemical studies on the ones from bacterial and eukaryotic origins (41). A structural understanding of type IA topoisomerases (except for reverse gyrase) in the

350 Archaea remains elusive. In this work, we present the crystal structure of Sso
 351 topo III, an archaeal type IA topoisomerase, at 2.1 Å resolution. The structure
 352 provides the first molecular view of this archaeal topoisomerase, which
 353 removes negative supercoils and decatenates DNA catenane (30, 32, 42). Sso
 354 topo III is toroidal in shape with a four-domain protein core closely resembling
 355 that found in other members of the topoisomerase IA family (6). A striking
 356 feature of Sso topo III is the presence at its carboxyl-terminus of a novel zinc
 357 finger-containing part that attaches to the hinge region of domain II. Intriguingly,
 358 the enzyme lacks the decatenation loop found in some of the known type IA
 359 topoisomerases capable of DNA decatenation. As revealed by mutational
 360 analysis, the zinc-binding motif is critical for effective decatenation of Sso topo
 361 III. Therefore, Sso topo III represents a novel type of topoisomerases which
 362 employ a DNA decatenation mechanism different from those of the known
 363 bacterial and eukaryotic topoisomerases III.

364 The carboxyl-terminal domain of a type IA topoisomerase varies widely in
 365 composition, length and shape (41, 43), as well as in orientations relative to
 366 the hinge region of the enzyme. Despite wide variations, the carboxyl-terminal
 367 domain exhibits ssDNA-binding activity, and is responsible for the high
 368 DNA-binding affinity of the enzyme. In the case of EcTOP1, domain IV extends
 369 into the carboxyl-terminal domain consisting of three zinc ribbon domains
 370 containing the tetracysteine motif and two zinc ribbon-like domains (39).
 371 Among the five domains, four domains bind to ssDNA with primarily π -stacking

372 and cation- π interactions. Similarly, TmTOP1 has a zinc ribbon motif, but
373 without a bound zinc ion, at its C-terminus (8). The deletion of the
374 carboxyl-terminal domain resulted in a significant loss of the relaxation activity
375 of TmTOP1, possibly due to the reduction in DNA-binding by the mutant
376 protein (44, 45). By comparison, the truncation of the carboxyl-terminal domain
377 almost abolished the relaxation activity of EcTOP3 (46), whose C-terminus
378 displayed a partially disordered structure (7, 14). Unlike EcTOP1, MtTOP1 has
379 four tandem novel folds, but not the zinc-binding motif, at the C-terminus (9).
380 Similar to EcTOP3, the partial truncation of the carboxyl-terminal domain
381 abolished the relaxation activity of MtTOP1. It is generally believed that the
382 carboxyl-terminal domains of these two topoisomerases have ssDNA-binding
383 activity, although they lack a zinc-binding motif. Our structure of Sso topo III
384 provides the first molecular view of the carboxyl-terminal domain of an
385 archaeal type IA topoisomerase (Fig. 1B). The carboxyl-terminal domain of
386 Sso topo III is unique, and harbours a zinc-binding motif (3Cys1His type) (Fig.
387 1D) that differs from the tetracysteine zinc-binding motif conserved in
388 prokaryotic type IA topoisomerases such as EcTOP1 (39). A structural
389 analysis of the EcTOP1-DNA complex demonstrated that a zinc ribbon domain
390 or zinc ribbon-like domain, mainly consisting of β -sheets, is capable of binding
391 ssDNA, implying that these domains may not bind double-stranded DNA. In
392 some enzymes involved in DNA transactions, the zinc-finger domains, which
393 contain α -helices, bind to double-stranded DNA (47). It will be interesting to

394 determine whether the carboxyl-terminal fold of Sso topo III can bind
395 double-stranded DNA. In Sso topo III, the zinc-binding motif is required for
396 efficient relaxation activity (Fig. 3 and 4). By comparison, TmTOP1 (44)
397 remained fully active in DNA relaxation when the zinc ribbon motif was deleted
398 while EcTOP1 (48) was inactive when the zinc ion was removed.

399 The first solved structure of a type IA topoisomerase indicates that domains
400 II and III may move away from domains I and IV in the strand passage reaction
401 (6). Further evidence for this movement has been provided by biochemical (19,
402 21), structural data (14, 15, 35) and single-molecule experiments (22-24). A
403 structural comparison between EcTOP3 and EcTOP1 indicates that there are
404 two unique insertions in EcTOP3 in the vicinity of its central hole (29) (Fig. 2A).
405 One insertion (the decatenation loop) is rich in basic amino acids within
406 domain IV of EcTOP3, and the other (the acidic loop) is abundant in acidic
407 amino acids and is located within domain II (2) (Fig. 2A). Decatenation by the
408 enzyme is driven primarily by the interaction between the decatenation loop
409 and the acidic loop, both of which line the central hole of the topoisomerase,
410 during its gate dynamics. Compared with the interaction in EcTOP1, that in
411 EcTOP3 presumably stabilizes and holds the open state for longer, thus
412 allowing sufficient time for EcTOP3 to catch duplex DNA and pass it through
413 the break in a single strand into the gate (24). In the eukaryotic Top3a-RMI
414 complex, RMI1 coordinates the gate dynamics of Top3a through its unique
415 decatenation loop, which has a functionally equivalent role to that of the

416 decatenation loop in EcTOP3 (11). Hence, a unified loop-mediated
417 decatenation mechanism for prokaryotic and eukaryotic topoisomerases III
418 has been proposed (11).

419 Our results demonstrated that the zinc-binding motif is essential for the DNA
420 decatenation activity of Sso topo III (Fig. 5C). We provided the following
421 evidence in support of the notion that the decatenation mechanism of Sso topo
422 III is distinct from the current loop-mediated decatenation mechanism
423 proposed for type IA topoisomerase: (i) The decatenation domain (domain V)
424 of Sso topo III is a novel zinc finger fold (Fig. 1D), whereas the decatenation
425 loop is present in domain IV of EcTOP3 (29) (Fig. 2A). (ii) The decatenation
426 domain (domain V) of Sso topo III is on the outer edge of the topoisomerase
427 gate and attaches to domain II through the hinge region by extensive
428 hydrophobic interactions (Fig. 1D and 2C), whereas the decatenation loop in
429 EcTOP3 lines the DNA gate and is away from the hinge region of the catalytic
430 core (24) (Fig. 2A). (iii) The 594ΔC mutant lacking domain V showed an
431 approximately 5-fold reduction in ssDNA-binding compared with wild-type Sso
432 topo III (Fig. S5). In contrast, the deletion of the decatenation loop from
433 EcTOP3 did not reduce its ssDNA-binding activity (29). Obviously, further
434 biochemical studies are needed to understand the molecular details of the
435 unlinking of covalently closed catenanes catalysed by Sso topo III.

436

437 MATERIALS AND METHODS

Plasmid and DNA constructs Using the standard PCR cloning strategy, Sso topo III gene from *S. solfataricus* genome was cloned into the *NdeI* and *XhoI* sites of the modified version of vector pET28a (Novagene), which harbored an N-terminal poly-histidine tag followed by a tobacco etch virus (TEV) protease cleavage site (ENLYFQ/G) (where ‘/’ donates the cleavage site). However, the growth of *E.coli* cells harboring wild-type Sso topo III gene were severely inhibited, possibly due to its toxicity. So, Sso topo III gene was mutated to the Sso topo III Y318F by replacing residue Tyr318 with Phe using the QuickChange™ Site-Directed Mutagenesis Kit (Stratagene) according to the instructions of manufacturer. Site-directed mutagenesis of the other Sso Topo III mutants used in this study was carried out according to modified QuickChange™ protocol (49). All the plasmid constructs and mutants were confirmed by DNA double strands sequencing. Primers for Sso Topo III site-directed mutagenesis are listed in Table S1.

Protein Expression and Purification The recombination Sso topo III Y318F was overexpressed as a fusion protein with an N-terminal poly-histidine tag in *E. coli* strain Rosetta2 (DE3) pLysS (Novagen). For each purification procedure, 10-l *E. coli* cultures harbouring fusion proteins were grown in the shaker at 37°C at 185 r.p.m. When their density reached an OD_{600nm} of 1.2, sodium citrate was added into cultures to a final concentration of 100 mM and temperature of the cultures dropped to 25°C, followed by the addition of isopropylb-D-thiogalactoside to a final concentration of 0.5 mM.

460 After growth for further 6 h at 25°C, the cultures were harvested, and the
 461 resulting cell sediments were suspended in a solubilization buffer containing
 462 20 mM Tris-HCl (pH 8.0), 2 mM potassium citrate, 2 mM MgCl₂, 10 mM
 463 imidazole and 500 mM NaCl supplemented with DNAase I (55 µg/ml,
 464 Amersco), followed by disruption with a French press at 12,000 p.s.i. for three
 465 cycles. To remove cell debris, cell lysates were centrifuged for 40 min at
 466 16,000 r.p.m. The resultant supernatant was collected and loaded onto an
 467 equilibrated Ni²⁺ Chelating Sepharose™ Fast Flow column (GE Healthcare).
 468 Purification procedure with the nickel column were performed as described
 469 before (50), except the insertion of an additional wash with high-salt of 1 M
 470 NaCl. After several wash, Sso topo III Y318F protein was eluted with a buffer
 471 containing 20 mM Tris-HCl (pH 8.0), 2 mM potassium citrate, and 300 mM
 472 NaCl supplemented with 300 mM imidazole. The eluate was digested using
 473 TEV protease to remove the poly-histidine tag, and dialyzed against buffer I
 474 (20 mM Tris-HCl, pH 7.5, 10 mM potassium citrate, 150 mM NaCl) overnight at
 475 4°C. The resultant eluate was filtered through a 0.45 µm filtration membrane
 476 and then loaded onto a 5 ml SOURCE 15S column (GE Healthcare)
 477 equilibrated with buffer I. The column was eluted with an 80 ml linear gradient
 478 from 150 mM NaCl to 500 mM NaCl, both in buffer I. The fractions, eluted at
 479 0.45-0.50 M NaCl, were pooled and concentrated to 4 mg ml⁻¹ in a buffer
 480 containing 20 mM Tris-HCl (pH7.5), 150 mM NaCl, 10 mM potassium citrate,

481 and 2 mM DTT for later usage. Purified protein was analysed by SDS-PAGE
482 for purity and quantified with Bradford assay using BSA as standards.

483 Expression and purification of the other Sso Topo III mutants (597ΔC and
484 CCCH/4A) were carried out according to the procedure described above, with
485 the exception of ion-exchange column chromatography in the final purification
486 step. The resultant nickel column eluate was loaded onto a 5 ml SOURCE 15Q
487 column (GE Healthcare), instead of 15S column, equilibrated with buffer I. The
488 column was eluted with an 80 ml linear gradient from 0.150 mM NaCl to 1.0 M
489 NaCl, both in buffer I. The fractions, eluted at 0.55-0.65 M NaCl, were pooled.

490 **Crystallization** Sso topo III Y318F was crystallized by the sitting-drop
491 vapor diffusion method, with 0.7μl drops of protein or protein-ssDNA complex
492 mixed with an equal volume of reservoir solution. Initial crystallization trials
493 screen clustered-needle-shaped crystals in reservoir solution containing 8%
494 (v/v) Tacsimate (pH 4.4), and 20% (w/v) Polyethyleneglycerol 3,350. Further
495 optimization of the crystallization condition led to the appearance of
496 rod-shaped crystals in the well in which 0.7μl protein solution was mixed with
497 an equal volume of reservoir solution consisting of 8% (v/v) Tacsimate (pH 4.4),
498 and 13.75% (w/v) Polyethyleneglycerol 3,350 at 12°C. The diffraction-level
499 crystals, by the microseeding method, appeared one week later and reached a
500 maximum size within 2 months. These crystals in the mother liquor were
501 exposed to air for 10 min, and transferred into a drop of paraffin and NVH mix
502 oil before being crycooled by plunging rapidly in liquid nitrogen.

503   **Crystallographic Data Collection, Structure determination**

504   **and Refinement** X-ray diffraction data were collected at the BL17U
 505   beamline of the Shanghai Synchrotron Radiation Facility, and indexed,
 506   integrated and scaled using the HKL2000 package (51). The initial phases
 507   were determined by molecular replacement using the program PHASER (52),
 508   with the structure of TmTOP1 (PDB ID: 2GAJ) as a searching model. The
 509   structure refinement was carried out with phenix.refine (53) and Refmac5 (54).
 510   Model building was carried out by iterative rounds of manual building with Coot
 511   (55). MolProbity (56) was used to validate the structure. Data collection and
 512   refinement statistics are listed in Table 1.

513   **Assays of decatenation and relaxation activities** DNA

514   relaxation assay was performed as described previously (30), except that the
 515   final concentration of $MgCl_2$ is 20 mM, instead of 10 mM, in the standard
 516   reaction mixture. Decatenation of kinetoplast DNA (kDNA) was carried out by a
 517   similar protocol except that 400 ng kDNA (www.topogen.com) was used as
 518   substrate in each standard reaction mixture. Reaction mixes were incubated at
 519   90 °C for 30 min unless specified temperature otherwise, and the
 520   enzyme-catalysed products were examined by electrophoresis in the indicated
 521   agarose gels.

522   **Data availability** Atomic coordinates and structure factors for the
 523   crystal structure of Sso topo (apo) have been deposited in the Protein Data
 524   bank under accession codes 6K8N.

525

526 **SUPPORTING INFORMATION**

527 Additional supporting information may be found online in the Supporting
528 Information section.

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548 **REFERENCE**

- 549 1. Wang JC. 1996. DNA topoisomerases. Annu Rev Biochem 65:635-92.
- 550 2. Champoux JJ. 2001. DNA topoisomerases: structure, function, and

- 551 mechanism. *Annu Rev Biochem* 70:369-413.
- 552 3. Vos SM, Tretter EM, Schmidt BH, Berger JM. 2011. All tangled up: how
553 cells direct, manage and exploit topoisomerase function. *Nat Rev Mol*
554 *Cell Biol* 12:827-41.
- 555 4. Pommier Y, Sun Y, Huang SN, Nitiss JL. 2016. Roles of eukaryotic
556 topoisomerases in transcription, replication and genomic stability. *Nat*
557 *Rev Mol Cell Biol* 17:703-721.
- 558 5. Forterre P. 2006. DNA topoisomerase V: a new fold of mysterious origin.
559 *Trends Biotechnol* 24:245-7.
- 560 6. Lima CD, Wang JC, Mondragon A. 1994. Three-dimensional structure
561 of the 67K N-terminal fragment of *E. coli* DNA topoisomerase I. *Nature*
562 367:138-46.
- 563 7. Mondragon A, DiGate R. 1999. The structure of *Escherichia coli* DNA
564 topoisomerase III. *Structure* 7:1373-83.
- 565 8. Hansen G, Harrenga A, Wieland B, Schomburg D, Reinemer P. 2006.
566 Crystal structure of full length topoisomerase I from *Thermotoga*
567 *maritima*. *J Mol Biol* 358:1328-40.
- 568 9. Tan K, Cao N, Cheng B, Joachimiak A, Tse-Dinh YC. 2016. Insights
569 from the Structure of *Mycobacterium tuberculosis* Topoisomerase I with
570 a Novel Protein Fold. *J Mol Biol* 428:182-93.
- 571 10. Cao N, Tan K, Zuo X, Annamalai T, Tse-Dinh Y-C. 2020. Mechanistic
572 insights from structure of *Mycobacterium smegmatis* topoisomerase I

- 573 with ssDNA bound to both N- and C-terminal domains. *Nucleic acids*
574 *research* 48:4448-4462.
- 575 11. Bocquet N, Bizard AH, Abdulrahman W, Larsen NB, Faty M, Cavadini S,
576 Bunker RD, Kowalczykowski SC, Cejka P, Hickson ID, Thoma NH. 2014.
577 Structural and mechanistic insight into Holliday-junction dissolution by
578 topoisomerase IIIalpha and RMI1. *Nat Struct Mol Biol* 21:261-8.
- 579 12. Goto-Ito S, Yamagata A, Takahashi TS, Sato Y, Fukai S. 2017.
580 Structural basis of the interaction between Topoisomerase IIIbeta and
581 the TDRD3 auxiliary factor. *Sci Rep* 7:42123.
- 582 13. Rodriguez AC, Stock D. 2002. Crystal structure of reverse gyrase:
583 insights into the positive supercoiling of DNA. *Embo j* 21:418-26.
- 584 14. Changela A, DiGate RJ, Mondragon A. 2001. Crystal structure of a
585 complex of a type IA DNA topoisomerase with a single-stranded DNA
586 molecule. *Nature* 411:1077-81.
- 587 15. Zhang Z, Cheng B, Tse-Dinh YC. 2011. Crystal structure of a covalent
588 intermediate in DNA cleavage and rejoining by *Escherichia coli* DNA
589 topoisomerase I. *Proc Natl Acad Sci U S A* 108:6939-44.
- 590 16. Berger JM, Fass D, Wang JC, Harrison SC. 1998. Structural similarities
591 between topoisomerases that cleave one or both DNA strands. *Proc*
592 *Natl Acad Sci U S A* 95:7876-81.
- 593 17. Brown PO, Cozzarelli NR. 1981. Catenation and knotting of duplex DNA
594 by type 1 topoisomerases: a mechanistic parallel with type 2

- 595   topoisomerases. *Proc Natl Acad Sci U S A* 78:843-7.
- 596   18.   Kirkegaard K, Wang JC. 1985. Bacterial DNA topoisomerase I can relax
- 597   positively supercoiled DNA containing a single-stranded loop. *J Mol Biol*
- 598   185:625-37.
- 599   19.   Li Z, Mondragon A, DiGate RJ. 2001. The mechanism of type IA
- 600   topoisomerase-mediated DNA topological transformations. *Mol Cell*
- 601   7:301-7.
- 602   20.   Cejka P, Plank JL, Dombrowski CC, Kowalczykowski SC. 2012.
- 603   Decatenation of DNA by the *S. cerevisiae* Sgs1-Top3-Rmi1 and RPA
- 604   complex: a mechanism for disentangling chromosomes. *Mol Cell*
- 605   47:886-96.
- 606   21.   Leelaram MN, Bhat AG, Godbole AA, Bhat RS, Manjunath R, Nagaraja
- 607   V. 2013. Type IA topoisomerase inhibition by clamp closure. *FASEB J*
- 608   27:3030-8.
- 609   22.   Dekker NH, Rybenkov VV, Duguet M, Crisona NJ, Cozzarelli NR,
- 610   Bensimon D, Croquette V. 2002. The mechanism of type IA
- 611   topoisomerases. *Proc Natl Acad Sci U S A* 99:12126-31.
- 612   23.   Terekhova K, Marko JF, Mondragon A. 2014. Single-molecule analysis
- 613   uncovers the difference between the kinetics of DNA decatenation by
- 614   bacterial topoisomerases I and III. *Nucleic Acids Res* 42:11657-67.
- 615   24.   Mills M, Tse-Dinh YC, Neuman KC. 2018. Direct observation of
- 616   topoisomerase IA gate dynamics. *Nat Struct Mol Biol* 25:1111-1118.

- 617 25. Liu LF, Wang JC. 1979. Interaction between DNA and Escherichia coli
618 DNA topoisomerase I. Formation of complexes between the protein and
619 superhelical and nonsuperhelical duplex DNAs. J Biol Chem
620 254:11082-8.
- 621 26. Tse Y, Wang JC. 1980. E. coli and M. luteus DNA topoisomerase I can
622 catalyze catenation of decatenation of double-stranded DNA rings. Cell
623 22:269-76.
- 624 27. DiGate RJ, Mariani KJ. 1988. Identification of a potent decatenating
625 enzyme from Escherichia coli. J Biol Chem 263:13366-73.
- 626 28. Terekhova K, Gunn KH, Marko JF, Mondragon A. 2012. Bacterial
627 topoisomerase I and topoisomerase III relax supercoiled DNA via
628 distinct pathways. Nucleic Acids Res 40:10432-40.
- 629 29. Li Z, Mondragon A, Hiasa H, Mariani KJ, DiGate RJ. 2000.
630 Identification of a unique domain essential for Escherichia coli DNA
631 topoisomerase III-catalysed decatenation of replication intermediates.
632 Mol Microbiol 35:888-95.
- 633 30. Dai P, Wang Y, Ye R, Chen L, Huang L. 2003. DNA topoisomerase III
634 from the hyperthermophilic archaeon Sulfolobus solfataricus with
635 specific DNA cleavage activity. J Bacteriol 185:5500-7.
- 636 31. Couturier M, Gadelle D, Forterre P, Nadal M, Garnier F. 2020. The
637 reverse gyrase TopR1 is responsible for the homeostatic control of DNA
638 supercoiling in the hyperthermophilic archaeon Sulfolobus solfataricus.

- 639 Molecular Microbiology 113:356-368.
- 640 32. Bizard AH, Yang X, Debat H, Fogg JM, Zechiedrich L, Strick TR,
641 Garnier F, Nadal M. 2018. TopA, the *Sulfolobus solfataricus*
642 topoisomerase III, is a decatenase. *Nucleic Acids Res* 46:861-872.
- 643 33. Capranico G, Marinello J, Chillemi G. 2017. Type I DNA
644 Topoisomerases. *J Med Chem* 60:2169-2192.
- 645 34. Schoeffler AJ, Berger JM. 2008. DNA topoisomerases: harnessing and
646 constraining energy to govern chromosome topology. *Q Rev Biophys*
647 41:41-101.
- 648 35. Feinberg H, Lima CD, Mondragon A. 1999. Conformational changes in
649 *E. coli* DNA topoisomerase I. *Nat Struct Biol* 6:918-22.
- 650 36. Morales R, Sriratana P, Zhang J, Cann IK. 2011. *Methanosarcina*
651 *acetivorans* C2A topoisomerase IIIalpha, an archaeal enzyme with
652 promiscuity in divalent cation dependence. *PLoS One* 6:e26903.
- 653 37. Holm L, Sander C. 1995. Dali: a network tool for protein structure
654 comparison. *Trends Biochem Sci* 20:478-80.
- 655 38. Yang J, Bachrati CZ, Ou J, Hickson ID, Brown GW. 2010. Human
656 topoisomerase IIIalpha is a single-stranded DNA decatenase that is
657 stimulated by BLM and RMI1. *J Biol Chem* 285:21426-36.
- 658 39. Tan K, Zhou Q, Cheng B, Zhang Z, Joachimiak A, Tse-Dinh YC. 2015.
659 Structural basis for suppression of hypernegative DNA supercoiling by
660 *E. coli* topoisomerase I. *Nucleic Acids Res* 43:11031-46.

- 661 40. Englund PT. 2014. A passion for parasites. *J Biol Chem* 289:33712-29.
- 662 41. Baker NM, Rajan R, Mondragon A. 2009. Structural studies of type I
663 topoisomerases. *Nucleic Acids Res* 37:693-701.
- 664 42. Chen L, Huang L. 2006. Oligonucleotide cleavage and rejoining by
665 topoisomerase III from the hyperthermophilic archaeon *Sulfolobus*
666 *solfataricus*: temperature dependence and strand annealing-promoted
667 DNA religation. *Mol Microbiol* 60:783-94.
- 668 43. Viard T, de la Tour CB. 2007. Type IA topoisomerases: a simple puzzle?
669 *Biochimie* 89:456-67.
- 670 44. Viard T, Lamour V, Duguet M, Bouthier de la Tour C. 2001.
671 Hyperthermophilic topoisomerase I from *Thermotoga maritima*. A very
672 efficient enzyme that functions independently of zinc binding. *J Biol*
673 *Chem* 276:46495-503.
- 674 45. Viard T, Cossard R, Duguet M, de La Tour CB. 2004. *Thermotoga*
675 *maritima*-*Escherichia coli* chimeric topoisomerases. Answers about
676 involvement of the carboxyl-terminal domain in DNA topoisomerase
677 I-mediated catalysis. *J Biol Chem* 279:30073-80.
- 678 46. Zhang HL, DiGate RJ. 1994. The carboxyl-terminal residues of
679 *Escherichia coli* DNA topoisomerase III are involved in substrate binding.
680 *J Biol Chem* 269:9052-9.
- 681 47. Krishna SS, Majumdar I, Grishin NV. 2003. Structural classification of
682 zinc fingers: survey and summary. *Nucleic Acids Res* 31:532-50.

- 683 48. Tse-Dinh YC. 1991. Zinc (II) coordination in Escherichia coli DNA
684 topoisomerase I is required for cleavable complex formation with DNA.
685 J Biol Chem 266:14317-20.
- 686 49. Liu H, Naismith JH. 2008. An efficient one-step site-directed deletion,
687 insertion, single and multiple-site plasmid mutagenesis protocol. BMC
688 Biotechnol 8:91.
- 689 50. Wang H, Liu X, Zhao J, Yue Q, Yan Y, Gao Z, Dong Y, Zhang Z, Fan Y,
690 Tian J, Wu N, Gong Y. 2018. Crystal structures of multicopper oxidase
691 CueO G304K mutant: structural basis of the increased laccase activity.
692 Scientific Reports 8:14252.
- 693 51. Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data
694 collected in oscillation mode. Methods Enzymol 276:307-326.
- 695 52. McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC,
696 Read RJ. 2007. Phaser crystallographic software. J Appl Crystallogr
697 40:658-674.
- 698 53. Afonine PV, Grosse-Kunstleve RW, Adams PD. 2005. A robust
699 bulk-solvent correction and anisotropic scaling procedure. Acta
700 Crystallogr D Biol Crystallogr 61:850-5.
- 701 54. Murshudov GN, Skubak P, Lebedev AA, Pannu NS, Steiner RA,
702 Nicholls RA, Winn MD, Long F, Vagin AA. 2011. REFMAC5 for the
703 refinement of macromolecular crystal structures. Acta Crystallogr D Biol
704 Crystallogr 67:355-67.

- 705 55. Emsley P, Cowtan K. 2004. Coot: model-building tools for molecular
706 graphics. *Acta Crystallogr D Biol Crystallogr* 60:2126-32.
- 707 56. Chen VB, Arendall WB, 3rd, Headd JJ, Keedy DA, Immormino RM,
708 Kapral GJ, Murray LW, Richardson JS, Richardson DC. 2010.
709 MolProbity: all-atom structure validation for macromolecular
710 crystallography. *Acta Crystallogr D Biol Crystallogr* 66:12-21.

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724 **TABLE AND FIGURES LEGENDS**

725 Table 1. Data collection and refinement statistics

Native Sso topo III	
Data collection	
Space group	P2 ₁
Cell dimensions	
a, b, c (Å)	110.7, 90.0, 156.2

α, β, γ (°)	90.0, 100.5, 90.0
Wavelength (Å)	0.98
Resolution (Å)	50-2.10 (2.14-2.10) ^a
Rmerge	0.057 (0.942)
$\langle I/\sigma(I) \rangle$	25.8 (2.0)
Completeness (%)	98.0 (94.5)
Redundancy	7.0
Refinement	
Resolution (Å)	50-2.10
No. reflections	171,737
Rwork/ Rfree	0.203/0.247
No. atoms	
Protein	21,559
DNA	-
ZN	4
Water	1020
B-factors	46.0

Rmsd bond length (Å)	0.008
Rmsd bond angle (°)	0.9
Ramachandran Plot	
Favoured (%)	97.8
Allowed (%)	2.2
Outliers (%)	0.0

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a. The values in parenthesis mean those of the highest resolution shell.

742

FIGURES LEGENDS

743

FIG 1 Overall structure of full-length Sso topo III in apo state.

744

A. Domain organization of Sso topo III. Sso topo III is composed of N-terminal

745

four domains (domain I-IV) and the unique carboxyl-terminal domain V.

746

Domains I-V are colored hotpink, green, cyan, orange, red, respectively.

747

B. C. Two views of Sso topo III. Domains I-V are colored as the legend to

748

Figure 1A. The close-up view (D) is highlighted by the solid box in B. Detailed

749 interactions (E and F) within the domains II/V interface is highlighted by the
750 solid box in C.

751 D. The close-up view shows the anomalous difference electron density map
752 (drawn in cyan mesh) around Zn (II) contoured at 15σ using the diffraction data
753 collected at the Zn absorption peak. The three Cys (C602, C605, C615) and
754 one His residue (H618) coordinating Zn (II) are shown in sticks. Zn (II) is
755 shown as a gray sphere.

756 E. Hydrophobic zipper between domain V residues from helix $\alpha 19$ and domain
757 II residues from the loop linking domain I and II. The hydrophobic interactions
758 are shown in space-filling presentations.

759 F. As in E but describing the corresponding hydrogen-bonding network. The
760 hydrogen bonds are shown as black dashed lines.

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763

764 FIG 2 Comparison of structural elements around the hinge region in Sso topo
765 III, EcTOP1, EcTOP3, HsTop3 α and HsTop3 β .

766 A. Overall structural comparison among Sso topo III (hotpink), EcTOP3 (green)
767 and HsTop3 α (cyan). For clarity, the RMI1 subunit of HsTop3 α complex (PDB
768 entry 4CHT) is not shown. Domain V of Sso topo III is indicated. The
769 decatenation loop and the acidic loop of EcTOP3 (PDB entry 1D6M) are
770 shown, and their movement during the gate dynamics is shown with opposing

771 arrows.

772 B. The close-up views around the hinge regions of HsTop3 α in Top3 α -RMI1
773 complex (PDB entry 4CHT), HsTop3 β in Top3 β -TDRD3 complex (PDB entry
774 5GVE) complexes, and EcTOP1, mainly showing the hydrophobic interactions.
775 The hydrogen bonds are shown as black dashed lines. For clarity, the ssDNA
776 molecule of EcTOP1 complex (PDB entry 4RUL) is not shown.

777 C. The close-up view around the hinge region of Sso topo III, showing the
778 hydrophobic interactions. For clarity, helix α 20 (residues 639-654) of Sso topo
779 III is not shown.

780

781 FIG 3 Relaxation activity of wild-type and mutant Sso topo III proteins.

782 pUC18 DNA was incubated at 75 °C for 30 min in relaxation reaction buffer (50
783 mM Tris-HCl (pH 8.8), 20 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA, 90 mM
784 sodium chloride, 30 μ gml⁻¹ BSA, and 12% (vol/vol) ethylene glycol)) with
785 wild-type Sso topo III (top panel), 597 Δ C (middle panel) and CCCH/4A (bottom
786 panel) at 500, 250, 62.5, 12, 6, 3, 1.5 and 0 fmol. Reactions were terminated;
787 the DNA products were separated in 1.4% agarose gels and visualized by
788 staining with ethidium bromide. Oc indicates open circle nicked or gapped
789 circular DNA; Sc denotes negatively supercoiled circular DNA; and Rel is the
790 relaxed topoisomers.

791

792 FIG 4 Time-course of DNA relaxation by wild-type and mutants Sso topo III

793 proteins.

794 DNA relaxation by wild-type Sso topo III (top panel), 597ΔC (middle panel) and
795 CCCH/4A mutant (bottom panel), were carried out over the indicated time
796 periods as described in the FIG 3. The molar ratio of protein to pUC18 DNA
797 was 1:1 in the reaction. Samples terminated at the indicated time-points.
798 Sample at 0-min point indicated untreated substrate pUC18 DNA. The reaction
799 products were analyzed in 1.4% agarose gels and visualized by staining with
800 ethidium bromide. Oc indicates open circle nicked or gapped circular DNA; Sc
801 denotes negatively supercoiled circular DNA; and Rel is the relaxed
802 topoisomers.

803

804 FIG 5 kinetoplast DNA (kDNA) decatenation by wild-type and mutants Sso
805 topo III proteins.

806 Decatenation assays were carried out as described for DNA relaxation assay
807 except that kDNA (400 ng) was used as substrate with wild-type Sso topo III,
808 597ΔC and CCCH/4A mutant at 1000, 500, 250, 62.5 and 12 fmol. The
809 reactions were performed at 90°C for 30 min and were terminated. Samples
810 were electrophoresed through a 2% agarose gel, and the DNA products were
811 visualized by staining with ethidium bromide. Lane CK corresponds to the
812 kDNA control incubated at 90°C without the enzyme. Minicircles denote
813 minicircle DNA released by Sso topo III.

814

815

FIG 1

FIG 2

A

C

B

FIG 3

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FIG 4

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FIG 5