Mammalian SMC3 C-terminal and Coiled-coil Protein Domains Specifically Bind Palindromic DNA, Do Not Block DNA Ends, and Prevent DNA Bending

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The C-terminal domains of yeast structural maintenance of chromosomes (SMC) proteins were previously shown to bind double-stranded DNA, which generated the idea of the antiparallel SMC heterodimer, such as the SMC1/3 dimer, bridging two DNA molecules. Analysis of bovine SMC1 and SMC3 protein domains now reveals that not only the C-terminal domains, but also the coiled-coil region, binds DNA, while the N terminus is inactive. Duplex DNA and DNA molecules with secondary structures are highly preferred substrates for both the C-terminal and coiled-coil domains. Contrasting other cruciform DNA-binding proteins like HMG1, the SMC3 C-terminal and coiled-coil domains do not bend DNA, but rather prevent bending in ring closure assays. Phosphatase, exonuclease, and ligase assays showed that neither domain renders DNA ends inaccessible for other enzymes. These observations allow modifications of models for SMC-DNA interactions.

Structural maintenance of chromosomes (SMC)† proteins are ubiquitous chromosomal components, evolutionary conserved from most prokaryotes to higher eukaryotes (for recent reviews, see Refs. 1–7). The four characteristic members of the SMC protein family, generally termed SMC1 to SMC4, are involved in several aspects of chromosome dynamics. They form heterodimers, which are contained in higher order multi-protein complexes serving specific biological functions. In eukaryotes, so far two types of heterodimers have been observed, SMC1/3 and SMC2/4. In prokaryotic organisms, the SMC proteins form homodimers that are crucial for successful chromosome partition during cell division (8–10). So far, four different biological roles have been assigned to eukaryotic SMC protein complexes. The two most common are chromosome condensation (11–13) and sister chromatid cohesion (14–16) during mitosis, exerted by complexes based on the SMC2/4 and the SMC1/3 heterodimers, respectively. In addition, SMC proteins function in sex chromosome gene dosage compensation in nematodes as an SMC2/4 heterodimer (17, 18), and, with SMC1/3 as necessary subunits of the protein complex RC-1, act in DNA recombination and repair reactions (19). Smc3p has been recently noted to also be required for meiotic sister chromatid cohesion and reciprocal meiotic recombination in yeast (20).

SMC proteins, with molecular masses between 110 and 170 kDa, share a unique structure that has been described in detail in a number of reviews (3–7). In brief, SMC proteins are characterized by two extended coiled-coil domains separated by a short hinge region of about 150 amino acids. The N- and C-terminal globular domains of about 100–150 amino acids are highly conserved and carry important motifs. The N-terminal domain contains an NTP binding motif (Walker A box; Ref. 21), which has been shown to bind the ATP analog azido-ATP (22), and the C-terminal domain contains a DBA box (21). While the N-terminal domain does not bind to DNA, the C-terminal domain does. The Saccharomyces cerevisiae SMC1p and SMC2p C-terminal domains have been analyzed in some detail with respect to their interaction with DNA (22). These domains show a strong preference of at least 100-fold for double-stranded DNA (dsDNA) substrates and a high specificity for such dsDNA molecules, which are able to adopt secondary structures. Synthetic cruciform DNA, as well as naturally occurring palindromic DNA sequences with the potential to form secondary structures, serve as the best substrates in gel shift experiments. Likewise, efficient competitors were scaffold-associated regions and budding yeast centromere DNA-derived fragments (22). Overall, there are good indications for a preference of yeast SMC C-terminal domains for specific DNA structures. Since studies on SMC binding to DNA are rather limited at present, many questions remain, for example whether the coiled-coil regions are directly involved in DNA binding and what effects SMC binding has on the substrate DNA.

Electron microscopy studies of the Bacillus subtilis SMC homodimer revealed that the two SMC molecules probably form an antiparallel dimer, with colocalization of the C and N termini at each end (8). The most frequently observed shape of the homodimer is that of a completely folded rod, but SMC molecules can also exist as extended rods of about 100 nm in length and partially folded dimers. This indicates that they can move around the central hinge. Such a structure, upon formation of a complex between SMC dimer and DNA, might allow bipolar attachment and formation of a flexible protein bridge between two DNA molecules (6, 7). Based on the high degree of evolutionary conservation, it is currently assumed, although not proven, that very similar structures are formed by the eukaryotic heterodimers.

Key to all currently known biological activities of SMC proteins are interactions with DNA. Therefore, we set out to study DNA binding and bending by the terminal domains of mammalian (bovine) bSMC1 and bSMC3 proteins. Since mutant studies on Schizosaccharomyces pombe SMC4/2 protein homologs Cut3/Cut14 indicated that the second coiled-coil region may be involved in interactions with DNA (23), we also in-
cluded the corresponding bovine SMC3 coiled-coil region protein in the analysis.

MATERIALS AND METHODS

Cloning, Overexpression, and Purification of bSMC1 and bSMC3 Protein Domains—Cloning of the full-length cDNAs for bSMC1 and bSMC3 was described before (24). For expression of individual SMC protein domains, the respective cDNA fragments were cloned into the appropriate vectors of the pQE30–32 series (Qiagen, Inc.), placing a His6 tag at the N terminus of the proteins. The bSMC1-N contains the N-terminal 200 amino acids (22.6 kDa), and the bSMC1-C contains the C-terminal amino acids 744–1232 (56.7 kDa) and thus contains most of the second coiled-coil region and the C-terminal domain. The bSMC3-N contains the N-terminal 150 amino acids (17.2 kDa), the bSMC3-C contains the C-terminal amino acids 1031–1216 (20.8 kDa), and the bSMC3 coiled-coil protein amino acids 638–801 (19 kDa). The positions of the domains relative to the full-length protein are indicated in Fig. 1.

Expression of the proteins was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside to logarithmically growing cultures of the respective Escherichia coli strains (host SG13009) and further incubation for 1.5 h at 37 °C. Lysis of the cells was achieved by lysozyme treatment, sonication in lysis buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, protease inhibitors (Complete tablet, Roche Molecular Biochemicals Inc.)), and one freeze-thaw cycle of the cell pellet. Only about one-fifth of the C-terminal proteins was soluble under native conditions. After centrifugation, the clear supernatant (fraction I) was applied to 5-ml nickel-nitritotrifluorocetate acid columns (Qiagen Inc.), and the proteins were eluted by a stepwise increase of imidazole (20, 40, 80, 160 mM imidazole in lysis buffer). Protein bSMC1-N eluted between 80 and 160 mM imidazole from the nickel column, was further purified on a 2-ml MacroQ FPLC (Bio-Rad) column after dialysis against buffer B (50 mM KCl, 20 mM Tris/HCl pH 7.5, at 4 °C, 0.5 mM dithiothreitol (DTT), 10% glycerol, protease inhibitors). Elution was performed with a gradient of 50–600 mM KCl in buffer B, and the homogenous protein eluted around 180 mM KCl. The bSMC1-C protein eluted in the 80 mM imidazole step and was diazoyzed against buffer B. Protein bSMC3-N eluted at 160 mM imidazole from the nickel column and was diazoyzed against buffer B before use. The bSMC3-C protein also eluted in the 80 mM imidazole step. The protein was further purified on a 112-ml Superdex75 FPLC gel filtration column (Amersham Pharmacia Biotech) in buffer C containing 20 mM EPPS, pH 7.4, 5% glycerol, 1 mM DTT, 100 mM KCl, and protease inhibitors as above. The protein eluted at about a 45-ml (40%) column volume, which indicates formation of aggregates (fraction III). Further purification was achieved on a MacroS FPLC column after 1:4 dilution of fraction III with buffer C without KCl. Elution was done with a gradient from 25 to 600 mM KCl in buffer C, and the protein was eluted around 200 mM KCl (fraction IV). The bSMC3 coiled-coil protein eluted from the nickel column in the 80 and 160 mM fractions, with the latter (fraction IV) containing less contaminants. For some experiments, the 160 mM fraction was further purified on a phenyl-Sepharose column after dialysis against buffer B with 1 mM NaCl, also used as loading buffer.

Fig. 1. Purification of the bSMC protein domains bSMC1-C, bSMC3-C, bSMC3 coiled-coil (cc), bSMC1-N, and bSMC3-N. Silver-stained SDS-polyacrylamide gels, loaded with the induced E. coli lysate (Ind. Lys., fraction I), the nickel column eluates (fraction II), and for bSMC3 C-terminal and bSMC3 coiled-coil with further chromatography fractions (fractions III and IV) as described under “Materials and Methods.” A diagram shows the domains relative to the full-length SMC protein.
buffer, and eluted by a gradient from 1000 to 10 mM NaCl in buffer B. The protein eluted in a broad peak between 400 and 100 mM NaCl (fraction III). The resulting protein fractions were analyzed by electrophoresis in reducing SDS-polyacrylamide (12%) gels and silver staining, and are shown in Fig. 1. Some proteins migrate at positions higher than expected from their molecular mass. Two or more independent protein preparations of each domain were used in the experiments, which were reproduced severalfold.

**Gel Retardation Assays—** These assays were performed in 10-μl reaction mixtures containing 0.03–0.05 ng (1000–1500 cpm) of 32P-labeled DNA in 20 mM HEPES (pH 7.5), 1 mM DTT, 100 μg/ml bovine serum albumin, and protein as indicated. After a 20-min incubation at room temperature, DNA-protein complexes were resolved by electrophoresis at 4 °C in nondenaturing 6% polyacrylamide gels in 0.5× TBE. Gels were fixed (30 min in 10% acetic acid, 10% ethanol), dried, and exposed for autoradiography. The following DNA substrates were used: a 422-bp AcoI M13mp18 DNA fragment, bp 5824–6261, a 139-bp polymerase chain reaction-generated M13mp18 DNA fragment, bp 6091–6230; and a 232-bp AcoII fragment from pUC19 (bp 1837–2059). Except for the 139-bp fragment, which was 5′-labeled with 32P, end-labeling through fill-in synthesis by Klenow DNA polymerase rendered all other ends blunt. In addition, the gel-purified, structure-tested cruciform DNA substrate CF101 (25), made by annealing of four 29-nt oligonucleotides (29 nt each) corresponding to the A strand and the complementary regions of the B and D strands of the cruciform DNA substrate were annealed, and, after 5′ 32P labeling, used as control substrates. Quantification of autoradiography signals was done using a Molecular Dynamics densitometer and area integration modes.

**DNA Ridge Closure** (Circularization) Assay for DNA Binding—A 5′-32P-labeled 189-bp EcoRI fragment from the Drosophila fz SAR region (26), previously shown to be efficiently bound by ySMC C-terminal proteins (22), was used. The assay was performed essentially as described (27–34). Reaction mixtures (10 μl) contained 20 mM HEPES (pH 7.5), 1 mM DTT, 100 μg/ml bovine serum albumin, 0.5–1 ng 189-bp fragment, and protein as indicated. After preincubation at room temperature for 20 min, DNA was digested by the addition of 0.1 volume of 10× T4 DNA ligase buffer (500 mM Tris-HCl, pH 7.5, 100 mM MgCl2, 10 mM DTT, 10 mM ATP, 250 μg/ml bovine serum albumin) and T4 DNA ligase (New England Biolabs Inc.) as indicated. After incubation for 45 min at room temperature, the reactions were stopped by heating to 65 °C for 15 min. For controls, some of the reactions were treated with 10 units of E. coli exonuclease III (New England Biolabs) at 37 °C for 30 min. A solution of stop solution (10% SDS, 50% glycerol, 0.25% xylene cyanol and bromphenol blue) was added, and the samples were electrophoresed in 6% nondenaturing polyacrylamide gels in 0.5× TBE buffer. After electrophoresis, the gels were fixed, dried, and exposed for autoradiography.

**Phosphatase, Exonuclease, and Ligase Assays—** To test the accessibility of DNA ends, the 5′-32P-labeled 189-bp fragment was preincubated with proteins as described above. The samples were then treated with calf intestinal alkaline phosphatase (New England Biolabs) at 37 °C for 30 min, and radioactivity of the trichloroacetic acid-precipitable product was measured.

For analyzing exonuclease degradation of the 139-bp DNA fragment, the DNA substrate was generated by polymerase chain reaction with 1 ng of M13mp18 DNA as template and inclusion of [α-32P]dATP in the reaction (oligonucleotide primers: 5′-GAGCGCAGCGCAATTAATG and 5′-CGTAACTCATGCATGCTGCATGCTG). The 139-bp DNA fragment was the only polymerase chain reaction product and was either gel-purified or purified by chromatography on a Sephadex G50 column. The specific activity was 2 × 107 cpm/μg DNA. Various amounts of SMC proteins were preincubated for 20 min at room temperature with 1 ng of the substrate as described for the gel retardation assay. One-tenth volume of 10× exonuclease III buffer (500 mM Tris-HCl, pH 8.0, 50 mM MgCl2) was added, and the mixture incubated for another 20 min at room temperature with 20 units of E. coli exonuclease III. An equal volume of formamide dye solution was added, the mixture was heated at 95 °C for 5 min, and products were analyzed by electrophoresis in 13% polyacrylamide, 8 μM urea gels. The gels were fixed, dried, and exposed for autoradiography.

For control of ligase activity in the presence of the SMC protein domains, various amounts of the proteins were preincubated with 1 ng of 32P-labeled substrate for 20 min at room temperature as described for the gel retardation assay. One-tenth volume of 10× T4 DNA ligase buffer and various amounts of T4 DNA ligase were added. After incubation for 45 min at room temperature, reactions were stopped by heating at 65 °C for 15 min. The samples were electrophoresed in 2% agarose gels in TAE buffer. After electrophoresis, the gels were dried onto DEAE paper and exposed for autoradiography.

**RESULTS**

**Mammalian SMC C-terminal Domains Bind DNA—** A previous study showed that the C-terminal but not the N-terminal domains of S. cerevisiae SMC1 and SMC2 proteins bind duplex DNA in gel shift assays. Single-stranded DNA (ssDNA) is only very weakly bound (22). We now investigated DNA binding of separate domains of mammalian bSMC1 and bSMC3 proteins. The proteins were cloned and overexpressed in E. coli, purified as described under “Materials and Methods” (Fig. 1), and tested in gel retardation assays. Results were quantified by densitometry. Direct comparison of the S. cerevisiae ySMC2-C domain and the bovine bSMC3-C in binding to a 230-bp dsDNA substrate derived from M13mp18 revealed similar binding efficiencies (Fig. 2). A slightly higher efficiency of the ySMC2-C was not reproduced with other preparations. The amount of DNA bound into the main DNA-protein complex (asterisk in Fig. 2) increased linearly upon titration of protein between 1 and 40 ng. With bSMC3-C, a second, minor band migrating at a lower position was observed, which increased in intensity only between 1 and 5 ng of protein in the reaction. Similar results were obtained with the bSMC1-C protein (not shown).

The yeast C-terminal SMC protein domains possess a preference to bind dsDNA substrates with secondary structures (22). We extended this observation by testing DNA binding of the mammalian bSMC1-C protein, which contains the C-terminal domain and a part of the second coiled-coil region (Fig. 1). A synthetic cruciform oligonucleotide DNA (CF101; Ref. 25) served as substrate in comparison with a linear double-stranded oligonucleotide derived from part of the same sequence (Fig. 3). The bSMC1-C protein, like the yeast SMC1 and SMC2-C terminal domains (22), binds with a preference of at least 40-fold to the cruciform DNA. Binding to the linear form was readily detectable only after prolonged autoradiographic exposure and resulted in a DNA-protein complex that migrated at a lower position in the gel (Fig. 3).

**Binding of the SMC Coiled-coil Domain to DNA—** In addition to the C-terminal domain, the bSMC1-C protein used in the experiments described above contains about three-quarters of the second coiled-coil region of SbSMC1. Earlier, mutant studies in S. pombe indicated that the second coiled-coil domain press...
ent in the SMC proteins Cut3 and Cut14, representing the SMC4 and SMC2 subtypes, interact with DNA (23). We therefore cloned a 170-amino acid stretch of the bSMC3 coiled-coil domain and purified the protein to test whether this region alone would bind DNA (Fig. 4A). In this experiment, increasing amounts of the C-terminal, the N-terminal, or the coiled-coil domain proteins were titrated into the binding reaction with a 139-bp M13mp18 dsDNA substrate derived from the 230-bp, palindrome-rich sequence. No DNA binding was seen with the bSMC3 N-terminal domain (and the bSMC1 N terminus; not shown), but both the coiled-coil and the C-terminal proteins bound the substrate. Increasing the amount of the C-terminal and coiled-coil domain increased the amount of DNA-protein complex. For the coiled-coil domain, the increase was linear up to 97 ng, as determined by densitometry. There was no indication for cooperative binding of the C-terminal or the coiled-coil domain. The main binding products (asterisk in Fig. 4) consisted of two bands running close to each other with the bSMC3 coiled-coil protein and one band with the bSMC3 C-terminal protein. Two less prominent protein-DNA complexes at lower positions were observed with both the C-terminal and the coiled-coil domain. The S. cerevisiae SMC2 C-terminal, bSMC3 C-terminal, and the bSMC3 coiled-coil proteins were similarly tested for DNA binding on various substrates of different length, including a larger (422-bp M13mp18) dsDNA fragment (Fig. 4B). Binding resulted again in one prominent band for the C-terminal proteins and two prominent bands for the coiled-coil domain, besides one band at a lower position. The nature of the minor band at lower positions, seen in Figs. 2 and 4, is unknown. Since we have not observed degradation of the DNA substrate or the protein in any of these and the following experiments, possible explanations may invoke either DNA structure or protein structure alterations upon binding.

The C-terminal domains of yeast SMC proteins have been shown to bind dsDNA with at least 100-fold preference over ssDNA. We observed the same preference for the mammalian C-terminal and coiled-coil domains (Fig. 5A). The bSMC3 C-terminal or coiled-coil proteins were incubated with 0.05 ng of the labeled 230-bp M13 DNA fragment, and increasing amounts of either unlabeled double-stranded or single-stranded full-length M13mp18 competitor DNA was added. While there was efficient competition with the dsDNA at 0.05 ng, much less competition was observed with up to 80 ng of ssDNA. Densitometric scanning showed the duplex DNA preference of both proteins to be several hundred-fold.

Since the C-terminal domains of yeast and mammalian SMC proteins bind preferentially cruciform DNA, or DNA substrates with palindromes and a high potential to form secondary structures such as stem loops, we tested whether the coiled-coil domain also shows this preference. Toward this end, we directly compared binding of the bSMC3 C-terminal and coiled-coil domain proteins to either the 230-bp M13 mp18 DNA substrate rich in palindromic sequences, or a 232-bp DNA substrate without palindromic sequences derived from a pUC plasmid (22) (Fig. 5B). Like for the C-terminal domain, the coiled-coil domain binds the palindromic, potentially secondary structure-rich DNA with high preference. A corresponding, weak signal was obtained from the pUC DNA substrate only after long exposure (not shown). Quantitation determined the preference for the 230-bp substrate to be at least 80-fold. While the possibility cannot be excluded that features other than the palindromic nature of the M13 DNA fragment such as an
unknown, unusual DNA structure or a particular DNA sequence are (co-)responsible for the preferential binding, this seems less likely. Both DNA fragments have a very similar G/C content (M13, 51.7%; pUC, 49.8%), about the same length, and no obvious sequence motifs except the palindromes. Neither here nor in earlier DNA binding experiments (22) was any sequence specificity observed. As noted earlier, the coiled-coil domain produced two protein-DNA complexes, which run closely together in the gel, besides a weak band at a lower position.

**SMC Protein Domains neither Bend DNA nor Block DNA Ends**—Proteins that bind DNA, however, can efficiently support intramolecular ligation, i.e. circle formation, of such fragments. We have used a 189-bp DNA fragment, which was derived from the Drosophila ftz SAR region (26), has been shown to be a good binding substrate for yeast SMC protein domains (22), and is readily bound by bSMC proteins as well (not shown). The circular ligation product can be observed by gel electrophoresis. Some preparations of this gel-purified, labeled DNA fragment contained a small amount of a larger DNA fragment, which did not react in any of the bending assays. The 189-bp substrate, because of its relatively large length, allows limited circularization by T4 DNA ligase without any other protein added. Thus, stimulatory and inhibitory effects can be observed. The identity of the circle product was confirmed by treatment with *E. coli* exonuclease III (Fig. 6). The product appeared only if T4 DNA ligase was added, and it was not degraded by exonuclease III treatment. The linear 189-bp DNA fragment, however, was completely degraded by the nuclease. We have used HMG1 protein as a positive control, omitted additional protein in the negative control, and tested the bSMC3 coiled-coil domain for the ability to promote circularization of the 189-bp DNA fragment. Without the addition of the coiled-coil domain, the ligase ligated a modest fraction of the 189-bp DNA substrate, increasing up to 3-fold with more ligase added (Fig. 7A). Without DNA ligase added, no circle formation was observed. The addition of 20 ng of HMG1 protein significantly increased the yield of ligated product at all ligase concentrations (up to 7-fold), similar to what has been described (32). To test the effect of the bSMC3 coiled-coil domain, increasing units of T4 DNA ligase were added to reactions containing two different amounts of the protein. The addition of the bSMC3 coiled-coil domain, however, caused the opposite: the amount of ligated product decreased to 0.3-fold. Less coiled-coil protein added (35 ng instead of 70 ng) yielded about 0.6-fold the amount of ligated product if compared with the control. There was only a minor increase in circles at the highest amount of ligase added.

Similarly, an independent preparation of *S. cerevisiae* SMC2 C-terminal protein, described in Ref. 22, did not promote bending but rather inhibited ring closure in a concentration-dependent manner (Fig. 7B). Increasing amounts of the protein titrated into the reaction almost completely abolished the

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**Fig. 5.** A, competition of binding of the bSMC3 coiled-coil protein to the 230-bp M13mp18 dsDNA fragment by either dsDNA or ssDNA. 140 ng of each protein was incubated with the labeled dsDNA substrate and increasing amounts of unlabeled competitor DNA, either full-length double- or single-stranded M13mp18 as indicated. B, binding of bSMC3 protein domains to palindromic and nonpalindromic DNA. Two amounts of either bSMC3 coiled coil or bSMC3-C were incubated with either the highly palindromic 230-bp M13 dsDNA or the 232-bp nonpalindromic pUC DNA fragment and analyzed by a gel retardation assay. –, no protein added.

**Fig. 6.** Ring closure assay product control. The 189-bp, end-labeled DNA fragment was incubated with various amounts (in units (U)) of T4 DNA ligase, and the reaction products were treated for 30 min at 37 °C with exonuclease III. C, ExoIII-resistant circular molecules; l, linear substrate.
generation of circles. Two amounts (40 and 80 ng) of the bSMC3-C protein were also tested in the circularization assay (Fig. 7C). The protein showed a similar inhibitory effect, which was dependent on protein concentration, and was at the 40 ng amount only partially alleviated by adding more units of the ligase. The efficiency of this anti-bending effect was similar to that of the bSMC3 coiled-coil protein used in parallel. In contrast, 32 ng of HMG1 protein rendered a large fraction of the substrate ligatable into the circular form.

To rule out nonspecific effects (i.e., effects not directly related to DNA bending), it is important to control for the accessibility of the DNA ends and the activity of the DNA ligase in these assays. For testing the accessibility of the ends, we added calf intestinal alkaline phosphatase to the complete reaction mixture. If the bSMC3 coiled-coil protein would bind to and block the ends, the 5'-32P would be resistant to the treatment. There was, however, efficient phosphate removal with or without bSMC3 coiled-coil protein in the reaction mixture (Table I).

While the phosphatase assay revealed accessibility of the 5' phosphate end, we also tested for accessibility of the nucleotides at the 3' ends. For this purpose, we used *E. coli* exonuclease III, which has a 3'-5' exonuclease activity involved in processing ends in certain DNA repair pathways (37). The 139-bp DNA substrate was uniformly internally labeled by inclusion of [$\alpha$-32P]dATP in a polymerase chain reaction used to synthesize the fragment. Therefore, DNA fragments shortened by exonuclease III remain visible in denaturing gel electrophoretic analysis. Various amounts of bSMC3 coiled-coil domain were preincubated with the DNA substrate, and then the nuclease was added (Fig. 8A). While mock-treated DNA appeared as a single band, the DNA, preincubated without the bSMC3 coiled-coil domain, was efficiently degraded by the exonuclease. Preincubation with the protein, however, rendered the DNA partially resistant in a manner dependent on protein concentration. The highest concentration (60 ng of bSMC3 coiled-coil) yielded a major degradation product of approximately 84 nt, 20 ng produced DNA fragments of ~84 and 73 nt, and 2 ng did not protect sufficient amounts of DNA to give a signal. Protection of one end of the substrate would allow the exonuclease to degrade one strand, while the other would remain full-length. Since there was very little of the input 139-nt DNA fragments left, this indicates that degradation took place at each end and thus removed about 30 nt. Similarly, the bSMC3 C-terminal protein protected the DNA substrate. Besides some full-length DNA, fragments of about 85 and 63 nt were generated. Since the masses of the two proteins do not differ much (20.8 and 19 kDa), the different length of the smaller protected fragments may be the consequence of different binding characteristics. Together, the DNA ends are accessible to other enzymes and not covered by the SMC protein.

The activity of T4 DNA ligase in the presence of the bSMC3 coiled-coil or C-terminal domains was assayed by incubating end-labeled DNA fragments with T4 DNA ligase and increasing amounts of the coiled-coil or C-terminal domain. One DNA fragment contained two incompatible cohesive ends to prevent intramolecularly ligated without the help of a bending protein. To efficiently resolve multimeric ligation products, the products were analyzed in 2% agarose gels. The identities of the circular and linear products were confirmed by exonuclease III treatment (not shown). While HMG1 efficiently facilitated circularization, the bSMC3 coiled-coil and C-terminal proteins did

### Table I

| Reaction conditions | Radioactivity in DNA pellet cpm |
|---------------------|---------------------------------|
| Untreated DNA       | 4590                            |
| With CIAP (0.05 units) | 170                         |
| With HMG1 (30 ng)   | 4000                            |
| With HMG1 (15 ng), CIAP (0.05 units) | 160                     |
| With HMG1 (30 ng), CIAP (0.05 units) | 150                     |
| With bSMC3-cc (70 ng) | 4720                         |
| With bSMC3-cc (35 ng), CIAP (0.05 units) | 170                     |
| With bSMC3-cc (70 ng), CIAP (0.05 units) | 160                     |

The 5'-32P-labeled 189-bp DNA was preincubated with or without protein (bSMC3-coiled-coil or HMG1) as indicated and then treated with calf intestinal alkaline phosphatase (CIAP) as described under “Materials and Methods.” The DNA was precipitated by trichloroacetic acid, and the radioactivity of the precipitate was measured.
not. Since there is no residual circle formation by the ligase alone, anti-bend activity cannot be assessed with this rather short substrate. The fragment became intermolecularly ligated into a dimer and higher oligomers in the presence of the bSMC3 coiled-coil, the C-terminus, or the HMG1 protein. Thus, the activity of T4 DNA ligase is not affected by the presence of SMC protein domains. This also confirms that these proteins do not render DNA ends inaccessible to other enzymes.

**DISCUSSION**

In this paper on mammalian SMC protein domains, we report hitherto undescribed interactions with DNA: (i) the ability of the coiled-coil region alone to bind DNA, (ii) the strong preference of this interaction for duplex DNA and DNA substrates containing secondary structures, (iii) the inability of the coiled-coil and the C-terminal domains to bend DNA, but rather to inhibit bending, and (iv) the absence of a DNA binding activity that blocks DNA ends.

An indication for an interaction of coiled-coil domains with DNA was revealed in mutant studies of *S. pombe* Cut3/Cut14 SMC proteins (SMC4/2 homologs). These proteins support reannealing of DNA complementary strands, but mutations within the coiled-coil regions of Cut3 (at amino acid 1147) and Cut14 (at amino acid 861) significantly reduced the activity of the heterodimer in that *in vitro* assay (23). Our use of the bSMC1-C protein, which contains both the C-terminal domain and a large part of the adjacent coiled-coil region, indicated that the coiled-coil region at least does not inhibit DNA binding by the C terminus. Testing an isolated stretch of the bSMC3 coiled-coil region, which is relatively far apart from the C-terminal domain, showed that the coiled-coil region alone binds DNA and thus contributes to the DNA binding properties of full-length SMC3 protein. Binding of the yeast and bovine C-terminal and coiled-coil SMC proteins was observed with DNA substrates ranging from 139 to 422 bp in length. Double-stranded DNA served far better as a competitor than ssDNA. For their biological function in sister chromatid cohesion, binding of SMC1 and SMC3 proteins to newly synthesized daughter DNA duplexes, and not to single-stranded regions in DNA seems logical. Since none of the DNA binding activities of SMC protein domains investigated so far were unique for any particular type of SMC1, SMC2, or SMC3, one may speculate that the properties reported here are of a general nature for the eukaryotic SMC protein family. The high degree of conservation between eukaryotic SMC proteins supports such a hypothesis. Differences contributing to the distinct biological roles of the two SMC heterodimers may for example be reflected in hitherto unknown characteristics of the SMC proteins themselves, the heterodimers, or the holocomplexes.

Proteins like those of the HMG family have been repeatedly shown to bind preferentially to secondary structured DNA, and to efficiently bend DNA in ring closure assays (28–35). In contrast, the SMC protein domains, although displaying a very similar DNA substrate binding specificity, do not promote circularization of a 189- or 123-bp DNA fragment and thus appear

**Fig. 8.** Exonuclease and ligation activity control for the DNA ring closure assay. A, exonuclease treatment of the 139-bp, internally labeled DNA fragment after preincubation with or without bSMC3 C-terminal (3-C) or coiled-coil (3-cc) protein as indicated. B, ligation of a 230-bp fragment with different cohesive ends in the presence or absence of bSMC3 C-terminal or coiled-coil protein as indicated. C, ligation of a 123-bp fragment with identical cohesive ends in the absence of added protein or the presence of HMG1 or bSMC3 coiled-coil or C-terminal protein. Analysis of products in B and C was in 2% agarose gels. C, circular DNA; d, dimer; l, linear fragment.

**Fig. 9.** Model for binding of the SMC1/3 heterodimer to the two sister chromatids. The folded heterodimers’ length is from Ref. 8. C/N and N/C indicate the presence of the two terminal domains at each end of the heterodimer. cc, coiled coil domain; H, hinge domain. A, binding to the sister chromatids only through the C-terminal domains of the SMC heterodimer; B, extended binding of the sister chromatids by the coiled-coil and C-terminal regions; C, a DNA double strand break in one sister chromatid. The sister chromatids and thereby the ends at the double strand break are held in position to facilitate recombinational repair. Besides their structural role, the SMC proteins may be actively involved in the repair reaction.
not to bend DNA. Moreover, they prevent bending and may stabilize an extended, rod-like, linear structure of the DNA. Since such a negative result may have been caused by nonspecific factors, we included several controls, which showed that the ends of the linear DNA fragment are not blocked by the SMC proteins and are accessible to proteins and that the DNA ligase is active in the presence of the various SMC protein preparations. It may be noted that, in the mammalian multiprotein complex RC-1, the SMC1 and SMC3 proteins associate with a DNA ligase, which is also active in the presence of the two SMC proteins (38). The likely ability of SMC proteins to promote straightening of DNA seems to be evolutionary conserved, since the C-terminal and the coiled-coil region of bSMC3 and the S. cerevisiae SMC2-C protein behaved similarly. For the function of the SMC1/3 heterodimer in sister chromatid cohesion, such straightening of DNA may be advantageous for local linear juxtaposition of the two duplexes. Avoiding bends and kinks may also reduce steric hindrances not only for establishing sister chromatid cohesion but also in subsequent chromosome condensation steps. An overbent α-form with ends protruding into distance too far apart to be ligated may be an alternative structure generated in the ring closure assay. However, not only would this require a very high degree of bending especially of the 123-bp substrate, but also the known sequence-independent bending proteins are active in the ring closure assay regardless of their ability to overbend and form loops (39).

The preference of SMC C-terminal and coiled-coil domains for DNA that has the potential to form secondary structures (i.e., palindromic DNA) or for synthetic cruciform DNA substrates appears to be also conserved from yeast (22) to mammalian SMC proteins. Together, this specificity was observed for the C-terminal and coiled-coil domains of SMC1, SMC2, and SMC3 proteins, which at least in part represent the two biologically relevant heterodimers SMC1/3 and SMC2/4. It is remarkable that such differently structured domains as the globular C terminus and the coiled-coil domains display similar DNA interaction specificities. How can the preferential binding to secondary structures be reconciled with the anti-bending activity of the proteins? Removing bends and straightening DNA may happen to just such DNA secondary structures, as the equilibrium gets shifted toward the nonfolded, linear structure at a palindromic sequence. Thus, targeting the anti-bend protein SMC to secondary structures may further help in establishing a straight DNA configuration, more amenable to sister chromatin cohesion, chromosome condensation, and perhaps DNA recombination.

As observed in the phosphatase, exonuclease, and ligase activity control assays in the bending experiments, the C-terminal and coiled-coil proteins do not interfere with the accessibility of dsDNA ends such as the 5’ protruding or blunt ends used in these assays. Thus, the SMC3 protein is not a DNA end-protecting protein. Limited protection of a stretch of interhelical DNA may happen to just such DNA secondary structures, as it holds the two sister chromatids closely together and keeps the DNA ends at such a break in position (Fig. 9C). This may support the initiation of recombination and repair of a double-strand break, which may have arisen during DNA replication or independent thereof (40). It remains possible, as has been proposed (19, 20), that the SMC proteins play both a structural and an active role in recombination and repair reactions. In any case, it will be important in future studies to determine how the DNA is bound and acted upon by full-length SMC proteins, the heterodimers, or holocomplexes.

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