The CSB Protein Actively Wraps DNA*

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Nancy Beerens‡§, Jan H. J. Hoeijmakers‡®, Roland Kanaar‡¶, Wim Vermeulen‡¶,
and Claire Wyman‡¶

From the Department of Cell Biology and Genetics and Radiation Oncology, Erasmus Medical Center,
P. O. Box 1738, 3000 DR Rotterdam, The Netherlands

The CSB protein is a member of the SWI2/SNF2 family of ATP-dependent chromatin remodeling factors and is essential for transcription-coupled DNA repair. The role of CSB in this DNA repair process is unclear, but the protein was found to remodel nucleosomes and alter DNA double helix conformation upon binding. Elucidating the nature of the change in DNA structure induced by CSB is of great interest for understanding the CSB mechanism of action. We analyzed the CSB-DNA complex by scanning force microscopy and measured a shortening of DNA contour length upon CSB binding in the presence of ATP. This DNA length reduction most likely results from DNA wrapping around the protein. Shorter DNA molecules were observed more frequently in the presence of non-hydrolyzable ATP analogues. These results suggest that DNA wrapping depends on ATP binding, whereas ATP hydrolysis results in unwrapping. We also provide evidence suggesting that CSB binds DNA as a dimer. DNA wrapping and unwrapping allows CSB to actively alter the DNA double helix conformation, which could influence nucleosomes and other protein-DNA interactions.

In the eukaryotic cell nucleus, DNA is packaged in the highly compact structure of chromatin. DNA processing events in the nucleus, such as transcription, replication, recombination, and repair, are restricted by this packaging. To facilitate the interaction of proteins with nucleosomal DNA, a variety of chromatin remodeling factors use the energy of ATP hydrolysis to locally alter chromatin structure (1, 2). ATP-dependent chromatin remodeling complexes are characterized by the presence of an ATPase subunit from the SWI2/SNF2 family. Because human Brg1 and hBrm and Drosophila ISWI have nucleosome remodeling activity on their own (3, 4), the ATPase subunits are suggested to be the catalytic core of the remodeling complexes. All SWI2/SNF2 proteins contain seven conserved motifs, similar to those found in DNA/RNA helicases. Members of this family possess DNA-stimulated ATPase activity but no classical helicase activity. The current evidence suggests that these factors use the energy of ATP hydrolysis to generate torsion in DNA and alter local DNA conformation (1, 5, 6). How remodeling enzymes generate torsion is not known, but rotation of the DNA helix by DNA tracking activity or a molecular wrench action has been proposed (1, 5, 7). This may alter nucleosome structure by rotation of DNA at the nucleosomal entry/exit sites, causing its over- or underwinding and/or looping on the nucleosome surface. Interestingly, many mechanistically distinct DNA repair pathways include SWI2/SNF2 proteins, such as CSB for transcription-coupled repair (TCR),1 Rad5 for postreplication repair, Rad16 for global genome repair, and Rad54 for recombination repair. This suggests that nucleosome remodeling is an essential aspect of many DNA repair pathways. Apparently, remodeling of chromatin structure during repair of distinct lesions cannot be provided by a universal component. Therefore, the SWI2/SNF2 proteins in each DNA repair pathway may provide additional pathway-specific functions.

The TCR pathway of nucleotide excision repair is responsible for the rapid repair of transcription-blocking lesions in DNA (8). The CSA and CSB proteins are specifically required for TCR, although their exact function in this process remains elusive. Inherited defects in these genes lead to Cockayne syndrome in humans (9). Several studies suggest that the Cockayne syndrome proteins have a subtle additional role in transcription (10, 11). The elongating RNA polymerase II (pol II) complex that encounters a lesion is thought to detect damage and to activate the repair machinery in a CSA/CSB-dependent manner. However, the stalled pol II may constitute a steric hindrance for the repair machinery and likely needs to retract or dissociate to allow repair of the damage (12). Some aspects of TCR are highly conserved from Escherichia coli to yeast and mammals (13). In E. coli, the transcription-repair coupling factor (TRCF) displaces the DNA polymerase stalled at a lesion and stimulates TCR by recruiting the repair machinery. A similar activity was proposed for CSB (14) because of its limited sequence similarities with TRCF. In agreement with this possible function, CSB was found to associate with pol II (15, 16). Because CSB is a member of the SWI2/SNF2 family (17), it was also suggested to be involved in chromatin remodeling at the damage site. CSA has many of the activities expected for a SWI2/SNF2 protein; it has DNA-stimulated ATPase activity but is not a classical helicase (18, 19). CSB remodels nucleosomes at the expense of ATP hydrolysis and interacts with core histones directly in vitro (20). In addition, CSB alters DNA double helix conformation upon binding. The mechanism by which CSB remodels nucleosomes is unknown, but by changing DNA conformation, CSB may disrupt the histone-DNA interactions, as well as the interaction of stalled pol II with damaged DNA. Resolving the nature of this CSB-induced change in DNA conformation will therefore provide important information on...
the mechanism of CSB action in both DNA repair and gene transcription.

CSB alters DNA conformation as detected in assays that fix and report changes in DNA linking number (Lk) (20). Lk is a topological parameter of double-stranded DNA that describes the number of times that the two strands of the DNA double helix wind around each other. Topoisomerases change DNA Lk directly by breaking, passing, and rejoining of DNA strands, but this activity has not been detected for CSB or other SWI2/SNF2 family members. The change in DNA conformation induced by CSB can be caused by the protein constraining twist or writhe. Proteins can affect writhe by wrapping the DNA around their surface (21), such as in nucleosomes (22). Proteins can affect twist by stretching the DNA helix in a protein filament (23), such as the recombinases RecA and Rad51 (24). Finally, the change in DNA conformation may result from the introduction of unconstrained supercoils in the DNA by a protein translocation mechanism. This was suggested for the yeast SWI2/SNF2-like remodeling enzyme Rsc (25) and for Rad54 (26, 27). However, the different mechanisms that induce changes in DNA conformation by affecting twist or writhe are not easily distinguished in bulk biochemical assays. They do result in architecturally different DNA-protein complexes and therefore can be distinguished by direct observation with scanning force microscopy (SFM). Analysis of DNA conformational changes and direct imaging of CSB-DNA complexes by SFM suggest that CSB constrains writhe by a DNA wrap. Wrapping of DNA was found to depend on ATP binding, whereas the constrained wrap is apparently lost upon ATP hydrolysis. This active DNA wrapping and unwrapping by CSB is discussed as a mechanism to disrupt nearby protein-DNA interactions.

**EXPERIMENTAL PROCEDURES**

**DNA Substrates**—The plasmid pDER1 (1.8 kb) was described previously (27). This plasmid was singly nicked in a 30-μl reaction containing 0.5 μg of DNA, 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM MgCl2, 360 μg/ml etidium bromide, and 1 μg/ml DNAse I at 30 °C for 30 min. The reaction was stopped by the addition of 0.1 volume of stop mix (5% SDS, 50 mM EDTA, 30 μg/ml proteinase K) and incubation at 65 °C for 30 min. DNA was purified by chloroform/phenol extraction, ethanol precipitation performed in H2O, and redissolved in H2O. The nicked plasmid substrate was generated by digestion of plasmid pDER1 with the PvuI restriction enzyme (Roche Applied Science) and purified using the GFX-PCR purification kit (Amersham Biosciences).

**Proteins**—The recombinant epitope-tagged CSB protein (HA-CSB-his6) was overexpressed using the baculovirus expression system and purified using the FLAG-agarose (Sigma) at 4 °C overnight. The FLAG-agarose was washed with H2O (glass-distilled, Sigma) and dried with a stream of filtered air. The purified CSB protein complexes were imaged in air at room temperature and humidity using a Nanoscope IIIa (Digital Instruments) operating in tapping mode with a type E scanner. Images were collected as 2 × 2 μm scans (512 × 512 pixels). The raw data were processed only by flattening to remove background slope using Nanoscope software. Silicon tips (Nanoprobes) were obtained from Digital Instruments.

**Immunoprecipitation**—DNA contour length was measured from SFM images using the Alex toolbox (29) in Matlab (The Math Works, Inc.). DNA contours were manually traced and subsequently smoothed. For DNA-protein complexes, the contour length was traced as the shortest possible DNA path through the bound protein. The volume of the DNA-bound protein complexes was measured from SFM images imported into Image-SXM (National Institutes of Health Image version modified by Steve Barrett, Surface Science Research Center, University of Liverpool, UK). The protein was manually traced, and its area and average height were measured, as described previously (30). A background volume determined from the same size area, including a segment of DNA, was subtracted. Volume measurements are given in arbitrary units and are used only to compare the relative sizes of proteins.

**RESULTS**

**CSB Changes DNA Double Helix Conformation Depending on ATP Binding**—The ability of CSB to change DNA conformation was demonstrated in topological assays. Singly nicked plasmid DNA was incubated with the purified CSB protein (19, 20), and subsequently, the nick was closed by the addition of DNA ligase. Ligation will fix changes in DNA conformation induced by the protein. Protein-induced changes in DNA conformation, evident as changes in Lk after ligation, were detected as changes in the electrophoretic mobility of the DNA. Ligation of the nicked plasmid in the absence of CSB resulted in three major topoisomers (Fig. 1, lane 1). In the absence of ATP, addition of 60 or 90 ng of CSB induced a subtle shift in the DNA topoisomer distribution (Fig. 1, lanes 2 and 3). The intensity of the lower band was decreased, whereas the intensity of a fourth slower migrating topoisomer was increased. In the presence of ATP, CSB induced
a more apparent shift in the distribution of the topoisomers, as the intensity of the fourth slower migrating topoisomer was further increased (Fig. 1, lanes 4 and 5). Interestingly, in the presence of CSB and the non-hydrolyzable ATP analogue AMP-PNP, the three major topoisomers were completely shifted toward slower migrating species (Fig. 1, lanes 6 and 7). The chloroquine included in the gel caused positive supercoiling of DNA. In the presence of ATP, CSB shifted the topoisomer population toward less positively supercoiled topoisomers, indicating that CSB introduced negative supercoiling (Fig. 1, lanes 4 and 5). In the presence of CSB and AMP-PNP, the DNA topoisomer distribution was dramatically shifted. All positive supercoiling introduced in the DNA by chloroquine in the gel was eliminated, resulting in negatively supercoiled topoisomers, which have a different mobility compared with the positively supercoiled topoisomers (Fig. 1, compare lanes 4 and 5 to 6 and 7). At lower protein to DNA ratios (Fig. 1, lanes 12–14), CSB introduced less negative supercoiling. On average, two negative supercoils were introduced at an approximate molar ratio of 7 CSB monomers/1 DNA molecule of 1.8 kb (Fig. 1, lane 14; 20 ng of CSB) in the presence of AMP-PNP. Although there is some residual activity in the absence of ATP, the introduction of negative supercoiling by CSB appears to depend on ATP-binding and is reduced when ATP hydrolysis is possible. These results demonstrate that CSB actively changes DNA conformation dependent on ATP-binding by the introduction of constrained negative supercoiling.

Architecture of the CSB-DNA Complex—The topological assays revealed that CSB-induced changes in DNA conformation were trapped as negative Lk because of the introduction of negative supercoiling upon CSB binding. This change in DNA Lk could result from changes in twist or writhe. Proteins can change writhe by wrapping DNA around their surface. Proteins can change twist by stretching the helix in a protein filament. To discriminate between these possibilities, we used SFM to directly visualize individual CSB-DNA complexes. CSB protein was incubated with a singly nicked circular DNA molecule in the absence or presence of ATP or AMP-PNP. Fig. 2 shows SFM images of CSB-DNA complexes formed in the presence of ATP. The appearance of the CSB-DNA complexes formed in the presence or absence of ATP or AMP-PNP was similar. Under all conditions, −12% of the DNA molecules was bound by CSB. The percentage of DNA bound by CSB was similar for nicked circular, closed circular, and linear DNA templates, indicating that CSB does not specifically bind to nicks or DNA ends. We found no evidence for protein-coated filaments or elongated DNA structures in these images. This indicates that CSB is unlikely to alter DNA conformation by changing twist. Interestingly, in the presence of ATP or AMP-PNP, many of the CSB-DNA complexes observed were indicative of DNA wrapping. The DNA did not appear to pass through the center of the protein but rather entered and exited the protein on one side (Fig. 2, B and C).

CSB Binding Shortens DNA Contour Length Dependent on ATP Binding—When CSB affects DNA structure by constraining writhe due to DNA wrapping, this results in a decrease in contour length of the DNA-protein complexes relative to protein-free DNA. We measured the contour length of free singly nicked circular DNA molecules and DNA molecules bound by CSB in the same deposition. These results are shown in Fig. 3 and are summarized in Table I. In the absence of ATP, there was no significant difference in the DNA contour length of CSB-bound DNA molecules and free DNA molecules (Fig. 3A). However, in the presence of ATP, a shift in the distribution and a significant reduction in the average DNA contour length was observed upon binding of CSB (Fig. 3B). The distribution of the DNA lengths suggests that two populations of CSB-bound complexes were present, one with and one without shortened contour length.

Because the CSB-induced change in DNA conformation was most pronounced in the presence of a non-hydrolyzable ATP analogue, AMP-PNP, in the topological assays, we also analyzed the DNA contour length under these conditions. There was a more pronounced shift toward shorter DNA contour lengths upon binding of CSB in the presence of AMP-PNP, indicating that more CSB-bound DNA molecules are present with a shorter contour length (Fig. 3, B and C). The average contour length measured for CSB-bound DNA molecules was 40 nm shorter in the presence of AMP-PNP. However, the
presence of CSB-bound DNA molecules that are not shorter will result in a slight underestimation of the shortening. The protein-free 1821-bp DNA molecule has an average contour length of 548 nm in our images. Thus 40 nm represents an average DNA contour length reduction of 125 bp as a result of CSB binding in the presence of AMP-PNP. Preliminary experiments with the CSB mutant K538R, containing a mutation in the ATP-binding domain that allows binding of ATP but not hydrolysis (20), suggested a similar DNA contour length reduction upon binding in the presence of ATP and AMP-PNP (results not shown). Although the path of DNA cannot be followed in these images, the loss of this relatively large amount of DNA can most easily be explained by DNA wrapping around the protein surface (see “Discussion”). These results suggest that CSB wraps on average 125 bp of DNA around its surface dependent on ATP binding but not hydrolysis. ATP hydrolysis may result in DNA unwrapping, because shorter DNA molecules were observed more frequently in the presence of AMP-PNP.

Size of the DNA-bound CSB Protein—The size of a protein can be estimated from SFM images by measuring its volume. This is especially useful for determining the size of DNA-bound proteins (30). Although the absolute dimensions of biomolecules in SFM images will vary with the deposition and the specific tip used (32), there is a linear relationship between SFM-measured volume and molecular mass (30, 33). Thus the size of a protein of interest can be determined by comparison of its volume to that of a protein size standard included in the same deposition. CSB has a molecular mass of 168 kDa, and we used E. coli RNA polymerase (450 kDa) and the Ku70/80 heterodimer (155 kDa) as internal standards. CSB was bound to singly nicked circular DNA, and the protein standard was bound to a linear DNA fragment in separate binding reactions. The reactions were mixed together immediately before deposition for imaging. Control reactions, in which CSB was bound to circular DNA and mixed with naked linear DNA just before deposition, did not result in protein transfer to the linear DNA (results not shown). Therefore, CSB could be distinguished from the protein standard by the DNA substrate to which it was bound. Fig. 4 shows CSB bound to circular DNA (Fig. 4, A and B, black arrows) together with RNA polymerase (Fig. 4A, white arrows) or with the Ku heterodimer (Fig. 4B, white arrow) bound to linear DNA. Fig. 4 shows the volume distribution for CSB relative to RNA polymerase (Fig. 4C) and Ku (Fig. 4D). The average measured volume and the estimated size of CSB are summarized in Table II.

### Table II

<table>
<thead>
<tr>
<th>Mean DNA contour length</th>
<th>No cofactor</th>
<th>ATP</th>
<th>AMP-PNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free DNA</td>
<td>549 ± 1.9 (198)</td>
<td>553 ± 1.7 (190)</td>
<td>543 ± 1.9 (145)</td>
</tr>
<tr>
<td>CSB-bound DNA</td>
<td>547 ± 2.8 (121)</td>
<td>522 ± 4.6 (118)</td>
<td>504 ± 4.6 (145)</td>
</tr>
</tbody>
</table>

*Mean DNA contour length in nm ± S.E. (number of molecules measured).*

Fig. 3. Contour length distribution of protein-free and CSB-bound DNA molecules. Histograms show the contour length of protein-free and CSB-bound DNA molecules in the absence (A) and the presence of ATP (B) and the presence of AMP-PNP (C). Black bars represent free DNA molecules, and gray bars represent DNA bound by CSB.
protein was transfected with a construct encoding a FLAG-tagged CSB protein. The transfected FLAG-CSB construct is expressed at a relatively low level compared with the stably expressed GFP-CSB protein (Fig. 5, lane 1). This cell extract was used for immunoprecipitation experiments with a FLAG-specific antibody. The GFP-CSB fusion protein was found to co-immunoprecipitate with the FLAG-tagged CSB protein (Fig. 5, lane 3). The two CSB variants were immunoprecipitated in a 1:1 ratio probably because of the relative overexpression of GFP-CSB and the resulting over-representation of FLAG-CSB/GFP-CSB interactions. An extract from mock-transfected cells was analyzed in parallel (Fig. 5, lane 2). Incubation of this extract with the anti-FLAG antibody did not result in immunoprecipitation of GFP-CSB (Fig. 6B, lane 7). The co-immunoprecipitation was repeated with similar results in the presence of ethidium bromide (100 µg/ml), which is known to disrupt protein-DNA interactions without affecting protein-protein interactions (results not shown). This indicated an interaction between two differentially tagged CSB molecules in mammalian cell extracts. These results, combined with the SFM volume measurements, suggest that CSB associates with itself and likely functions as a dimer.

**TABLE II**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cofactor</th>
<th>Volume</th>
<th>SEM</th>
<th>No. molecules</th>
<th>Relative volume</th>
<th>Relative size</th>
<th>kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA pol</td>
<td></td>
<td>1176</td>
<td>62</td>
<td>63</td>
<td>1</td>
<td>1</td>
<td>268</td>
</tr>
<tr>
<td>CSB</td>
<td></td>
<td>699</td>
<td>54</td>
<td>63</td>
<td>0.59</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>RNA pol</td>
<td>AMP-PNP</td>
<td>1783</td>
<td>79</td>
<td>98</td>
<td>1</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>CSB</td>
<td>AMP-PNP</td>
<td>1077</td>
<td>50</td>
<td>98</td>
<td>0.60</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>Ku</td>
<td>AMP-PNP</td>
<td>450</td>
<td>24</td>
<td>95</td>
<td>1</td>
<td>284</td>
<td></td>
</tr>
<tr>
<td>CSB</td>
<td>AMP-PNP</td>
<td>324</td>
<td>7</td>
<td>220</td>
<td>1</td>
<td>284</td>
<td></td>
</tr>
<tr>
<td>CSB</td>
<td>AMP-PNP</td>
<td>629</td>
<td>13</td>
<td>220</td>
<td>1.94</td>
<td>301</td>
<td></td>
</tr>
</tbody>
</table>

× a.u., arbitrary units.
DISCUSSION

The CSB Protein Actively Wraps DNA—We demonstrated that CSB changes DNA double helix conformation by introducing constrained negative supercoiling in topological assays. This activity was surprisingly more efficient in the presence of AMP-PNP, suggesting that the CSB-induced change in DNA conformation depends on ATP binding but not hydrolysis. These results are consistent with previous studies demonstrating that the CSB mutant K538R efficiently induces negative supercoiling in topological assays (20). This mutation in the ATP binding pocket of CSB prevents ATP hydrolysis but still allows ATP binding. SFM analysis of CSB-DNA complexes revealed that CSB-bound DNA was shorter in the presence of both ATP or AMP-PNP. We measured a DNA contour length shortening of −125 bp upon binding of CSB in the presence of AMP-PNP. Such a relatively large DNA shortening is unexpected to be caused by DNA compaction within the protein. Neither would DNA compaction easily explain the constrained negative supercoiling induced by CSB. Alternatively, CSB could make two discrete contacts within the DNA molecule generating a loop, with translocation resulting in the accumulation of supercoiling. A protein translocation mechanism generates unconstrained supercoiling and predicts the generation of both positive and negative supercoils in DNA (see also Fig. 6B), whereas CSB was found to introduce only constrained negative supercoiling. In addition, the loop size would vary dramatically depending on the DNA cross-over site stabilized by CSB. The architecture of the CSB-DNA complexes was extremely uniform in our SFM images (Fig. 2), and circular DNA molecules divided into two domains of variable size by a CSB-mediated cross-over were not observed. Therefore, the measured shortening most likely results from wrapping of a defined amount of DNA around the CSB surface, constraining a negative supercoil. The DNA entry/exit configuration of the CSB-DNA complex in the SFM images was also consistent with DNA wrapping (Fig. 2, B and C). We measured a shorter DNA

![FIG. 5. Immunoprecipitation of CSB. A, cells stably expressing the GFP-CSB fusion protein were transfected with a construct expressing FLAG-tagged CSB. Immunoprecipitation (IP) was performed with an antibody specific for the FLAG-tag. Whole cell extracts of the GFP-CSB cell line (mock, lane 2), the GFP-CSB cell line transfected with FLAG-CSB (input TF, lane 1), and the precipitated proteins (IP TF, lane 3) were analyzed by immunoblotting using a CSB-specific antibody. Purified recombinant CSB was analyzed in parallel as a size control (rec. CSB, lane 4). B, to demonstrate the specificity of the anti-FLAG antibody, the mock-transfected cell extract (input mock, lane 5) was immunoprecipitated with this antibody (IP mock, lane 7). The FLAG-CSB-transfected cell extract (input TF, lane 6) and immunoprecipitation of this extract with the anti-FLAG antibody (IP TF, lane 8) were analyzed in parallel.](http://www.jbc.org/content/early/2017/08/11/jbc.M117.786855/F5.large.jpg)

![FIG. 6. A, model of the CSB-DNA complex. A CSB dimer wraps 125 bp of DNA around its surface in ATP-bound state. DNA is wrapped left-handed around the protein in slightly more than one complete turn. B, models for the introduction of torsion by DNA rotation. In this figure, rotation of the DNA is constrained by the gray blocks on the DNA ends, creating topologically closed domains. In the tracking model, a remodeling protein translocates along the DNA helix in an ATP-dependent manner. When free rotation of the protein is prevented, this will cause rotation of the DNA axis, generating a potentially large change in positive supercoiling (+SC) ahead of movement and negative (−SC) behind. In the wrench action model, the protein constantly holds tight to a specific site on the DNA but changes its orientation with respect to the DNA, dependent on ATP. This results in a relatively small change in both positive and negative supercoiling. In the wrapping model, the CSB protein wraps DNA around its surface in the ATP-bound state, constraining one negative supercoil. The wrap is released upon ATP hydrolysis, introducing one unconstrained negative supercoil in the DNA.](http://www.jbc.org/content/early/2017/08/11/jbc.M117.786855/F6.large.jpg)
The dynamic process of DNA wrapping and unwrapping by CSB thus appears to be regulated by ATP binding and hydrolysis.

Architecture of CSB Bound to DNA—SFM size measurements suggested that CSB binds DNA as a dimer, because we consistently measured a volume ~1.6-fold larger than a monomer. This measured volume, larger than a monomer but smaller than a dimer, could be explained by the presence of a mixed population of monomers and dimers. However, the volume distributions do not obviously display two populations. Alternatively, the discrepancy between measured volume and molecular weight could be caused by the specific geometry of the CSB-DNA complex; two subunits may form a very compact dimer. Finally, the protein density of CSB or its interaction with the SFM tip may differ from that of the protein standards used. In the presence of AMP-PNP, the DNA wrapped around the CSB surface may contribute to the measured volume. We measured only a small increase in size, within the margin of the standard error of the experiments. The large size of the CSB protein (168 kDa) may make it difficult to measure the relatively small increase in size caused by the DNA. In addition, the DNA may lie within a groove in the protein and not significantly add to the measured volume. In immunoprecipitation experiments, two differentially tagged forms of CSB were found to co-immunoprecipitate. These combined results suggest that CSB associates with itself and likely functions as a dimer.

The results of this study are combined in the model of the CSB-DNA complex in Fig. 6A. The dimeric CSB protein wraps, on average, 125 bp of DNA around its surface, dependent on ATP binding. The introduction of negative supercoiling in topological assays indicates left-handed wrapping of DNA. The ATP binding. The introduction of negative supercoiling in topological assays. These results indicate that DNA wrapping by CSB is dependent on ATP binding, whereas ATP hydrolysis results in DNA unwrapping. The dynamic process of DNA wrapping and unwrapping by CSB thus appears to be regulated by ATP binding and hydrolysis.

DNA Wrapping by CSB as a Mechanism for Chromatin Remodeling—The ability of CSB to actively wrap and unwrap DNA may be the mechanism underlying its chromatin remodeling activity. Currently, the introduction of torsion in DNA is believed to be a key component of ATP-dependent chromatin remodeling (1, 5, 6). How remodeling enzymes generate torsion is not known, but current models involve rotation of the DNA helix. To generate torsion by DNA rotation, the free rotation of the DNA must be constrained to prevent dissipation of stress. SWI2/SNF2-dependent DNA loops have been detected by microscopy, suggesting that this protein generates topologically closed domains (38). Two mechanisms were proposed for DNA rotation by SWI2/SNF2-like proteins. One mechanism is tracking along the DNA helix (Fig. 6B), as has been described for other members of the SF2 family of helicases (39) and was demonstrated for the Rad54 protein involved in recombination repair (26, 27, 40). ATP-dependent DNA translocation will generate positive supercoiling ahead of movement and negative behind, provided that the free rotation of the protein is constrained (41). The other mechanism is a molecular wrench action (Fig. 6B), as has been suggested for TFIH in promoter melting (42), where the remodeling protein changes its rotational position while remaining bound to a specific site on the DNA. Both positive and negative supercoiling can be introduced by a wrench action. CSB may remodel nucleosomes by a mechanism involving DNA wrapping and unwrapping, introducing a negative twist (Fig. 6B).

Although our data do not yet address how DNA wrapping by CSB is used to disrupt chromatin structure, it is plausible that it affects DNA-histone interactions. Because both CSB and nucleosomes wrap approximately the same amount of DNA left-handed around their surface, CSB could replace a nucleosome topologically. ATP hydrolysis would result in release of the wrap transferring the supercoiling constrained by the nucleosome to the local DNA, although a previous study failed to detect released nucleosomes (20). Alternatively, CSB may generate torsion to remodel nucleosomes by the active wrapping and unwrapping of linker DNA. This requires that free rotation of CSB is constrained, which could be achieved by binding to both DNA and the nucleosome. Indeed, CSB was found to interact directly with histone proteins (20). Thus CSB could cause rotation of the DNA at the nucleosomal entry/exit site by active DNA wrapping. ATP hydrolysis was shown previously to be required for chromatin remodeling by CSB, whereas negative supercoiling and DNA wrapping were demonstrated to depend on ATP-binding (Ref. 20 and this study). This suggests that active DNA wrapping and unwrapping upon ATP hydrolysis are required for chromatin remodeling by CSB.

DNA Wrapping by CSB as a Mechanism to Disrupt Protein-DNA Interactions—By altering DNA double helix conformation, CSB may disrupt histone-DNA interactions, as well as the interaction of other proteins with DNA. In the prokaryote E. coli, the TRCF protein is required for TCR. TRCF binds and displaces the stalled RNA polymerase (43) or rescues arrested transcription complexes into productive elongation (44, 45). Interestingly, TRCF was also suggested to wrap DNA around its surface. DNase I footprinting of TRCF on DNA results in alternating protected and hypersensitive regions, consistent with DNA being wrapped around the protein (46). DNA wrapping by CSB may similarly serve to remove or displace stalled pol II from the site of damage and to allow TCR. Indeed, CSB was found to interact with pol II (15, 16). A recent in vitro study suggested that dissociation of stalled pol II is not dependent on CSB, whereas repair of the DNA damage could occur in the presence of the stalled transcription complex (47). However, the repair of damage in vivo is generally thought to require the removal or displacement of the stalled pol II. CSB also plays a non-essential role in the transcription process itself (15). DNA wrapping by CSB may be important for both its function in DNA repair and gene transcription. The dual role of CSB could provide an explanation for the fact that the various DNA repair pathways require specific SWI2/SNF2 family members. These proteins can all affect chromatin structure, but their additional functions may demand specialization. The DNA wrapping ac-
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tivity of CSB differentiates this protein from the other SWI2/ SNF2 proteins. Although these proteins are members of the same family, they appear to alter DNA conformation by very different mechanisms.

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REFERENCES

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