Histone Tail-independent Chromatin Binding Activity of Recombinant Cohesin Holocomplex

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Cohesin, an SMC (structural maintenance of chromosomes) protein-containing complex, governs several important aspects of chromatin dynamics, including the essential chromosomal process of sister chromatid cohesion. The exact mechanism by which cohesin achieves the bridging of sister chromatids is not known. To elucidate this mechanism, we reconstituted a recombinant cohesin complex and investigated its binding to DNA fragments corresponding to natural chromosomal sites with high and low cohesin occupancy in vivo. Cohesin displayed uniform but nonspecific binding activity with all DNA fragments tested. Interestingly, DNA fragments with high occupancy by cohesin in vivo showed strong nucleosome positioning in vitro. We therefore utilized a defined model chromatin fragment (purified reconstituted mononucleosome) as a substrate to analyze cohesin interaction with chromatin. The four-subunit cohesin holocomplex showed a distinct chromatin binding activity in vitro, whereas the Smc1p-Smc3p dimer was unable to bind chromatin. Histone tails and ATP are dispensable for cohesin binding to chromatin in this reaction. A model for cohesin association with chromatin is proposed.

The essential function of sister chromatid cohesion (SCC) is in cell proliferation is to facilitate accurate chromosome segregation (1, 2). Cohesion between chromatids is established only during DNA replication, when a four-subunit cohesin complex is assembled (3–5) consisting of Smc1p (6), Smc3p, Scc3p (5), and Scc1p/Mcd1p (1, 2) in Saccharomyces cerevisiae. Essentially, the same four subunits comprise cohesin complexes in all eukaryotes (3, 5, 7–9). Although the essential role of cohesin is in mitotic and meiotic chromosome segregation, the cohesin complex is also involved in a number of important, albeit non-essential, chromatin-mediated processes (10–18).

Despite the multitude of cohesin functions in vivo, little is known about the mechanism of cohesin association with its DNA/chromatin target. It is believed that the SMC core of the cohesin complex (Smc1p-Smc3p) forms either a DNA-binding bridge (19) between the two sister DNA molecules or “embraces” two sister chromatids (20). The role of the non-SMC cohesin subunits (Scc1p/Mcd1p and Scc3p) is to promote the establishment (20, 21) and breakage (22) of these SCC links in the course of the cell cycle. However, experimental evidence for direct cohesin-DNA interaction is rather limited (21, 23). The inability to detect sequence-specific cohesin-DNA interactions supports the suggestion that the interaction of cohesin with DNA is more topological in nature (24). However, even the most recent data cannot explain the S-phase requirements (4) for the de novo formation of an SCC site, the mechanisms that determine the placement of heritable cohesin sites in vivo (25, 26), or the specificity of the transient (centromeric) SCC links (27, 28) versus the stable SCC sites on chromosome arms. The obscurity of molecular mechanisms of cohesin activity in vivo highlights the necessity of reconstituting cohesin activity in vitro. Chromatin cannot be ignored while designing such an in vitro model, as it was recently shown that cohesin binding to heterochromatin in vivo is dependent on a specific site of histone H3 methylation (16, 29, 30). Even though this histone modification is not found in S. cerevisiae (31), it is conceivable that chromatin structure may play an active role in the establishment of SCC and/or in the placement of cohesin-binding sites (25).

In this report we characterize cohesin interaction with chromatin in vitro and show that chromatin is likely the preferred substrate for chromatin binding over naked DNA. We also demonstrate that cohesin-chromatin interaction is independent of histone tails and does not protect linker DNA. This allows us to propose a model for the binding of two chromatin fibers by the cohesin holocomplex, which partially agrees with the embrace-like model of SCC establishment (24).

EXPERIMENTAL PROCEDURES

Estimation of Minimal Cohesin-binding Site in Yeast—Yeast strains were of S288c or W303 background. The 2-YPH499bp3/pAS630/1 strain (smc3::URA3) with a yeast DNA-only circular minichromosome pAS630/1 (3,629 bp) was used for chromatin immunoprecipitation (ChIP) (32). pAS630/1 was constructed from a shuttle plasmid pAS630 (3,629 bp) which was used for chromatin immunoprecipitation (ChIP) (32). pAS630/1 was constructed from a shuttle plasmid pAS630 (3,629 bp) by deleting the majority of non-yeast DNA (XbaI-SpeI digest), followed by ligation and direct transformation into yeast cells. pAS630 was YCPlac111 with an XbaI linker inserted into the AatII site. Under selective conditions (media without leucine), the pAS630/1 copy number increased to an average of five copies per cell (as determined by quantitative PCR), probably due to a negative effect of minichromosome size on the LEU2 expression. This copy number increase allowed us to separate the ChIP signal generated by the plasmid sequences, some of which there also present in the 2-YPH499bp3 chromosomes (numbers 1, 3, 4, 7, and 12), by diluting the ChIP DNA so that chromosomal
cohesin-bound sequences were not amplified (verified by the CEN8 vector and ChIP).

For native chromatin purification (Fig. 1, B and C) 2 liters of yeast BY4733bΔp (SCC3;12His:3HA-Ura3) (30 °C, OD600 = 1.5) were used for native preparation (33). Nuclei were treated with micrococcal nuclease (MN) (1 × 10^6 for 15 min at 37 °C) in 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM β-mercaptoethanol, 0.5 mM AEBSF, 4 mM CaCl2, and 10% glycerol. Nuclei were spun down (15,000 × g for 10 min at 4 °C), and nuclear extract was separated on sucrose gradient (5–30%), for 16 h at 35,000 × g at 4 °C in 20 mM Tris-HCl, pH 7.4, and 150 mM NaCl. For Fig. 1C, the sucrose gradient fraction 15 was dialyzed (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5 mM AEBSF, and 0.01% Triton X-100) and incubated with anti-βt AdvI affinity resin (12 h for 4 °C). The resin was poured into a column, washed with washing buffer and eluted with elution buffer (see below). Serum depleted of specific anti-Mcd1 antibodies was used as a control. Antibodies—Antibodies to Scclp were prepared as described (34) using a synthetic peptide (SynPep) CENPEPNNKIQKAKSNQTQRE-KAPQPNSREDT as immunogen. Other antibodies were from commercial sources or were described previously (2, 6).

Recombinant Cohesin Purification—Recombinant cohesin subunits were first individually expressed both with a His6 tag (pFastBAC-HTb) and without (pFastBAC) Recombinant proteins were tested for solubility in EBX200 (50 mM Hepes-KOH, pH 7.5, 200 mM NaCl, 100 mM KCl, 50 mM NaF, 5 mM Na3PO4, 10 mM sodium β-glycerophosphate, 2.5 mM MgCl2, 10% glycerol, Complete protease inhibitor mixture (Roche Applied Science) 0.01% Triton X-100) and incubated with anti-Mcd1 affinity resin (12 h for 4 °C). The resin was poured into a column, washed with washing buffer and eluted with elution buffer (4°C) indicated above. Serum depleted of specific anti-Mcd1 antibodies was used as a control. The eluates were used to native gel electrophoresis (10% acrylamide). The purity of the purified cohesin was estimated from the cohesin bands by Coomassie staining. The cohesin bands were cut out of the gel, and cohesin bands were excised from the gel and eluted with 500 mM imidazole, and then applied to a HR10/30 Superose-6 column in EBX200 buffer.

DNA Binding Assay—For Fig. 2A, four 350-bp fragments of DNA, corresponding to in vivo sites with variable strength of cohesin binding, were made by PCR and cloned. The fragments were excised from the plasmids, labeled, and compared in an agarose gel electrophoretic mobility assay (EMSA). The following fragments were used as DNA probes: EBX200 proximal site (site 548.7 in Ref. 25), primers 5′-TGAGGAAAAATTTAAAGAACGCTGTGGA-3′ and 5′-TGCCCAACATGATCGGCTGATTTAATG-3′; DMC1-distal site (549.5 in Ref. 25), primers 5′-TGAGGAGCCTCGGTAGCCATTGGTG-3′ and 5′-TACGATGACCAGTAAATTTAAGAA-3′; CEN4-CDEI proximal site (Fig. 1A), primers 5′-CTTTTTAATCTTATTAGGCTGATTTTCTG-3′ and 5′-TACTATTTATGTGATTTTCTG-3′; FAU1 (5′-TCCGCGCCGTTGTTGCGGATGATGTTGTTGTTGTTGATTG-3′ and 5′-CTACACGCCGCTAAGTGGCGAGACGGAAGGAGG-3′). Because cohesin binds ATP and has a weak ATPase activity for 16 h at 35 °C, 20% sucrose gradient as described above. Recombinant Cohesin and Chromatin

As a first step toward reconstitution of cohesin activity in vitro, we estimated the minimal size of chromatin fragments bound to cohesin in living cells. This parameter has never been previously determined, as relevant ChIP data on cohesin-binding sites available have a resolution of no finer than 1 kb (25, 26, 42). That does not provide sufficient guidance for design of an adequate binding target for the in vitro studies. To characterize the minimal unit of SCC, we followed two approaches. First, we created a small minichromosome containing essentially no plasmid or bacterial sequences, probably extremely topologically constrained in vitro due to its small size (3.6 kb). This approach allowed us to limit the extensive (several kb) spreading of cohesin from the peak at the centromere, which was previously observed in native chromosomes (25), and to reveal the minimal-size cohesin sites essential for proper segregation. Fig. 1A shows the results of the Smc3p-HA ChIP of this minichromosome. The most robust cohesin association was restricted to a short (300 bp) CDEI-adjacent region of the minichromosomal CEN4 (Fig. 1A, white box). Two other binding sites were also detected (Fig. 1A) and also limited to 300 bp in both cases. As a second approach, we purified and analyzed

RESULTS

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the chromatin-bound cohesin pool (Fig. 1, B and C). After digestion of yeast chromatin with MN (Fig. 1B) to saturation and purification of the resulting nucleoprotein complexes using sucrose gradient centrifugation and immunoaffinity chromatography, we analyzed fractions for the presence of the cohesin subunits, the chromatin marker Hmo1p (Fig. 1C), and DNA. Analysis of the gradient fractions indicates that cohesin preferentially associated with the higher molecular weight chromatin fractions within the peak containing dinucleosomal DNA (fractions 15 and 16, Fig. 1C). Cohesin-containing chromatin fractions also contained only a small fraction of Hmo1p, as was expected in view of the sparse spacing of SCC sites in vivo (43). After immunoaffinity purification of cohesin from fraction 15 using an anti-Mcd1p antibody resin, it was observed that cohesin was still associated with Hmo1p (even as the latter was partially depleted), indicating persistent association of cohesin with the dinucleosome-sized chromatin fragments.

Thus, we established that chromatin, digested to dinucleosomal length, still co-purified with cohesin and, therefore, the size of a minimal cohesin binding unit in vivo is likely comparable with the size of two nucleosomes or even smaller. We used this result as a guide for the design of DNA probes in all subsequent experiments.

Because active cohesin is tightly associated with chromatin in yeast, we purified a recombinant cohesin complex using the baculovirus expression system. After achieving the maximal expression levels of individual subunits (Fig. 1D), they were co-expressed in insect cells, and the resulting yeast recombinant cohesin was purified (Fig. 1E). Poor solubility of Scc1p/Mcd1p was the main factor limiting the yield of recombinant holocomplex, as untagged Scc1p/Mcd1p expressed alone was virtually insoluble. Yet, >20% of recombinant Scc1p/Mcd1p was found to be soluble when co-expressed with other subunits, indicating that assembly of cohesin did occur upon co-expression. After purification in a complex that was >95% pure, cohesin subunits were present in a near 1:1:1:1 ratio (Fig. 1E), according to the gel density scan. Subsequent purification of the complex resulted repeatedly in progressive loss of the Scc3p subunit, consistent with the findings for Schizosaccharomyces pombe cohesin purification (7).

Human cohesin was previously shown to have nonspecific
DNA binding activity in vitro (44). We therefore tested the recombinant yeast cohesin complex for DNA binding (Fig. 2A). Instead of the random DNA fragments used in preceding studies, we used the four probes corresponding to yeast genomic sites with the known SCC potential. Unfortunately, only very few genomic sites have been verified as strong cohesin-negative sites with the known SCC potential. We therefore tested the specificity of recombinant cohesin toward DNA, to deposit and position nucleosomes. We found that the SCC sites (CEN4 and DMC1, proximal and distal) were good positioning sequences for either mono or dinucleosomes, whereas the site that does not bind cohesin in vivo (PAU1) showed little nucleosome positioning (Fig. 2B). This observation raises a possibility that phased nucleosomes are the natural substrates for cohesin binding in vivo.

Based on the fact that naked DNA evidently does not carry enough information to determine its cohesin-binding potential (Fig. 2A), we tested chromatin-binding properties of recombinant cohesin in vitro. We used a nucleosome-positioning sequence of 5 S rRNA gene from X. borealis (38, 39) to generate a defined chromatin substrate (dinucleosome) for cohesin binding. The choice of dinucleosome was determined by its similarity to a natural chromatin fragment, i.e. existence of two nucleosomes separated by linker DNA, and by our estimates of a minimal cohesin-binding site (Fig. 1A and B). The dinucleosome assembled on this 394-bp DNA fragment (Fig. 3A) was purified to ensure homogeneity. We repeated the purification of recombinant cohesin and tested the fractions from the last chromatography step (size exclusion) for dinucleosome binding. The recombinant cohesin (Fig. 3) demars robust DNA binding activity (Fig. 2A) that was ATP-independent but sensitive to heat inactivation (not shown). All four genomic sites associated with recombinant cohesin regardless of their binding to cohesin in vivo, yet did not form a defined complex of a specific size (Fig. 2A) at any DNA-protein ratio. This indicates that cohesin does not establish a stoichiometric complex with naked DNA in vitro (see also Fig. 5). This DNA binding was also sensitive to poly(dI-dC) and, therefore, was not sequence-specific (Fig. 2A). Thus, despite its substantial DNA binding activity, cohesin cannot discriminate in vitro between the DNA sequences corresponding to functional SCC sites and DNA derived from the sites with no cohesin binding in vivo.

These results indicate that some additional factors might be involved in the selection and formation of SCC sites in vivo. An important factor potentially facilitating formation of an SCC site in vivo is chromatin itself (25, 30). Indeed, cohesin was found in a complex with chromatin in our experiments (Fig. 1C). Also, at least in the case of pericentromeric SCC, cohesin must cohabit with the underlying phased nucleosomes (45). In chromosomal regions elsewhere, cohesin was also shown to target chromatin with phased nucleosomal structure, i.e. heterochromatin (16, 17, 30, 49). We tested the ability of the DNA fragments, used in Fig. 2A to analyze binding of cohesin to DNA, to deposit and position nucleosomes. We found that the SCC sites (CEN4 and DMC1, proximal and distal) were good positioning sequences for either mono or dinucleosomes, whereas the site that does not bind cohesin in vivo (PAU1) showed little nucleosome positioning (Fig. 2B). This observation raises a possibility that phased nucleosomes are the natural substrates for cohesin binding in vivo.

To verify by independent means that the observed dinucleosome gel shift was indeed dependent on cohesin, we attempted a super-shift experiment with cohesin-specific antibodies. The super-shifted complexes were not satisfactorily reproducible, however, probably due to the competing effects of antibody addition such as disruption of the cohesin-chromatin complex versus non-disruptive binding as well as the relatively small contribution of antibody binding to the mass of the complex. To overcome this technical difficulty, we utilized the known property of Esp1 protease to cleave Scc1p/Mcd1p (40) and thus disrupt SCC in vivo (22). We established that activated Esp1 isolated from yeast cells (see “Experimental Procedures”) was able to cleave Scc1p/Mcd1p in vitro in the context of cohesin...
holocomplex.\textsuperscript{2} Therefore, we subjected the cohesin-chromatin binding reactions to Esp1p and found that Esp1p treatment disrupted the cohesin-chromatin complex (Fig. 3C).

Next, we addressed the following questions regarding the mode in which adhesin binds the chromatin probe. (i) Is a stoichiometric complex formed between the dinucleosome and cohesin? (ii) What is the relative strength of this complex compared with the cohesin-DNA complex? (iii) Is there any contribution of ATP in the dinucleosome binding? (iv) Finally, does cohesin interact with histone tails or linker DNA? To assess the possible stoichiometry of cohesin-chromatin interaction, the binding of cohesin to dinucleosomes was titrated with decreasing amounts of cohesin. As a result, we established that, at a given ratio (see “Experimental Procedures”), a specific complex between cohesin and the positioned dinucleosome template was formed (Fig. 4A, lanes 3–5). No specific complex with a mononucleosomal fragment was formed (not shown) at any cohesin-chromatin ratio. The dinucleosome-cohesin complex migrated more slowly than two dinucleosomes joined by DNA ligase (Fig. 4A, chromatin ladder). One possible explanation is that the cohesin complex joined two dinucleosomes (Fig. 5). Alternatively, it may indicate that cohesin binding dramatically changes dinucleosome conformation, resulting in retarded mobility. Formation of this complex was extremely resistant to competition by the naked DNA template (Fig. 4A, lanes 4 and 5), which was used in a 30-fold excess over the dinucleosomal DNA (nearly 500-fold excess over the cohesin-bound dinucleosome), suggesting that the chromatinized fragment is not only able to limit the number of cohesin complexes bound to the probe but is also a preferred substrate for cohesin binding compared with naked DNA (Fig. 4B). As the dinucleosome has some protruding DNA stretches (Fig. 3A), binding of

\textsuperscript{2} A. Strunnikov, unpublished data.
cohesin to them could mask the stoichiometric complex in the gel, resulting in some smearing of the signal (Fig. 4A, lane 2). This smear is eliminated upon the addition of competitor DNA (Fig. 4A, lane 5), indicating that naked DNA is indeed responsible for it. The bigger secondary complex (Fig. 4A, open circle) had variable prominence and, thus, was not investigated further. The presence of ATP in the reaction buffer had no notable effect on the cohesin-chromatin interaction, yet we found that ATP pre-bound to cohesin may have a role in maintaining the integrity of the complex (see Supplemental Data in the on-line version of this paper).

We also tested whether cohesin associated with histone tails, which potentially provide a sizable interaction surface for chromatin-binding proteins and are indirectly involved in cohesin association with heterochromatin (16, 46). However, cohesin did not show any specific association with the purified recombinant amino-terminal histone tails (47) compared with the glutathione S-transferase-only control (Fig. 4C). To test this in a chromatin context, we generated a tailless dinucleosomal template. As shown in Fig. 4A, lanes 6–8, the absence of histone tails did not alter the chromatin-binding activity of cohesin but proportionally reduced the size of the specific chromatin-cohesin complex.

We also examined whether de novo chromatin binding is the property of the cohesin holocomplex or whether the Smc1p-Smc3p heterodimer, a core of a hypothetical chromatin fiber-embracing ring (24), can also display this activity. The purified Smc1p-Smc3p heterodimer, however, did not form any specific complex with chromatin and displayed only traces of binding activity (Fig. 4D), indicating that binding to chromatin requires the fully assembled cohesin. Besides, as the Smc1p-Smc3p heterodimer and cohesin holocomplex were purified repeatedly in an identical fashion, the absence of chromatin-binding activity in the Smc1p-Smc3p preparations serves as an additional control for the cohesin-specific nature of the observed gel shift activity. This result demonstrates that distinct in vitro chromatin binding properties of the full cohesin complex and the Smc1p-Smc3p dimer are in good agreement with their chromatin association dynamics during SCC establishment in vivo.

The dependence on chromatin for establishing a specific cohesin-DNA ratio revealed in the above experiments may imply that chromatin structure organizes/limits access of cohesin to DNA. We tested accessibility of linker DNA to nuclease digestion after cohesin binding (Fig. 4E). Accessibility to the EcoRI site (Fig. 3A) in the linker DNA and protection from digestion by MN (Fig. 4E) were not changed in the presence of saturating cohesin concentration. This indicates that cohesin does not bind linker DNA tightly enough to impede access of other DNA-binding proteins. This conclusion supports the recently proposed idea that a stable cohesin-chromatin interaction relies primarily on the topological constraints of the complex rather than on the specific interaction with DNA or proteins (24). Our data showing that cohesin does not directly recognize DNA sequence (Fig. 2A), does not protect linker DNA (Fig. 4E), and does not bind histone tails (Fig. 4, A and C), but yet forms a specific complex with a dinucleosome provide strong supporting evidence for an analogous model (Fig. 5B).

**DISCUSSION**

In recent years, cohesin has been shown to interact with other chromatin proteins (16, 30, 46, 48–51). Yet, direct interaction of cohesin with chromatin in vitro has not been studied, and the mechanism for euchromatic targeting of cohesin, particularly in budding yeast, remains an open question. A range of models has been proposed for the structure of SCC sites (20, 24, 52). These models, however, do not take into account any cohesin-chromatin interaction. One of the most advanced models postulates that chromatin fibers are “embraced” by a large cohesin ring (20), thus implying that the cohesin-chromatin interaction is more dependent on topology than physical contact (24). This view agrees well with the observed tight association of cohesin with chromatin in vivo (5, 49), but it gives no hints regarding the reproducible placement of SCC sites in vivo. The data presented in this report suggest that chromatin specifically organizes access of cohesin to DNA, and, therefore, cohesin depends on chromatin structure for establishing a stoichiometric cohesin-DNA ratio. Published data on cohesin complex architecture and molecular dimensions (20, 53) support the idea that only few chromatin fibers can possibly fit into one embracing cohesin complex. It is conceivable that the relatively large cohesin ring observed in solid phase (53) could be much more tight in the three-dimensional context of natural chromatin so that no more than two fibers (corresponding to sister chromatids) are able form a complex with cohesin (Fig. 5B). The fact that a specific complex is formed between cohesin and the dinucleosome substrate (Fig. 4A) also implies that cohesin is immobilized when it embraces two chromatin fibers. This interaction mode (Fig. 5B) provides a very simple way to form the SCC links in vivo (24) (Fig. 5C). In view of this model, it is not surprising that binding of cohesin to naked DNA is not stoichiometric (21) (Figs. 2A and 5A). As DNA binding activity of cohesin is quite weak, the naked DNA strands, with a diameter much smaller than the chromatin fiber, cannot be immobilized by cohesin and therefore produce an aggregate instead of a specific complex (Fig. 5A).

We also attempted to investigate the rules that govern the stereotypic placement of cohesin binding sites in S. cerevisiae (25). For in vitro analysis we chose a limited number of SCC sites, which have been validated by their analysis in cohesin mutants (25). We found that the cohesin complex itself does not carry a recognition mechanism that allows it to discriminate between the occupied sites and the sites that do not bind cohesin in vivo (Fig. 2A) and, thus, is not a sequence-specific DNA-binding complex. An unexpected finding that the true native SCC sites position nucleosomes suggests that a particular nucleosomal organization may be required to specify sites of cohesin binding to chromatids. It is presently unclear whether positioned nucleosome structure is sufficient to form an SCC site in vivo. Yet, positioned nucleosomes have been associated with the established sites of cohesin binding in

**FIG. 5. Proposed modes of cohesin association with DNA and chromatin.** A, non-stoichiometric binding of cohesin to naked DNA in vitro. B, a model of cohesin immobilization to dinucleosomal substrate in vitro. C, the corresponding model of cohesin binding to chromatin and the establishment of SCC in vivo. Structure of cohesin is according to Refs. 20 and 53. Light gray, Smc1p and Smc3p; dark gray, Scc1p/Mcd1p; dashed line, Scc3p.
Taking into account available data on chromatin interaction with cohesin, it is clear that an understanding of the SCC-site formation is hardly possible without knowing the exact mode of cohesin-chromatin binding. This task, however, could be difficult because of functional and likely structural polymorphism in cohesin-chromatin association, both in S. cerevisiae and Metazoa. In budding yeast this specialization exists at several levels, one of the most evident being the divergence of cohesin binding sites in the pericentromeric regions (27, 28, 57) and the chromosome-arm SCC sites that need to be cleaved to open in anaphase (22). Moreover, data accumulated on the involvement of cohesin in the non-SCC functions (10, 11, 13, 16) indicate that cohesin has more than one mode of targeting and/or binding chromatin. We can hypothesize that the activity recapitulated in this report is the basic chromatin-embracing SCC activity displayed by cohesin in vivo. It agrees well with the recent data on cohesin structure (24). It seems surprising, however, that this activity in vitro is independent of DNA replication, which is critical for SCC establishment in vivo (4). In that regard, we could speculate that a high local concentration of paired sister chromatid fibers (required for their entrapment by cohesin), which can only be achieved in vivo in the process of DNA replication, in an in vitro experiment is reached through other means, e.g. through molar excess of very short chromatin fragments, absence of histone modifications, etc.

Independence of cohesin chromatin-association activity from histone tails shown in this work agrees well with the primary essential function of cohesin, i.e. to establish links between sister chromatids. As histone tails can significantly change properties of the corresponding chromatid, cohesin must have a way to bypass this variable to establish SCC. This view is reinforced by the recent characterization of the cohesin-like SMC complex from bacilli (58), where the chromatin structure also determines the molecular configuration of the SCC site itself.

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Recombinant Cohesin and Chromatin

S. cerevisiae, namely centromere regions (25, 45), rDNA (43, 54, 55), and HMR (17, 45, 56).

In vivo replication, which is critical for SCC establishment
