Supporting Information

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SI Materials and Methods

Yeast Strains, Media, and Cell Synchronization. Yeast strains used in this study are listed in Table S1. All strains are derivatives of the A364A genetic background. Plasmid information is also given within the strain table, written as a part of the relevant strain containing the plasmid. Exponentially dividing cultures were arrested in G1 using 15 nM alpha factor (Sigma) or in S using 130 mM hydroxyurea (HU) (Sigma) or G2/M using 1.5 mg/mL nocodazole (Sigma) for 3 h in YePD (1% yeast extract, 2% peptone, and 2% glucose, and 0.01 mg/mL adenine).

DNA Substrates. pIO1 was constructed by inserting a 2.09-kb SmaI-KpnI fragment containing LacO repeats into pTNT (Promega). Yeast chromosomal DNA fragments described in Table S2 were amplified by PCR from yeast genomic DNA and cloned into pIO1 sequentially to the lacO array KpnI site. Substrates were prepared by PCR with one of the following sets of pTNT universal primers: 5′-biotin-teg-GGCTAGAGTACTTAATACG ACTCAC and 5′- GTTTAGAGGCCCCAAGGGGT TATGC (linear substrates, -L); Or 5′-biotin-teg-GGCTAGAGTACTTAATACGAC-TCAC and 5′-biotin-teg-GTTTAGAGGCCCC AAGGGGTTAT-GC (closed substrates, -C).

Substrate Preparation. Streptavidin-coupled Dynabeads (M-280, Invitrogen) were washed 3 times with DBB [50 mM Tris-HCl (pH 7.5, 1 M NaCl, 1 mM EDTA)] and resuspended in 1 volume of DBB. Five hundred nanograms biotin-labeled DNA was added for every 20 μL beads and beads were incubated at room temperature for 2 h with gentle shaking. For no DNA control, 5 μL of 0.1 M Spermidine (Fluka) was added to the beads. Beads were washed 5 times with PBB [50 mM Hepes pH 8.0, 10% glycerol, 100 mM KCl 2.5 mM MgCl2, 0.4% NP-40, 5 mM 2-mercaptoethanol, 50 mM Na Botyrate, protease inhibitor cocktail (Roche)] and resuspended in 55 μL PBB for every 20 μL beads. Five microliters of BSA 100 mg/mL (Sigma), poly-dIdC:dIdC (Sigma) and acetyl-coA (Roche) were added for every 20 μL beads and beads were split to 70-μL reactions.

Protein Extract Preparation and Cohesin Binding. Cells were grown in 5 mL YePD for 8–10 h at 30 °C, diluted into 0.5 L, and grown for 16 h at 30 °C to a density of approximately 0.6×10^7 cells/mL then arrested in S (unless mentioned otherwise). Cells were washed once with water and pellets from 1×10^9 cells were frozen in liquid nitrogen and stored at −80 °C. To prepare extracts, cell pellets were thawed briefly on ice. Three hundred fifty microliters cold PBB and chilled 0.6 g of 425–600 μm glass beads (Sigma) were added, then cells lysed by bead beating at 3×30 s pulses using the Biospec™ mini bead beater. The soluble fraction was separated from the insoluble fraction by two consecutive centrifugations of $100 \times g$ for 5 min and $16{,}000 \times g$ for 15 min at 4 °C. Thirty microliters of extract were added to each reaction tube containing 70 μL of the reaction mix as described above. Tubes were incubated for 30 min at 30 °C (unless mentioned otherwise) with occasional shaking. The reaction was spun down for 2 s, and beads were immobilized using a magnet (Invitrogen). Beads were washed 3 times with PBB or PBB-0.5 (PBB, 0.5 M KCl) and once with PBB. DNA-bound proteins were eluted from the DNA by adding 30 μL of Laemmli sample buffer and boiling the samples for 5 min. Proteins were separated on SDS-PAGE, transferred to PVDF membrane (Perkin-Elmer) and analyzed by Western blot as described in ref. 1. Densitometry of protein bands was done with IPLab image processing software (Scanalytics, Inc.). Binding was normalized by comparing the bound protein levels to the binding levels of the circular substrate washed with 0.1 M salt.

The amount of DNA bound to the beads was quantified by stripping the DNA from the beads by adding to the beads in 200 μL [50 mM Tris pH 8.0, 2% SDS, 1 μL proteinase K (14–22 mg/ml, Roche)] and incubating for 2 h at 42 °C. DNA was extracted by phenol:chloroform:isoamyl alcohol and ethanol precipitation. DNA was analyzed by agarose gel electrophoresis.

Immunodepletion of Scc2p-3V5. Extract from JH5257 cells arrested by HU was prepared as described above. 5.75 μgr anti-V5 (Invitrogen) was added to the extract and the reaction was incubated on ice for 1 h. No antibody was added for mock-depleted control. Antibodies were collected on protein A agarose beads (Roche) for 1 h at 4 °C. Beads were separated from the lysate by three consecutive centrifugations. Depleted extract was used to assemble cohesin/DNA complexes as described above.

In Vitro Deletion. Δ1a and Δ1b were created by PCR of pIO2 with one universal oligo 5′-biotin-teg-GTTTAGAGGCCCCAAGG-GGTTATGC and an internal oligos 5′-biotin-teg-A GTGAGG-GACTAAAAG TCAATACTG or GAACTGCGCTGTTGG-TGGCCCAGAG, respectively. pIO23 was constructed by cutting pIO2 with KpnI and MluI, fill-in and re-ligation. Δ1c was amplified from pIO23 as described under *DNA Substrates*.

Half kilobase deletion was done by QuikChange II XL site-directed mutagenesis kit (Stratagene) according to the manufacture procedure with the following oligos: Δ2a: 5′-TGCTCCT-CTGGGTTGTGCTATTGTC, 5′-CTGCAGTTTCATCAATG-TGGA CAGC. Δ2b CGCAACAAAATCTTTTAAGTACGCG, GACAATAGCACAACCCAG AGGAGCA

In Vivo Deletion and ChIP. Deletion of chromosome III coordinates 101,220–102,333 was done by PCR-based integration of hisG::URA3::hisG cassette flanked with LoxP sites into the genome to create YIO604/Δcd::URA3. To pop out the URA3 insertion, cells were transformed with pFVL57 Cre recombinase and grown in YeP supplemented with 2% galactose (Sigma). The deletion was confirmed by PCR and sequencing. YΔ2a (chromosome III coordinates 101,282–101,789) and YΔ2b (chromosome III coordinates 101,739-102,333) were constructed as above. ChIP was performed as described before (2).

In Vitro ChIP. Cohesin binding assay was performed as described above. After the last wash with PBB, beads were resuspended in 100 μL PBB and proteins were cross-linked to the DNA by adding formaldehyde to a final concentration of 1% for 30 min at room temperature. Beads were washed 3 times with PBB and resuspended in 0.5 mL PBB. DNA was sheared to about 500-bp fragments by six sonication bursts for 10 s each, using the Branson 250 sonicator with a 3/16” tapered microtip, at constant duty cycle and 1.5 output setting. Beads were immobilized on a magnet, and the soluble fraction was transferred to a new tube and the total volume was increased to 1 mL with PBB and 5 μL of anti-HA antibodies (12CA5, Roche) was added. Samples were rotated over night at 4 °C. Antibodies were collected on 30 μL protein A agarose beads (Roche), consecutively washed for 5 min. with 1 mL of PBB, PBB0.5, $LiCl_2$ detergent (0.25 M LiCl 1%, NP-40, 1% DOC, 1 mM EDTA, 10 mM Tris-HCl pH 8.1), and twice with TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Proteins were eluted in 300 μL (1% SDS, 0.1 M $NaHCO_3$). Reversal of cross-linking

and recovery of DNA were performed following the in vivo ChIP procedure above. One reaction was used as input control. Beads were resuspended in 0.5 mL PBB, sonicated, and treated as described above and kept at −20 °C. Reversal of cross-linking and DNA recovery was performed by following the in vivo ChIP procedure above.

1. Onn I, Guacci V, Koshland DE (2009) The zinc finger of Eco1 enhances its acetyltransferase activity during sister chromatid cohesion. *Nucleic Acids Res* 37:6126–6134.
2. Heidinger-Pauli JM, Unal E, Guacci V, Koshland, D (2008) The kleisin subunit of cohesin dictates damage-induced cohesion. *Mol Cell* 31:47–56.

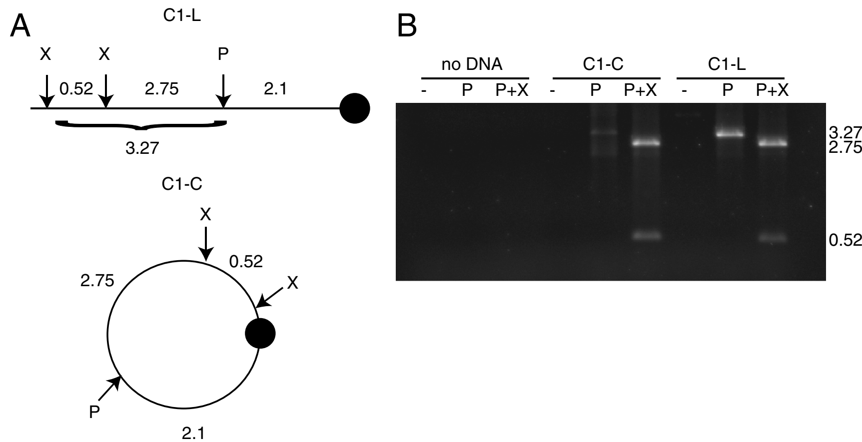

Fig. S1. *DNA labeled with 2 biotins form a substrate with closed topology*. (*A*) Schematic of linear and closed substrates of PstI (P) and XhoI (X) restriction sites and the expected fragment sizes in kb are indicated. (*B*) The DNA substrates C1-C and C1-L (Fig. 1*B*) were cleaved with Pst I (P) or Pst I and Xho I (P + X). Beads were collected on a magnet and the DNA content of the soluble fraction was analyzed by agarose gel electrophoresis.

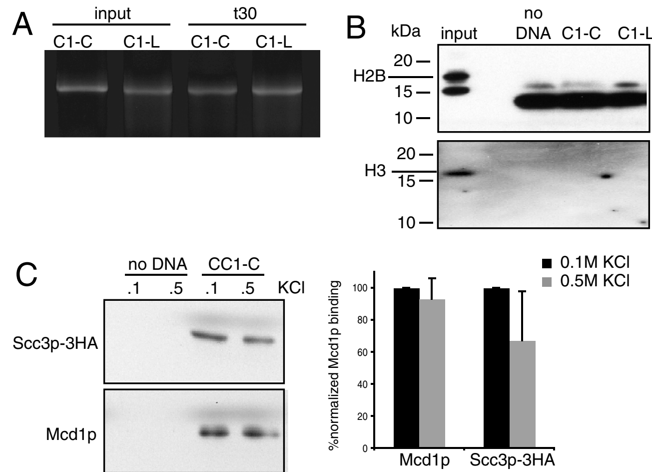

Fig. S2. *DNA substrates are not degraded*. (*A*) C1-L and C1-C were recovered from beads after incubation with protein extract from 3131-13V under the standard reaction conditions. (*B*) Protein extract was prepared from JH5257 cells arrested in S and mixed with C1-L or C1-C under the standard assay conditions with the following modification: Unbound proteins were washed with 0.1 M KCl. Bound proteins were analyzed with antibody against histones H2B and H3 (Active Motif). Proteins were normalized to the DNA on the beads. (*C*) Protein extract was prepared from VG3176-2A cells arrested in G1 or S and mixed with C1-C under the standard assay conditions. Bound proteins were analyzed with antibodies against HA (Scc3p-3HA) and Mcd1p (*Lower*).

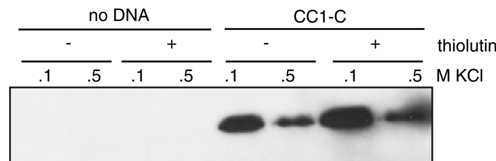

Fig. S3. *Cohesin enrichment on MAT CAR is not affected by CARC1 deletion*. ChIP analysis of the MAT CAR from 2185-6B, YIO6041, and YIO604.

Fig. S4. *Transcription does not affect cohesin binding in vitro*. Protein extract was prepared from YIO71 cells arrested in S and mixed CC1-C under the standard assay conditions with the following modification: Extract was supplemented with 3 μg/mL thiolutin (Axxora). Bound proteins were analyzed with antibodies against Mcd1p.

Table S1. Yeast strains

3131-V13	*MATa pep4Δ::g418::TRP1 trp1-1 leu2-3,112 ura3-52 his3-11,15 bar1 GAL+*
YIO5031	*MATapep4Δ::g418::TRP1 trp1-1 leu2-3,112 ura3-52 his3-11,15 bar1 GAL+ SMC3 SMC3-6HA::URA3*
YIO5032	*MATa pep4Δ::g418::TRP1 trp1-1 leu2-3,112 ura3-52 his3-11,15 bar1 GAL+ SMC3 SMC3-6HA K38I::URA3*
YIO71	*MATa pep4Δ::g418::TRP1 trp1-1 leu2-3,112 ura3-52 his3-11,15 bar1 GAL+ SMC3Δ::clonat SMC3-6HA::URA3*
2185-6B	*MATa ura3-52 lys2-801 his3Δ200 bar1 gal MCD1-6HA*
YIO604/ Δcd::URA3	*MATa ura3-52 lys2-801 his3Δ200 bar1 gal MCD1-6HA URA3::Δchr.III101,220-102,333*
YIO6041/Δcd	*MATa ura3-52 lys2-801 his3Δ200 bar1 gal MCD1-6HA Δchr. III 101,220-102,333pFVL57::HIS3*
YIO6051/Δc	*MATa ura3-52 lys2-801 his3Δ200 bar1 gal MCD1-6HA Δchr.III 101,282-101,789 pFVL57::HIS3*
YIO6052/Δd	*MATa ura3-52 lys2-801 his3Δ200 bar1 gal MCD1-6HA Dchr.III 101,739-102,333pFVL57::HIS3*
YIO971	*MATa pep4Δ::g418::TRP1 trp1-1 leu2-3,112 ura3-52 his3-11,15 bar1 GAL+ MCD1-TEV-3HA SMC3Δ::clonat SMC3-6HA::URA3*
JH5257	*MATa LacO(DK)-NAT:Lys4 pHIS3-GFPLacI-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1 GAL+ SCC2-3V5-G418*
VG3176-2A	*MATα SCC3-3HA-HIS3MX trp1-1 leu2-3,112 ura3-52 lys2-801 his3-11,15 bar1 GAL+*

Table S2. In vitro substrates

Name	Coordinates	Plasmid
CC1	Chr. III 99,096–102,380	pIO2
CC2	Chr XII 441,109–444,191	pIO30
N1	Chr. III 95,500–98,488	pIO15
N2	Chr. III 204,025–206,994	pIO25
N3	pBR322 45-4215	pIO29