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Cohesin-Dependent Association of Scc2/4 with the Centromere Initiates Pericentromeric Cohesion Establishment

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Summary

Cohesin is a conserved ring-shaped multiprotein complex that participates in chromosome segregation, DNA repair, and transcriptional regulation [1, 2]. Cohesin loading onto chromosomes universally requires the Scc2/4 "loader" complex (also called NippedBL/Mau2), mutations in which cause the developmental disorder Cornelia de Lange syndrome in humans [1–9]. Cohesin is most concentrated in the pericentromere, the region surrounding the centromere [10–15]. Enriched pericentromeric cohesin requires the Ctf19 kinetochore subcomplex in budding yeast [16–18]. Here, we uncover the spatial and temporal determinants for Scc2/4 centromere association. We demonstrate that the critical role of the Ctf19 complex is to enable Scc2/4 association with centromeres, through which cohesin loads and spreads onto the adjacent pericentromere. We show that, unexpectedly, Scc2 association with centromeres depends on cohesin itself. The absence of the Scc1/Mcd11/Rad21 cohesin subunit precludes Scc2 association with centromeres from anaphase until late G1. Expression of SCC1 is both necessary and sufficient for the binding of cohesin to its loader, the association of Scc2 with centromeres, and cohesin loading. We propose that cohesin triggers its own loading by enabling Scc2/4 to connect with chromosomal landmarks, which at centromeres are specified by the Ctf19 complex. Overall, our findings provide a paradigm for the spatial and temporal control of cohesin loading.

Results and Discussion

Scc2 Association with Centromeres Depends on the Ctf19 Complex

Cohesin is highly enriched throughout the pericentromere, yet the Scc2/4 cohesin loader shows strong enrichment only within the core ~125 bp centromere [10, 19–21] (see Figures S1A and S1B available online). Enrichment of Scc2 at centromeres, but not at a control rRNA site [21, 22], requires Ctf19 complex components [17] (Figures S1C and S1D). Scc2 turns over rapidly near kinetochores [20] and does not stably associate with the Ctf19 complex (Figures S1E, S1F, and Table S3). Even when the Ctf19 complex was purified from cells producing a version of Smc3 (Smc3E1155Q) blocked at an early step in cohesin loading [20, 23], virtually the entire kinetochore was a specific effect of tethering Scc4-lacI nearby, because cohesion at CEN4 was not improved when Scc4-lacI was produced in strains without lacOs, where CEN4 was visualized with TetR-GFP bound to tetO arrays (to which Scc4-lacI cannot bind) (Figure 1H). These results indicate that a failure to recruit Scc2 to centromeres, thereby abrogating cohesin loading, causes defective centromeric cohesion in chl4.1 cells.

Centromere-Tethered Scc4 Rescues the Cohesion Defect of chl4.1 Cells

Although CEN4-tethered Scc4-LacI led to only a localized increase in Scc1, cohesin recruited to the lacOs might rescue the cohesion defect of chl4.1 cells at CEN4. We produced LacI-GFP in wild-type and chl4.1 cells carrying CEN4-proximal lacOs and tested the effect of Scc4-lacI production on CEN4-GFP separation as cells progressed from G1 into metaphase (Figure 1G). Analysis of budding indicated similar cell-cycle timing in all strains, but chl4.1 cells separated sister centromeres prematurely and to a greater extent than wild-type cells (Figure 1G). Remarkably, production of Scc4-LacI reduced the frequency of separated foci in chl4.1 cells to a level close to that of wild-type. Similarly, Scc4-LacI production improved the cohesion of wild-type CEN4 (Figure 1G). This was a specific effect of tethering Scc4-lacI nearby, because cohesion at CEN4 was not improved when Scc4-lacI was produced in strains without lacOs, where CEN4 was visualized with TetR-GFP bound to tetO arrays (to which Scc4-lacI cannot bind) (Figure 1H). These results indicate that a failure to recruit Scc2 to centromeres, thereby abrogating cohesin loading, causes defective centromeric cohesion in chl4.1 cells.
lacO

Recruitment of Scc1 and Scc2 by Scc4-LacI is dependent on showing relative levels of Scc2-6HIS-3FLAG, Scc4-LacI, Scc1-6HA, and Pgk1 (loading control) in the samples analyzed from a representative experiment.

The mean of four independent experiments is shown with error bars representing standard error (*p < 0.5, two-tailed paired t test). Anti-FLAG, anti-lacI, anti-HA, and anti-Pgk1 (D) immunoblots for Scc1 and Scc2 ChIP. qPCR analysis (A–C) using the indicated primer sets on chromosome IV (A), III (B), or V (C). The mean of four independent experiments is shown with error bars representing standard error.

Figure 1. The Ctf19 Complex Promotes Cohesion Establishment by Enabling Association of the Scc2/4 Loader with Centromeres

(A–D) Artificial recruitment of Scc2/4 to a centromere leads to a modest increase in Scc1 levels at the tethering site. An approximately 10 kb array of lacO repeats was integrated approximately 500 bp to the right of CEN4 in strains that were otherwise wild-type (AM10285), pGAL-SCC4-lacI (AM10287), or chl4Δ (AM10289), or chl4Δ pGAL-SCC4-lacI (AM10291) and carried SCC2-6HIS-3FLAG and SCC1-6HA as well as a no-tag control (AM8387). Strains were precultured in raffinose-containing medium and then arrested in nocodazole and benomyl for 2.5 hr in medium containing raffinose and galactose before harvesting for Scc1 and Scc2 ChIP. qPCR analysis (A–C) using the indicated primer sets on chromosome IV (A), III (B), or V (C). The mean of four independent experiments is shown with error bars representing standard error (*p < 0.5, two-tailed paired t test). Anti-FLAG, anti-lacI, anti-HA, and anti-Pgk1 (D) immunoblots showing relative levels of Scc2-6HIS-3FLAG, Scc4-LacI, Scc1-6HA, and Pgk1 (loading control) in the samples analyzed from a representative experiment.

(E and F) Recruitment of Scc1 and Scc2 by Scc4-LacI is dependent on lacOs. Strains with a 10 kb array of lacO repeats ~500 bp from CEN4 (E) or without lacOs (F) and with the indicated genotypes were grown and analyzed as in (A), and Scc1 and Scc2 ChIP is shown for the indicated primer sets. Strains with lacOs (E) are as in (A). Strains without lacOs (F) all carried SCC2-6HIS-3FLAG and SCC1-6HA and are otherwise wild-type (AM8413), pGAL-SCC4-lacI (AM11066), chl4Δ (AM8415), chl4Δ pGAL-SCC4-lacI (AM11065). Note that the presence of lacO arrays adjacent to CEN4 reduces cohesion association with IV-c2. Mean of three independent experiments is shown and error bars indicate standard error.

(legend continued on next page)
De Novo Cohesin Loading at the Centromere
Because neither cohesin (Scc1) nor its loader (Scc2) are localized to chromosomes in G1-arrested cells [19, 24], we asked whether the Ctf19 complex is required for de novo cohesin loading upon cell-cycle entry. Scc2 levels are unchanged throughout the cell cycle, whereas Scc1 appears only upon cell-cycle entry [19, 24] (Figures 2A and 2B). In G1-arrested cells, as expected, neither Scc1 nor Scc2, associated with five chromosomal sites tested (Figures 2C and 2D). However, upon cell-cycle entry, both Scc1 and Scc2 were rapidly recruited to chromosomes, though their pattern was distinct. Scc2 was most enriched at the centromere, was not detected in the pericentromere or at a “high-cohesin” chromatid arm site, and was present at only very low levels at a tRNA site (Figure 2C). In contrast, Scc1 associated with the centromere, pericentromere, and an arm site, and low levels were also observed at the tRNA site (Figure 2D). Both Scc1 and Scc2 associated with centromeres immediately upon cell-cycle entry (Figures 2C and 2D). However, association of Scc1 with sites distant from its loader (i.e., pericentromeres, chromosomal arm) occurred later. This is consistent with the idea that cohesin loads onto defined chromosomal sites, such as centromeres, and then subsequently translocates away from these sites into the adjacent part of the chromosome [10, 20]. Taken together with the high specificity of Scc2 at centromeres [20], it seems likely that Scc2/4 dissociates from the chromosome following the loading reaction.

Deletion of CHL4 abolished the recruitment of both Scc1 and Scc2 to centromeric, but not chromatid, sites (Figures 2C and 2D). Consistent with the idea that pericentromeric cohesin is derived mainly from that loaded at centromeres, cohesin accumulation within the pericentromere was also greatly reduced in the chl4Δ mutant. Interestingly, however, low levels of cohesin appeared in the pericentromere after a delay (Figure 2D). Although this could be explained by weak loading activity in the pericentromere, we favor the idea that it travels from loading sites outside the pericentromere [10].

Scc2 Association with Kinetochores Is Dependent on Chl4
We confirmed the cell-cycle regulation of Scc2 association with centromeres and its dependence on the Ctf19 complex by time-lapse microscopy of live cells carrying Scc2-GFP and the kinetochore marker Mtw1-tdTomato (Figures 2E-2H; Movies S1, S2, S3, and S4). In wild-type cells, Scc2-GFP showed enrichment at kinetochores from late G1 until metaphase, though nuclear localization was observed at all cell-cycle stages (Figures 2E and 2F). Interestingly, the kinetochore-associated Scc2-GFP signal disappeared in anaphase (Figures 2E and 2F), though nuclear localization was subject to temporal control and was not observed in early G1 cells (Figures 2E and 2F). What controls the timing of Scc2 association with centromeres? We ruled out DNA replication as a possible cause, given that cohesin can associate with unreplicated chromosomes [25] (Figure S2). Another possibility is that the cohesin subunit, Scc1, which is absent in G1 and cleaved in anaphase [24, 26, 27] (Figure 2A) is required for Scc2 association with centromeres. Previous experiments using temperature-sensitive versions of cohesin subunits are difficult to interpret, given that we found that centromeric Scc2 levels are affected by temperature even in wild-type cells [19] (Figure S3).

Instead, we analyzed Scc2 association with chromosomes after Scc1 depletion. Endogenous SCC1 was placed under the methionine-repressible promoter and Scc2 localization was analyzed as cells entered the cell cycle in the presence of methionine to prevent SCC1 expression. We confirmed that Scc2 was present at all time points, that Scc1 was successfully depleted in pMET-SCC1-19MYC cells, and that both strains entered the cell cycle with similar timing (Figures 3A and 3B). Although Scc2 was loaded onto the centromere in wild-type cells, its levels did not rise above background in Scc1-depleted cells (Figure 3C). Similar results were obtained in metaphase-arrested cells (Figures 3D and 3E). We conclude that SCC1 expression is required for the association of Scc2 with centromeres.

SCC1 Expression Is Required for Scc2 Association with Centromeres
Is Scc1 expression the key event that triggers cohesin loading upon cell-cycle entry? If so, ectopic production of Scc1 in G1-arrested cells would be predicted to trigger Scc2 association with the centromere and cohesin loading. To allow expression of SCC1 in G1, we integrated an additional copy of SCC1 carrying a 3HA tag under control of the galactose-inducible promoter (pGAL-SCC1-3HA). As expected, in G1-arrested cells grown in raffinose, Scc1-3HA was absent, though Scc2 was produced (Figure 4B) and neither protein localized to chromosomes (Figure 4A). However, addition of galactose to induce pGAL-SCC1-3HA during the G1 arrest drove Scc1-3HA production and, despite residual separase activity in early G1 cells, robust full-length Scc1 production (Figures 4B and 4A). Remarkably, Scc1 production led to the association of both Scc2 and Scc1-3HA with the centromere (Figures 4A and 4B). Similar to the loading of endogenous cohesin, ectopic G1-loaded Scc1-3HA was found in the pericentromere (Figure 4A). Furthermore, ectopic G1-loaded Scc1-3HA also associated with a chromosome arm site where endogenous cohesin normally resides (Figure 4A), suggesting that Scc1 production in G1 triggers transient Scc2 association with sites throughout the genome, though we are only able to detect it reliably at the centromere among the sites tested. Taken together with the data shown in Figure 3C, these findings

(G) Tethering Scc2/4 to centromeres rescues the centromeric cohesin defect of chl4Δ cells. Strains with approximately 10 kb lacO arrays integrated adjacent to CEN4, producing LacI-GFP and carrying pMET-CDC20, were arrested in G1 in raffinose-containing medium lacking methionine by treatment with alpha factor before being released into medium containing raffinose, galactose, and methionine to induce a metaphase arrest and induce Scc4-LacI. Samples were taken at the indicated time points after release from alpha factor, and the number of GFP foci per cell and budding was scored. A representative experiment is shown. Strains used were AM10570 (wild-type), AM10571 (pGAL-SCC4-lacI), AM10572 (chl4Δ), and AM10573 (chl4Δ pGAL-SCC4-lacI).

[H] Scc4-lacI requires lacO arrays at CEN4 to rescue the cohesin defect of chl4Δ cells. To visualize CEN4, while preventing Scc4-lacI binding, tetO arrays were integrated approximately 2.4 kb right of CEN4 in pMET-CDC20 strains producing TetR-GFP. Wild-type (AM4643), SCC4-lacI (AM1162), chl4Δ (AM4644), and chl4Δ SCC4-lacI (AM11183) were treated as described in (G), and a representative experiment is shown. See also Figure S1.
Figure 2. Scc2 Associates with Centromeres from Late G1 until Anaphase

(A–D) Sites of association of Scc1 and Scc2 as cells progress into the cell cycle and their dependence on Chl4. Strains AM1176 (no tag), AM8414 (Scc2-6HIS-3FLAG), and AM8415 (Scc2-6HIS-3FLAG, chl4Δ) were arrested in G1 in alpha factor at room temperature, then released into medium containing benomyl and nocodazole at 18°C. Samples were extracted prerelease (G1) and at 15, 30, 45, and 60 min following release for analysis of Scc1-6HA and Scc2-6His-3FLAG levels by western blotting (A), analysis of DNA content by FACS (B), and association of Scc1-6HA and Scc2-6HIS-3FLAG Chromatin IP

(chromosome IV

C) no tag ■ wild type ▲ chl4Δ

CEN pericentromere arm tRNA arm

0 0.005 0.01 0.015 enrichment/input

G1 15 30 45 60 G1 15 30 45 60 G1 15 30 45 60 G1 15 30 45 60

(E–G) merge late G1 S phase/G2

Wild type

Scc2-GFP

Mtw1-tdTomato

DIC 0 14 42 56

G

 late G1 S phase/G2

merge

Wild type

Scc2-GFP

Mtw1-tdTomato

DIC 0 14 42 56

(H) merge metaphase anaphase

Wild type

Scc2-GFP

Mtw1-tdTomato

DIC 0 7 14 21

chl4Δ

merge metaphase anaphase

Wild type

Scc2-GFP

Mtw1-tdTomato

DIC 0 7 14 21
show that SCC1 expression upon cell-cycle entry is both necessary and sufficient for Scc2 association with centromeres, at least, and cohesin loading throughout the genome. These findings refute the view that the Scc2/4 complex is established on centromeres prior to binding cohesin and rather suggest that Scc2 associates with chromosomes only during the act of cohesin loading.

Production of Scc1 Promotes the Association of Cohesin with Its Loader

The Smc1 and Smc3 subunits of cohesin associate to produce a V-shaped structure, but association of the Scc1 subunit is required to close the ring and allow its association with a V-shaped structure, but association of the Scc1 subunit is required to close the ring [28] and allow its association with chromosomes [20]. We hypothesized that cohesin ring formation upon Scc1 expression in G1 might allow Scc2/4 binding, enabling the entire complex to associate with chromosomes and the cohesin loading reaction to occur. This idea predicts that cohesin subunits associate with the Scc2/4 loader only in the presence of Scc1. To test this possibility, we immuno-precipitated Scc2-6His-3FLAG either from wild-type cells or enables the entire cohesin ring to associate with its loader. To test whether other cohesin subunits are required for Scc2 association with centromeres, we tagged Smc3 with the auxin-inducible degron (aid) [29]. Treatment of G1-arrested Smc3-aid cells with auxin (NAA), triggered Smc3-aid degradation and greatly reduced the levels of Scc1 and Scc2 recruited to centromeres upon ectopic Scc1 production (Figures 4D and S4B). These results indicate that cohesin ring formation promotes the association of the Scc2/4 cohesin loader with centromeres.

Ectopic Scc2 Loading at Centromeres Requires Chl4

We asked whether the spatial information provided by the Ctf19 complex is also required for Scc2 to associate with centromeres during ectopic Scc1-induced G1 loading. Although Scc1 production in G1 triggered Scc2 binding to the centromere in wild-type cells, it failed to do so in chl4Δ cells (Figures 4E and 4F). However, levels of ectopically produced Scc1 at a chromosomal arm site were similar in wild-type and chl4Δ cells, indicating that only the
Figure 4. SCC1 Expression Is Sufficient for Scc2 to Associate with Centromeres and for Scc1 Loading in G1
(A and B) Strains AM9335 (SCC2-6HIS-3FLAG) and AM9334 (SCC2-6HIS-3FLAG pGAL-SCC1-3HA) were arrested in G1 in raffinose-containing medium with alpha factor for 3 hr. Samples were extracted for anti-FLAG, anti-HA, and anti-Pgk1 immunoblotting (B) and ChIP-qPCR (A; raffinose), then 2% galactose
(legend continued on next page)
centromeric loading site was affected by chl4. Interestingly, low levels of Scc1 were also detected in the pericentromere in chl4 cells under these conditions (Figure 4E), again supporting the idea that cohesin loaded at sites distant from the centromere can travel into the pericentromere in chl4 cells.

Live-cell microscopy of wild-type and chl4 cells ectopically expressing SCC1 confirmed that Scc2 can associate with kinetochores at all cell-cycle stages in a Chl4-dependent manner (Figure 4G). We conclude that Scc1 production is the limiting factor for cohesin loading during the cell cycle.

Requirements for Cohesin Loading

We have defined the requirements for cohesin loading onto centromeres by Scc2/4. We show that cohesin loading at centromeres is defined temporally by the availability of the Scc1 subunit of cohesin and spatially by the Ctf19 complex. Scc2/4 is not prebound to centromeres, but rather Scc2/4 and cohesin bind coordinately. It seems likely that cohesin ring formation is similarly required for Scc2/4 association with its other, much weaker, sites of association on chromosome arms. This suggests a model that invokes Scc2/4 as an accessory factor that both connects cohesin with spatial landmarks and enables its release onto the chromosome (Figure 4H). This draws parallels to the loading of sliding clamps during DNA replication where prebinding of the loader opens the clamp and triggers DNA binding, thereby stimulating the ATPase activity of the loader and release of the clamp [30]. Cohesin itself, rather than its loader, hydrolyzes ATP; nevertheless, this is important for cohesin association with loading sites but rather its translocation away from these sites [20]. Our findings imply the requirement for a docking site for the Scc2/4-cohesin complex to perform this loading reaction. Clearly, the predominant docking site is at centromeres, defined by the Ctf19 complex, though weaker, less-defined sites must exist on chromosome arms [22]. Presumably, new cohesin loading sites must also be set up in response to a need to generate new cohesive domains to drive cohesin-dependent alterations in transcription or repair of DNA lesions [5-7]. The ability to spatially and temporally regulate cohesin loading might be especially critical in these contexts where dynamic chromatin interactions are required.

Supplemental Information

Supplemental Information includes four figures, three tables, and Supplemental Experimental Procedures, and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.02.022.

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