A SIR-independent role for cohesin in subtelomeric silencing and organization

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Cohesin is a key determinant of chromosome architecture due to its DNA binding and tethering ability. Cohesin binds near centromeres and chromosome arms and also close to telomeres, but its role near telomeres remains elusive. In budding yeast, transcription within 20 kb of telomeres is repressed, in part by the histone-modifying silent information regulator (SIR) complex. However, extensive subtelomeric repressed domains lie outside the SIR-binding region, but the mechanism of silencing in these regions remains poorly understood. Here, we report a role for cohesin in subtelomeric silencing that extends even beyond the zone of SIR binding. Clusters of subtelomeric genes were preferentially derepressed in a cohesin mutant, whereas SIR binding was unaltered. Genetic interactions with known telomere silencing factors indicate that cohesin operates independent of the SIR-mediated pathway for telomeric silencing. Mutant cells exhibited Mpk1-dependent Sir3 hyperphosphorylation that contributes to subtelomeric derepression to a limited extent. Compaction of subtelomeric domains and tethering to the nuclear envelope were impaired in mutant cells. Our findings provide evidence for a unique SIR-independent mechanism of subtelomeric repression mediated by cohesin.

cohesin | silencing | telomere | chromatin organization | transcriptome

Chromatin is organized in a nonrandom orderly manner within the nucleus, and this organization affects its functions. The primary configuration of chromatin in the form of basic histone-containing nucleosome arrays is further reorganized by association with nonhistone proteins such as SMC (structural maintenance of chromosomes) proteins that bring about higher-order folding states by loop extrusion and compaction (1, 2). Cohesin is a key architectural chromosomal protein complex of the SMC family (3). Smc proteins are conserved, essential chromosomal structural proteins that engage in diverse chromosomal transactions (1, 4). Cohesin consists of a heterodimer of Smc1 and Smc3, forming a V-shaped structure having coiled-coil arm regions attached to ATP-binding head domains bridged by Mcd1/Scc1 associated with Scc3/Irr1 (3, 5, 6). Its primary role in sister-chromatid cohesion (7, 8) has been extensively investigated. Cohesin binds chromosomes at centromeres and specific sites along the arms termed CARs (cohesin-associated regions) that are spaced ∼10 kb apart and frequently occur in intergenic regions (9–11).

Deficiency of cohesin is associated with cancer (12, 13) and serious developmental defects in humans (14–16), underscoring the importance of understanding the complete spectrum of its roles in the cell. Studies in budding yeast have revealed that in addition to sister-chromatid cohesion, cohesin is also required for mitotic chromosome condensation (2, 7), DNA double-strand break repair (17–19), progression of replication forks (20), and barrier activity of tDNA boundary elements (21, 22). Inactivation of cohesin in budding yeast has also been shown to affect the expression levels of a small number of genes (23). While a role for cohesin in establishment of silencing in budding yeast was also investigated earlier, no evidence indicating a requirement of cohesin for silencing was found. Instead, it was found that cohesin hinders the establishment of silencing at the silent mating locus, *HMR* (24). Since changes in gene expression due to cohesin dysfunction have been postulated to contribute to developmental defects associated with cohesinopathies, it is important to understand the various mechanisms by which cohesin modulates gene expression.

While cohesin binds to sites near chromosomal subtelomeric regions in *Saccharomyces cerevisiae* (10, 11, 25), a requirement for its function near telomeres has not been explored extensively. Telomeres are protective repetitive regions present at chromosome ends maintained by the activity of telomerase (26). Early studies in *Drosophila melanogaster* investigating variegated expression of eye-color genes in particular mutant lines revealed that telomeres exert position effects on genes inserted nearby (position effect variegation) such that their expression is repressed (27). In *S. cerevisiae*, telomere-proximal regions also exhibit telomere position effect, repressing the expression of reporter genes adjacent to telomeres in a Sir2/3/4 (SIR) complex-dependent manner (28, 29). The activity of the SIR complex compacts and shields the underlying DNA from the transcriptional machinery, resulting in silencing (30). Within the nucleus, telomeres are usually situated at the nuclear periphery near the nuclear envelope (31, 32). The peripheral subnuclear zone favors silencing that may contribute further to telomere silencing (32–35).

Recent genome-wide studies have shown that genes located up to ∼20 kb from a telomere are expressed at a lower level; however, only a small subset of these genes are silenced in a SIR-dependent manner (36, 37). This raises the possibility that additional mechanisms may exist to achieve repression in subtelomeric regions.

Significance

Cohesin is a conserved chromatin-associated SMC complex crucial for higher-order chromosome architecture. Cohesin mutations in humans are associated with severe developmental defects thought to arise from altered gene expression. We report that cohesin is an important determinant of subtelomeric gene repression in budding yeast. The conserved histone deacetylase Sir2, along with other silencing factors, is required for telomere silencing. However, silencing in extensive subtelomeric regions is SIR-independent, but poorly understood. We found that cohesin deficiency results in preferential derepression of multiple subtelomeric genes and its contribution to silencing is Sir-independent. Interestingly, cohesin mutants are defective in nuclear envelope tethering and compaction of subtelomeric regions. Our findings suggest that cohesin may directly contribute to subtelomeric silencing by altering telomere organization.

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Here, we report a requirement for cohesin in transcriptional repression of subtelomeric genes. We further demonstrate that the contribution of cohesin to telomere silencing is SIR-independent. Cohesin dysfunction also impairs telomere tethering to the nuclear envelope and is associated with a more open chromatin configuration near telomeres.

Results

Telomere Silencing Defect in a Cohesin Mutant. To test whether cohesin affects telomere-proximal silencing, a temperature-sensitive (ts) allele of the *MCD1* gene, *mcd1-1*, was introduced into a silencing reporter strain, CCFY101 (a kind gift from Kurt Runge, Cleveland Clinic, Cleveland), having the *URA3* reporter inserted adjacent to the right telomere of chromosome V (Fig. 1*A*). The *mcd1-1* mutant is viable at 23 °C but displays a strong ts phenotype resulting in inviability at 37 °C (7). Only a very slight growth defect is observed at 31 °C (Fig. 1*A*, *Top*), but at this semipermissive temperature almost complete abrogation of silencing of the telomere-proximal reporter *URA3* was observed, as evidenced by failure of the *mcd1-1* silencing reporter strains to grow on plates containing 5-fluoroorotic acid (5-FOA) that selects against $Ura3^+$ cells (Fig. 1*A*, *Bottom*). We further confirmed this result in two other telomere silencing reporter strains, ROY783, harboring a synthetic telomere on chromosome VR having *URA3* inserted close to the telomere (21) (*SI Appendix*, Fig. S1*A*), and YEF452 (38), having *URA3* near the telomere of the left arm of chromosome XI (*SI Appendix*, Fig. S1*B*). Once again, the telomere VR-proximal *URA3* was strongly derepressed at 31 °C but not at 23 °C, while the chromosome XIL–proximal *URA3* was only mildly derepressed in *mcd1-1* at 31 °C when assayed on 5-FOA plates (*SI Appendix*, Fig. S1 *A* and *B*, respectively). We also assayed RNA levels of the telomere-proximal *URA3* reporter in derivatives of CCFY101 that were modified such that the endogenous *URA3* locus is replaced by *KanMX4* and these strains hence completely lack sequences homologous to *URA3* elsewhere in the genome. Real-time PCR analysis of RNA isolated from wild-type or *mcd1-1* derivatives of these strains showed an increase (3.58-fold, $P = 0.001$) in the levels of *URA3* transcripts in the mutant (Fig. 1*A*, *Bottom*), confirming that enhanced 5-FOA sensitivity detected in the growth assays may indeed be due to an increase in *URA3* transcripts resulting from derepression of transcription of the reporter and not due to differential sensitization of the mutant to 5-FOA due to altered nucleotide metabolism (39).

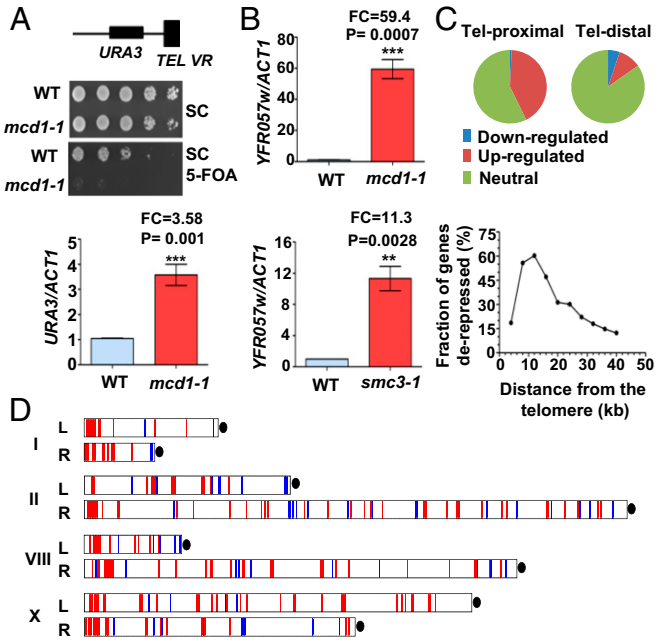

Fig. 1. Cohesin represses subtelomeric gene expression. (*A*) Schematic of modified telomere-VR in CCFY101 (*Top*). Spot tests to analyze silencing of telomere-proximal *URA3* at 31 °C (*Middle*) in wild type (SLY2199) and *mcd1-1* (SLY2200). RT-qPCR of *URA3* in wild type (SLY2420) and *mcd1-1* (SLY2422) (*Bottom*). (*B*) RT-qPCR of *YFR057w* in wild type (SLY2420) and *mcd1-1* (SLY2422) (*Top*), or wild type (ROY951) and *smc3-1* (ROY1060) (*Bottom*). Error bars represent SEM; means of $n \geq 3$ experiments are plotted. $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ (two-tailed unpaired *t* test). FC, fold change. (*C*) Pie chart comparing the proportion of affected/unaffected genes in subtelomeric (within 20 kb from the end) with telomere-distal regions (*Top*). The graph shows the distribution of the fraction of derepressed genes with respect to distance from the telomere (*Bottom*). The endpoint of each 4-kb interval is plotted on the *x* axis. (*D*) Chromosomal display of changes in gene expression in *mcd1-1* for selected chromosomes. Affected (≥2-fold, adjusted $P \leq 0.05$) genes (red, derepressed; blue, repressed) and unaffected (white) genes are ordered as per their position from the telomere to the centromere (black circles).

We also examined the effect of the *mcd1-1* mutation on silencing of naturally occurring subtelomeric genes. We compared the transcript levels of one such subtelomeric gene located near a SIR-binding peak in the genome (*YFR057W*), whose silencing was previously reported to be abrogated significantly in *sir* mutants (36). Real-time qPCR analysis of RNA from wild-type or *mcd1-1* cells grown at 31 °C showed a substantial increase in transcripts corresponding to *YFR057W* in the mutant (Fig. 1*B*, *Top*) (59.4-fold, $P = 0.0007$), while only a modest increase (2.43-fold) was observed at the permissive temperature (23 °C) (*SI Appendix*, Fig. S1*C*). Likewise, significant derepression of *YFR057W* transcripts was also observed in *smc3-1* (Fig. 1*B*, *Bottom*) (11.3-fold, $P = 0.0028$), a mutant defective in another subunit (Smc3) of cohesin, indicating that the observed silencing defect is not limited to dysfunctional Mcd1 in *mcd1-1* but represents a more general role of the cohesin complex.

While mapping the transcriptional landscape of budding yeast chromosome ends, it was reported that genes present within ∼20 kb of chromosome ends are expressed at a lower level compared with the rest of the genome, but only a small subset (∼6%) of these subtelomeric genes are repressed in a SIR-dependent manner (36, 37). We examined expression levels of two other subtelomeric genes, *SEO1* and *YHL044w*, that were also derepressed in *mcd1-1* (*SI Appendix*, Fig. S1*D*) (24.10-fold, $P = 0.0067$; 7.43-fold, $P = 0.0004$) and *smc3-1* (*SI Appendix*, Fig. S1*E*) (3.84-fold, $P = 0.0304$; 3.06-fold, $P = 0.0005$). The expression of these genes was unaffected in *sir2Δ* here (*SI Appendix*, Fig. S1*F*) and in the earlier study (36). We also found that asynchronous *mcd1-1* cells showed a cell-cycle distribution profile indistinguishable from wild type at 31 °C (*SI Appendix*, Fig. S2*A*) and derepression of subtelomeric genes was also observed in nocodazole-arrested *mcd1-1* cells (*SI Appendix*, Fig. S2*B*), confirming that the changes in gene expression were not an indirect consequence of cell-cycle arrest in the mutant. G1-arrested wild-type and mutant cells had comparable *YFR057w* transcripts (*SI Appendix*, Fig. S2*C*), indicating that cohesin does not repress subtelomeric gene expression in G1, possibly because Mcd1 is limiting in G1.

We extended the differential gene expression analysis comparing *mcd1-1* with wild type at a genome-wide scale by RNA sequencing. Approximately 42% of genes located within 20 kb of telomeres exhibited significantly increased levels of transcripts (≥2-fold; Dataset S1), whereas only ∼10% of genes located elsewhere in the genome were similarly derepressed (Fig. 1*C* and *SI Appendix*, Table S1). In contrast, only 0.69% of subtelomeric genes were down-regulated (Dataset S1), whereas 5.21% of telomere-distal genes were down-regulated (Fig. 1*C* and *SI Appendix*, Table S1); a majority of the telomere-distal genes were unaffected.

We also compared the observed fraction of derepressed subtelomeric genes in the mutant with the genome-wide average using the χ^2 test. Notably, genes repressed by cohesin are significantly (at significance level $\alpha = 0.0001$) enriched within subtelomeric regions between 4 and 24 kb from the chromosome

ends (Fig. 1*C*, *Bottom* and *SI Appendix*, Table S2). Repression of genes within 4 kb from the end is also impaired (18.57% relative to the genome-wide average of 11.44%, $\alpha = 0.05$; *SI Appendix*, Table S2) in *mcd1-1*, albeit to a lesser extent. When we analyzed the effects on gene expression with respect to position across individual chromosomes, we observed enrichment of clusters of derepressed genes in the subtelomeric regions in *mcd1-1*, as shown for several representative chromosomes (Fig. 1*D*) and all chromosomes (*SI Appendix*, Fig. S3). Notably, down-regulated genes in *mcd1-1* were scarce near chromosome ends; more strikingly, clusters of repressed genes were absent near ends (Fig. 1*D* and *SI Appendix*, Fig. S3).

Association of Silencing Proteins and Modified Histones with Telomeric Heterochromatin in *mcd1-1*. Formation of silent chromatin at telomeres depends on the SIR proteins Sir2, Sir3, and Sir4 (29). To rule out the possibility that expression of SIR proteins may be limiting in *mcd1-1*, resulting in derepression of silencing, we compared their steady-state levels in cell lysates by western blotting and found comparable levels of Sir2, Sir3-6HA, and Sir4-9myc in wild-type and mutant cells (*SI Appendix*, Fig. S4*A*). We then assayed the association of SIR proteins with *YFR057w*, one of the native subtelomeric genes that was strongly derepressed in the mutants (Fig. 1*B*), by chromatin immunoprecipitation (Fig. 2*A*). Surprisingly, there was no significant reduction in binding of Sir2, Sir3, or Sir4 to the *YFR057w* ORF (Fig. 2*A*) in *mcd1-1*. The binding of Sir2 to a site upstream (1,456 bp) and downstream (870 bp) of *YFR057w* (or 2.5 kb and 6 bp from the end, respectively) was unaltered (*SI Appendix*, Fig. S4*B*, *Left* graph). Binding and spreading of Sir3 1,456 and 3,126 bp upstream of *YFR057w* (or 2.5 kb and 4 kb from the end, respectively) was also not significantly altered (*SI Appendix*, Fig. S4*B*, *Right* graph). In addition, no defect in Sir2 binding to *YFR057w* was observed in *smc3-1* (*SI Appendix*, Fig. S4*C*).

We also examined telomere silencing-associated histone marks at *YFR057w* in *mcd1-1*. Not much increase in H4K16ac, a hallmark of euchromatin, was observed in *mcd1-1* (1.69-fold), whereas *sir2*Δ (defective in deacetylation of H4K16) showed a substantial increase in H4K16ac relative to wild type (5.55-fold) (Fig. 2*B*, *Middle* and *SI Appendix*, Fig. S4*D*, respectively). In addition, *mcd1-1* showed no change in H3K4me3, an active chromatin mark (Fig. 2*B*, *Left*), and not much increase in H3K79me2 (1.45-fold) (Fig. 2*B*, *Right*). However, association of cohesin with the CAR (25) about 0.5 kb from the end of telomere VIR, near *YFR057w*, was decreased by 38% in the mutant (*SI Appendix*, Fig. S4*E*). Interestingly, this was accompanied by enhanced binding of Cet1 (by 43%) (*SI Appendix*, Fig. S4*F*), a component of the active RNA Pol II complex to *YFR057w*, suggesting that enhanced transcript levels of these genes may arise in part from enhanced transcription as a result of increased RNA Pol II occupancy to some extent.

Genetic Interaction of Cohesin with Silencing Factors Reveals a SIR-Independent Contribution of Cohesin to Telomere Silencing. Since we did not observe any difference in association of Sir proteins with derepressed subtelomeric genes, and Sir proteins were silencing-competent in *mcd1-1* (*SI Appendix*, Fig. S5 and *Text*), we wondered whether the role of cohesin in subtelomeric silencing could be Sir-independent. To test whether the silencing defect in *mcd1-1* was indeed SIR-independent, we compared the transcript levels of *YFR057w* in *mcd1-1 sir2*Δ and *mcd1-1 sir4*Δ double mutants with *mcd1-1*, *sir2*Δ or *sir4*Δ, and wild-type cells. We found that *YFR057w* transcripts were significantly more abundant in the double mutants relative to either of the *sir* mutants, *mcd1-1*, or wild-type cells (Fig. 2 *C* and *D*). Likewise, comparison of *YFR057w* transcript levels in the *smc3-1 sir2*Δ double mutant with *smc3-1*, *sir2*Δ, and wild-type cells also yielded similar results (Fig. 2*E*), confirming that cohesin can indeed contribute to subtelomeric silencing independent of Sir proteins. Similar results were obtained

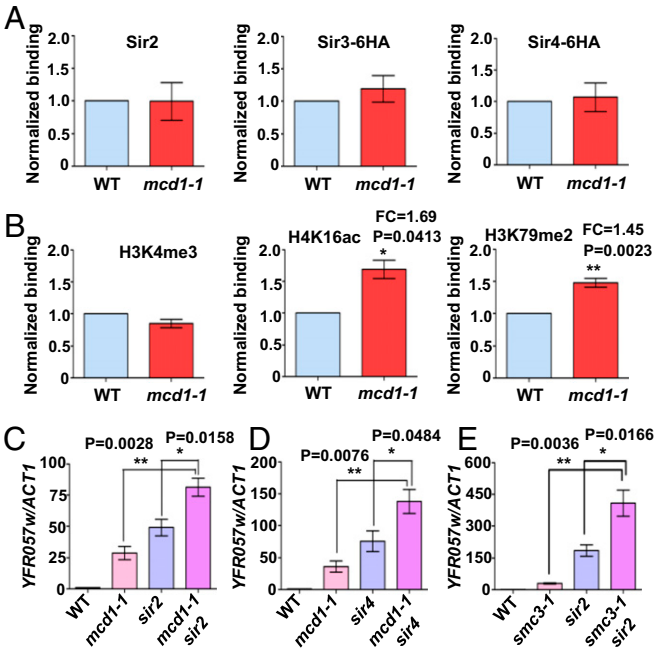

Fig. 2. Cohesin dysfunction-associated derepression of subtelomeric genes is SIR-independent. (*A*) Binding of Sir proteins at *YFR057w* by ChIP-qPCR. Comparison of Sir2 binding in WT (SLY2420) and *mcd1-1* (SLY2422), Sir3-6HA in WT (SLY2595) and *mcd1-1* (SLY2597), and Sir4-6HA in WT (SLY2587) and *mcd1-1* (SLY2589). (*B*) Analysis of histone modifications at *YFR057w*. ChIP-qPCR of H3K4 trimethylation (*Left*) and H4K16 acetylation (*Middle*) in WT (SLY2420) and *mcd1-1* (SLY2422), and H3K79 dimethylation in WT (SLY2764) and *mcd1-1* (SLY2767) (*Right*). Fold enrichment is relative to *PHO5*; wild-type values are normalized to 1 (*$P \leq 0.05$ and **$P \leq 0.01$, paired *t* test). (*C* and *D*) RT-qPCR of *YFR057w* in wild type (SLY2201), *mcd1-1* (SLY2202) compared with *sir2* (SLY2531) and *mcd1-1 sir2* (SLY2533) (*C*), and *sir4* (SLY2541) and *mcd1-1 sir4* (SLY2543) (*D*). (*E*) RT-qPCR of *YFR057w* in wild type (ROY951), *smc3-1* (ROY1060), *sir2* (SLY2747), and *smc3-1 sir2* (SLY2749). Means of $n \geq 3$ experiments are plotted; bars represent SEM. *$P \leq 0.05$ and **$P \leq 0.01$ (two-tailed unpaired *t* test).

with *yku70*, a mutant defective in Yku70 that participates in the SIR-mediated silencing pathway by recruiting silencing proteins (*SI Appendix*, Fig. S6 and *Text*).

Activation of Mpk1-Mediated Stress Signaling in *mcd1-1*. Our interesting yet confounding observation of a disruption of subtelomeric silencing uncoupled from defects in SIR recruitment and formation of histone activation marks in the cohesin mutant resembles earlier observations made for subtelomeric derepression during the stress response (40, 41). A hallmark of rapamycin (that induces stress by targeting target of rapamycin kinases)-induced derepression of telomeric silencing is Mpk1-mediated Sir3 hyperphosphorylation (41). To test whether a similar stress response may be activated in *mcd1-1*, we examined Sir3 phosphorylation. Indeed, *mcd1-1* cells had enhanced slow-migrating electrophoretic forms of Sir3 similar to the rapamycin-induced hyperphosphorylated forms (Fig. 3*A*) (41) whose accumulation was Mpk1-dependent (Fig. 3*B*). In addition, the phosphorylated form of Mpk1 was more abundant in the mutant, indicating its activation in *mcd1-1* (Fig. 3*C*). Mutant cells exhibited other properties of Mpk1-mediated stress signaling, such as derepression of *PAU3* (Fig. 3*D*), a member of the subtelomeric *PAU* gene family, encoding cell-wall mannoproteins. Deletion of *MPK1* in *mcd1-1* only slightly diminished *YFR057w* transcripts relative to *mcd1-1* (Fig. 3*E*). These observations indicate that while Mpk1-mediated signaling was activated in *mcd1-1*, it only contributes to derepression to a very limited extent; there was a substantial additional contribution of cohesin to subtelomeric

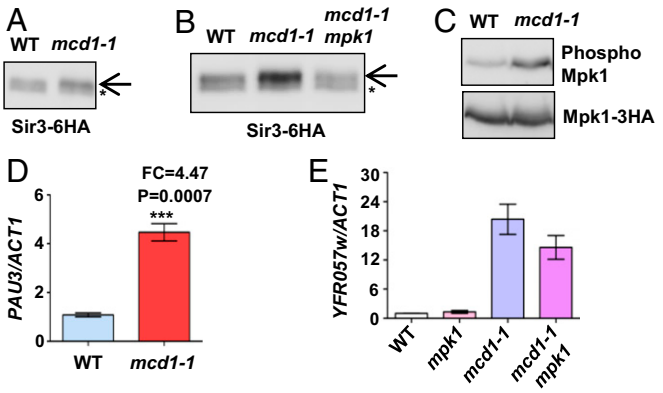

Fig. 3. Activation of a stress-responsive silencing regulatory pathway in *mcd1-1*. (*A* and *B*) Mpk1-mediated Sir3 hyperphosphorylation in *mcd1-1*. (*A*) Western blot showing Sir3-6HA phosphorylation in WT (SLY2662) and *mcd1-1* (SLY2664). (*B*) Sir3-6HA phosphorylation in WT (SLY2662), *mcd1-1* (SLY2664), and *mcd1-1 mpk1* (SLY2668). Arrows denote the modified form; asterisks denote the unmodified form. (*C*) Mpk1 phosphorylation in WT (SLY2794) and *mcd1-1* (SLY2796). (*D*) RT-qPCR of *PAU3* in WT (906-1A) and *mcd1-1* (955-7D). (*E*) RT-qPCR of *YFR057w* in wild type (SLY2764), *mpk1* (SLY2869), *mcd1-1* (SLY2767), and *mcd1-1 mpk1* (SLY2871). Means of $n \geq 3$ experiments are plotted; bars represent SEM. $^{***}P \leq 0.001$ (two-tailed unpaired *t* test).

silencing, independent of the stress pathway-induced Sir3 hyperphosphorylation.

Analysis of Telomere Tethering in *mcd1-1*. Given our failure to detect significant changes in molecular composition of subtelomeric heterochromatin, we examined the effect of cohesin dysfunction on telomere tethering. The sister-chromatid tethering function of cohesin near a telomere and at an arm site was indeed impaired in *mcd1-1* at 31 °C (*SI Appendix*, Fig. S7 *A* and *B* and *Text*). To explore additional potential mechanisms for derepression of telomere-proximal genes, we examined telomere tethering to the nuclear envelope. The *mcd1-1* mutation was integrated in a strain having a Tel-VR–proximal GFP-based chromosomal tag (42) that also expresses Nup49-GFP that illuminates the nuclear envelope, facilitating precise delineation of the nuclear boundary (Fig. 4*A*). The percentage of nuclei having tethered versus untethered configuration was enumerated in G2/M-arrested cells at 31 °C; G1 cells and anaphase cells were excluded from the analysis. We found a modest but statistically significant increase in untethering of Tel-VR from the nuclear envelope in *mcd1-1* (from 26% in the wild type to 41% in the mutant) (Fig. 4*A*, *Right*). Hence, our findings indicate that cohesin contributes to telomere cohesion and nuclear membrane tethering of telomeres.

Assessment of Chromatin Accessibility in *mcd1-1*. Cohesin also has a role in chromosome condensation (2, 7). Earlier studies demonstrated that telomeres have a special compact organization particularly refractory to DNA-modifying enzymes (30). We compared compaction in wild-type and *mcd1-1* cells by quantifying Dam-methylase (an enzyme that methylates adenine within a GATC sequence) accessibility of subtelomeric chromatin near Tel-VIR (30). Subtelomeric chromatin (at *YFR057w* and *SEO1*) in the mutant appeared to be more accessible as it was more susceptible to cleavage by Dpn1, a methylation-dependent restriction enzyme, indicating a relatively open organization; the same region was equally sensitive to Sau3A1, a methylation-insensitive restriction endonuclease (Fig. 4*B* and *SI Appendix*, Fig. S7*C*). These findings indicate that subtelomeric chromatin in *mcd1-1* assumes a more open conformation. Interestingly, the same region was even more accessible in the *mcd1-1 sir2* double mutant, indicating their independent contributions to closed chromatin configuration near telomeres (Fig. 4*B*). A telomere-distal euchromatin region (at *YFL015c*) was also more accessible in *mcd1-1* (*SI Appendix*, Fig. S7*D*), indicating that open configuration in the mutant is not limited to subtelomeric regions.

Discussion

Cohesin's expanding repertoire of functions includes not only its original well-studied role in sister-chromatid cohesion but several other chromosome transactions. Cohesin dysfunction resulting in altered gene expression has been implicated as a contributory factor to developmental defects in cohesinopathies. Hence, understanding the multiple mechanisms by which cohesin regulates gene expression has high relevance. Here we report a previously unappreciated role

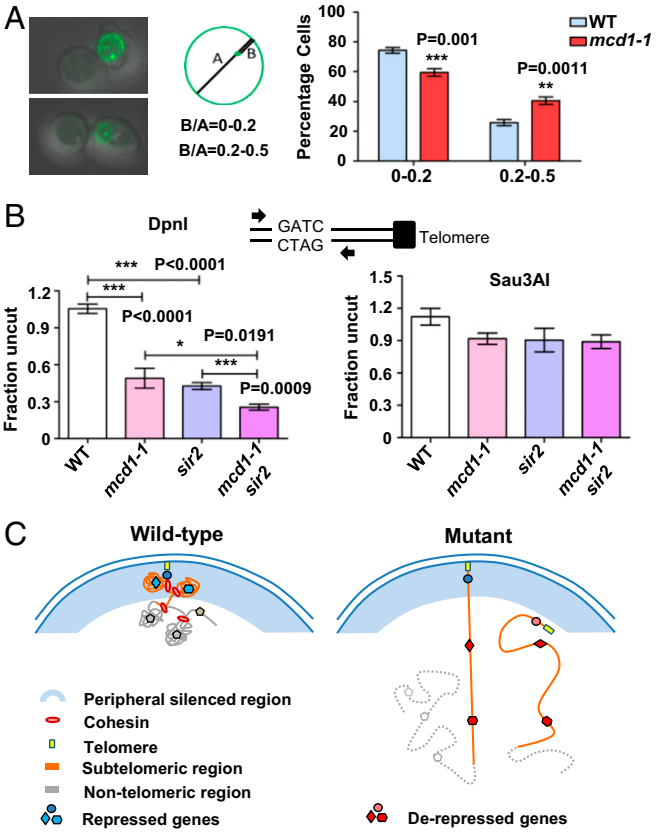

Fig. 4. Telomere organization defects in *mcd1-1*. (*A*) Telomere tethering to the nuclear periphery is defective in *mcd1-1*. Tethering of GFP-tagged TEL-VR was measured in nocodazole-arrested WT (SLY2442) and *mcd1-1* (SLY2445) strains expressing *NUP49-GFP*. Representative images showing tethered (*Top*) and untethered (*Bottom*) configurations. A B/A value of 0 to 0.2 was considered as tethered, whereas 0.2 to 0.5 was considered untethered (A, nuclear diameter; B, distance of the telomere dot from the nuclear envelope). Graph shows the percentage cells having tethered versus untethered telomeres. (*B*) Assessment of chromatin accessibility at *YFR057w* by Dam-methylase accessibility assay in wild type (SLY2873), *mcd1-1* (SLY2875), *sir2* (SLY2877), and *mcd1-1 sir2* (SLY2879) by digestion with DpnI (*Left*) or Sau3AI (*Right*) followed by qPCR. Schematic depicts locations of primers (arrows) near TEL-VIR relative to the cutting site. Means of $n \geq 3$ experiments are plotted; bars represent SEM. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$ (two-tailed unpaired *t* test). (*C*) Model depicting cohesin's role in subtelomeric silencing. In wild-type cells, cohesin-mediated chromatin compaction may place subtelomeric genes within the peripheral silent compartment and shield them from the transcriptional machinery. In the mutant, decompaction may localize the subtelomeric genes outside the peripheral silent compartment and also render them more accessible to the transcriptional machinery (*Left*). In the mutant, untethering of the chromosome from the nuclear envelop may also occur, derepressing both telomeric and subtelomeric genes (*Right*).

for cohesin in repression of expression of subtelomeric genes in budding yeast that is SIR-independent.

A Role for Cohesin in Transcriptional Repression of Subtelomeric Genes. Assembly of heterochromatin domains occurs by a conserved mechanism that involves action of histone-modifying silencing proteins and the association of silencing proteins with the modified histones (43). In budding yeast, telomere silencing depends upon the SIR complex that associates with telomeres and silenced subtelomeric regions and forms a heterochromatin domain (29). Interestingly, two genome-wide studies revealed that while the zone of subtelomeric silencing extends to ∼20 kb from the end, only a very small fraction (∼6%) of telomere-proximal repressed genes were silenced in a SIR-dependent manner (36, 37). The mechanism for repression of genes in the zone not overlapping with Sir-binding regions is poorly understood. Here, we found that repression of subtelomeric genes (both SIR-dependent and -independent) is decreased in cohesin mutants, indicating a requirement for cohesin in subtelomeric silencing. Silencing at the mating loci *HMR* and *HML*, as well as ribosomal DNA, was also impaired in the cohesin mutant, albeit to a lesser extent (*SI Appendix*, Fig. S8 and *Text*). This contrasts with an earlier observation on the role of cohesin in silencing in budding yeast at the silent mating locus *HMR*, where it was inferred that cohesin hinders the establishment of silencing at *HMR* (24). Our findings establish cohesin as an important determinant of repression in the extensive zone of subtelomeric heterochromatin that is SIR-independent and extends beyond SIR complex-binding regions.

The Role of Cohesin in Subtelomeric Silencing Is Independent of the SIR Complex. Derepression of subtelomeric genes in the cohesin mutant was observed in SIR-binding as well as nonbinding regions. Interestingly, based on genetic interactions with *sir* mutants, we found that the contribution of cohesin to telomere silencing is largely SIR-independent. SIR proteins retain their association with desilenced subtelomeric loci (e.g., *YFR057w*) in the cohesin mutants. This contrasts with observations in *Schizosaccharomyces pombe*, where loss of silencing was observed in a region located between telomere heterochromatin and arm euchromatin (that has combined eu/heterochromatin histone marks) in the *rad21* mutant (44), but in this case the fission yeast silencing protein Swi6 and a silencing-associated histone mark, H3K9me, were also observed to be depleted in this region. Unexpectedly, in our study, an active chromatin-associated histone mark, H3K4me3, is unaltered in the cohesin mutant in the derepressed subtelomeric regions relative to wild-type cells, and H4K16ac and H3K79me2 are also only marginally increased in the mutant. The requirement of cohesin for subtelomeric repression in both budding and fission yeasts suggests that this may be a general role of cohesin in many eukaryotes.

Enhanced transcription of subtelomeric genes in the cohesin mutant without a change in binding of silencing proteins or histone modifications is intriguing. Interestingly, there is some precedence for activation of subtelomeric genes without a decrease in Sir protein association or alterations in histone modifications. For example, a silencing defect in the absence of depletion of Sir proteins or silencing-associated histone modifications was noted in the case of activation of a heat shock-inducible transgene system (40) where >200-fold activation of transcription occurred without much change in Sir3 binding. In the same study, transcription activation (150-fold) of the natural subtelomeric gene *YFR057w* by the drug cycloheximide occurred without substantial changes in histone occupancy or modification. Other instances of activation of a subtelomeric gene unaccompanied by changes in binding of silencing proteins have been reported in the case of transcriptional activation of a subtelomeric *URA3* reporter by growth of cells in medium lacking uracil (45), where there was an increase in H4 acetylation while Sir3 and Rap1 binding was retained. Interestingly, Kitada et al. (46) reported that the epigenetically variegated ON vs. OFF states of the reporter do not differ with respect to Rap1 or Sir3 binding or deacetylation of H4K16.

Since Sir protein binding at derepressed telomeres was unaltered, we wondered whether there may be a change in a posttranslationally modified form of the silencing proteins. It has been reported that Mpk1-mediated Sir3 phosphorylation is induced upon stress or rapamycin treatment, which correlates with reduced subtelomeric silencing (41). Indeed, Sir3 was hyperphosphorylated in the cohesin mutant in an Mpk1-dependent manner, indicating that the Mpk1-mediated stress pathway was activated in this mutant. However, our findings indicate that while elevated Sir3 phosphorylation contributes to the silencing defect in the cohesin mutant, this is to a limited extent.

Cohesin Is Required for Tethering and Compaction of Telomere-Proximal Regions. Cohesin's role in multiple chromosomal processes depends on its association with chromosomes and its ability to tether DNA molecules. Telomere cohesion was indeed impaired in our study under conditions where telomeric silencing was reduced in the cohesin mutant. In this study, we also examined cohesin's role in telomere localization to the perinuclear compartment that favors silencing. Yeast telomeres cluster at the nuclear periphery and are anchored to the nuclear envelope (32, 33). The nuclear membrane-adjacent peripheral nuclear space has been described as a subcompartment of the nucleus that favors silencing of genes localized in this region (34, 35). Given our failure to detect changes in SIR binding and gene activation-specific histone marks, we examined the effect of cohesin dysfunction on telomere tethering to the nuclear envelope to explore additional potential mechanisms of derepression. We found that telomere tethering to the nuclear envelope was moderately impaired in the cohesin mutant, indicating a role for cohesin in telomere tethering that may influence telomere-proximal gene repression.

In budding yeast, cohesin also helps in chromosome arm compaction or condensation. Cohesin's role in mitotic chromosome condensation was first revealed by characterization of the *mcd1* mutant (7) that showed defects in both cohesion and condensation. Recent genome-wide studies analyzing the conformation of mitotic chromosomes in yeast (2) have also revealed the role of cohesin in compaction via formation of intra-arm loops. In this study (2), the *scc1-73* mutant displayed fewer loops and diminished short-distance (<100-kb) contacts indicative of defective mitotic compaction at the restrictive temperature. Our current study reveals that cohesin dysfunction is also associated with a less compact subtelomeric chromatin organization. Interestingly, the effect of cohesin on compaction of subtelomeric domains was also independent of SIR-mediated compaction in the same region. These defects in organization of repressed subtelomeric chromatin domains are likely to contribute to the transcriptional derepression of subtelomeric genes observed in the cohesin mutants (as depicted in the model; Fig. 4*C*).

A Model for Cohesin's Role in Transcriptional Repression of Subtelomeric Genes. The proposed model for cohesin-mediated subtelomeric gene repression based on our findings is shown in Fig. 4*C*. In wild-type cells, cohesin-mediated compaction may not only shield subtelomeric genes, rendering them inaccessible to the transcriptional machinery, but may also place them within the peripheral nuclear subcompartment that favors silencing. In mutant cells, the cohesin dysfunction-associated condensation defect may open up chromatin organization, making genes more accessible to the transcriptional machinery, and may also result in their relocation outside the peripheral silent compartment. In the case of chromosome ends that remain tethered to the nuclear envelope, telomeric genes may remain silent while subtelomeric genes may become derepressed (shown for the chromosome on the left in the mutant; Fig. 4*C*).

Our results also reveal a modest impairment of telomere tethering to the nuclear envelope in the cohesin mutant (Fig. 4*A*). Untethering of the chromosome end from the envelope coupled with decompaction may derepress telomeric genes as well as subtelomeric genes (shown for the untethered chromosome on the right in the mutant; Fig. 4*C*). This is consistent with our results summarized in the graph in Fig. 1*C* and *SI Appendix*, Table S2 that show only a modest impairment of repression of telomeric genes that are within 4 kb from the end (18.57% for telomeric genes compared with the genome-wide average of 11.44%, at significance level $\alpha = 0.05$; *SI Appendix*, Table S2) and a substantial derepression of subtelomeric genes (ranging from 30 to 60% for genes within 4 to 24 kb from the end, at significance level $\alpha = 0.0001$; *SI Appendix*, Table S2) in the mutant. The effects on expression of nontelomeric regions are not depicted here, although cohesin's role in compaction is not limited to subtelomeric regions.

In conclusion, our work reveals an alternative way by which cohesin can modulate gene expression. The requirement for cohesin in subtelomeric silencing suggests another mechanism by which the expression of multiple (subtelomeric) genes can be altered in a cohesin mutant. We propose that cohesin contributes to repression of subtelomeric genes by chromatin compaction that renders them inaccessible to the transcriptional machinery, and positions these genes that are adjacent to tethered chromosome ends within the peripheral silent compartment. Our findings establish cohesin as an important determinant of extended subtelomeric gene repression that extends up to 20 kb from budding yeast chromosome ends, well beyond the zone of Sir binding, and provide evidence for a Sir-independent mode of repression mediated by cohesin over extended chromosomal domains.

Materials and Methods

For RT-qPCR, RNA was isolated using the Qiagen RNeasy Mini Kit, and qPCR of cDNA was carried out on a Bio-Rad IQ5 real-time PCR machine using Bio-Rad iTaq Universal SYBR Green Supermix. ChIP was performed as described earlier (10). RNA sequencing was done using Illumina's HiSeq X by Clevergene Biocorp and Genotypic Technology; the data reported have been deposited in the Gene Expression Omnibus (GEO) database (47). Confocal microscopy was performed using the Zeiss LSM 880 (Airyscan) confocal microscope and analyzed using ZEN 2.1 (Black) software. Detailed experimental procedures are provided in *SI Appendix, Materials and Methods*.

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