Processive translocation of cohesive and non-cohesive cohesin *in vivo*

Running Title: Processive translocation of cohesin

Melinda S. Borrie and Marc R. Gartenberg^{1,2}

Department of Biochemistry and Molecular Biology
Robert Wood Johnson Medical School
Rutgers, The State University of New Jersey
Piscataway, NJ 08854

¹Member of The Rutgers Cancer Institute of New Jersey, New Brunswick, NJ 08901

²Correspondence: marc.gartenberg@rutgers.edu

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Abstract

Cohesin is a central architectural element of chromosome structure that regulates numerous DNA-based events. The complex holds sister chromatids together until anaphase onset and organizes individual chromosomal DNAs into loops. *In vitro*, cohesin translocates along DNA and extrudes loops in an ATP-dependent fashion. *In vivo*, cohesin redistributes in response to transcription as if pushed by RNA polymerase. Direct evidence of processive genomic translocation by the complex, however, is lacking. Here, obstacles of increasing size were tethered to DNA in yeast to detect translocation. The obstacles were built from a GFP-lacI core fused to one or more mCherries. Cohesin translocation was initiated from an upstream gene. A chimera with four mCherries blocked cohesin passage in late G1. During M phase, the threshold barrier to passage depended on the state of cohesion: non-cohesive complexes were also blocked by four mCherries whereas cohesive complexes were blocked by only three mCherries. That synthetic barriers alter cohesin redistribution demonstrates that the complex translocates processively on chromatin *in vivo*. The approach provides a relative measure of the maximum size of the protein chamber(s) that embraces DNA during cohesin translocation. The data indicate that the cohesive embrace is more restrictive than the embrace of non-cohesive complexes.

Introduction

Cohesin is a central architectural element of chromosome structure that functions by bringing distant DNA sites together (1). The protein complex was first characterized for its role in sister chromatid cohesion. Cohesin is now known to participate in most if not all fundamental chromosomal processes, including transcription, replication and repair. Recent work has shown that the complex also organizes chromosomes into large DNA loops that contribute to the segmentation of the genome into self-associating domains. Mis-regulation of cohesin has been identified as the cause of certain developmental diseases known collectively as cohesinopathies (2). Mutations in cohesin subunits have also been associated with human cancers.

The evolutionarily conserved core cohesin complex contains two SMC subunits (Smc1 and Smc3) and an α kleisin subunit Mcd1 (also known as Scc1/Rad21), which form a protein ring that can embrace DNA topologically (3). In yeast, the complex accumulates densely across tens of kilobases surrounding centromeres (4, 5). Along chromosome arms, the complex binds between convergently transcribed genes. Chromosome conformation capture (3C) techniques have shown that such sites participate both in sister chromatid cohesion and chromosomal loop formation (6-9). Cohesin performs these same architectural functions in higher eukaryotes (10-12).

Cohesin loading onto yeast chromosomes begins each cell cycle in late G1. Initially, the interaction is dynamic. The complex cycles on and off DNA because loading is balanced by an unloading factor, WAPL (Wpl1 or Rad61 in yeast). During S phase, Eco1 halts cohesin loss by acetylating the Smc3 cohesin subunit, which antagonizes Wpl1 (13-17). Eco1 travels with the DNA replication fork, presumably to couple the stabilization of cohesin complexes directly with the creation of sister chromatids (18).

A second dynamic property of cohesin is the ability to redistribute on chromosomes. Cohesin accumulates at genomic sites that are often great distances from where the complex initially loads (19). RNA polymerase was seen as a central driver of repositioning because cohesin on dormant or weakly transcribed genes was repositioned downstream by transcriptional induction (4, 5, 20). Translocation of complexes by processive movement along the chromatin contour best

explains repositioning, particularly given cohesin's topological embrace around DNA. Indeed, cohesin was shown to move along DNA *in vitro*, and transcribing RNA polymerase was shown to bias the directionality of movement (21-23). More recent biochemical work showed that cohesin, in combination with an auxiliary loading factor, possesses an intrinsic motor activity that enables translocation of the complex along naked and nucleosomal DNA in an ATP-dependent fashion (24, 25). Importantly, these studies showed that translocating cohesin can extrude DNA loops, an activity that is thought to form the large chromosomal loops that typify genomes (26-28). In support of the loop extrusion model, the size and distribution of chromosomal loops are altered by factors that influence the residence time of cohesin on chromatin (7, 8, 29-31). However, cohesin complexes have rarely been characterized during the act of translocation *in vivo*.

We recently obtained evidence for cohesin translocation on chromosomal DNA in yeast using a system involving transcriptional induction of a cohesin bound gene (32). Upon induction of the gene, cohesion of the locus was lost. If, however, the locus was first converted to an extrachromosomal circle, cohesion persisted. The results are most readily explained by cohesin complexes that redistribute by processive translocation because such complexes cannot migrate off circularized DNA. In the present study, the properties of translocating cohesin complexes were further studied by measuring their ability to bypass synthetic obstacles of increasing molecular size. This work provides direct evidence that cohesin translocates processively on DNA in response to transcription *in vivo*. Moreover, the data reveal that the fate of encounters with barriers depends on the cohesive state of the complex: a barrier sufficient to trap cohesive complexes is not large enough to trap non-cohesive complexes.

Results

Nuclear membrane tethering of DNA blocks cohesin translocation

Our previous study found that DNA circularization, as well as downstream convergently oriented genes, prevented transcription-driven cohesion loss (32). While DNA circles capture complexes that might remain mobile, we inferred that downstream genes act as barriers to cohesin passage. Intriguingly, DNA-bound lac repressors did not behave similarly. We hypothesized that the bacterial protein (lacI fused to GFP) was smaller than the chamber of the complex through which DNA passes. If cohesin truly redistributes by translocation *in vivo*, then DNA-bound proteins of sufficient size should block passage of the complex. To begin to explore this concept, we used a GFP-lacI chimera linked to FFAT, a tetrapeptide motif that associates with the inner nuclear membrane (33). When tethered to DNA and the nuclear membrane simultaneously, GFP-FFAT-lacI essentially represents an immobile obstacle of infinite size (Figure 1A).

Translocation assays were performed on an engineered genomic locus (Figure 1B) that contains the 256 lac operator array downstream of *URA3*, a gene that can be induced 3-5 fold by withholding uracil. Previous work showed that cohesive complexes associate with uninduced *URA3* until transcriptional induction triggers their departure. Cohesin levels at the gene do not diminish, however, because new complexes reload during the experimental interval (32). Here, chromatin immunoprecipitation coupled with quantitative real-time PCR (ChIP-qPCR) was used to monitor arrival of cohesin at a downstream site immediately preceding the lac operator array (hereafter referred to as the 5' lacO site).

URA3 transcription was induced after cells were arrested in M phase by depletion of Cdc20. When GFP-lacI bound the array, cohesin at 5' lacO was low both before and after *URA3* induction (Figure 1C). The result is consistent with the inability of GFP-lacI to block translocating cohesin complexes. Binding of GFP-FFAT-lacI, on the other hand, increased the level of cohesin at 5' lacO following transcription. This finding serves as a proof-of-principle that large immobile objects block translocation of cohesin complexes in yeast. In *B. subtilis*, tethering a DNA-bound repressor to the plasma membrane similarly blocked translocation of bacterial SMC proteins (34).

Large DNA-bound proteins block cohesin translocation

To determine if large DNA-bound proteins could similarly block cohesin passage, a series of size-calibrated obstacles was generated by fusing increasing numbers of mCherry to GFP-lacI (Figure 2A-C). This red fluorescent protein is ideally suited as a size standard because it has a compact monomeric structure unlike its tetrameric progenitor, DsRed (35-37). Like GFP-lacI, the mCherry chimeras bind lacO arrays, albeit with reduced efficiency, as demonstrated by weaker GFP foci and higher background nuclear fluorescence (Figure S1).

Accumulation of cohesin at 5' lacO was measured in M phase-arrested cells following three different *URA3* induction protocols (Figure 2D). In the first, *URA3* was never induced. Accordingly, cohesin levels at 5' lacO remained low and comparable to the cohesin-free control site, which is represented by a dashed line in Figure 2E. In the second protocol, *URA3* was induced following imposition of an M phase arrest. In this case, accumulation of cohesin at 5' lacO depended on the identity of the GFP-lacI chimera used (Figure 2C). When GFP-lacI was fused to one or two mCherries, cohesin levels did not rise above the baseline. By contrast, when GFP-lacI was fused to three or four mCherries cohesin accumulated at 5' lacO. To be certain that an aberrant property of mCherry was not responsible, the experiment was repeated with a chimera bearing three copies of monomeric yeGFP in place of the three mCherries. Similar results were obtained (Figure S2). These results indicate that DNA bound proteins of sufficient size block passage of translocating cohesin complexes.

In the final experimental protocol, *URA3* was induced at the outset of asynchronous growth. Previously we found that a downstream convergently-oriented gene could not block translocation of cohesive complexes in this scenario but the basis for this phenomenon was not understood (32). When *URA3* was induced from the outset here, most of the mCherry chimeras similarly failed to accumulate cohesin at 5' lacO. Some binding, however, was detected when GFP-lacI was fused to four mCherries (Figure 2E). That the timing of transcriptional induction influences the size of an effective obstacle further reinforces the notion that an aberrant property of mCherry is not responsible for blockade. Rather, the data suggests that different sized barriers act as obstacles under different conditions. Additional experiments elaborate on this concept below.

Trapped cohesin complexes remain cohesive

Translocation of cohesin along a single chromatid during loop extrusion or along a pair of chromatids during sister chromatid cohesion could yield the ChIP signals seen thus far. To determine whether the complexes at 5'lacO hold sister chromatids together, we evaluated cohesion of the chromosomal domain bearing the locus. In our strains, target sites for the R site-specific recombinase flank the entire region (Figure 3A). Induction of the recombinase during an M phase arrest generates a pair of DNA circles, one from each sister chromatid. Owing to the bound GFP-lacI chimeras, a single dot of fluorescence appears if the DNA circles are cohesed whereas a pair of dots appears if they are not (Figure S1). *URA3* transcription was induced according to the three protocols described above and cohesion was evaluated at the end of the growth protocol (Figure 3B). In the absence of induction, cohesion of DNA circles was observed in all strains due to the presence of cohesive cohesin on *URA3* (Figure 3C; (32)). When transcription was induced following M phase arrest, cohesion was greatly reduced in strains with chimeras bearing one or two mCherries or none at all. This result indicates that these chimeras are too small to block translocating cohesin. By contrast, cohesion persisted when *URA3* transcription was induced in strains expressing chimeras with three and four mCherries. Thus, consistent with the results in figure 2, DNA-bound chimeras equal to or larger than GFP-lacI-3xmCherry block passage of translocating cohesive cohesin. We conclude that at least some of the trapped cohesin complexes detected by ChIP are engaged in sister chromatid cohesion.

When transcription was induced from the outset of cell growth, cohesion of the DNA circles was abolished in all strains, irrespective of the chimera expressed. This result recapitulates the earlier observation that convergent genes downstream of *URA3* also do not trap cohesive complexes under identical induction conditions (32). These data suggest that the ability of an obstacle to block passage of cohesin depends on the interplay between transcription and cell cycle stage. Data supporting this view is presented below.

A larger barrier is required to block cohesin translocation in late G1 phase

The studies above examined translocation of cohesin on newly replicated chromatids in M phase-arrested cells. To study translocation on unreplicated chromatin, cells were arrested in late G1 by expressing a non-degradable allele of Sic1, a Cdk1 inhibitor that prevents S phase entry

(38). By definition, the chromatin-bound complexes at this stage of the cell cycle are not cohesive. The steps of the growth protocol are depicted in figure 4A and validation of the growth arrest is shown in figure S3. Transcriptional induction of *URA3* following late G1 arrest caused accumulation of cohesin at 5'lacO only in a strain expressing GFP-lacI-4xmCherry. In strains expressing GFP-lacI-3xmCherry or GFP-lacI alone, cohesin accumulation matched the background levels of the uninduced controls (Figure 4B). This data shows that different size barriers are required to block cohesin at different stages of the cell cycle. Whereas three mCherries were enough to block translocation in M phase, at least four mCherries were required in late G1.

We hypothesized that the four mCherry threshold seen in figure 4B was related to the same threshold seen during M phase arrest after induction from the outset of growth (Figure 2E). To understand this line of reasoning, recall that cohesin binding resets after each anaphase passage when separase activity subsides and Mcd1 synthesis resumes (39-41). If *URA3* is already transcribing when cohesin binding resumes in late G1 then the ensuing translocation of the complex would occur on an unreplicated chromosome, at least until S phase. Put simply, translocation before DNA replication would bypass the three mCherry limitation normally seen in M phase cells. To test this idea, *URA3* was induced from the outset of growth and barrier activity of the obstacles was analyzed by ChIP after cells were arrested in late G1. Figure 4C shows that cohesin accumulated at 5'lacO in transcribing cells that expressed a 4xmCherry obstacle but not the 3xmCherry variant or GFP-lacI alone. These data indicate that when *URA3* is induced from the outset of growth, transcription triggers translocation of cohesin before DNA replication, leading to mobile complexes that pass obstacles the size of GFP-lacI-3xmCherry but not GFP-lacI-4xmCherry.

Translocation of complexes that arrive after DNA replication

Cohesin loads continuously onto chromosomes from late G1 to anaphase (18, 32, 42). Complexes that arrive after DNA replication fail to acquire S phase-dependent acetylation and are turned over by Wpl1 (43, 44). To analyze the translocation properties of these late arriving complexes, *URA3* transcription was induced while cells were arrested in late G1 and then the cultures were released for growth into a subsequent M phase arrest. ChIP at this stage thus

reports on those cohesin complexes migrating to 5'lacO both before and after S phase. Formally, this induction protocol also records translocation events during DNA replication of the gene, a time span that is likely too short to affect the results significantly. The novel arrest-and-release procedure was achieved by inducible clearance of AID-tagged, “non-degradable Sic1”, using the auxin analog 1-naphthaleneacetic (Figure S3). The ChIP data of figure 4D show that when *URA3* transcription was induced, cohesin accumulated at 5'lacO in cells that expressed 4xmCherry obstacle but not the 3xmCherry variant or GFP-lacI alone. That the barrier limit does not revert to 3xmCherries indicates that late arriving, non-cohesive cohesin is not blocked by the smaller barrier.

Eco1 and Wpl1 modulate cohesin's encounters with barriers

We next tested the influence of regulators of cohesin, Eco1 and Wpl1, on the ability of the complex to translocate past synthetic obstacles. The proteins were depleted by clearance of AID-tagged alleles during a G1 arrest, then cells were grown to the subsequent M phase arrest before inducing *URA3* (Figures 5A). Validation of protein clearance is shown in figure S4. When Eco1 was depleted the smallest obstacle, GFP-lacI alone, blocked cohesin translocation (Figure 5B). This unexpected blockade was dependent on the synthetic barrier: when Eco1 was depleted from a strain lacking GFP-lacI, transcription-dependent accumulation of cohesin at the 5'lacO site was abolished (Figure S5). It appears that Eco1, presumably through acetylation of Smc3, facilitates translocation of cohesin past an array of DNA-bound GFP-lacI.

To explore the phenomenon further, we examined cohesin translocation in a strain with an *smc1-D1164E* allele. This mutation diminishes ATP-hydrolysis by cohesin, creating chromatin-bound complexes that do not require Eco1 for protection against Wpl1 unloading (15, 45, 46). Importantly, this mutation reduces Smc3 acetylation by Eco1 (46). In this strain, *URA3* transcription during an M phase arrest also caused cohesin to accumulate at GFP-lacI alone (Figure 5C). These results reinforce the notion that acetylation of Smc3 by Eco1 influences how cohesin navigates the GFP-lacI array.

When Wpl1 was depleted, two notable changes occurred. First, depletion of the protein abolished the 3xmCherry barrier, yielding a slight increase in accumulation at the 4xmCherry barrier

(Figure 5D). The small signal amplitude may be related to earlier observations that cohesin levels are lower at enrichment sites in the absence of Wpl1 (8, 16, 17). These data suggest that cohesin translocation is less restrained in the absence of Wpl1. The second notable change in figure 5D is that accumulation of cohesin at the 4x chimera occurred even in the absence of *URA3* induction. This feature is not unique to M phase-arrested cells. During late G1 arrest when cohesin accumulates at the 4xmCherry chimera, loss of Wpl1 also eliminated the requirement for *URA3* induction (Figure S6A). Elimination of Wpl1 does not cause mis-regulation of *URA3* transcription. RT-qPCR analysis showed that induction profiles of the gene were relatively unchanged (Figure S4D and S6B). Instead, it seems that basal transcription of *URA3* is sufficient to cause cohesin accumulation at synthetic obstacles in the absence of Wpl1. If cohesin binds less specifically in Wpl1 mutants (47), and the residence time on DNA is longer (43), basal transcription of *URA3* might be sufficient to mobilize mis-targeted complexes toward the synthetic barriers downstream.

When Eco1 and Wpl1 were co-depleted, cohesin bypassed the 3xmCherry chimera yet the complex was blocked efficiently by the larger 4xmCherry chimera (Figure 5E). Cohesin accumulation did not require *URA3* induction, as seen before when depleting Wpl1 alone. Interestingly, the accumulation of cohesin at GFP-lacI, seen when depleting Eco1 alone, was abolished when both proteins were co-depleted. Apparently, the impact of Wpl1 depletion is dominant when both proteins are absent. Perhaps with a longer DNA residence time conferred by Wpl1 loss, cohesin eventually bypasses GFP-lacI but not the larger GFP-lacI-4xmCherry barrier.

Interpretation of the cohesin translocation results in the absence of Eco1 and Wpl1 requires knowing the cohesive state of the complexes under study. Thus, we measured cohesion of the *URA3* locus in the absence of the regulators. The experiments were performed without induction of the gene to assess baseline levels of cohesion before mobilization. Depletions were achieved during G1 arrest and then cells were grown to an M phase arrest before DNA circles were excised. As expected, depletion of Eco1 abolished cohesion (Figure 5F). Co-depletion of Eco1 and Wpl1 also yielded a severe cohesion defect, as anticipated from earlier work (See (48), and references there in). Wpl1 depletion was previously shown to have an intermediate effect on chromosomal cohesion (16, 17, 48). Here, in the context of a specific chromosomal segment

290 unlinked from flanking genomic sequences, depletion of Wpl1 also abolished cohesion. These
291 data show that the *URA3* locus used in our studies is devoid of cohesive cohesin when Eco1 and
292 or Wpl1 are depleted. Cohesin translocation under these conditions occurs on only one
293 chromatid even though both sisters are present.

Discussion

Despite the intense study of cohesin dynamics and loop formation of chromosomal DNA, few studies have caught cohesin in the act of translocation *in vivo*. Here, we show that synthetic barriers of sufficient size cause ectopic accumulation of the complex downstream of an inducible gene (Figure 2). That a DNA-bound obstacle built entirely of non-yeast proteins blocks the passage of cohesin proves that the complex translocates processively along DNA inside live cells. While our work was underway, a study using nuclease-dead Cas9 as a barrier arrived at a similar conclusion (49). Our study goes further in two significant ways. First, we show that both cohesive and non-cohesive complexes translocate on chromosomes. Second, we show that the two types of complexes are blocked by different size barriers.

Although transcription triggered translocation in this study, it is not yet clear whether a transcribing polymerase is required once the complex gains mobility. Both ATP-dependent and independent movement of cohesin on DNA have been described *in vitro* (21-25). In the former case, cohesin translocated while associated with NIPBL (Scc2 in yeast), a cofactor that stimulates the ATPase activity of the complex (50). Scc2 has also been implicated in cohesin redistribution *in vivo* (50). Further work is required to determine whether NIPBL/Scc2 is required for the processive movement described here.

Synthetic obstacles of increasing size serve as a molecular ruler of the embrace of cohesin around DNA. Crosslinking studies have defined one large DNA chamber formed by the interactions of core subunits (Smc1, Smc3 and Mcd1), as well as two smaller DNA chambers partitioned from the larger chamber by the engaged heads of the Smc proteins (Figure 6A, (51)). Synthetic obstacles of sufficient size could block transport of DNA through one or more of these chambers. Significant conformational changes are likely to accompany translocation that could narrow the DNA chamber(s) further, as proposed for the related condensin complex (52). Our work now provides an effective upper size limit (measured in mCherries) for cohesin's DNA embrace while translocating on single chromatin fibers and pairs of sister chromatids.

An exciting and unexpected finding of this study is that the threshold size of an impassible barrier changes depending on cell cycle stage. In late G1 an obstacle bearing four mCherries blocks translocation whereas only three mCherries are needed in M phase (Figures 2 and 4). Chromosomal loops are formed before DNA replication (7, 53), presumably by loop extruding complexes that interact in a pairwise fashion with a given DNA segment. Additional complexes might also translocate at this stage via single interactions with a given DNA segment (24, 54). We propose that one or both of these classes of cohesin complexes are blocked by the 4xmCherry threshold during late G1 arrest (Figure 6B). Importantly, during S phase passage a new class of cohesin complexes emerges: one that can translocate while engaged in sister chromatid cohesion (Figure 3). We propose that it is this class of complex, cohesive cohesin, that cannot bypass the 3xmCherry barrier during M phase arrest (Figure 6C). ChIP signals immediately upstream of the lacO array may nevertheless register the accumulation of heterogenous mixture of complexes in M phase because the stalling of a cohesive complex may cause the subsequent accumulation of all others.

The view that only non-cohesive complexes are trapped by the 4xmCherry barrier is supported by the set of experiments in which *URA3* was induced from the outset of growth. In this protocol, only the 4xmCherry barrier limited cohesin passage, even when examined in M phase-arrested cells that contain cohesive cohesin (Figure 2E and 4C). The inability to trap cohesin with the 3xmCherry barrier can be understood when considering cohesin loading relative to ongoing transcription. During the induction from outset protocol, complexes are triggered to migrate as soon as they begin loading in G1, reaching barriers before becoming cohesive in S phase. Similarly, complexes that load and migrate after S phase during this induction protocol also do so without being cohesive (Figure 4D). Thus, the induction from outset experiments only measure translocation of non-cohesive cohesin. In agreement with the late G1 arrest studies, these complexes are also blocked by the 4xmCherry barrier. Nevertheless, a feature of these experiments remains unresolved. During the induction from outset protocol, complexes that translocate into 4xmCherry barriers early in the cell cycle do not become cohesive as cells progress to an M phase arrest. In other words, complexes that can be detected by ChIP at 4xmCherry barriers do not yield cohesion under this induction protocol (Figures 2E and 3C). An

intriguing possibility is that complexes arrested at barriers before S phase (perhaps those dedicated to extrusion) do not readily convert to cohesive complexes during S phase passage.

Both Eco1 and Wpl1 influence the size and distribution of chromosome loops in yeast, as do their homologs in higher eukaryotes (7, 8, 24, 29, 30, 55). Whereas Wpl1 restricts loop size by limiting the residence time and location of cohesin on DNA, Eco1 is thought to restrict loop size through a mechanism(s) independent of cohesion establishment. A simple hypothesis holds that conditions which favor more extensive loop extrusion would also favor translocation past smaller synthetic barriers. Indeed, simultaneous depletion of Eco1 and Wpl1 or just Wpl1 alone increased the threshold size of a barrier in M phase cells from 3xmCherries to 4xmCherries (Figure 5D and 5E). However, by using DNA circularization we showed that the locus under study was devoid of cohesive cohesin in the absence of Eco1 and Wpl1 (Figure 5F). Thus, a simpler explanation may hold: the increase in chimera size from 3xmCherries to 4xmCherries reflects the switch to a barrier limit that applies when only non-cohesive complexes are present. If cohesin only binds single chromatids at *URA3* in the absence of Wpl1 (with or without Eco1), then it is not surprising that the barrier limit matches that for translocation on unreplicated chromosomes in wild-type cells. Despite the presence of sister chromatids the complex translocates on single chromatids under these conditions.

Lastly, depletion of Eco1 alone yielded an entirely unexpected result: GFP-lacI lacking mCherries was sufficient to impede cohesin passage (Figures 5B and S6). The ChIP signal was highly reproducible, statistically significant, and reliant on GFP-lacI expression. Moreover, the result was reinforced with an *smc1-D1164E* mutant that hinders Smc3 acetylation by Eco1, as well as the intrinsic ATPase of cohesin (Figure 5C). Further work is needed to understand how the acetyltransferase facilitates translocation past the synthetic barriers and whether similar events occur at any natural barriers within chromosomes.

Maps of mammalian chromosomal loops often contain “stripes” that are thought to be a signature of translocating cohesin complexes (56). A typical stripe encompasses interactions between a loop anchor and the rest of the looped domain, as if a cohesin complex that reaches one loop anchorage point extrudes intervening DNA until reaching the other loop anchorage point. Fine

384 structure mapping shows that stripes contain embedded dots, possibly from paused translocating
 385 cohesin complexes (57). The synthetic barriers described here likely mimic the natural
 386 impediments (CTCF and other proteins that bind nucleosome depleted regions) that punctuate
 387 and terminate stripe patterns. It is not yet clear, however, whether the trapped complexes in our
 388 experiments are stably arrested or merely delayed in their passage. Either way, the data show that
 389 cohesin translocates on DNA inside living cells in a manner that would give rise to stripes.

Materials and Methods

Yeast strains and plasmids

Strains generated for this study were confirmed by PCR and/or DNA sequencing. Strains are listed in Table S2 and primers are listed in Table S1. GFP-lacI with a linked SV40 NLS was expressed from either a single or tandem integration of plasmid pGVH60, as noted in the strain table. These integrants were modified by PCR-mediated gene tagging using multimeric repeats of mCherry in plasmids pMAM12 and pMAM44 or 3xycGFP(A206R) in plasmid pMAM88. Strains with tandem pGVH60 thus yielded simultaneous expression a GFP-lacI chimera and GFP-lacI alone. Strains co-expressing GFP-lacI/GFP-lacI-mCherry chimeras, however, produced identical results to strains expressing the mCherry chimeras alone (Compare figures 4C and S7). Integrating plasmid pXDA2-FFAT was assembled by in vivo recombination from pAFS144-FFAT and pXRA2 (58). pXDL2-GAL1p-Sic1(4m)-AID*-9xmyc was similarly assembled from pXRL2 (58), pRS306-GAL-Mt-4A-SIC1-HA-HIS6-Act (38) and pKan-AID*-9myc (59). Other genomic AID alleles were constructed by PCR tagging with the 3xmAID-5xflag module in pST1933 (NBRP ID: BYP8880; (60)). OsTir1 was from integrated pTIR5 (*ADH1p-OsTir1-CaADH1t*, Thomas Eng thesis, U.C. Berkeley) or integrated pMSB27 (*ADH1p-OsTir1*(yeast codon optimized)), which was derived by in vivo recombination from pXRT1 (58) and pMK200 (NBRP ID: BYP7569).

Cell growth and arrest

To arrest cells in M phase for ChIP, methionine was added (Cf = 2mM) to mid-log cultures grown in SC-met to shut-off *MET3p-cdc20*. After 2.5 hours (roughly 80% of the cells adopted a dumbbell shape), cells were washed twice with water and resuspended in SC media lacking uracil to induce *URA3*. Two hours later the cultures were fixed for ChIP, according to (32). To arrest cells in M phase for analysis of cohesion, cultures were grown in SC-met plus dextrose for 6-8 hours and then diluted 200-400 fold into SC-met plus raffinose for overnight growth. When cells reached mid-log the following morning, methionine was added. 150 minutes later, cells were washed twice and resuspended in SC media lacking uracil. Two hours later, galactose was added (Cf = 2%) to induce circularization by the R recombinase. Cells were fixed with paraformaldehyde after two additional hours. To arrest cells for ChIP in late G1, dextrose grown cultures were diluted 200-400 fold into SC-met plus raffinose for overnight growth. When the

cultures reached mid-log the following morning, alpha factor was added (Cf = 10 μ M), and 90 minutes later galactose was added to induce expression of *GALIp-SIC1(4m)-AID*-9xmyc*. After one additional hour, cultures were washed twice with water and resuspended in SC-met plus galactose and pronase E (Cf = 0.1 mg/ml) to release from pheromone arrest. One hour later, the cells were washed twice and resuspended in galactose lacking methionine and uracil. Two hours later, the cultures were fixed for ChIP. For release from late G1 arrest, cells were grown into the arrest as described above but with the following exceptions. After cells were washed to remove alpha factor, they were resuspended in galactose media that lacked uracil but contained methionine and pronase E. Two hours later, dextrose (Cf = 2%) was added to stop expression of *Sic1(4m)-AID*-9xmyc* and 1-naphthaleneacetic acid (NAA, Cf = 100 μ M) was added to degrade the existing protein. Two hours later when cells accumulated in M phase, cultures were fixed for ChIP. For depletion of the cohesin regulators, strains with the various AID alleles were treated with alpha factor for 90 minutes before adding NAA for 60 additional minutes. After release from alpha factor arrest, cells were resuspended in media containing methionine for arrest in the subsequent M phase. 90 minutes later, cells were washed twice before resuspending with media lacking uracil. Two hours later, cells were fixed for ChIP. For cohesion assays involving protein depletions, cells were grown similarly except that cultures were diluted into SC-met plus raffinose for overnight growth. In addition, galactose was added once cells reached the M phase arrest and cells were fixed two hours later.

Other techniques

ChIP, RT-qPCR and fluorescence microscopy were performed as described in (32). For ChIP, the mean and SD of three or more biological replicates are presented, unless specified otherwise. All values were normalized to an unlinked cohesin-bound site (549.7 kb on Chr IV). Statistical significance was determined by pairwise Student's t tests relative to the induced 0x mCherry strain, unless specified otherwise. Immunoblots were probed with anti-GFP (G1544, Sigma), anti-myc (9E10, Roche), anti-mini-AID (M214-3, MBL), anti-Pgk1 (ab113687, Abcam) or anti-Rad23 (from the Madura laboratory), and secondary antibodies (W401B and W402B, Promega). ChIP was performed with anti-TAP and protein A Dynabeads (CAB1001 and 10001D, Invitrogen). A ChemiDoc instrument (BioRad) was used for gel imaging.

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Competing Interests

The authors declare that no competing interests exist.

Figure legends

Figure 1 – Anchoring DNA to the inner nuclear membrane blocks cohesin translocation. **A)**

Schematic of transcription-triggered cohesin translocation and blockage by membrane anchorage. Only one repressor is shown for clarity. NE = nuclear envelope. **B)** Schematic of the locus used to test translocation. Binding of Mcd1 is evaluated at the 5' lacO site immediately upstream of the lacO array. **C)** ChIP of Mcd1-TAP. Strains MSB114 and MRG6947 were arrested in M phase by Cdc20 depletion and then *URA3* transcription was induced. Values were normalized to a cohesin bound site (see Materials and Methods). Measurements at a cohesin-free site (534 kb on Chr IV) were averaged for all experiments of the figure panel and shown as a dashed line. p values for pairwise Student's t tests are presented relative to benchmark sample (designated with a dash). ns, not significant.

Figure 2 – Synthetic barriers of sufficient size block cohesin translocation. **A)** Schematic of

transcription-triggered cohesin translocation and blockage by GFP-lacI chimeras. **B)** Organization of the GFP-lacI chimeras with mCherries. **C)** Immunoblot of GFP-lacI chimeras. Strains expressing MSB174, MSB179, MSB180, MSB181 and MRG7102 were used. The lower molecular weight bands marked with * likely represent partial proteolytic cleavage of mCherry (61). **D)** Experimental flow chart. Transcription was either induced from the outset of growth, after M phase arrest or not at all. **E)** ChIP of Mcd1-TAP. Cohesin binding at the 5' lacO site was evaluated in strains MSB114, MRG6810, MRG6812, MSB165.1 and MRG7060.

Figure 3 – Synthetic barriers of sufficient size block translocation of cohesive cohesin. **A)** An

assay for cohesion at a specific chromosomal location. The GFP-tagged region of interest is converted to extrachromosomal circles by site specific recombination. Half-filled boxes represent target sites for the inducible R recombinase. **B)** Experimental flow chart. **C)** Cohesion analysis of extrachromosomal circles. Strains MSB35.2, MRG6806, MRG6808, MSB167 and MRG7060 were used. p values for pairwise χ^2 tests are presented relative to a benchmark condition for each strain (designated with a dash). Error bars represent the standard error of proportion. n = the number of cells examined.

Figure 4 – Synthetic barriers to cohesin translocation before and after S phase. **A)** Experimental flow chart. **B)** Barriers to translocation in late G1-arrested cells. ChIP of Mcd1 was performed in strains MRG7027, MRG7026 and MRG7059 that were arrested by expression of non-degradable Sic1. *URA3* transcription was induced after cell cycle arrest. **C)** Same as **B** except transcription was induced from the outset of growth. Two trials were performed for each strain. **D)** Barriers to translocation in cells progressing from G1 to M phase. Details of the cell cycle arrest, release and re-arrest are described in Materials and Methods. Strains MRG7033, MRG7032 and MRG7282 were used.

Figure 5 – The impact of cohesin regulators on translocation past synthetic barriers. **A)** Experimental flow chart. **B)** Translocation in the absence of Eco1. 3xmAID alleles of Eco1 were depleted by the addition of 100 μ M NAA to strains MRG7301.1, MRG7300.1, MRG7302.1, as described in Materials and Methods. p values for pairwise Student's t-tests are presented for the transcriptional induction of each strain. **C)** The impact of *smc1-D1164E* on translocation past barriers. Strains MRG7151, MRG7146 and MRG7159 were arrested in M phase before transcription was induced, as in figure 2. **D)** Translocation in the absence of Wpl1. Strains MRG7327.1, MRG7329.1, MRG7324.1 were used. **E)** Translocation in the absence of Eco1 and Wpl1. Strains MRG7332.1, MRG7356.1, MRG7353.1 were used. **F)** Cohesion of the *URA3* locus in the absence of Eco1 and Wpl1. 3xmAID alleles were depleted during G1 arrest before release into M phase arrest when DNA circles were excised. Strains MSB203, MSB204, MSB205 and MSB206 were used.

Figure 6 – Cohesin translocation blocked by synthetic barriers of sufficient size. **A)** Chambers of cohesin. The large topological compartment created by interactions of Smc3 and Smc1 with Mcd1 is bisected into two smaller chambers by engagement of the Smc3 and Smc1 heads (filled circles, (51)). The chamber(s) used during translocation have not yet been defined. **B** and **C)** Proposed limits of cohesin translocation. Non-cohesive complexes translocating on single chromatids (in late G1 or M phase) cannot translocate past a barrier larger than GFP-4xmCherry. Both loop extruding and singly bound cohesin complexes are depicted. Cohesive complexes during M phase cannot translocate past a barrier larger than GFP-lacI-3xmCherry. Cohesin is represented by a generic ring without reference to chamber(s).

Figure S1 – Representative images of cells expressing GFP-lacI chimeras. **A)** Strains MSB35.2, MSB167 and MRG7060 were photographed after M phase arrest. Fluorescent protein chimeras accumulate in the nucleus owing to a C-terminal SV40 NLS. Binding of GFP-lacI alone to the lacO array yielded green foci with little background fluorescence. When chromatids separate, two dots are seen (yellow arrows). Fusion of mCherries to GFP-lacI increases background nuclear fluorescence, suggesting that the C-terminal addition to lacI interferes with chimera binding. Nevertheless, sufficient binding occurs to yield discrete foci. **B)** In the absence of a lacO array (strains MRG7372, MRG7373 and MRG7374), no foci are seen, indicating that GFP-lacI-mCherry chimeras do not intrinsically aggregate.

Figure S2 – GFP-lacI with three copies of yeGFP also blocks cohesin translocation. Strains MSB165.1 and MRG7320 were used. Each of the three yeGFP multimers carries the A206R mutation that abolishes weak dimerization of GFP (62). Cultures were grown as described in figure 2. 3x yeGFP(A206R) yields the same result as 3x mCherry, indicating that residual multimerization of mCherry or some other mCherry artifact is not responsible for barrier activity. The requirement for different sized obstacles at different cell cycle stages further indicates that mCherry multimerization is not driving barrier activity in these studies (compare figures 2 and 4).

Figure S3 – Stepwise cell cycle arrests using controlled expression on “non-degradable” Sic1. **A)** Cell viability following induction and clearance of Sic1(4m)-AID*-9xmyc. Strains bearing a *GAL1p-SIC1(4m)-AID*-9xmyc* allele (MRG7027 and MRG7033) were spotted in 10-fold serial dilutions on the indicated plates. Galactose-induced lethality due to Sic1(4m)-9xmyc-AID* expression is over-ridden by 100 μ M 1-naphthaleneacetic acid (NAA), which triggers degradation of AID chimeras by OsTir1. Methionine was omitted from all plates for *MET3p-cdc20* expression. **B)** Validation of Sic1(4m) protein expression levels. The same strains were grown according to the arrest and release protocol described in the Materials and Methods. Lanes 1 and 5 = asynchronous growth in dextrose; lanes 2 and 6 = initial late G1 arrest in galactose media, one hour after removing alpha factor; lanes 3 and 7 = extended arrest at late G1 in galactose media, three hours after removing alpha factor; lanes 4 and 8 = two hours after adding

NAA. Note that the myc-labelled Sic1(4m)-AID* band is cleared in lane 8 allowing this strain to advance to an M phase arrest. **C)** Validation of late G1 arrest by flow cytometry. Strain MRG7027 was grown into a permanent late G1 arrest as described in Materials and Methods, and used in figure 4B. DNA content was assessed in asynchronous culture, after late G1 arrest and then after subsequent *URA3* induction. **D)** Validation of sequential cell cycle arrests. Top row of panels - Dot plots of flow cytometry data (peak fluorescence vs fluorescence intensity). Strain MRG7033 was used. Cells with clear 1N and 2N DNA content are shown in navy blue and green, respectively. After a prolonged time in late G1 arrest, a large fraction of cells (red) fall outside the gated area due to an elongated bud morphology (due to Sic1-suppressed Cdk activity, (63)) that adversely affects peak fluorescence. Nevertheless, the data show that cells shown in red shift from 1N to 2N DNA content during progression from the late G1 to M arrests. Bottom rows of panels - Direct visualization of cells and cell nuclei. The percentage of cells that meet the specified morphological criteria of each cell cycle arrest are listed below the images. DNA was stained with Hoechst dye after ethanol fixation. n = number of cells examined.

Figure S4 – Depletion of cohesin regulators and their impact on *URA3* induction. A) Cell viability following depletion of Eco1 and Wpl1. Strains MRG7270.1, MRG7301.1, MRG7327.1, MRG7332.1 and MRG7355.1 bearing the indicated 3xmAID alleles were spotted in 10-fold serial dilutions on SD +leu,+his plates containing or lacking 100 μ M NAA. Depletion of Eco1 causes a loss of viability that is rescued by co-depletion of Wpl1. Similar results were obtained whether Wpl1 was depleted or the *WPL1* gene was deleted, indicating that depletion phenocopies the null mutation. **B)** Camptothecin sensitivity following Wpl1 depletion. Strains MRG7269.1, MRG7329 (isolate 1), MRG7329 (isolate 2) and MRG6830.1 were spotted on SC-met containing or lacking 20 μ g/ml camptothecin (CPT) and 100 μ M NAA in 5-fold serial dilutions. Strains lacking Wpl1 are sensitive to the DNA damaging agent (See (47) and references therein). The middle panel shows that addition of the 3xmAID slightly diminishes the function of Wpl1 and the right panel shows that depletion of the protein with NAA phenocopies the null mutation. **C)** Depletion of 3xmAID-tagged proteins. Strains MRG7301.1, MRG7327.1, and MRG7332.1 were used. Cells were grown to mid-log in SC-met and treated with either 100 μ M NAA or DMSO for 60 minutes. The tagged proteins were immunoblotted with an antibody to mAID. **D)** RT-qPCR analysis of *URA3* mRNA following depletion of mAID-tagged proteins. Strains MRG7270.1,

MRG7301.1, MRG7327.1, and MRG7332.1 were grown according to the protocol described in figure 5A. Transcript levels were measured relative to *ACT1* mRNA and normalized to a single trial of the uninduced wt control. The data show that loss of Wpl1 and or Eco1 does not induce *URA3* prematurely.

Figure S5 – Barrier activity requires GFP-lacI chimeras. Strain MSB200 (expressing no GFP-lacI chimera) was grown into an M phase arrest following Eco1 depletion during the preceding G1 arrest. *URA3* was then induced. Omission of a GFP-lacI chimera abolishes transcription-dependent cohesin accumulation at 5'lacO.

Figure S6 –A synthetic barrier to cohesin translocation in late G1-arrested *wpl1Δ* cells. **A)** ChIP of Mcd1-TAP. Strains MRG7235.1, MRG7232.1 and MRG7238.1 were grown according to the protocol described in figure 4A. Loss of Wpl1 causes accumulation of cohesin at the 4xmCherry barrier without *URA3* transcriptional induction. **B)** RT-qPCR analysis of *URA3* mRNA levels. Strains MRG7027 and MRG7235.1 were grown asynchronously before RNA extracts were prepared. Values normalized as in figure S4. Loss of Wpl1 does not alter the basal level of *URA3* transcription or the ability to induce the gene.

Figure S7 – Barrier activity in strains expressing homogeneous GFP-lacI-mCherry chimeras. *URA3* was induced in strains MRG7115 and MRG7122 after arrest in late G1 by Sic1(4m)-AID*-9xmyc. Binding of cohesin at the 5'lacO was analyzed by ChIP of Mcd1-TAP. Barrier function requires 4x mCherries, similar to the results found with strains co-expressing GFP-lacI-mCherry chimeras along with GFP-lacI alone in figure 4.

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Figure 2

Figure 5

Figure S1