nature portfolio

Peer Review File

Condensin Accelerates Long-Range Intra-Chromosomal Interactions

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is an interesting study of genome organization in yeast to discern dynamic aspects of condensin and cohesin. The experiments are clearly described and of the highest quality. The paper is well-written and well-organized.
The authors take advantage of the forkhead binding protein system to cross-link genomic loci tagged with lacO or TetO operator sequences. The ability to engineer this allows accurate assessment of cross-linked loci and quantitate encounter times. This is an ingenious strategy that the authors have put to good use.
The major comments are in terms of the novelty of the findings. The major contribution is the estimation of kinetics of loop extrusion, which they find in remarkable agreement to that found in vitro studies (~2 kb/s). They also find that condensin is the major driver, rather than cohesin, which while different from that in mammalian chromosomes, is not a surprise in yeast from prior studies.
Specific comments:
The authors state that loops are relatively sparse and only 10% of the genome is in loops at any given moment. How does this relate to the abundance of condensin, which has been estimated to be bound about 1 molecule every 10 kb. If 10% of the genome is in loops (~10^6 bp), and there is a loop every 10 kb, then each loop is only 100bp? Alternatively, only a fraction of bound condensin is extruding loops. I'd like to see the authors comments on the fraction of the genome in loops and their thoughts on the number of active condensin molecules relative to total bound.
The authors attempt to compartmentalize cohesin and condensin functon(lines 400-500). They state that cohesin drives genome-wide compaction of the mitotic chromosomes, citing fellow Hi-C papers. This may be the case, but this function of cohesin is non-essential. Heidinger-Pauli JM, et al. (Curr Biol. 2010. PMID: 20451387) demonstrate that the bulk of cohesin can be depleted to 13% without effecting chromosome segregation. The essential function of cohesin seems to be in the centromere bottlebrush, that is required for building tension over micron scales (J Cell Sci. 2022 Feb 15;135(4):jcs259532. doi: 10.1242/jcs.259532.). From trying to understand mechanism of action, I'm not sure the division of labor section sheds any particular insight.
Finally, the authors talk about the spatial segregation of chromosomes. This is difficult to understand. Unlike the situation in mammalian cells, where the chromosomes are individualized on a micron scale (easily distinct in the light microscope), in yeast all 16 chromosomes gathered at their centromeres and pulled to the spindle poles. In other words, the entire genome moves toward the pole as one. The chromosomes are individual entities, but spatial separation as the authors suggest (line 448), is not something that is obvious in yeast.
In summary, this is an important contribution in estimating the rate of loop extrusion using an engineered pairing system. The authors would be advised to relate the findings to the particulars of yeast mitosis, highlighting similar mechanisms in mammalian cells rather than potential differences in cohesin and condensin functions.

Reviewer #2

(Remarks to the Author)
In this work the authors employed a method they previously developed, namely Chemically Induced Chromosomal Interaction (CICI) to assess the kinetics of 3D interactions between pairs of fluorescently-labelled loci in live budding yeast cells. Measuring the fluctuations in the distance separating the two reporter-loci as a function of the time, before and after experimentally inducing their physical interactions, allows for assessing the kinetics of chromosomal encounters. Using this experimental system in G1-arrested budding yeast cells, the authors provide evidence that while the dynamics of all loci can

be well-described by the Rouse polymer model, the kinetics of encounters (i.e. encounter times) of intrachromosomal pairs deviates from this model, being significantly shorter than those of inter-chromosomal pairs. Using targeted auxin-induced degradation, the authors provide evidence that condensin is responsible for this acceleration in G1. Hi-C experiments are employed to further assess whether or not condensin drives long-range intrachromosomal contacts in budding yeast cells arrested in the G1 phase of the cell cycle. Using polymer physics and modelling, the authors further suggest that loop extrusion by condensin can explain such acceleration and delineate bio-physical parameters for condensin-mediated loop extrusion in vivo.

Overall this is an interesting study that has the potential to unveil a role for condensin in 3D genome organisation during the G1 phase of the cell cycle in budding yeast. However, and although I am not an expert in statistical physics and biopolymer simulation, I noticed several major points of concern regarding key experiments on which the manuscript is grounded. I suggest that those points should be addressed to strengthen the work and warrant publication.

Major points

Point n°1. Measurement of distances

Regarding the method used to extract the distances between the LacO and TetO dots from images in the CICI experiment. I understand from the Material and Methods chapter (lines 497-498) that distances have been measured on 2D projections of Z-stacks and not in 3D. Can the author confirm/infirm please? I think this is a key point since it seems to me that such 2D distances cannot by essence reflect the nuclear volume explored by the loci and would underestimate RMSD measurements. I think the authors should clarify this point.

Point n°2. Simulation

In this work, chromosomal condensins have been modelled as extruding DNA symmetrically (line 579), while there is experimental evidence that budding yeast condensin is an asymmetric and unidirectional extruder (PMID: 29472443; 35653469). This challenges the relevance of the modelling performed in this study. The authors should justify their choice and, importantly, describe the consequence of an asymmetrical vs symmetrical loop extrusion on the results of their simulation.

Point n°3. Hi-C analyses of the role played by condensin during the G1 phase of the cell cycle

The authors used SMC4-AID to deplete condensin in G1-arrested cells and to assess the consequence on 3D genome organisation by Hi-C. In Fig. S5 A-B, the Hi-C profiles of WT vs Smc4-AID -IAA vs SMC4-AID +IAA indicate that most of the difference between the WT and the SMC4-AID strain stem from the AID tag itself, while IAA adjunction in G1-arrests to deplete SMC4 has a very limited effect, if any. Crucially, for all the subsequent analyses aiming at assessing the impact of condensin in G1, the authors compare data from WT vs SMC4-AID +IAA. But since most of the difference is already present in the SMC4-AID, i.e. before auxin adjunction, I do not see how the authors can assign the effects they observed to the G1 phase. Those effects may well stem from the preceding interphase and/or from mitosis. In that context, I am afraid that the comparison between WT vs SMC4-AID +IAA performed in Fig. 4, Fig. 5, Fig. S5 and Fig. S6 do not allow to assess the role played by condensin in the G1 phase of the cell cycle.

Point n°4. LacO and TetO arrays

Arrays made of 256 LacO repeats and 192 TetO repeats are likely to constitute a roadblock for condensin (PMID: 31204167; 38446663; 40560727). Condensin stalling at those arrays, in turn, is likely to change/influence in cis the local chromosomal 3D dynamics (PMID: 38446663). 1) Can the author assess whether or not condensin accumulate at those sites (ideally in G1 or at least in G2/M to ease condensin detection)? 2) Can the authors introduce such a stalling in their modelling to further assess its consequence ?

Other points

Related to Figure 2 and Fig. S1, regarding the RMSD measurements made for intra-arm vs inter-arm vs inter-chromosomal loci pairs:

4.1) I find it difficult to determine to which extent the scaling factors and exponents obtained are similar or different between comparable pairs of loci. I suggest that the authors should better explain to the reader the meaning of those parameters and how they compare/contrast between conditions and pairs of loci. For example, I see in Fig. 2C a clear difference in the scaling factor of pair 1 vs the comparable pair 8 (i.e. intra-arm vs inter-arm) and also between pair 3 and its cognate pair 9 (intra-arm vs inter-chromosome). Whether those differences are significant or not should be clearly stated.

4.2) It could be expected that loop extrusion would modify the diffusion dynamics of loci and therefore that the RMSD values calculated for intra-arm pairs of loci in the "– rapa, slow" condition differ, at least slightly, from those RMSD of inter-chromosome pairs. It seems to me that intra-arm pairs 1 to 6 do exhibit a smaller scaling factor than others. Likewise, I see a difference in the exponent and scaling factors of pair 3 vs pair 9. Can the author better address those difference and better explain to the reader how to compare and contrast results on intra-arm vs inter-arm. This would help assessing the data shown in Fig. S3 and related to the impact of condensin depletion on RMSD.

Related to Fig.3 on the impact of cohesin vs condensin on CFT

By auxin mediated depletion of cohesin or condensin in late G1, the authors conclude that condensin accelerates intra-chromosomal interactions in G1 while cohesin plays no role. However, the depths of depletion achieved for cohesin vs condensin are not comparable, cohesin being notably less depleted. Since the authors claim that intra-chromosomal encounters measured by CICI are accelerated by few loop extruders per Mb, I think they cannot rule out the possibility that traces of cohesin remaining in their experiments may contribute to CICI formation. I suggest that the authors should consider/discuss this possibility.

Cohesin depletion has no effect on the dot distance and CFT of pair1 but the dot distance of pair4 is significantly increased in SMC1-AID (thought the RMSD look similar). Why is that? Same question for pair 4 in SMC4-AID vs WT.

Fig. 1E. In population averaged- dot distances, one would expect distances in +rapa condition to reach a plateau, which is

not visible. Why is that? What is the fraction of cells showing CICI as a function of time ?
Fig. 1G. I would have expected the mean RMSD measured after CICI formation (+ rapa condition) to be of similar value between Fig.1G and Fig. 1F, which is not the case. Why is that?
Fig. 3B. Axis legends are inverted between the left and right panels.
Figure 4C. ChIP exo data of condensin. There is experimental evidence that most condensin peaks detected by ChIP-seq reflect the stalling of condensin against transcriptional barriers (PMID: 38446663). The abundance and amplitude of condensin ChIP-seq peaks on a given chromosome is therefore likely to depend on the transcription programme. It is not mentioned which phase of the cell cycle those data shown in Fig. 4C correspond to? Are they from G1 arrested-cells or not? If not, then what is their relevance to the current work?

Reviewer #3

(Remarks to the Author)
The work by Zou et al describes how the SMC condensin complex promotes long-range cis contacts along budding yeast G1 chromosomes. The work is supported by the CICI approach, developed by the authors, which exploits the fusion proteins LacI-FKBP12 and TetR-FRB to generate stable interactions through dimerization of FKBP12-FRB. Collision frequencies between 13 pairs of loci monitored under wt and condensin mutant conditions were supported by Hi-C contact maps of condensin-depleted cells, and polymer simulations.
The presence of condensin on yeast chromosomes in G1 (and at later stages of the cell cycle) is long documented (D'Ambrosio, 2008). A series of works has now shown that loop extrusion takes place on chromosome III, linking mating-type loci and explaining the particular contacts between the chromosome arms of this chromosome. I regret that the authors do not really present their work in this broader context, rather going very quicky on these important results (e.g. Dinda et al., 2023; and the pre-publication Piveteau et al., 2024, which should be discussed and cited).
The present study generalizes the observations, which is a good thing. To some extent, the results are expected, as in previous studies, researchers focus their efforts on the two chromosomes that behave as outliers because they carry either the rDNA or the mating type loci. But it's important to document a phenomenon that is in fact more general, albeit with little (visible) impact (so far) given the absence of genomic landmarks on most chromosomes.
I find the discussion interesting but sometimes a little forced, with attempts to make the connection between what happens in metazoans and yeast. For example, regarding the “canonical division of labor”, the authors fail to mention that condensins play a key role during the anaphase of mitosis (and not just cohesin). Bridging or highlighting the differences or similarities between yeast and metazoans provides interesting insights, but can also sometimes seem a little disconnected. For example, the authors could have discussed more what happens in species closer to S. cerevisiae, such as S. pombe, with regard to the condensin/cohesin interaction.

Major comments

Model
In the model, I'm uncertain whether the condensin is directional or bidirectional. The literature suggests that the condensin is a directional loop extruder (Shaltiel 2022, Kim 2020, Piveteau 2024,...) In the present work, it seems that the model is a bidirectional motor. If so, why? I would suggest clarifying this point and, if not already done, applying a unidirectional model in the present work, to better reflect the current understanding of the condensin loop extrusion process.

Controls
It seems to me that in studies exploiting budding yeast, it is a necessity (and easy) to present FACS analysis of synchronized cell populations. This seems particularly necessary when the study focuses on a specific cell cycle stage of a cell population. I understand that for microscopic analysis, FACS analysis is not essential, since individual cells and the number of foci provide sufficient evidence that the cells are in G1. But for genome-wide assays, I think it's important to adhere to these minimum controls, and want the authors to produce them.

Moreover, for the moment, the protocol for Hi-C experiments does not include a G1 synchronization step: "Yeasts were incubated in 200 ml of SCD medium until OD660 reached 0.3, (unless I'm mistaken). I assume - I hope - that this is an error, but it must be corrected or clarified.

Nor have I seen any western validation (apart from a mention in the method) that AID-based condensin depletion was complete. It is also essential, in my opinion, to show this control, given that the conclusion is based largely on this experiment.

Replicates

The changes between the WT, Smc4-AID – IAA, Smc4-AID + IAA, are really minor to the eye (but for chromosome XII) (S5A, S5B). This observation make me wonder how many replicates have been generated and analyzed for each condition, and how these replicates were handled (are they merged for the figures ?) In the present state, it is unclear. At least one replicate (experimental and analysis) must be produced for each condition (given there are so few of them this should not be an issue).

Image processing
The author do z-stacks but then merge them to measure 2D distances. Can they justify this approach and cite the corresponding literature ? It cannot introduce distortion in the comparison with the model ?

Condensin blocks
Condensin can be blocked by Rap1LacI networks (Analikwu 2025). It is quite possible that the LacOTetO networks used in CICI also affect condensin extrusion and lead to an overestimation of the long-distance contacts observed. Can the author show or point to data suggesting that this is not the case? Or at least discuss possible consequences for their analysis of this phenomenon and the impact with the modeling comparison ?

Minor comments
Please be consistent in the axis legends, scales, etc. (ie 4D vs S5D, ratio y axis of 4B, etc.)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have adequately addressed the reviewers comments.

Reviewer #2

(Remarks to the Author)
Review n°2 Nat comm

Point n°1. Measurement of distances
I agree that continuous co-localization over four consecutive time points is a convincing way to identify CICI formation. Yet, the problem remains that we ignore to which extent working on 2D projections, instead of 3D data, impacts dot distance measurements and RMSD values. It seems to me that the authors could (easily?) image a WT cell population with a short (resolutive) spacing between Z-stacks and compare such 3D results with those from 2D projections. I think this is an important validation to perform, unless there is a technical limitation that I missed.

Point n°2. Simulation
I thank the authors for clarifying this point. However, and as the authors clearly state in their rebuttal letter, the conclusion that one-sided and two-sided extrusion are equivalent is valid in the absence of barriers, a condition that is unlikely to apply to Condensin in vivo. Therefore, for the sake of clarity, I suggest that the authors clearly state in the main text that their conclusion is valid in the absence of barriers.

Point 3. Hi-C analyses
I am still puzzled by the way Hi-C data are compared, and I remain convinced that the authors should compare and contrast SMC4-AID – IAA vs SMC4-AID vs +IAA. Comparing WT (-IAA) vs SMC4-AID +IAA, as currently done in the core manuscript, is inappropriate because it does not allow to distinguish the effects of the AID tag from the effects of auxin.
I agree that the P(s) data now provided in Fig. S5E show no difference between WT and SMC4-AID -IAA, but P(s) are averages and therefore may not reveal regions of increased sensitivity to the AID tag, as exemplified by the rDNA. Lines [257-258] the authors state that condensin depletion leads to a greater reduction in Hi-C signals on ChrXV (16% over 200 kb) than ChrIV (12% over 200 kb). Could such increased sensitivity of ChrXV stem from the AID tag rather than the depletion per se ?
Moreover, the FACScan profiles of cell populations processed for Hi-C, now shown in Fig. S5E (a request of reviewer #3), are clearly different between the WT and the SMC4-AID samples, while the SMC4-AID – IAA and +IAA are much more similar. Those differences further advocate for comparing what must be compared, namely WT vs SMC4-AID (-IAA) and SMC4-AID – IAA vs +IAA.

Related to that, FACS data now shown in Fig. S5E suggest the presence of almost 40% of G2 cells in the SMC4-AID cell populations under G1-arrest. Is this credible or is it an artefact due to increased background staining upon arrest? Why is the SMC4-AID strain behaving differently? I think the authors should comment on this as it casts doubt on the synchrony of the cell populations that have been analysed.

Point 4. LacO and TetO arrays as barriers
I remind the authors that Rap1 arrays impair condensin-mediated loop extrusion in single molecule assays, i.e. without recruiting a large SAGA protein complex, arguing that being or not a roadblock to Condensin is unlikely to be a matter of size.
I thank the authors for the Hi-C analysis and ChIP-seq data. I agree that it is hard to see any difference around the LacO and TetO repeats in the Hi-C 2D maps. However, I am less convinced by the ChIP-seq results. When I compare the height of peaks in the IP fractions or replicates #1 and #2 vs the input I see a modest enrichment in the IP fraction #1, but not in IP#2. Notwithstanding the fact that only one input is provided. Thus, the validity of those ChIP-seq experiments remains unclear to me. I suggest that those ChIP-seq data should (1) be further assessed by measuring/verifying the enrichment of SMC4-HA at previously described Condensin-binding sites, such as tRNA genes and rDNA repeats (D'ambrosio et al. 2008), and (2) that

those data are included in sup materials to support the claim that TetO and LacO repeats pause no barrier to Condensin.
I am also surprised that the Amp gene is transcribed in budding yeast. Can the author comment on that.

Minor point
Fig. 3D, Pair 5 : WT and SMC4-AID+IAA show almost identical CFT, higher than SMC4-AID -IAA. I am therefore surprised that the comparison WT vs SMC4-AID-IAA and is shown as n.s. while the difference comparison SMC4-AID -IAA vs +IAA sis shown as **. Can the authors check this please.

The remain points that I raised have been addressed. I thank the authors for that.

Reviewer #3

(Remarks to the Author)
The authors have addressed the major points I raised previously and I thank them for that.
Although I would have liked the study to provide more functional information regarding "why condensin loops along G1 chromosomes?", and for some of the experimental conclusions to be more robust, I believe this study brings useful and interesting elements to the discussion.

I have a couple of minor points though to be addressed:

- Fig 4B: the authors must put in sup the correct control with the WT -IAA slope added to the present graphes, ie related to Chr. IV right arm. They only show the full genome analysis in Fig. S5E.

- FACS analysis: arrested cells cannot be grown, as written. And is it OD 0.3 or 0.4? Not a big deal but there is a lack of consistency in the experimental methods that is a bit ennoying.

- Line 197, 405: a discrete, stable, condensin-mediated loop was experimentally characterized in yeast anaphase between the rDNA and centromere in Lazar-Stefanita 2017 and subsequent works, this should be mentionned as it is different from the statement that the region inbetween the cen and the rDNA is more "self-condensed". If I am not mistaken this long loop is the first experimentally characterized in budding yeast.

- I understand why the authors removed the disucssion about segregation and "division of labor" (especially given that there are hints that cohesin could also impact segregation), but I think reminding the reader that condensin also plays a critical role in mitosis is important, notably since it seems to promote segregation (check out Stephane Marcand's - ie Guerin, 2019; Analikwu, 2025 - work for instance, and the outcome of condensin depletion in anaphase).

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
I thank the authors for the detailed explanation of the technical reasons that led them to work on 2D projections and for the updated work and sup data on the barrier vs non-barrier effect of LacO and TetO repeats in their experimental systems.
I have no further comment and congratulate the authors for their work.

We thank all three reviewers for carefully reading our manuscript and providing thoughtful feedback. We are encouraged by the positive comments and have revised the manuscript accordingly. Below is a detailed point-by-point response, with **reviewer comments shown in black** and **our replies in blue**. In our responses, all sentences describing changes made to the manuscript are underlined. **In the revised manuscript, the corresponding changes are highlighted in red.**

Reviewer #1 (Remarks to the Author):

This is an interesting study of genome organization in yeast to discern dynamic aspects of condensin and cohesin. The experiments are clearly described and of the highest quality. The paper is well-written and well-organized.

The authors take advantage of the forkhead binding protein system to cross-link genomic loci tagged with lacO or TetO operator sequences. The ability to engineer this allows accurate assessment of cross-linked loci and quantitate encounter times. This is an ingenious strategy that the authors have put to good use.

The major comments are in terms of the novelty of the findings. The major contribution is the estimation of kinetics of loop extrusion, which they find in remarkable agreement to that found in vitro studies (~2 kb/s). They also find that condensin is the major driver, rather than cohesin, which while different from that in mammalian chromosomes, is not a surprise in yeast from prior studies.

We thank the reviewer for the overall positive evaluation.

Specific comments:

1. The authors state that loops are relatively sparse and only 10% of the genome is in loops at any given moment. How does this relate to the abundance of condensin, which has been estimated to be bound about 1 molecule every 10 kb. If 10% of the genome is in loops (~10^6 bp), and there is a loop every 10 kb, then each loop is only 100bp? Alternatively, only a fraction of bound condensin is extruding loops. I'd like to see the authors comments on the fraction of the genome in loops and their thoughts on the number of active condensin molecules relative to total bound.

   The estimate of one condensin molecule per ~10 kb likely derives from dividing the ~12 Mb yeast genome by the total number of condensin molecules (~1000-2000). However, this estimate is likely to be off because of the following considerations: First, not all condensin molecules are necessarily chromatin-bound and/or extruding loops at a given time. In fact, FRAP recovery of condensin subunit Brn1 in G1-arrested cells is very fast[1] (half-time of 1.99 ± 1.37 s; for comparison, remodeler RSC complex, which transiently associate with chromatin, has a FRAP recovery half-life of 7.8s), indicating that most condensin complexes probably do not remain bound long enough to extrude long loops. Second, condensin is not uniformly distributed across the genome, but highly enriched at specific loci such as centromeres, rDNA, and other specialized regions. Overall, we expect the density of condensin to be low over regular chromosome arms. From our simulation, the average loop size is 120-220 kb, but the average distance between the loops are 1-2 Mb, leading to the conclusion that ~10% of the genome is in loops. We updated the discussion on page 13.

2. The authors attempt to compartmentalize cohesin and condensin function (lines 400-500). They state that cohesin drives genome-wide compaction of the mitotic chromosomes, citing fellow Hi-C papers.

This may be the case, but this function of cohesin is non-essential. Heidinger-Pauli JM, et al. (Curr Biol. 2010. PMID: 20451387) demonstrate that the bulk of cohesin can be depleted to 13% without effecting chromosome segregation. The essential function of cohesin seems to be in the centromere bottlebrush, that is required for building tension over micron scales (J Cell Sci. 2022 Feb 15;135(4):jcs259532. doi: 10.1242/jcs.259532.). From trying to understand mechanism of action, I'm not sure the division of labor section sheds any particular insight.

The study by Heidinger-Pauli et al., as the reviewer pointed out, reported that reducing cohesin levels affects chromosome condensation, a phenotype likely related to cohesin's role in loop extrusion. How such condensation defects influence chromosome segregation, however, is a distinct question that lies beyond the scope of our current work. To avoid overstating this point, we rephased the original paragraph on page 13 to: "We found that condensin, rather than cohesin, mediates loop extrusion in G1-phase yeast. Conversely, previous studies have reported that cohesin, not condensin, drives the genome-wide compaction of mitotic chromosomes in yeast, although condensin still plays critical roles in regions like the nucleolus[2,3]. These findings are in striking contrast to the well-established roles of these complexes in metazoans and fission yeast, where cohesin is the primary loop extruder during interphase, and condensin is mainly responsible for mitotic chromosome compaction[4,5]. The reason behind this functional divergence is unknown."

3. Finally, the authors talk about the spatial segregation of chromosomes. This is difficult to understand. Unlike the situation in mammalian cells, where the chromosomes are individualized on a micron scale (easily distinct in the light microscope), in yeast all 16 chromosomes gathered at their centromeres and pulled to the spindle poles. In other words, the entire genome moves toward the pole as one. The chromosomes are individual entities, but spatial separation as the authors suggest (line 448), is not something that is obvious in yeast.

We thank the reviewer for pointing this out. In light of our answer above, that chromosome segregation is beyond the scope of the current study, we decided to delete the last paragraph of the discussion.

Reviewer #2 (Remarks to the Author):

In this work the authors employed a method they previously developed, namely Chemically Induced Chromosomal Interaction (CICI) to assess the kinetics of 3D interactions between pairs of fluorescently-labelled loci in live budding yeast cells. Measuring the fluctuations in the distance separating the two reporter-loci as a function of the time, before and after experimentally inducing their physical interactions, allows for assessing the kinetics of chromosomal encounters. Using this experimental system in G1-arrested budding yeast cells, the authors provide evidence that while the dynamics of all loci can be well-described by the Rouse polymer model, the kinetics of encounters (i.e. encounter times) of intrachromosomal pairs deviates from this model, being significantly shorter than those of inter-chromosomal pairs. Using targeted auxin-induced degradation, the authors provide evidence that condensin is responsible for this acceleration in G1. Hi-C experiments are employed to further assess whether or not condensin drives long-range intrachromosomal contacts in budding yeast cells arrested in the G1 phase of the cell cycle. Using polymer physics and modelling, the authors further suggest that loop

extrusion by condensin can explain such acceleration and delineate bio-physical parameters for condensin-mediated loop extrusion in vivo.

Overall this is an interesting study that has the potential to unveil a role for condensin in 3D genome organisation during the G1 phase of the cell cycle in budding yeast. However, and although I am not an expert in statistical physics and biopolymer simulation, I noticed several major points of concern regarding key experiments on which the manuscript is grounded. I suggest that those points should be addressed to strengthen the work and warrant publication.

We thank the reviewer for the overall positive evaluation.

Major points

Point n°1. Measurement of distances

Regarding the method used to extract the distances between the LacO and TetO dots from images in the CICI experiment. I understand from the Material and Methods chapter (lines 497-498) that distances have been measured on 2D projections of Z-stacks and not in 3D. Can the author confirm/infirm please? I think this is a key point since it seems to me that such 2D distances cannot by essence reflect the nuclear volume explored by the loci and would underestimate RMSD measurements. I think the authors should clarify this point.

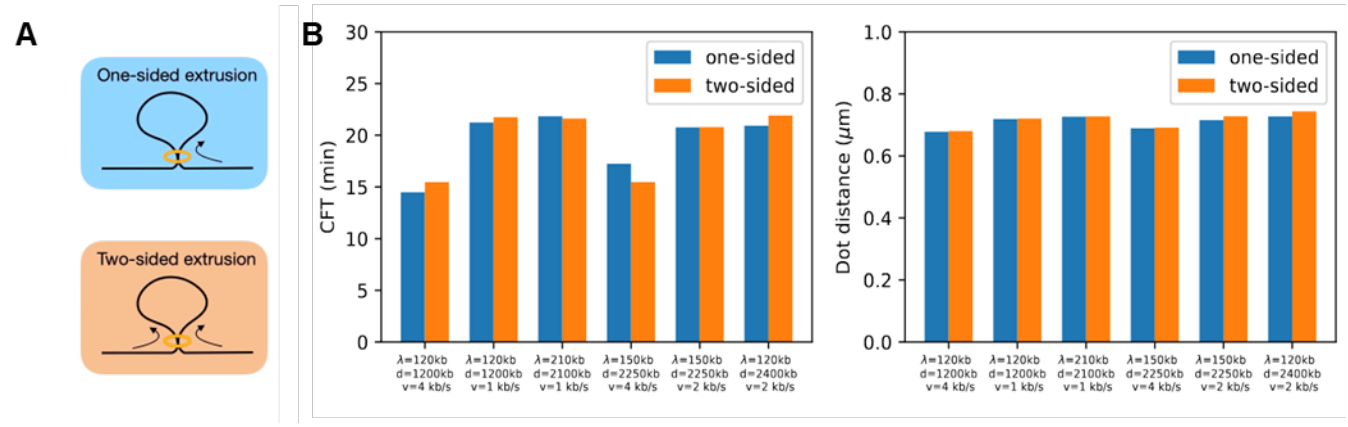
Yes, we reported the inter-dot distances based on their projection in the x–y plane. We used this method because of poor spatial resolution along the z-axis, which introduces greater measurement errors in 3D than in 2D. Our polymer model takes this into account by projecting all the simulated distances to the x-y plane, which indeed yields smaller RMSD values. We recognize that this approach introduces some false-positive co-localizations, particularly for dots that are aligned in x–y but separated in z. However, this limitation is mitigated by defining CICI formation based on continuous co-localization over four consecutive time points, which reduces the chance of misidentifying transient overlaps. We clarified this on page 16 in the Methods section.

Point n°2. Simulation

In this work, chromosomal condensins have been modelled as extruding DNA symmetrically (line 579), while there is experimental evidence that budding yeast condensin is an asymmetric and unidirectional extruder (PMID: 29472443; 35653469). This challenges the relevance of the modelling performed in this study. The authors should justify their choice and, importantly, describe the consequence of an asymmetrical vs symmetrical loop extrusion on the results of their simulation.

We thank the reviewer for raising this important point. Indeed, recent studies have shown that budding yeast condensin extrudes DNA asymmetrically and unidirectionally. In our simulations, we modelled condensin as a symmetric extruder. We chose this simplified implementation because, in the absence of barriers, one-sided and two-sided extrusion are effectively equivalent: what determines the dynamics of contact formation is the average loop size generated by an extruder of a given processivity and the density of extruders. Specifically, whether both DNA strands are reeled in or only one, the distribution of loop lengths over time remains the same, and consequently the statistics of first-passage times remain unchanged.

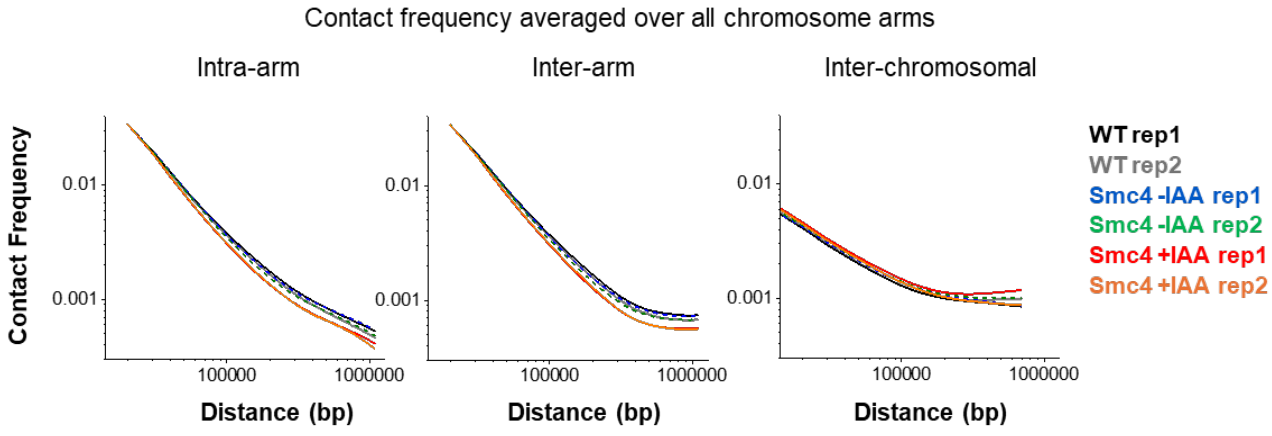
To confirm this point explicitly, we performed additional simulations using one-sided extrusion across six representative parameter sets. In all cases, search statistics and the average distances between probes remained unchanged compared to the two-sided case (see figure below). We have added a new supplemental Figure S9 illustrating these comparisons and updated the manuscript text accordingly on page 11.



Point n°3. Hi-C analyses of the role played by condensin during the G1 phase of the cell cycle

The authors used SMC4-AID to deplete condensin in G1-arrested cells and to assess the consequence on 3D genome organisation by Hi-C. In Fig. S5 A-B, the Hi-C profiles of WT vs Smc4-AID -IAA vs SMC4-AID +IAA indicate that most of the difference between the WT and the SMC4-AID strain stem from the AID tag itself, while IAA adjunction in G1-arrests to deplete SMC4 has a very limited effect, if any. Crucially, for all the subsequent analyses aiming at assessing the impact of condensin in G1, the authors compare data from WT vs SMC4-AID +IAA. But since most of the difference is already present in the SMC4-AID, i.e. before auxin adjunction, I do not see how the authors can assign the effects they observed to the G1 phase. Those effects may well stem from the preceding interphase and/or from mitosis. In that context, I am afraid that the comparison between WT vs SMC4-AID +IAA performed in Fig. 4, Fig. 5, Fig. S5 and Fig. S6 do not allow to assess the role played by condensin in the G1 phase of the cell cycle.

The assessment that “most of the difference between the WT and the SMC4-AID strain stem from the AID tag itself, while IAA adjunction in G1-arrests to deplete SMC4 has a very limited effect, if any” is not entirely accurate. Certain genomic regions, such as the rDNA locus, are indeed highly sensitive to condensin levels and already show strong effects in the Smc4-AID strain without IAA. However, for regular chromosome arms, the differences in contact frequency between WT and Smc4-AID –IAA are minor, and the majority



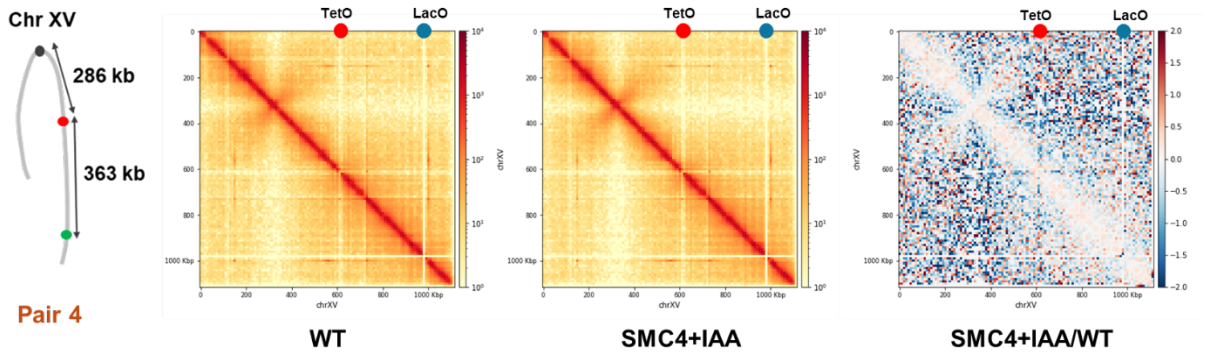
of changes occur between Smc4-AID ±IAA (figure above). In addition, Smc4-AID strain also shows significant difference in CICI formation kinetics with or without IAA (Figure 3E). These observations support the conclusion that condensin contributes to 3D chromosome organization specifically during G1. We have now included the figure above in Figure S5E. We also made this point clear on page 8 by stating: “Hi-C signals show minor changes in the Smc4-AID strain compared to WT in the absence of IAA, likely reflecting the basal degradation of Smc4. More pronounced changes in Hi-C profiles is observed after IAA treatment (Figure S5C-E).”

Point n°4. LacO and TetO arrays

Arrays made of 256 LacO repeats and 192 TetO repeats are likely to constitute a roadblock for condensin (PMID: 31204167; 38446663; 40560727). Condensin stalling at those arrays, in turn, is likely to change/influence in cis the local chromosomal 3D dynamics (PMID: 38446663). 1) Can the author assess whether or not condensin accumulate at those sites (ideally in G1 or at least in G2/M to ease condensin detection)? 2) Can the authors introduce such a stalling in their modelling to further assess its consequence ?

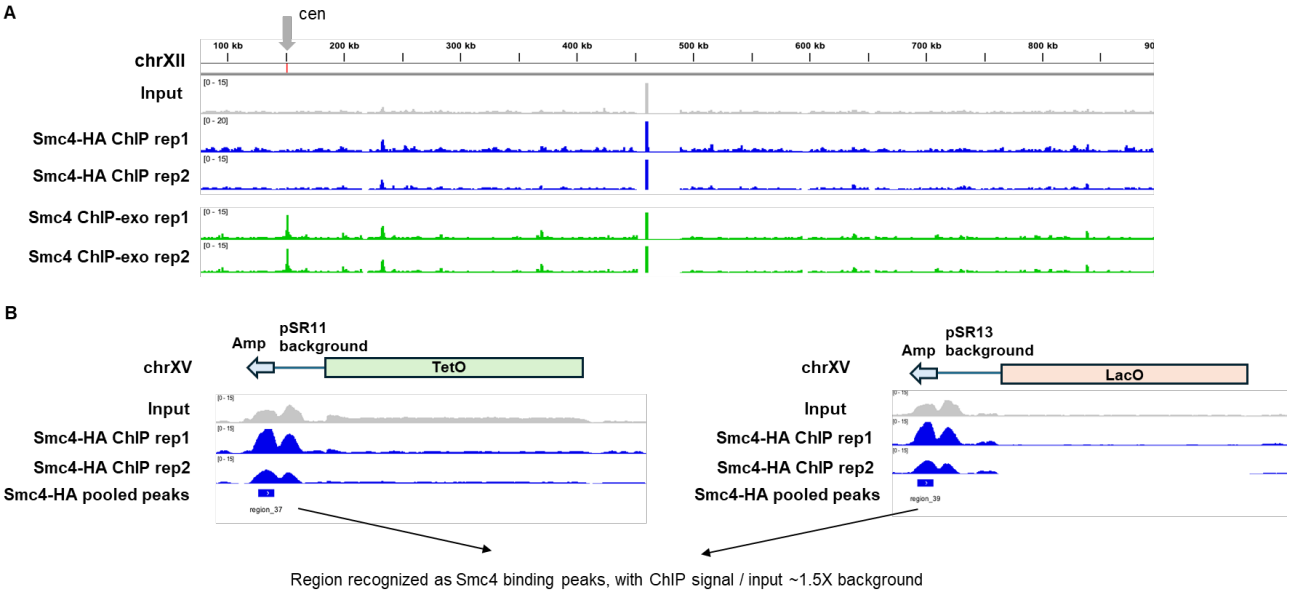
We thank the reviewer for raising this point. We agree that persistent stalling of loop extruders may significantly impact our model and is important to consider. Notably, the cited studies predominantly involve impediments associated with large endogenous protein assemblies (e.g., transcription machinery, telomere-associated complexes, or Rap1 arrays that can recruit SAGA). By contrast, the LacO/TetO arrays in our system are bound by exogenous factors (LacI and TetR) that are comparatively small, and it is not obvious that conclusions from those contexts translate directly to LacO/TetO.

We have taken two approaches to address this concern. First, we reanalyzed our Hi-C data using a custom genome build that includes the LacO and TetO arrays (our original analysis mapped to the WT genome). We focused on the Hi-C signals near the edge of the two arrays, where the sequencing data are mappable. If the arrays acted as robust roadblocks to loop-extruding condensin, we would expect to see *condensin-dependent* contact stripes emanating from these loci, like the ones observed from RE and rDNA locus[6]. We did not observe such features: there are no stripes, and the contact frequencies near the arrays are essentially identical in WT and Smc4-AID +IAA (see below). This data argues against condensin stalling with extruded loops at the arrays.

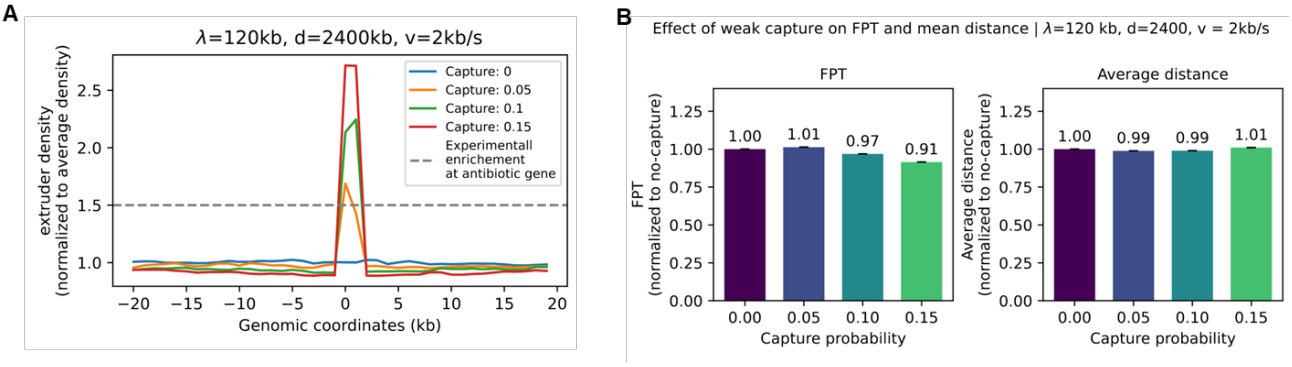


To directly detect potential stalling of condensin, we also performed Smc4-HA ChIP-seq in a G1-arrested CICI strain in the absence of auxin. Multi-mapped reads within the LacO/TetO repeats were assigned randomly over the arrays. Genome-wide Smc4 occupancies largely recapitulate published profile in cycling

cells[7] (Panel A below), with the notable exception of reduced condensin enrichment at centromeres, a pattern consistent with prior observation in G1[8]. Importantly, we detect no elevation (if anything, slight depletion) of Smc4 signal over the arrays (Panel B below), again indicating no stalling of condensin. We did detect a modest (~1.5X) increase in Smc4 signal immediately outside the array, over the ampicillin-resistance gene. Because this gene is highly expressed, it could impose a weak impediment to condensin translocation.



To test whether this small enrichment could influence the search time and distance between the arrays, we extended our simulations to include weak condensin pausing. Using the same extrusion framework, we introduced transient capture at the arrays with 5%, 10%, or 15% capture probability and a 60 s dwell time (the approximate time required for a single RNA polymerase to traverse the gene). These conditions produced a ~1.5–2.5X increase in simulated condensin density at the arrays, which is comparable to, or slightly higher than, the ~1.5X enrichment observed in the ChIP-seq data (Panel A below). Notably, only the highest capture probability (15%) leads to modest changes in FPT (~10%), whereas the effect completely disappears at 5% capture, the level that best reproduced the experimental accumulation (Panel B below). These results support that condensin pausing at the gene is too limited to accelerate the search processes of the arrays and validate our model assumption that the arrays can be treated as non-stalling barriers.



Other points

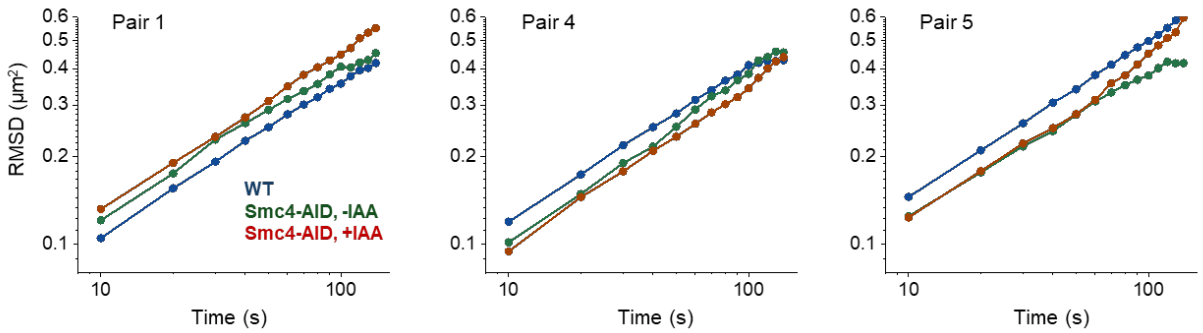
Related to Figure 2 and Fig. S1, regarding the RMSD measurements made for intra-arm vs inter-arm vs inter-chromosomal loci pairs:

4.1) I find it difficult to determine to which extent the scaling factors and exponents obtained are similar or different between comparable pairs of loci. I suggest that the authors should better explain to the reader the meaning of those parameters and how they compare/contrast between conditions and pairs of loci. For example, I see in Fig. 2C a clear difference in the scaling factor of pair 1 vs the comparable pair 8 (i.e. intra-arm vs inter-arm) and also between pair 3 and its cognate pair 9 (intra-arm vs inter-chromosome). Whether those differences are significant or not should be clearly stated.

4.2) It could be expected that loop extrusion would modify the diffusion dynamics of loci and therefore that the RMSD values calculated for intra-arm pairs of loci in the "– rapa, slow" condition differ, at least slightly, from those RMSD of inter-chromosome pairs. It seems to me that intra-arm pairs 1 to 6 do exhibit a smaller scaling factor than others. Likewise, I see a difference in the exponent and scaling factors of pair 3 vs pair 9. Can the author better address those difference and better explain to the reader how to compare and contrast results on intra-arm vs inter-arm. This would help assessing the data shown in Fig. S3 and related to the impact of condensin depletion on RMSD.

The two questions above are related, and we answer them together here. While some of the differences in the fitted parameters, particularly between intra-arm vs inter-arm / -chromosomal pairs, are statistically significant (as evidenced by non-overlapping error bars), the biological variation is relatively modest. The fitted scaling factors range from 0.039 - 0.053 $\mu m^2/s$, and the power-law exponents range from 0.41 to 0.54, which are close to the prediction of the Rouse model.

Several factors may contribute to these differences: 1) loci vary in their distances to structural anchors such as the centromere or telomere, which can impose differential constraints on their motion, 2) loci pairs with shorter genomic distances in between tend to reach saturation in RMSD at an earlier time point, which would affect the power-law fitting, and 3) whole chromosome arms may have large-scale collective motion relative to each other (for example, due to telomere movement). Such drift can be effectively canceled out in RMSD for intra-arm pairs, but not for inter-arm and inter-chromosomal ones. In fact, if there is a small upshift of the RMSD curve for the latter cases, we would expect to see a larger scaling factor and smaller exponent in the fit, which is consistent with the data in Figure 2C. We added some of the discussion above to page 5.



For the effect of condensin depletion on MSD, we were hoping to find some effects like in Mach et al.[9], where cohesin depletion leads to increased chromosome mobility. While pair 1 indeed shows such changes, the effect is small (especially between Smc4-AID ±IAA), and other pairs (e.g., pair 4 and pair 5)

do not show consistent trends. Therefore, we conclude that condensin depletion does not have major impact on chromosome dynamics under our experimental conditions.

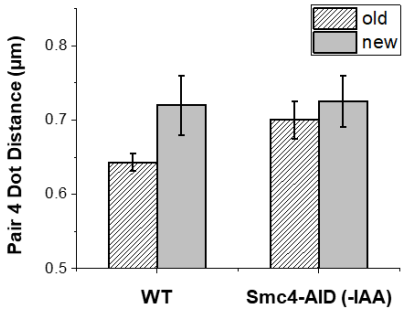
Related to Fig.3 on the impact of cohesin vs condensin on CFT

By auxin mediated depletion of cohesin or condensin in late G1, the authors conclude that condensin accelerates intra-chromosomal interactions in G1 while cohesin plays no role. However, the depths of depletion achieved for cohesin vs condensin are not comparable, cohesin being notably less depleted. Since the authors claim that intra-chromosomal encounters measured by CICI are accelerated by few loop extruders per Mb, I think they cannot rule out the possibility that traces of cohesin remaining in their experiments may contribute to CICI formation. I suggest that the authors should consider/discuss this possibility.

The observation that condensin depletion largely eliminates the difference in CFTs between intra- and inter-chromosomal pairs (Figure 3E) lead to the conclusion that condensin is primarily responsible for the accelerated establishment of intra-chromosomal interactions. We agree that we cannot formally exclude the possibility that cohesin contributes to this process in a minor way. In the manuscript, we weakened some of the statements like “condensin, but not cohesin, as the complex mostly responsible for these rapid intra-chromosomal interactions” “Condensin, but not cohesin, is primarily responsible for accelerated intra-chromosomal interactions in G1 cells” “cohesin does not play a major role for the rapid CICI formation observed between intra-chromosomal loci”.

Cohesin depletion has no effect on the dot distance and CFT of pair1 but the dot distance of pair4 is significantly increased in SMC1-AID (thought the RMSD look similar). Why is that? Same question for pair 4 in SMC4-AID vs WT.

We thank the reviewer for this observation. To address this point, we re-measured the dot distance of pair 4 in both the WT and Smc4-AID strains. The new measurements for the Smc4-AID strain were consistent with our previously reported values. However, the new data for the WT strain revealed a significantly longer average distance, comparable to that observed in the Smc4-AID strains (plot on the right). This new WT distance is also consistent with the pair4 distance observed in Smc1-AID strain (Figure 3B).



To understand the discrepancy with our earlier WT measurement, we re-examined the original dataset and identified a subset of measurements collected very early in the project, where the cell size on average was significantly smaller, likely due to a shorter G1 arrest time. We found the same issue for pair 9, where WT distances also appeared shorter than those of the AID strains. We therefore removed these early measurements with abnormally small cell sizes for both pair 4 and pair 9 and reanalyzed dot distance, RMSD, and CFT. Importantly, this correction only slightly impacts RMSD and CFT values (plot below) and does not alter any of the conclusions presented in the manuscript. We updated related panels in Figures 2 and 3 incorporating this new analysis.

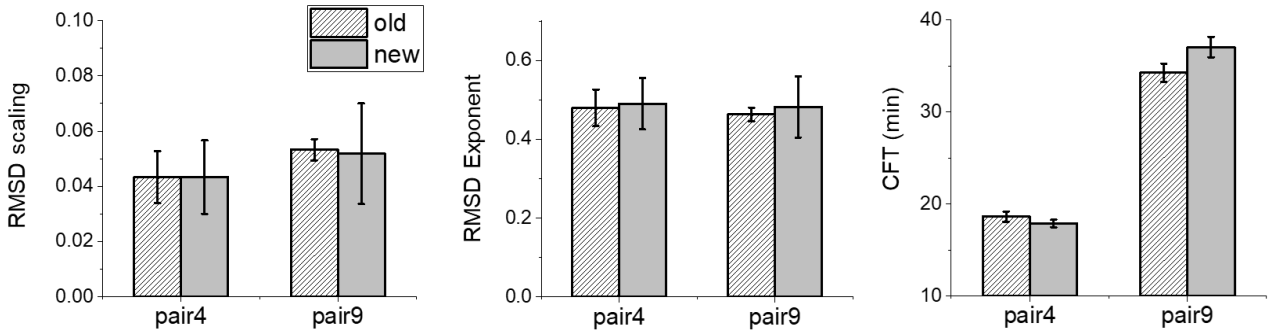


Fig. 1E. In population averaged- dot distances, one would expect distances in +rapa condition to reach a plateau, which is not visible. Why is that? What is the fraction of cells showing CICI as a function of time ?

Figure 1E x axis is in log scale, and therefore the long time points appear compressed. The same plot in linear scale is shown below, which clearly shows the plateau. The fraction of cells that form CICI as a function of time is shown in Figure 1H.

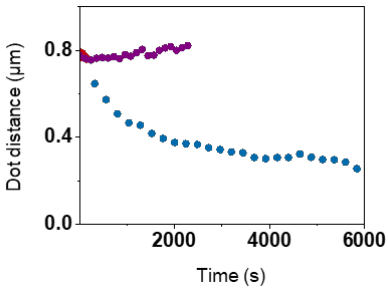


Fig. 1G. I would have expected the mean RMSD measured after CICI formation (+ rapa condition) to be of similar value between Fig.1G and Fig. 1F, which is not the case. Why is that?

We thank the reviewer for pointing this out. There was indeed an error in Figure 1G. We forgot to account for the difference in pixel size between the fluorescence and phase contrast images, which was off by a factor of two, and consequently, the RMSD is off by a factor of four. We have now corrected this error in Figure 1G. All other panels have already taken this factor into account, and they are correct.

Fig. 3B. Axis legends are inverted between the left and right panels.

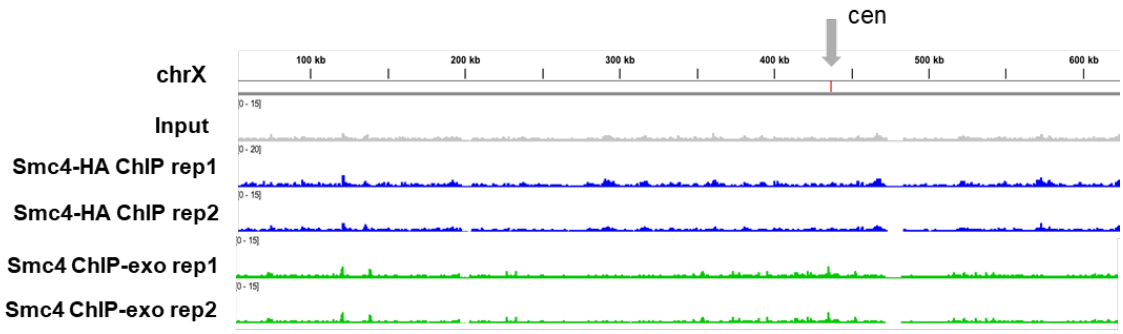
This has been corrected.

Figure 4C. ChIP exo data of condensin. There is experimental evidence that most condensin peaks detected by ChIP-seq reflect the stalling of condensin against transcriptional barriers (PMID: 38446663). The abundance and amplitude of condensin ChIP-seq peaks on a given chromosome is therefore likely to depend on the transcription programme. It is not mentioned which phase of the cell cycle those data shown in Fig. 4C correspond to? Are they from G1 arrested-cells or not? If not, then what is their relevance to the current work?

The quoted study above is from *fission yeast*, where condensin shuttles between the cytosol and nucleus throughout the cell cycle, and chromosome condensation during metaphase depends primarily on condensin[5]. In contrast, budding yeast condensin remains constitutively nuclear with constant level between G1 and metaphase[10], and the genome-wide distribution of Smc4 is largely indistinguishable in G1 and M phases, except that condensin is more enriched at the centromere during mitosis[8]. Given these observations, we do not expect substantial differences in Smc4 ChIP-seq profiles across the cell cycle.

To address the reviewer's concern directly, we performed ChIP-seq for Smc4-HA in G1-arrested cells. The resulting profiles closely match the ChIP-exo patterns obtained in asynchronous cultures (in addition to

the example provided in response to Question #4, another example is shown below). Overall, Smc4 shows little site-specific enrichment along chromosome arms. Although transcription can influence condensin localization to some extent, the effect is very small and unlikely to matter at the length scale (hundreds of kbs) that we focus on in this study.



Reviewer #3 (Remarks to the Author):

The work by Zou et al describes how the SMC condensin complex promotes long-range cis contacts along budding yeast G1 chromosomes. The work is supported by the CICI approach, developed by the authors, which exploits the fusion proteins LacI-FKBP12 and TetR-FRB to generate stable interactions through dimerization of FKBP12-FRB. Collision frequencies between 13 pairs of loci monitored under wt and condensin mutant conditions were supported by Hi-C contact maps of condensin-depleted cells, and polymer simulations.

The presence of condensin on yeast chromosomes in G1 (and at later stages of the cell cycle) is long documented (D'Ambrosio, 2008). A series of works has now shown that loop extrusion takes place on chromosome III, linking mating-type loci and explaining the particular contacts between the chromosome arms of this chromosome. I regret that the authors do not really present their work in this broader context, rather going very quicky on these important results (e.g. Dinda et al., 2023; and the pre-publication Piveteau et al., 2024, which should be discussed and cited).

We thank the reviewer for reminding us of D'Ambrosio, 2008, which was a beautiful study and is now cited (page 6). The other two studies have already been cited in the manuscript.

The present study generalizes the observations, which is a good thing. To some extent, the results are expected, as in previous studies, researchers focus their efforts on the two chromosomes that behave as outliers because they carry either the rDNA or the mating type loci. But it's important to document a phenomenon that is in fact more general, albeit with little (visible) impact (so far) given the absence of genomic landmarks on most chromosomes.

We thank the reviewer for their positive evaluation. Many of the points raised by Reviewer #3 overlap with those from Reviewer #2, and we refer the reviewer to our responses provided in that section.

I find the discussion interesting but sometimes a little forced, with attempts to make the connection between what happens in metazoans and yeast. For example, regarding the “canonical division of labor”, the authors fail to mention that condensins play a key role during the anaphase of mitosis (and not just cohesin). Bridging or highlighting the differences or similarities between yeast and metazoans provides interesting insights, but can also sometimes seem a little disconnected. For example, the authors could

have discussed more what happens in species closer to S. cerevisiae, such as S. pombe, with regard to the condensin/cohesin interaction.

As mentioned in our reply to Reviewer #1, we rewrote the paragraph related to the “division of labor” to avoid over-generalization (page 13) and also got rid of the last paragraph about chromosome segregation. We did not make this discussion entirely about the comparison between *S. cerevisiae* and *S. pombe*, as these two species are also highly divergent (estimated ~400 mil years of evolutionary distance). Condensin in fission yeast only goes into nucleus during mitosis and is mostly responsible for chromosome compaction during mitosis[5]. In this sense, condensin function in fission yeast is more similar to that in mammalian cells than in budding yeast.

Major comments

Model

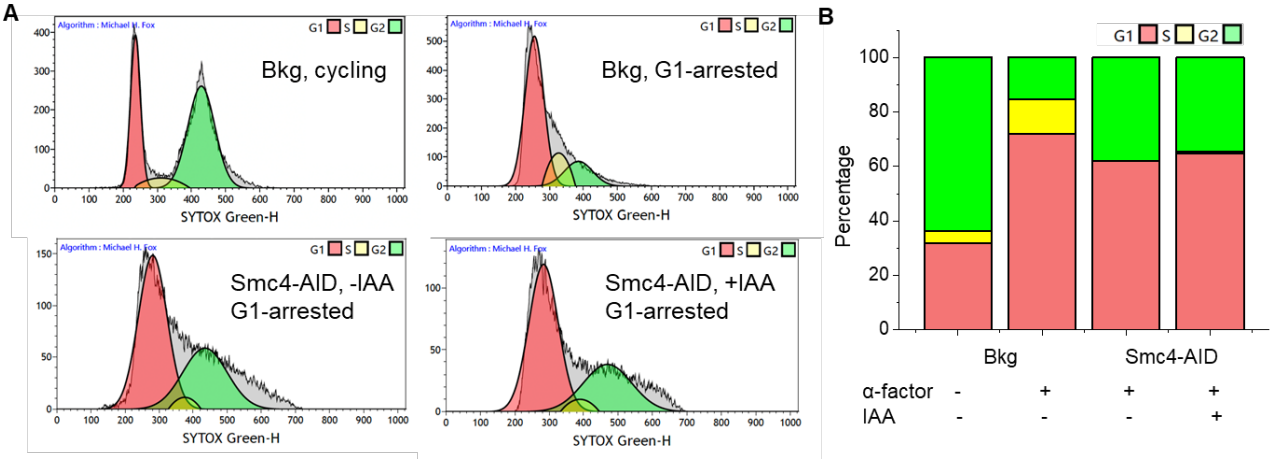
In the model, I'm uncertain whether the condensin is directional or bidirectional. The literature suggests that the condensin is a directional loop extruder (Shaltiel 2022, Kim 2020, Piveteau 2024,...) In the present work, it seems that the model is a bidirectional motor. If so, why? I would suggest clarifying this point and, if not already done, applying a unidirectional model in the present work, to better reflect the current understanding of the condensin loop extrusion process.

Please see our response to Reviewer #2, point #2.

Controls

It seems to me that in studies exploiting budding yeast, it is a necessity (and easy) to present FACS analysis of synchronized cell populations. This seems particularly necessary when the study focuses on a specific cell cycle stage of a cell population. I understand that for microscopic analysis, FACS analysis is not essential, since individual cells and the number of foci provide sufficient evidence that the cells are in G1. But for genome-wide assays, I think it's important to adhere to these minimum controls, and want the authors to produce them.

We have conducted the FACS measurement, as the reviewer suggested (see plots below). For the background strain (rapamycin resistant with doubling time 2.5-3 hr[11]), our treatment of α-factor for 2.5 hr results in ~70% of cells in G1 (in comparison to ~30% in G1 in cycling cells). The cell cycle profiles are very similar for Smc4-AID strains in -IAA and +IAA conditions, with slightly lowered G1 population of 62% and 65%, respectively. Some cells with high DNA content may reflect segregation failure due to reduced



condensin level[12]. These data are now added to Figure S5, and the corresponding methods are included.

Moreover, for the moment, the protocol for Hi-C experiments does not include a G1 synchronization step: "Yeasts were incubated in 200 ml of SCD medium until OD660 reached 0.3, (unless I'm mistaken). I assume - I hope - that this is an error, but it must be corrected or clarified.

Thank you for pointing this oversight. The arrest step is added on page 17.

Nor have I seen any western validation (apart from a mention in the method) that AID-based condensin depletion was complete. It is also essential, in my opinion, to show this control, given that the conclusion is based largely on this experiment.

Smc1 and Smc4 depletion levels in ±IAA conditions in comparison with WT cells are shown in Figure 3A.

Replicates

The changes between the WT, Smc4-AID – IAA, Smc4-AID + IAA, are really minor to the eye (but for chromosome XII) (S5A, S5B). This observation make me wonder how many replicates have been generated and analyzed for each condition, and how these replicates were handled (are they merged for the figures ?) In the present state, it is unclear. At least one replicate (experimental and analysis) must be produced for each condition (given there are so few of them this should not be an issue).

For each condition, we generated two independent biological replicates. To better illustrate reproducibility, we have now replotted Figure 4B and S5E to show the individual curves from each replicate. Because these curves represent averages across entire chromosomes, the associated error bars are very small, and the two replicates overlap closely.

Image processing

The author do z-stacks but then merge them to measure 2D distances. Can they justify this approach and cite the corresponding literature ? It cannot introduce distortion in the comparison with the model?

Please see our response to Reviewer #2, point #1.

Condensin blocks

Condensin can be blocked by Rap1LacI networks (Analikwu 2025). It is quite possible that the LacOTetO networks used in CICI also affect condensin extrusion and lead to an overestimation of the long-distance contacts observed. Can the author show or point to data suggesting that this is not the case? Or at least discuss possible consequences for their analysis of this phenomenon and the impact with the modeling comparison ?

Please see our response to Reviewer #2, point #4.

Minor comments

Please be consistent in the axis legends, scales, etc. (ie 4D vs S5D, ratio y axis of 4B, etc.)

The scales are modified accordingly.

1. Thadani, R., Kamenz, J., Heeger, S., Munoz, S. & Uhlmann, F. Cell-Cycle Regulation of Dynamic

Chromosome Association of the Condensin Complex. *Cell Rep* **23**, 2308-2317 (2018).
2. Schalbetter, S.A. et al. SMC complexes differentially compact mitotic chromosomes according to genomic context. *Nature cell biology* **19**, 1071-1080 (2017).
3. Lazar-Stefanita, L. et al. Cohesins and condensins orchestrate the 4D dynamics of yeast chromosomes during the cell cycle. *The EMBO journal* **36**, 2684-2697 (2017).
4. Kakui, Y., Rabinowitz, A., Barry, D.J. & Uhlmann, F. Condensin-mediated remodeling of the mitotic chromatin landscape in fission yeast. *Nat Genet* **49**, 1553-1557 (2017).
5. Hirano, T. Condensins: universal organizers of chromosomes with diverse functions. *Genes Dev* **26**, 1659-78 (2012).
6. Piveteau, V. et al. Condensin loop extrusion properties, roadblocks, and role in homology search in S. cerevisiae. *bioRxiv*, 2024.09.12.612585 (2024).
7. Rossi, M.J. et al. A high-resolution protein architecture of the budding yeast genome. *Nature* **592**, 309-314 (2021).
8. D'Ambrosio, C. et al. Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. *Genes Dev* **22**, 2215-27 (2008).
9. Mach, P. et al. Cohesin and CTCF control the dynamics of chromosome folding. *Nat Genet* **54**, 1907-1918 (2022).
10. Wang, B.D., Eyre, D., Basrai, M., Lichten, M. & Strunnikov, A. Condensin binding at distinct and specific chromosomal sites in the Saccharomyces cerevisiae genome. *Mol Cell Biol* **25**, 7216-25 (2005).
11. Du, M., Zou, F., Li, Y., Yan, Y. & Bai, L. Chemically induced chromosomal interaction (CICI) method to study chromosome dynamics and its biological roles. *Nature communications* **13**, 757 (2022).
12. D'Ambrosio, C., Kelly, G., Shirahige, K. & Uhlmann, F. Condensin-dependent rDNA decatenation introduces a temporal pattern to chromosome segregation. *Curr Biol* **18**, 1084-9 (2008).

We thank all reviewers for evaluating our manuscript another round and providing constructive feedback. Below is a detailed point-by-point response, with **reviewer comments shown in black** and **our replies in blue**. In our responses, all sentences describing changes made to the manuscript are underlined. **In the revised manuscript, the corresponding changes are highlighted in red.**

Reviewer #1 (Remarks to the Author):

The authors have adequately addressed the reviewers comments.

We thank the reviewer for approving our revision.

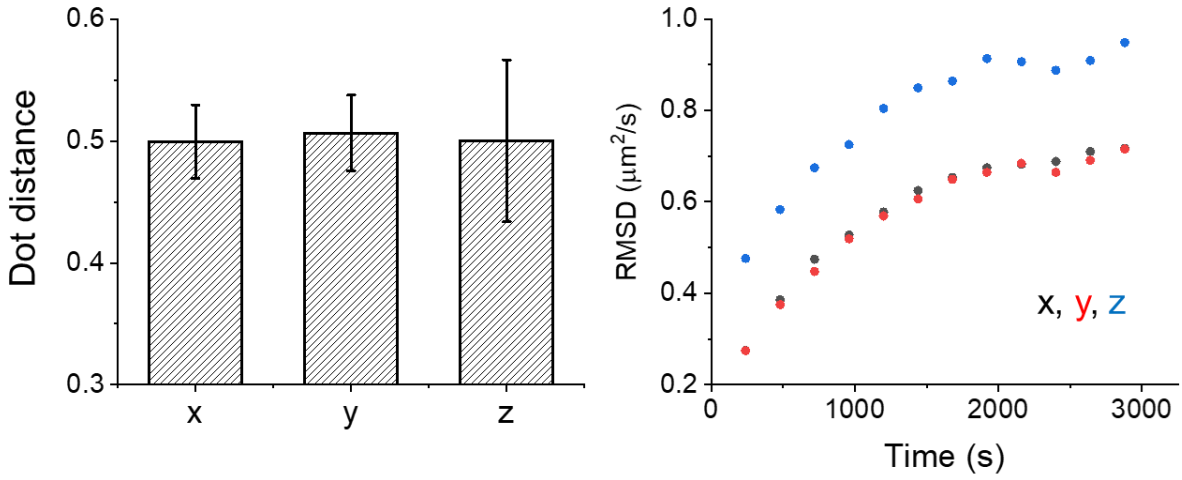
Reviewer #2 (Remarks to the Author):

Point n°1. Measurement of distances: I agree that continuous co-localization over four consecutive time points is a convincing way to identify CICI formation. Yet, the problem remains that we ignore to which extent working on 2D projections, instead of 3D data, impacts dot distance measurements and RMSD values. It seems to me that the authors could (easily?) image a WT cell population with a short (resolutive) spacing between Z-stacks and compare such 3D results with those from 2D projections. I think this is an important validation to perform, unless there is a technical limitation that I missed.

We agree with the reviewer that, in principle, getting full 3D distance would be valuable. However, there are technical limitations in our current imaging setup that make 3D position measurements less reliable than 2D projections.

Specifically, the axial (z) resolution of our microscope is significantly worse than the lateral (x–y) resolution, and our z-stacks are acquired with relatively large step sizes (0.6 μm). These large z steps are necessary due to the imaging sequence: we acquire the full z-stack in the green channel followed by the red (we cannot alternate channels at each z position, because channel switching in our system relies on mechanical rotation of a filter wheel, which is slow and subject to wear). To reduce the time delay between the two channels, which is critical for accurately assessing co-localization, we have to limit the number of z stacks by using larger z steps.

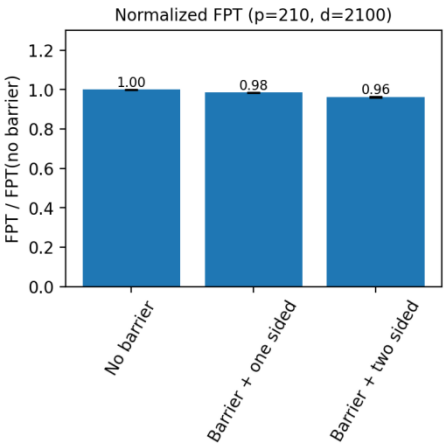
As a consequence, the z positions of individual dots cannot be determined with high precision and are associated with substantial measurement uncertainty. To quantify this effect, we analyzed a representative movie under -CICI condition by decomposing the inter-dot distances into x, y, and z components. The z position of the dot is determined by the z plane with maximum dot intensity. As shown in the left panel, while the mean distances along the three axes are comparable, the standard deviation of the z position is >2X than that in x or y. Because RMSD is sensitive to measurement noise ($RMSD^2_{z,measured} = RMSD^2_{z,true} + \sigma_z^2$, where σ_z^2 represents the variance due to localization uncertainty), this leads to an artificial inflation of the RMSD in the z direction (right panel). Under these conditions, including z information would degrade, rather than improve, the accuracy of distance and RMSD measurements.



Point n°2. Simulation: I thank the authors for clarifying this point. However, and as the authors clearly state in their rebuttal letter, the conclusion that one-sided and two-sided extrusion are equivalent is valid in the absence of barriers, a condition that is unlikely to apply to Condensin in vivo. Therefore, for the sake of clarity, I suggest that the authors clearly state in the main text that their conclusion is valid in the absence of barriers.

We thank the reviewer for emphasizing the importance of clarifying the role of barriers. We agree that the equivalence of one-sided and two-sided extrusion is not general and depends on the property of barriers. In the presence of a *strong*, *long-lived* barrier, a symmetric (two-sided) extruder can continue extruding from the unblocked side, while an asymmetric (one-sided) extruder would stall completely. However, in the context of our study, our data argue against the presence of such strong barriers. As shown in our previous rebuttal, our ChIP-seq and Hi-C data indicate LacO and TetO arrays and their neighboring sequences are at most *weak* barriers for condensin (more explanation below). Our previous simulations demonstrated that the effect of such a weak barrier on condensin search dynamics is minimal.

To further support this conclusion and to probe the potential impact of undetected barriers located between (rather than flanking) the arrays, we performed additional simulations incorporating weak barriers (5-10-15% capture probability) at random positions between the arrays (1 barrier per 100kb, i.e ~3 per array on average) under both one-sided and two-sided extrusion (see figure below). The results further confirm that the two models produce similarly weak and nearly equivalent effects on first-passage times.



Normalized first-passage times (FPTs) for p=210 and d=2100. FPTs are normalized to the no-barrier case (set to 1). Introducing weak barriers at random positions (1 per 100kb, i.e ~3 per array on average) has only a minor effect on search times, with nearly identical FPTs for one-sided and two-sided extrusion.

In contrast, however, this distinction is important in mammalian genomes, where strong extrusion barriers such as CTCF are prevalent. As suggested by the reviewer, we added explanations in the main text (page 11) to clarify the impact of barriers and emphasis the importance of extrusion dynamics in mammalian genomes where strong barriers are present. We also added FPT simulation in the presence of weak barriers into a new supplementary Figure S10.

Point 3. Hi-C analyses: I am still puzzled by the way Hi-C data are compared, and I remain convinced that the authors should compare and contrast SMC4-AID – IAA vs SMC4-AID vs +IAA. Comparing WT (-IAA) vs SMC4-AID +IAA, as currently done in the core manuscript, is inappropriate because it does not allow to distinguish the effects of the AID tag from the effects of auxin.

I agree that the P(s) data now provided in Fig. S5E show no difference between WT and SMC4-AID -IAA, but P(s) are averages and therefore may not reveal regions of increased sensitivity to the AID tag, as exemplified by the rDNA. Lines [257-258] the authors state that condensin depletion leads to a greater reduction in Hi-C signals on ChrXV (16% over 200 kb) than ChrIV (12% over 200 kb). Could such increased sensitivity of ChrXV stem from the AID tag rather than the depletion per se ?

Moreover, the FACS can profiles of cell populations processed for Hi-C, now shown in Fig. S5E (a request of reviewer #3), are clearly different between the WT and the SMC4-AID samples, while the SMC4-AID – IAA and +IAA are much more similar. Those differences further advocate for comparing what must be compared, namely WT vs SMC4-AID (-IAA) and SMC4-AID – IAA vs +IAA.

Related to that, FACS data now shown in Fig. S5E suggest the presence of almost 40% of G2 cells in the SMC4-AID cell populations under G1-arrest. Is this credible or is it an artefact due to increased background staining upon arrest? Why is the SMC4-AID strain behaving differently? I think the authors should comment on this as it casts doubt on the synchrony of the cell populations that have been analyzed.

We used consistent protocols for all the cell-cycle arrest experiment (total 2.5 hr arrest time). It is possible that, due to partial SMC4 degradation in the SMC4-AID strain, cells pass slower through mitosis and therefore arrest less at G1. It is also possible that there are other phenotypic changes that lead to increased staining. The important point here is that SMC4-AID +IAA to -IAA show essentially the same FACS profile.

We agree with the reviewer that, given the FACS profile difference, it is important to compare ±IAA conditions. We updated Figure 4B, 4D, S5E, and S5F to include the measurements under -IAA and direct +IAA/-IAA comparison. In all cases, the average contact frequency in SMC4-AID -IAA is more similar to the WT than SMC4-AID +IAA, and there is a consistent, albeit slightly smaller, reduction of long-range interaction when comparing +IAA to -IAA.

Point 4. LacO and TetO arrays as barriers: I remind the authors that Rap1 arrays impair condensin-mediated loop extrusion in single molecule assays, i.e. without recruiting a large SAGA protein complex, arguing that being or not a roadblock to Condensin is unlikely to be a matter of size.

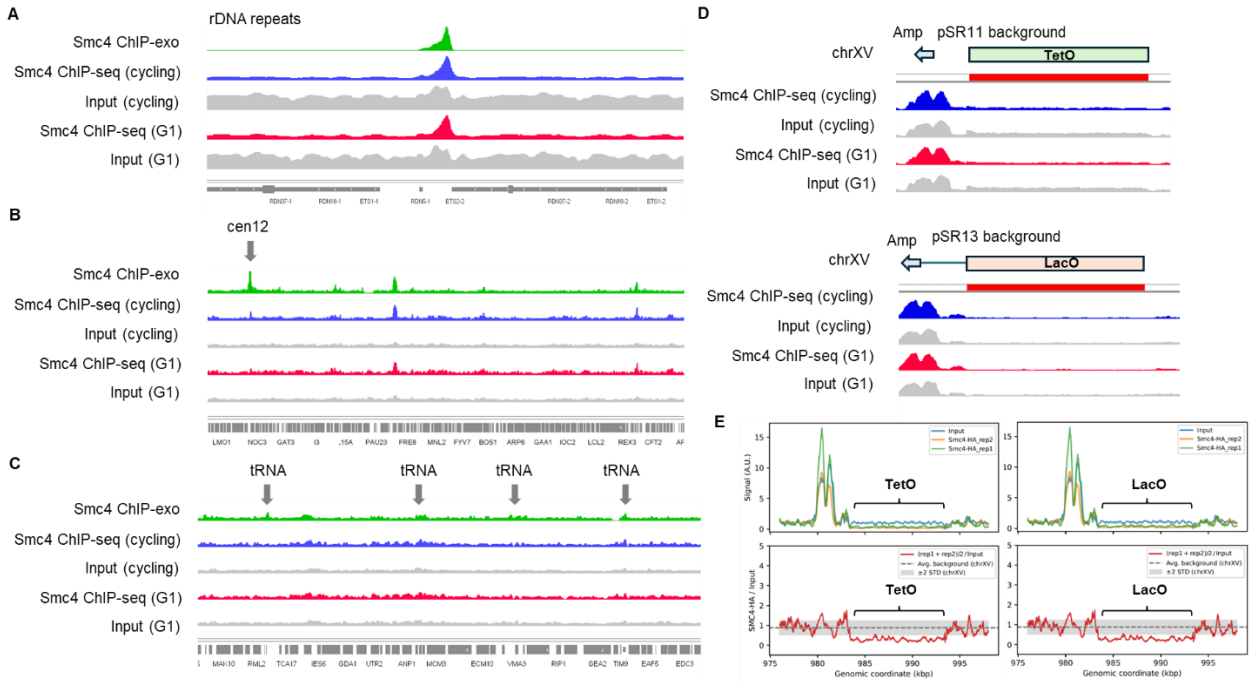
I thank the authors for the Hi-C analysis and ChIP-seq data. I agree that it is hard to see any difference around the LacO and TetO repeats in the Hi-C 2D maps. However, I am less convinced by the ChIP-seq results. When I compare the height of peaks in the IP fractions or replicates #1 and #2 vs the input I see a modest enrichment in the IP fraction #1, but not in IP#2. Notwithstanding the fact that only one input is provided. Thus, the validity of those ChIP-seq experiments remains unclear to me. I suggest that those ChIP-seq data should (1) be further assessed by measuring/verifying the enrichment of SMC4-HA at previously described Condensin-binding sites, such as tRNA genes and rDNA repeats (D'ambrosio et al. 2008), and (2) that those data are included in sup materials to support the claim that TetO and LacO repeats pause no barrier to Condensin.

We re-examined our ChIP-seq with exactly this in mind. As a positive control, we observed clear enrichment of SMC4–HA at a specific locus in the rDNA repeats (Panel A below), consistent with previously measured condensin localization (ChIP-exo data shown on the top row). Because condensin binding at centromeres is known to be lower in G1, we also repeated the ChIP-seq in cycling cells. Higher Smc4 enrichment is indeed detected at some centromeres (Panel B), but the signal is still lower than in ChIP-exo. This is expected because our rapamycin-resistant CICI strain has a slow cell cycle and spends a much larger fraction of time in G1. Although tRNA enrichment of Brn1 was reported in D'Ambrosio 2008, their enrichment is much lower than that at centromeres. We do not observe significant Smc4 enrichment at tRNA loci, this is consistent with previous ChIP-exo results (Panel C).

Importantly, we do not detect significant SMC4-HA peak at either the LacO or TetO insertion sites across replicates (Panel D & E), and for the point at hand, we also do not see any accumulation of SMC4-HA in the genomic interval between the two arrays. Together, these observations argue against stable condensin pausing/stalling at the LacO/TetO repeats, or in between them, which support our interpretation that treating these arrays as non-barriers. As the reviewer pointed out, this conclusion is also supported by our Hi-C data. Following reviewer's advice, we added the condensin ChIP-seq results and loop extrusion simulation in the presence of weak barriers into a new supplementary Figure S10.

One possible reason why Rap1 and LacI arrays have been reported to stall condensin in previous studies, but not in our system, is that LacI and TetR are expressed at much lower levels than the typical FROS system. For CICI to work efficiently, it is essential to keep the LacI and TetR fusion proteins at low concentrations. High levels of freely diffusing LacI-FKBP12 and TetR-FRB would dimerize with their chromatin-bound counterparts, thereby preventing direct interactions between the two arrays. We therefore drive the expression of these proteins with REV1 promoter, which is much weaker than HIS3 promoter used in common FROS system (Du et al., NAR, 2019). As a

result, the arrays are likely to be partially occupied, resembling the “sparse array” configuration described by Analikwu et al., which was shown to be less effective at blocking condensin.



I am also surprised that the Amp gene is transcribed in budding yeast. Can the author comment on that.

We do not fully understand this either. The LacO and TetO array is introduced using pSR13 and pSR11 from Dr. Gasser’s group (Rohner S et al., Yeast, 2008). In this construct, Amp gene should be driven by a bacterial promoter (a weak σ^{70}-type promoter upstream of the coding sequence can be identified). We do not understand why this gene appears to be expressed in yeast (in a separate line of investigation, we also detected high level of histone turnover over the Amp gene, again supporting its expression). Understanding the mechanism of Amp expression in yeast is beyond the scope of the current study.

Minor point

Fig. 3D, Pair 5 : WT and SMC4-AID+IAA show almost identical CFT, higher than SMC4-AID -IAA. I am therefore surprised that the comparison WT vs SMC4-AID-IAA and is shown as n.s. while the difference comparison SMC4-AID -IAA vs +IAA sis shown as **. Can the authors check this please.

As stated in the Figure 3 caption, “Y error bars represent 95% confidence interval of the CFT fit, … For CFT, statistical significance was determined using bootstrap resampling with replacement for 100000 iterations … ***P<0.001, **P<0.01, *P<0.05, n.s. non-significant.” Based on this definition, the *P*-value is 0.071 between WT and -IAA, and 0.0098 between +IAA and -IAA, so the label is correct.

The remain points that I raised have been addressed. I thank the authors for that.

Reviewer #3 (Remarks to the Author):

The authors have addressed the major points I raised previously and I thank them for that. Although I would have liked the study to provide more functional information regarding "why condensin loops along G1 chromosomes?", and for some of the experimental conclusions to be more robust, I believe this study brings useful and interesting elements to the discussion.

We thank the reviewer for the overall approval.

I have a couple of minor points though to be addressed:

- Fig 4B: the authors must put in sup the correct control with the WT -IAA slope added to the present graphes, ie related to Chr. IV right arm. They only show the full genome analysis in Fig. S5E.

We updated Figure 4B, 4D, S5E, and S5F to include the measurements under -IAA and direct +IAA/-IAA comparison.

- FACS analysis: arrested cells cannot be grown, as written. And is it OD 0.3 or 0.4? Not a big deal but there is a lack of consistency in the experimental methods that is a bit annoying.

In our Hi-C analysis, we grow the cells to OD 0.3 and arrested in G1 with 5 μM alpha factor for 2.5 hrs. During this time, some cells are still dividing, and some are growing in volume, leading to increased OD. The final OD is ~0.4, similar to what was used in the FACS analysis. This is made clearer in the revision.

- Line 197, 405: a discrete, stable, condensin-mediated loop was experimentally characterized in yeast anaphase between the rDNA and centromere in Lazar-Stefanita 2017 and subsequent works, this should be mentioned as it is different from the statement that the region in between the cen and the rDNA is more "self-condensed". If I am not mistaken this long loop is the first experimentally characterized in budding yeast.

We added this information more explicitly on page 6.

- I understand why the authors removed the discussion about segregation and "division of labor" (especially given that there are hints that cohesin could also impact segregation), but I think reminding the reader that condensin also plays a critical role in mitosis is important, notably since it seems to promote segregation (check out Stephane Marcand's - ie Guerin, 2019; Analikwu, 2025 - work for instance, and the outcome of condensin depletion in anaphase).

We thank the reviewer to point out these two interesting papers. Reading Analikwu et al. gave me some idea why the arrays in our system do not seem to block condensin (see reply to Reviewer #2, point 4). We modified the following sentence and referenced these two papers on page 14: "previous studies have reported that cohesin, not condensin, drives the genome-wide compaction of mitotic chromosomes in yeast, although condensin still contributes to the compaction[1,2]."

1. Analikwu, B.T. et al. Telomeres stall DNA loop extrusion by condensin. *Cell Rep* **44**, 115900 (2025).
2. Guerin, T.M. et al. Condensin-Mediated Chromosome Folding and Internal Telomeres Drive Dicentric Severing by Cytokinesis. *Mol Cell* **75**, 131-144 e3 (2019).