SMC complexes differentially compact mitotic chromosomes according to genomic context

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Structural maintenance of chromosomes (SMC) protein complexes are key determinants of chromosome conformation. Using Hi-C and polymer modeling, we study how cohesin and condensin, two deeply conserved SMC complexes, organize chromosomes in the budding yeast *Saccharomyces cerevisiae*. The canonical role of cohesin is to co-align sister chromatids, while condensin generally compacts mitotic chromosomes. We find strikingly different roles for the two complexes in budding yeast mitosis. First, cohesin is responsible for compacting mitotic chromosome arms, independently of sister chromatid cohesion. Polymer simulations demonstrate that this role can be fully accounted for through *cis*-looping of chromatin. Second, condensin is generally dispensable for compaction along chromosome arms. Instead, it plays a targeted role compacting the rDNA proximal regions and promoting resolution of peri-centromeric regions. Our results argue that the conserved mechanism of SMC complexes is to form chromatin loops and that distinct SMC-dependent looping activities are selectively deployed to appropriately compact chromosomes.

The extreme length of chromosomal DNA requires organizing mechanisms to both promote functional interactions between distal loci and ensure faithful chromosome segregation. Determining the unifying principles of functional organization requires an understanding of how organizing mechanisms have converged and diverged across evolution. In metazoans, the polymer organization of both local interphase domains and entire mitotic chromosomes is well described by the presence of chromatin looping in *cis*. The action of the SMC complexes cohesin and condensin is thought to be central to the formation of chromatin loops. Understanding how SMCs differentially orchestrate chromatin looping to develop functionally distinct chromatin structures in interphase and mitosis is a key question in cell biology.

In metazoans, SMCs play defined roles through the cell cycle. In interphase, cohesin-dependent *cis*-looping is required for partitioning interphase chromosomes into domains. During mitosis, metazoan chromosomes undergo chromosome-wide compaction leading to cytologically resolvable and longitudinally compacted structures. Both *in vitro* and *in vivo* studies suggest that condensin promotes the formation of *cis*-loops during this process. Condensin is required for chromosome compaction in meiotic extracts and cells. In early mitosis, cohesin is unloaded from chromosome arms, with condensin I complexes binding chromatin only following nuclear envelope breakdown. Therefore, condensin is considered central for chromatin looping during mitosis while cohesin's looping activity is assumed to be confined to interphase.

Mitotic compaction is also detected in organisms such as the budding yeast *Saccharomyces cerevisiae* that have distinct mitotic contexts from those found in metazoans. Budding yeast have significantly shorter chromosomes than metazoans and segregate duplicated chromosomes without nuclear envelope breakdown. Condensin is present within nuclei throughout the cell cycle and condensin is maintained along chromosome arms until its cleavage in anaphase. Budding yeast cells also rapidly progress from S to M without a definable G2 stage, even undertaking mitosis-associated functions such as kinetochore bi-orientation, before DNA replication is completed. The extent of compaction generally achieved in budding yeast in this compressed time frame appears significantly less than in metazoans. Resolvable and longitudinally compacted mitotic chromosomes are not readily apparent in metaphase-arrested cells. The only region to undergo readily visible compaction is the approximately 1-megabase (Mb)-long array of ribosomal DNA repeats. This array condenses in early mitosis before becoming further longitudinally compacted post anaphase. Pre-anaphase compaction at the rDNA array requires the concerted action of both cohesin

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and condensin\(^{17,20-22}\). A similar action of cohesin and condensin has been proposed to occur along chromosome arms\(^{14,20,23}\). However, interpreting locus-specific microscopic studies in cohesin and condensin mutants is impeded by the reduced mitotic compaction of the loci and the loss of sister chromatid arm cohesion that occurs in these backgrounds\(^{19,20,24}\). Therefore, fully assessing the role of cohesin and condensin in mitotic compaction along chromosomal arms requires an alternative approach to analyse mitotic structure.

Hi-C and computational modelling are ideal methodologies to study mitotic chromosome structure in budding yeast. Budding yeast have a relatively small and simple genome, a relative lack of repetitive regions, and are genetically tractable. Furthermore, their defined nuclear geometry makes them ideal for computational modelling of the chromosome structure underlying Hi-C contact maps\(^{25,26}\).

Here we use Hi-C and modelling to show that mitotic chromosome compaction in budding yeast is accounted for by cis-looping. Surprisingly, mitotic compaction of chromosome arms requires cohesin, but not sister chromatid cohesion or condensin. Therefore, our analysis indicates the deep conservation of chromosome compaction by SMC complexes while also demonstrating the divergent use of different SMC complexes in different contexts.

**RESULTS**

To study the mitotic organization of budding yeast chromosomes genome-wide, we used Hi-C on synchronized populations of budding yeast cells arrested in G1 or in metaphase (M) (Fig. 1a and Supplementary Fig. 1a). We fixed the synchronized populations with formaldehyde and prepared Hi-C libraries to assess chromatin conformation in each condition (Fig. 1a). We obtained on average 60 million unique valid pairs (pair-wise chromatin contacts) for each library (Supplementary Table 1). We binned contacts at 10 kb resolution and removed intrinsic biases using iterative correction\(^{27}\).

Hi-C contact maps in both G1 and M displayed the main features of budding yeast nuclear organization reported previously in asynchronous populations\(^{25,26}\); a Rabl-type organization with strong centromere clustering and arm-length-dependent telomere clustering (Fig. 1b,c and Supplementary Fig. 1b). However, comparison of the G1 and M contact maps (Fig. 1b,c) and inspection of their log\(_2\) ratio (Fig. 1d) showed the global reduction of contacts between the arms of different chromosomes (inter-chromosomal contacts) as compared with contacts formed along chromosome arms (intra-chromosomal contacts). This change was not simply caused by normalization, since no loss of inter-chromosomal contacts was observed between centromeres (Fig. 1d). Rather, chromosome arms were resolved from one another in mitosis relative to their interphase state.

Concurrently, Hi-C contact maps changed locally along chromosome arms. In M cells, the frequencies of intra-chromosomal contacts <100 kb apart were markedly increased relative to G1 while longer-range intra-chromosomal contacts were reduced (Fig. 1d). Close analysis of chromosome arm regions did not reveal any distinct domain structure across chromosome arms in M cells relative to G1 (Supplementary Fig. 1c)—rather, general, chromosome-wide, increased frequencies of contacts <100 kb were apparent. We next analysed the changes of intra-arm contact probability, \(P(s)\), with chromosomal distance \(s\) for all loci in the genome (Fig. 1e). \(P(s)\) analysis demonstrated that the G1 \(P(s)\) decayed at a similar rate at all distances, while M had a markedly slower decay in \(P(s)\) at short distances (<100 kb), suggesting chromosome compaction at this scale\(^{20}\), followed by a more rapid loss of \(P(s)\) for larger genomic distances. Analysis of \(P(s)\) of each individual chromosome arm confirmed that these changes occurred uniformly across all chromosomes (Supplementary Fig. 1e). Interestingly, the two regimes of \(P(s)\) that we observed in budding yeast M are reminiscent of Hi-C from mammalian mitotic cells, which also displayed an initial slow decay in contact frequency followed by a more rapid decay at longer distances\(^{29}\). Therefore, Hi-C analysis provides a distinct description of mitotic chromosome compaction in budding yeast chromosomes, demonstrating that all chromosomes undergo intra-chromosomal compaction in mitosis relative to their G1 state.

We next developed polymer models to test what changes to chromosomal structure can underlie the observed changes in the G1 and M Hi-C maps. Following previous simulations\(^{26,30}\) of yeast interphase Rabl organization, we modelled the genome as 16 long polymers confined to a spherical nucleus (Fig. 2a–e, Methods). Chromosomes are tethered by the centromeres to the spindle pole body, telomeres are held at the nuclear periphery, and the whole genome is excluded from the nucleolus, located opposite the spindle pole body (Fig. 2a).

Following previous analysis of 3C and imaging data\(^{31}\), we modelled the chromatin fibre as a polymer of 20 nm monomers (Fig. 2c), each representing 640 bp (~4 nucleosomes), with excluded volume interactions and without topological constraints, subject to Langevin dynamics in OpenMM\(^{22,33}\). We additionally introduced intra-chromosomal (cis-) loops of varying number and coverage, that is, the fraction of the genome spanned by all loops combined (Fig. 2b), motivated by previous models of mammalian mitotic\(^{29,34}\) and interphase\(^{4}\) chromosomes. Since changes occurred relatively uniformly along chromosome arms in M Hi-C maps, we introduced cis-loops stochastically from cell to cell at sequence-independent positions. For each combination of loop coverage and number, we collected conformations, simulated Hi-C maps and \(P(s)\) curves (Fig. 2d,e).

Comparison of simulated and experimental \(P(s)\) curves allowed us to identify changes in chromosome organization following G1→M transition (Fig. 2e). We found that in silico models with ~10 loops per megabase, ~35 kb each, covering ~35% of the genome, closely reproduced the \(P(s)\) for experimental mitotic Hi-C data in M (Fig. 2i–k and Supplementary Fig. 2a). In contrast, experimental G1 Hi-C data were best reproduced by models without cis-loops (coverage = 0.0, Fig. 2f–h). Interestingly, introducing sister chromatids to the best-fitting G1 models, either with or without sister chromatid cohesion between cognate loci, could not account for the differences we observe between G1 and M chromosomes (Supplementary Fig. 2b). Instead, the introduction of cis-loops into budding yeast chromosomes by a mitosis-specific activity best accounts for the compaction differences we observe between G1 and M chromosomes.

Next, we sought to identify the factors responsible for formation of these cis-loops in yeast mitosis. Mitotic compaction at the budding yeast rDNA array requires the concerted action of both cohesin and condensin\(^{21,22}\). In situ hybridization of individual loci has suggested that a similar process could occur on chromosome arms\(^{19,20,21}\). Cohesin accumulates on chromatin only in S and early mitosis, while condensin is activated by CDK in S and M phase\(^{15,21,35,36}\). Therefore, both SMC complexes are relatively inactive in G1 and active in M, consistent with both these complexes promoting chromosome
Figure 1 Budding yeast chromosomes are compacted in mitosis. (a) Experimental procedure to synchronize cells in either G1 or M, with and without cohesin and condensin. The green spots in the schematic on the yeast nucleus (blue) represent spindle pole bodies. (b) Hi-C contact heat map from G1 cells synchronously arrested at 37 °C with alpha factor. The Hi-C contact map for chromosomes XIII to XVI is shown as representative of the whole genome. Contact maps for each condition were assembled from two independent experiments. The side bars show the relative size of chromosomes XIII, XIV, XV and XVI, with the black dots on the chromosomes showing the locations of centromeres on each chromosome. (c) Hi-C contact heat map from cells arrested in M phase by depletion of Cdc20 at 37 °C. Both Hi-C maps have been normalized by iterative correction at 10 kb resolution. The heat map colour scale represents log10 number of normalized contacts. The Hi-C contact map for chromosomes XIII to XVI is shown as representative of the whole genome. (d) M/G1 log2 ratio of the data displayed in b and c. The black guidelines show ends of chromosomes. The pale green lines indicate the bin containing the centromere. (e) Contact probability, \( P(s) \), as a function of genomic separation, \( s \), for G1- and M-phase cells averaged over all chromosomes. The \( P(s) \) from each of the two independent experiments for each condition are shown.

We first examined the role of cohesin in budding yeast mitotic chromosome condensation using the scc1-73 ts allele. Under the restrictive conditions of 37 °C, the scc1-73-encoded protein (S525N) loses its affinity for Smc1/3, resulting in loss of cohesin complex functions37,38. Disruption of cohesin function using scc1-73 led to a disappearance of the characteristic mitotic features, as determined by Hi-C (Fig. 3a–c), despite cells being maintained in metaphase by the depletion of Cdc20 (Supplementary Fig. 1a) and cultured in the same conditions as wild-type cells. Indeed, the two-regime M phase \( P(s) \) disappeared, becoming closer to that of G1 (Fig. 3c),
with diminished short-distance (<100 kb) contacts and more frequent longer-range and inter-chromosomal contacts (Fig. 3a–c and Supplementary Fig. 3a). In contrast with the changes in the intra-arm organization, we found the Rabl conformation was maintained, as indicated by the persistence of centromere clustering contacts (Fig. 3a,b). Loss of cohesin activity also resulted in loss of short-distance (<100 kb) contacts and more frequent longer-range and inter-chromosomal contacts in the post-rDNA regions of chromosome XII (Supplementary Fig. 3a).

**Figure 2** Polymer simulations of the yeast genome support compaction by intra-chromosomal loops in mitosis. (a–e) Overview of simulations. (a) Illustration of geometric constraints used in simulations: confinement to a spherical nucleus, clustering of centromeres (blue), localization of telomeres to the nuclear periphery (yellow), and exclusion of chromatin from the nucleolus (grey crescent). (b) Intra-chromosomal (cis)-loops, generated with a specified coverage and number per yeast genome. (c) Chromatin fibre, simulated as a flexible polymer. (d) Simulated contact maps are generated in simulations with the above constraints. (e) Contact probability curves for 150 loops, and a range of coverage. Shown here are contact probability curves for 150 loops, and a range of coverage. (f) Goodness of fit for simulated versus experimental intra-arm contact probability curves for each experimental replica of G1 and M. (g) Contact probability curves for best-fitting G1 simulations (coverage = 0.0, that is, no loops) versus experimental M Hi-C. (h) Three sample conformations from the ensemble generated in the no-loops simulations; one chromosome highlighted in light brown (from left to right: XI, V, III), with its centromere in blue, telomeres in yellow, and the rest of the genome in grey. Selected conformations are shown at higher magnification.

This region is isolated from the centromere by the rDNA array and not subject to indirect effects resulting from cohesin action at the centromere. Consistently, modelling indicated that Hi-C maps for cohesin loss of function were well fitted by simulations with many fewer loops than wild-type mitotic Hi-C maps (Fig. 3d). Together our results indicate that cohesin is required for mitotic compaction in budding yeast, in a manner consistent with cohesin-dependent looping along chromosome arms.
As a stringent test of the cis-looping function of cohesin, we next assessed whether cohesin could still compact mitotic chromosomes when no sister chromatid cohesion was present. We examined mitotic cells generated without a preceding round of DNA replication using a cdc45 degron allele. Cells depleted of Cdc45 fully activate CDK without replicating DNA (Fig. 4a and Supplementary Fig. 4a) and enter anaphase if not arrested in mitosis (Supplementary Fig. 4b), acting in an apparently identical manner to cdc6 mutants. Therefore, this process generates mitotic chromosomes without sister chromatids.

Confirming our hypothesis regarding a role of cohesin in chromosome compaction, unreplicated mitotic chromosomes had contact frequencies distinct from G1, exhibiting $P(s)$ with two regimes, similar to wild-type M phase Hi-C, and consistent with the presence of cis-loops (Fig. 4b,c). Moreover, this difference was cohesin-dependent (Fig. 4b,d and Supplementary Fig. 4c,d). Loss of cohesin function also resulted in the concurrent loss of <100 kb intra-chromosomal contacts and increase in inter-chromosomal contacts between arms (Fig. 4d), as observed in cells with normal DNA replication. Therefore, cohesin activity was required for the mitotic resolution observed in mitotic cdc45 cells. Collectively, our data (Figs 3 and 4) and simulations (Supplementary Fig. 2b) strongly indicate a function for cohesin in budding yeast chromosome compaction, independent of, and in addition to, its accepted role in sister chromatid cohesion.

We next considered the role of condensin in budding yeast mitotic chromosome structure. We first examined the consequence of degrading the condensin subunit Smc2 in mitosis using a degron allele of SMC2. We degraded Smc2 protein in G2/M before arresting the cells in M phase (Fig. 1a). In contrast to cohesin, loss of condensin activity had surprisingly mild effects on mitotic intra-arm chromosome organization (Supplementary Fig. 5a).

We considered the possibility that we were not completely ablating condensin function with the degron allele, and engineered a system predicted to cause a close-to-null condensin inactivation. We used a conditional depletion/expression system to express an enzymatically dead form of Smc2 ($\text{smc2K38I}$) in G2/M cells while also depleting active degron-tagged Smc2 before arresting the cells in M phase (Supplementary Fig. 5b). Despite the increased penetrance of this allele, we did not observe any chromosome-wide loss of compaction.
or chromosome resolution (Fig. 5a,b) in metaphase-arrested cells. Indeed, in contrast with cohesin depletion, the two regimes of mitotic $P(s)$ persisted in condensin-depleted cells (Fig. 5c). Close examination of chromosome arms did not reveal loss of intra-chromosomal contacts (Supplementary Fig. 5c). Consistently, simulations did not support great differences in the amount of coverage by cis-loops (Fig. 5d).

We conclude that condensin activity is not required for the chromosome compaction we observe along mitotic chromosome arms in cells arrested before anaphase.

In contrast to the genome-wide role of cohesin, visual inspection of Hi-C maps revealed that condensin activity was relevant for higher-order chromosome structure in specific genomic contexts. We observed condensin-dependent changes at centromeres and condensin-dependent compaction of the region between CENXII and the rDNA array on ChrXII. First, at centromeres, loss of condensin action led to an increased isolation of CEN-proximal regions from loci further down the chromosome arms in cis (Fig. 6a), concurrent with increased contacts between centromeres in trans (Figs 5b and 6b). Others have shown that condensin has a focused role at centromeres in budding yeast41,42. The genome-wide visualization provided by Hi-C also suggests that condensin promotes resolution between the clustered centromeric regions. Condensin II has a similar role in neural stem cells43, suggesting that budding yeast condensin is functional for condensin II-like roles. Second, the pre-rDNA region, between centromere XII and the rDNA repeats, exhibited specific condensin-dependent compaction, with higher contact frequency at the same distance as compared with arm regions of other chromosomes in wild type (Fig. 6c–e). While the repeated structure of the rDNA makes it

Figure 4 Mitotic cohesin-dependent conformational changes are independent of sister chromatid cohesion. (a) FACS analysis of DNA content and budding analysis of cdc45-td (C) and cdc45-td scc1-73 (CH) cells following release from G1 arrest into a nocodazole-enforced mitotic block. Budding index (BI) confirmed that mitotic cells had activated CDK while FACS of DNA-stained cells confirmed no DNA replication has taken place. Representative images shown from one of two independent experiments comparing C with CH. (b) Contact probability, $P(s)$, versus genomic separation, $s$, for Hi-C of mitotic cdc45-td (C), mitotic cdc45-td scc1-73 (CH) and WT G1 cells (G1). The $P(s)$ from each of the two independent experiments for each condition are shown. (c) Log$_2$ ratio of the C (cdc45-depleted cells arrested in mitosis with nocodazole) contact data set over the G1 data set (C/G1). Contact maps for the ratio plot were assembled from two independent experiments for each condition. (d) Log$_2$ ratio of the –cohesin C data set over the C data set (CH/C).
refractory to direct analysis by Hi-C, we assume these changes are linked to the previously characterized loss of condensin-dependent compaction across the rDNA repeats. Condensin acted in a distinct manner from cohesin across the pre-rDNA region. Loss of condensin led to loss of contacts >100 kb (Fig. 6c–e) and left intact the <100 kb contacts that were affected by cohesin loss (Supplementary Fig. 3b). In contrast to the pre-rDNA region, the post-rDNA region (from the rDNA repeats to the telomere) remained remarkably similar to wild-type mitotic cells following loss of condensin activity (Fig. 6c–e). This suggests that proximity to the centromere is a key facet of condensin-dependent changes in pre-anaphase cells.

Finally we tested, and ruled out, the previously reported condensin-dependent transfer RNA gene clustering. We did not observe any general preferential contact patterns associated with tRNA pairs, neither in wild-type nor in mutant cells (Supplementary Fig. 6a–c). We conclude that previously reported condensin-dependent tRNA gene clustering shown by fluorescent in situ hybridization (FISH) was possibly an indirect consequence of condensin action localized at the nuclear organizing hubs of yeast: the centromere cluster and the rDNA array. This potential for condensin to reorganize genomes globally by acting at a few specific locations could account for earlier reports of condensin-dependent changes in pre-anaphase chromosome structure. These FISH and live-cell studies focused on centromere–proximal loci that could be disproportionately affected by condensin-dependent changes within the centromere cluster. This issue highlights the usefulness of visualizing genome conformation with a genome-wide methodology such as Hi-C.

Figure 5 Condensin action is not required for mitotic cis-looping along chromosome arms. (a) Hi-C data collected from M-phase cells following disruption of condensin with the smc2td GAL1-smc2K38I allele (abbreviated to MD). Chromosomes XIII to XVI are shown as representative of the whole genome. Contact maps were assembled from two independent experiments. (b) Log2 ratio of the –condensin M data set over the WT M data set (MD/M). (c) Contact probability (P(s)) for M and MD cells. The P(s) from each of the two independent experiments for each condition are shown. (d) Goodness of fit for simulated versus experimental intra-arm P(s), as in Fig. 2, for condensin-depleted cells.

DISCUSSION

In summary, our results support surprisingly different mitotic activities for both cohesin and condensin in budding yeast from those anticipated from their canonical functions in metazoans. For cohesin, our results indicate a genome-wide role in compacting mitotic chromosomes through the formation of intra-arm loops. For condensin, our results argue for a focused mitotic role in organizing centromeres and the vicinity of the rDNA locus. While cohesin has been previously reported to organize metazoan interphase chromosomes through looping, our data additionally indicate that cohesin-dependent looping can be utilized to compact entire chromosomes in preparation for chromosome segregation. This functional coherence over long evolutionary timescales and contrasting cellular contexts argues for a fundamentally dual function of cohesin, both for the formation of DNA loops in cis, and holding sisters together in trans.
A compacting role for cohesin in addition to sister chromatid cohesion is consistent with numerous otherwise puzzling observations in budding yeast and prior in situ hybridization analysis of yeast mitotic chromosomes.\(^{22}\) Cohesin becomes maximally loaded onto chromosomes only following bulk DNA replication.\(^{36}\) Certain alleles of cohesin support rDNA condensation, but are defective in arm cohesion.\(^{49-51}\) Two populations of chromatin-bound cohesin are detected on mitotic chromosomes, one stable, one dynamic.\(^{52,53}\) Such behaviour would be consistent with the dynamic population of cohesin being engaged in chromatin looping and the stable population with sister chromatid cohesion (Supplementary Fig. 6d). However, other models would also be consistent with our data (Supplementary Fig. 6e). The lack of a definable G2 stage in budding yeast means we do not yet know whether cohesin-dependent compaction is initiated as soon as cohesin is loaded following passage through START\(^ {15}\) or is initiated only during the early stages of mitosis. The action of cohesin in interphase in other organisms suggests that compaction initiates on loading.\(^ {45,47,48}\) However, the phosphorylation of cohesin prior to anaphase\(^{44}\) does provide a pathway for a mitosis-specific activity.

In contrast to cohesin, condensin is not required for the chromosome-wide compaction prior to anaphase. Instead, condensin has a more focused role to prevent excessive clustering of centromeres and compacting the regions between the rDNA array and its proximal centromere, in addition to its well-established role across the rDNA repeats. While this paper was in press another study has also reported that cohesin is required for the normal conformation of mitotic chromosomes and that condensin is required for restructuring the pre-rDNA region.\(^{35}\)

A general role of cohesin in mitotic chromosome compaction acting alongside a focused activity of condensin would appear to be at odds with their roles during mitosis in higher eukaryotes. In metazoans, cohesin is removed from mitotic chromosome arms during prophase,\(^ {11}\), while condensin appears to act across whole chromosomes during mitosis, leading to the formation of densely looped, compacted chromosomes.\(^ {29}\) A key difference between the two SMC-mediated mitotic chromatin states is the density of chromatin looping. Our modelling predicts that loops cover 30–40% of mitotic budding yeast chromosomes, significantly lower than the 100% coverage predicted for mammalian mitotic chromosomes.\(^ {29}\) We speculate that cohesin-dependent looping is generally sufficient for the lower level of metaphase compaction required for chromosome segregation in budding yeast. In this model, budding yeast condensin activity provides an auxiliary compaction mechanism, deployed when compaction provided by cohesin-dependent looping is either insufficient or inappropriate for segregation. Indeed, the focused action of condensin at and adjacent to the rDNA is consistent with the exceptional segregation.
requirements of this region in yeast; the rDNA accumulates excessive levels of sister chromatid intertwines and requires extra longitudinal compaction to segregate its exceptional length. This is additionally consistent with ‘adaptive hypercondensation’, where condensin is deployed along other chromosomal arms specifically in anaphase as an emergency measure to resolve persistent entanglements. In this framework, the longer and more repetitive chromosomes of higher eukaryotes not only require functional compaction during interphase, imposed via cohesin, but will also require the additional compaction offered by condensin through mitosis.

Understanding how different SMC complexes promote distinct chromatin looping states is a crucial question for the future. There are clear differences in the form and function of mitotic chromosomes in metazoans and budding yeast. Potentially these differences reflect the functional consequences of compaction via either cohesin- or condensin-promoted looping. We speculate that the conserved mechanism of SMC action has been adapted within the different complexes to cope with the varying requirements for chromatin looping in different organisms and contexts. Unravelling how the baton of SMC function has been passed through evolution presents a fascinating topic for future research, and promises to shed light on the pleiotropic consequences of mutations to these key chromosome organizers in human disease.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

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AUTHOR CONTRIBUTIONS

S.A.S. performed all cell culture and generated Hi-C libraries. A.G. analysed sequenced libraries and Hi-C data sets. G.F. modelled chromosome conformation of the budding yeast nucleus with help from A.G. J.M.B. guided the modelling of the Hi-C data. J.B. conceived and coordinated the study. J.B., G.F. and A.G. wrote the manuscript with input from S.A.S., L.M. and J.D.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Methods

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Cell fixation and Hi-C library preparation. Cell fixation and Hi-C library preparation were carried out as described previously34 with the variation that cells were fixed at 37°C. Conditions and number of replicates used for each state are shown in Supplementary Table 1.

FACS, nuclear morphology, western blotting and antibodies used. The protocols for FACS, analysis of nuclear morphology and western blotting have been described previously35. Smc4 phosphoSer4 antibody was a kind gift from D. D’Amours (IRIC, Montreal, Canada). Anti-HA antibody (12CA5 mouse monoclonal IgG2a, K. Roche, Fisher Scientific 10026563). Anti-V5 antibody (MCA1360, abD Serotec) used for ChIP.

Chromatin immunoprecipitation (ChIP). Fixed cells were defrosted and resuspended in 100 μl ChIP buffer (150 mM NaCl, 50 mM Tris HCl, 5 mM EDTA, 0.5% NP-40 (I GEPAI), 7% Triton X-100, complete Tablets, Mini EDTA-free EASY pack (Roche)). Cells were lysed in a FASTPREP machine, 6 rounds of 30 s at 6.5 power, with 1 ml of 0.5 mM silica beads on the bottom. The resultant lysate was separated by centrifugation and made up to 1 ml with ChIP buffer. Sonicated for 15 x 30 s (Bioruptor Pico, Diagenode). One hundred microliters of sonicate was processed as an input control; 200 μl was incubated with 12.5 μg/ml anti-V5 antibody (MCA1360, abD Serotec). For immunoprecipitation (IP), tubes were agitated for 1 h 30 min at 4°C. Forty-five microliters of magnetic beads (Dynabeads protein G, Life Technologies), washed three times in 1 ml ChIP buffer, were added and the tubes incubated at 4°C for 2 h.

Magnetic beads were isolated and washed four times in ChIP buffer, and a fifth time in ChIP buffer minus protease-inhibiting supplement. To reverse crosslinking, magnetic beads were incubated with 10% Chelex 100 resin beads (BioRad 142-1253), in purified water at 95°C for 30 min. Samples were spun down and the supernatant was kept at −20°C prior to analysis by qPCR. Input controls were precipitated using 0.1x volume 3 M NaAc pH 5.2 and 2.5x volume 100% ethanol and then crosslinking reversed as before, followed by purification with Nucleospin PCR clean up kit and eluted in nucleic-acid-free purified water.

The immunoprecipitated DNA was analysed using 2X AB-1323/B Abolutoluate QPCR SYBR Green Low ROX Mix and processed in an MX3005p qPCR machine. Primers used for RT-PCR of DNA at CEN4 were (forward) TGGCTTGCAAAAGG TGCACTGCTTAGTAT-3' and (reverse) 5'-CATTTTGCCGCCGTCTCAGTGTTG-3'.

Data were analysed using the ‘percentage input method’ where the CT values obtained from the ChIP are divided by the CT values obtained from the input control. To adjust the CT value of input samples to 100%, 6.644 (log of 100) was subtracted from it. Then the following formula was used to calculate the percentage input for each IP sample: 2^(-ΔCT ChIP input CT value – IP CT value) x 100.

Computational analysis of Hi-C maps. Mapping and filtering contacts. We mapped sequenced reads pair to the W303 yeast genome using Bowtie 2.1.0 and the previously described method of iterative mapping36. To generate lists of contact pairs, we assigned each mapped side of a read pair to a HindIII fragment, and removed pairs with both sides assigned to the same HindIII fragment, reads with unmapped sides, and PCR duplicates.

Aggregating contact maps. To generate Hi-C contact maps, we aggregated the filtered contact lists into 10 kb genomic bins using the cooler Python package for Hi-C data at 2% final concentration and 15 min later. We filtered out low-coverage genomic bins using the MAD-max (maximum allowed median absolute deviation from the median coverage) filter on the total number of interactions per bin, set to 7.4 median absolute deviations (corresponding to 5 standard deviations in the case of a normal distribution). We also removed the contacts within the first two diagonals of the contact maps as they are contaminated by uninformative Hi-C artefacts, unligated and self-ligated DNA fragments. Finally, we iteratively corrected the resulting maps to equalize genomic coverage.

Contact map analyses. We calculated the curves of intra-arm contact probability P(s) versus genomic separation, s, from the 10 kb contact maps using 15 logarithmically spaced bins spanning distances between 20 kb to 1 Mb. We excluded chromosomes IV and XII from these analyses as well as the bins within 40 kb distance from the nearest centromere and telomere. The post-RNAseq scalings were generated using the same approach on the region between the rDNA locus on chromosome XII and the telomere on chromosome arm. We generated the centromere and rDNA pileups by averaging the contact maps of regions surrounding the respective genomic features. To exclude a possible contribution of telomeric conformations, we used only genomic features that were separated from the telomeres by more than 200 kb.

Polymer models. We modelled the yeast genome as 16 polymers, subject to additional constraints imposed by a Rabl chromosome organization and intra-chromosomal loops with a specified number and coverage (Fig. 2a–e). We then obtained conformations from simulations and calculated simulated contact maps. For a range of loop parameters, intra-arm P(s) was calculated from these contact maps and compared with experimental P(s) to determine the best-fitting parameter sets for each experimental condition, using the average fold deviation between the experimental and simulated P(s) as the goodness-of-fit: exp(mean(log(P(s)/exp(P(s))/sim2))/2).

Polymer fibre. Following ref. 31 we simulate the yeast genome as 16 chromatin fibres of 20 nm monomers, each monomer representing 640 bp (~4 nucleosomes). Polymer connectivity was implemented by connecting adjacent monomers with harmonic bonds using a potential U = 100 + (r − 1)2 (energy in units of kT (Boltzmann constant, k, × temperature, T), distances in monomers). The stiffness of the fibre was implemented using a three-point interaction term, with the potential U = 2.5 × (1 − cos(s)). Excluded volume interactions between monomers were implemented using a soft-core repulsive potential with a maximum repulsion U(0) = 1.5 that goes to zero at a distance of 1.05 (that is, U(1.05) = 0), as in ref. 4.

Geometric constraints. Geometric constraints were implemented as in ref. 30 with slight modifications. Confinement to the nucleus was imposed with a harmonically increasing potential when monomer radial position exceeds the confinement radius (1,000 nm). Centromere tethering to the SPB was imposed with a harmonically increasing potential when monomer radial position exceeds the confinement radius (250 nm, centred 800 nm below the centre of the nucleus). Telomere tethering to the nuclear periphery was imposed with a harmonic potential pushing them to the periphery when their radial position was less than 0.95 of the nucleus radius. Radii were defined from the nucleolus was imposed via a harmonically increasing potential from an offset sphere (1,200 nm below the centre of the nucleus along the centre–SPB axis, 1,600 nm radius). Finally, both sides of the rDNA locus on chromosome XII were attracted to the periphery of the nucleolus, imposed with harmonic potentials. All harmonic potentials for geometric constraints have strengths of 1 kT per unit distance (in monomers).

Intra-chromosomal loops. To generate sets of intra-chromosomal (cis-) loops with a desired coverage and number, we used one-dimensional loop extrusion simulations (see refs 3.4) using a genome-wide lattice at monomer resolution with boundaries at chromosome telomeres, centromeres, and the rDNA locus on ChrXII. This ensures that loops are generated within chromosomes, are non-overlapping, do not cross centromeres, and do not cross the rDNA locus. Note that other than these requirements, we allow positions of loops to be variable along the chromosome arm. In polymer simulations, intra-chromosomal loops are then imposed with harmonic bonds (as in refs 4.65).

Statistics and reproducibility. No statistical methods were used to predetermine sample size. Data from two independent experiments were pooled for contact heat maps. The supplementary condensin depletion condition using smo26d MDm2c2 is represented from one data set. The replicates of the conditions are shown individually for all P(s) histograms demonstrating the high reproducibility of the data. Details of individual libraries are available in Supplementary Table 1.

Code availability. All libraries used for analysis and simulations are publicly available at https://bitbucket.org/mirnylab/hiclib and https://bitbucket.org/mirnylab/openx-polymers.

Data availability. Full details of libraries and raw sequencing are available at GEO under accession number: GSE87311. Genomic sequencing of each test strain is
available from the authors on request. All other data that support the conclusions are available from the corresponding author on reasonable request.

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Supplementary Figure 1 Experimental set up and confirmation of Rabl conformation of yeast nucleus arrested in G1 and M. a) At collection for Hi-C analysis an aliquot of cells was fixed and DNA stained with DAPI. Only experiments that had >94% large budded cells were taken for further processing. Budding index demonstrates CDK activation in yeast cells. Mitotic cells were then assessed as to whether they had maintained the pre-anaphase arrest – as indicated by a single nucleus. Or had proceeded into anaphase – as indicated by 2 split nuclei. Abbreviations for states are as used in text specifically M - cdc20 arrested, MH – cohesin depleted (scc1-73) cdc20 arrested, MD – condensin depleted (smc2td GAL1-smc2K38I) cdc20 arrested. R1 and R2 refer to replicate 1 and replicate 2, respectively. Therefore two independent experiments were conducted for each state. b) Telomeres (40kb) of all chromosome arms have been grouped according to arm length. The interaction frequency between the 8 shortest and 8 longest arms relative to each other has been analyzed. c) Zoom into contact heatmaps from G1 and M datasets for selected regions on (top) ChrXV (0-330kb (CENXV is at 330kb)) and (bottom) the post-rDNA region of ChrXII (660 kb to 940 kb). Each block represents 10kb bin. d) Zoom into log2 ratio of M over G1 contact maps for selected regions on (top) ChrXV (0-330kb (CENXV is at 330kb)) and (bottom) the post-rDNA region of ChrXII (660 kb to 940 kb). e) Overlaid P(s) curves for each individual chromosome arm taken from G1 (green) or M (blue) cells. All contact maps shown were assembled from two independent experiments.
Mitotic chromosome conformation can be accounted for by addition of intra-chromosomal loops, but not by sister-crosslinks. 

**a)** The family of $P(s)$ curves for 150 loops, and a range of different coverage levels (left). And the family of $P(s)$ curves for coverage=0.4, and a range of number of loops (right).

**b)** Simulations with sister-crosslinks imposed with the indicated frequency (12kb, 192kb, none) at random positions along chromosome arms in different simulations. Importantly, sister-crosslink simulations do not display two phases in their $P(s)$, unlike experimental M-phase $P(s)$ curves (grey, two replicas).
Supplementary Figure 3 Mitotic chromosome compaction requires cohesin function. a) (Top) Zoom into contact heatmaps from MH data for selected regions on (left) ChrXV (0-330kb (CENXV is at 330kb)) and (right) the post-rDNA region of ChrXII (660 kb to 940 kb). (Bottom) Zoom into log2 ratio of MH over M contact maps for selected regions on (left) ChrXV (0-330kb (CENXV is at 330kb)) and (right) the post-rDNA region of ChrXII (660 kb to 940 kb). b) (Left) Log2 (MH/M) ratio of contacts for ChrXII. Regions where contact frequency was higher in MH (-cohesin) than M (wt cohesin) are shown in red, regions where contact frequency was lower in MH than M in blue. The post-rDNA region is highlighted by the orange bar. (Right) Contact probability, P(s), as a function of genomic separation, s, specifically for the post-rDNA region of ChrXII for the replicate experiments of MH and M. All contact maps shown were assembled from two independent experiments.
Supplementary Figure 4  Cohesin-dependent compaction is independent of sister chromatid cohesion. a) FACS for DNA content (left) and Western blotting (right) showing that cdc45 and cd45 scc1-73 cells enter mitosis without DNA replication, with CDK phosphorylating condensin on Smc4 Serine 4 (Smc4 S4P) with the same kinetics as wildtype cells (*unspecific band). Picture of Ponceau stained blot confirms equal loading of Western (right, bottom). Western blotting for to confirm CDK activation in cells was from one experiment. b) Plot of nuclear morphology examining number of DAPI stained cells from the indicated timepoints that have undergone nuclear division. % of single nucleus (rectangles), double nuclei (checked line with squares) and cells with an anaphase nucleus are shown (gray line). c) (Top) Zoom into contact heatmaps from cdc45 mitotically arrested cells, C, (top) and cohesin depleted mitotically arrested cells, CH, (bottom), for selected regions on (left) ChrXV (0-330kb (CENXV is at 330kb)) and (right) the post-rDNA region of ChrXII (660 kb to 940 kb). d) Zoom into log2 ratio of CH over C contact maps for selected regions on (left) ChrXV (0-330kb (CENXV is at 330kb)) and (right) the post-rDNA region of ChrXII (660 kb to 940 kb). Arrows indicate a prominent track of cohesin-dependent contacts seen also in supplementary Fig. 3a). All contact maps shown were assembled from two independent experiments.
**Supplementary Figure 5** Mitotic conformation following depletion of Smc2 and characterization of smc2K38I allele. a) (Left) Hi-C data collected from M phase cells following disruption of condensin with smc2-td allele to deplete Smc2 (MDsmc2). Chromosomes XIII to XVI are shown as representative of the whole genome. (Middle) Log2 ratio of smc2 depleted M dataset over wt M dataset (MDsmc2/M), respectively. (Right) P(s) of M versus MDsmc2. Data set assembled from one Hi-C data set. b) Description and characterization of the smc2-td GAL1smc2K38I allele used in Figures 5 and 6. i) Western blot showing degradation of the degron tagged Smc2 protein and the concurrent GAL1 induced expression of Smc2K38I mutant in both nocodazole and cdc20 arrested metaphase state. Expression examined by Western blot in one experiment ii) FACS analysis of DNA content following degradation of smc2-td with/without expression of Smc2K38I. Representative profiles shown from one of two independent experiments. Expression of Smc2K38I increases the aneuploidy of cells generated following one cell division (right) as shown by increased number of cells with more than 2C and less than 1C DNA content. Profiled cells also contain V5-tagged Brn1 (as in (iii)). iii) ChIP analysis of condensin complex enrichment as assayed by ChIP with Brn1-V5 at CEN4, in wildtype cells (wt), smc2 degron cells (smc2-td), or smc2-td combined with expression of smc2K38I shown in a boxplot format with all data points shown (sample size n = 11, 5, 3 experiments, respectively). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, data points are plotted as open circles. The increased penetrance of the smc2K38I phenotype with regard to aneuploidy and chromatin binding suggests that this allele approximates the null state. c) (Left) Zoom into contact heatmaps from condensin depleted mitotically arrested cells, MD, for ChrXV (0-330kb) (CENXV is at 330kb). (Right) Zoom into log2 ratio of MD over M contact maps for ChrXV (0-330kb) (CENXV is at 330kb).
Supplementary Figure 6 Changes between \textit{in cis} and \textit{in trans} tRNA-tRNA loci contacts in the different datasets. 

\textbf{a} The map of average contact probability between tRNA pairs located on the same chromosomal arm and separated by 80kb-120kb. To avoid indirect clustering effects, we selected tRNA-tRNA pairs located more than 100kb away from a centromere or a telomere (90 pairs in total). 

\textbf{b} Same as in (a), but for tRNA pairs located on the same chromosomal arm, but separated by 180kb-220kb (50 pairs in total). 

\textbf{c} Same as in (a) and (b), but for tRNA pairs located on different chromosomes (8290 pairs in total). 

\textbf{d) Speculative models of how cohesin complexes have a dual role in both generating chromatin loops \textit{in cis} and sister chromatin cohesion \textit{in trans}. Cohesin complexes act in chromatin loop formation and sister chromatid cohesion independently. In this model distinct populations of cohesin complexes are engaged in chromatin loop formation and sister chromatid cohesion. We speculate that loop forming complexes will exhibit dynamic binding of chromatin whereas cohesive cohesin complexes will be stably bound to chromatin. 

\textbf{e) Chromatin loop formation occurs alongside sister chromatid cohesion.} Cohesin complexes could simultaneously act in sister chromatid cohesion and in loop formation. This model would require that both cohesive and non-cohesive complexes could promote chromatin loops. In the “handcuff” model of cohesive cohesin this would require two loop-promoting cohesin complexes being brought together.
## Supplementary Table 1

Summary of Hi-C libraries generated in this study. R1 and R2 refer to Replicate 1 and Replicate 2 respectively. Full details of libraries and raw sequencing available at GEO number: GSE87311. Abbreviations for libraries (also used in main text): M - *cdc20* arrested, MH - cohesin depleted (*scc1-73*) *cdc20* arrested, MD*smc2* condensin depleted *cdc20* arrested, (smc2td) MD - condensin depleted and *smc2k38I* expressed (smc2td GAL1-smc2k38I) *cdc20* arrested, C - *cdc45-td* mitotic arrest (nocodazole) and CH *cdc45-td* and *scc1-73* mitotic arrest (nocodazole). R1 and R2 refer to replicate 1 and replicate 2 respectively.

| Sample   | Total reads from each side | unique valid pairs |
|----------|---------------------------|--------------------|
| wt G1 R1 | 241,459,680               | 52,249,342         |
| wt G1 R2 | 245,245,920               | 75,685,505         |
| wt M R1  | 192,027,518               | 64,944,673         |
| wt M R2  | 427,487,395               | 85,672,725         |
| MH R1    | 56,917,488                | 23,371,565         |
| MH R2    | 166,890,033               | 57,512,008         |
| MD*smc2* R1 | 111,130,137           | 44,619,350         |
| MD R1    | 232,928,309               | 88,073,200         |
| MD R2    | 196,668,125               | 74,468,608         |
| C R1     | 71,309,748                | 20,264,415         |
| C R2     | 246,282,342               | 95,845,558         |
| CH R1    | 64,251,477                | 15,532,255         |
| CH R2    | 245,646,429               | 95,363,974         |
**Supplementary Table 2** Yeast strains and genotypes used during this study. Full genome wide shotgun sequencing of strains used available on request

| Database | Yeast Strain and Genotypes |
|----------|-----------------------------|
| cdc20-td (wt) | MATa ade2-1 ura3-1 his3-11, trp1-1, can1-100 UBR1::pGAL-myc-UBR1 (HIS3), leu2-3 LEU2::pCM244 x3 cdc20-td CDC205' upstream -100 to -1 replaced with kanMX-tTA (tetR-VP16)-tetO2 - Ub -DHFRts - Myc -linker) |
| scc1-73 cdc20-td | cdc20-td + scc1-73 TRP1 |
| smc2-td GAL1-smc2K38I cdc20-td | cdc20-td + SMC2 5' upstream -100 to -1 replaced with kanMX-tTA (tetR-VP16)-tetO2 - Ub -DHFRts - 3xHA extended linker), trp1-1::pFA6 TRP1- GAL1-smc2K38I-HA-TRP1 |
| smc2-td cdc20-td | cdc20-td + SMC2 5' upstream -100 to -1 replaced with kanMX-tTA (tetR-VP16)-tetO2 - Ub -DHFRts - 3xHA extended linker) |
| cdc45-td Δ1 | UBR1::pGAL-myc-UBR1 (HIS3), CDC45::cdc45-td (CUP1p-Ub-DHFRts-HA-CDC45)(TRP1) |
| cdc45-td scc1-73 | UBR1::pGAL-myc-UBR1 (HIS3), CDC45::cdc45-td (CUP1p-Ub-DHFRts-HA-CDC45)(TRP1), scc1-71, trp1::hphNT1 |
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1. Sample size
   Describe how sample size was determined.
   - No statistical methods were used to predetermine sample size

2. Data exclusions
   Describe any data exclusions.
   - No data was excluded. All Hi-C libraries generated are included in the analyses.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   - All the Hi-C libraries were replicated other than smc2td (MDsmc2, Supp. Fig 5a). This data was biologically replicated using the smc2K38I allele (which also generates loss of condensin function). Two Hi-C libraries of smc2K38I were generated and results shown.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   - Samples were not randomized

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - Investigators were not blinded

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

   - [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - [ ] A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
   - [ ] A statement indicating how many times each experiment was replicated
   - [ ] The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - [ ] A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - [ ] The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted
   - [ ] A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - [ ] Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study. All code is publicly available at https://bitbucket.org/mirnylab/openmm-polymer and https://bitbucket.org/mirnylab/hiclib.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The Nature Methods guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There is no restriction on materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Smc4 phospoS4 antibody was a kind gift from Damien D’Amours - characterized in Robellet et al Genes and Dev, 2015, 29 (4) 426-439. Anti-HA antibody (12Ca5 mouse monoclonal IgG₂k, Roche, Fisher scientific 10026563). Anti-V5 antibody (mouse monoclonal MCA1360, abD Serotec).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used in the study.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.
Flow Cytometry Reporting Summary

Data presentation

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation. Sequential 70% EtOH fixation, RNAase treatment, pepsin digest followed by propidium iodide staining for DNA content

6. Identify the instrument used for data collection. Becton Dickinson FACScaliber

7. Describe the software used to collect and analyze the flow cytometry data. CELLQUEST version 3.3

8. Describe the abundance of the relevant cell populations within post-sort fractions. We acquired 30000 cells

9. Describe the gating strategy used. For the DNA content experiment of yeast cells a gating strategy is not applicable. The assay sets up conditions to assay cell with 1C and 2C content of propidium iodide cells in the exponential population (as shown in the figure) and then uses this as the comparison for all subsequent test samples acquired under identical conditions. The point of the assay is to show how propidium content increases as cells go through S phase before decreasing as cell divide resulting in cells with half the content. I have ticked the box to confirm that a gating strategy is not required in this case. In this assay Data presentation points 3 and 4 are also not applicable - the histograms show the population of PI stained events and how the distribution of that population varies over the time course of one cell cycle.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒