RNA polymerase backtracking drives the accumulation of fission yeast condensin at active genes

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Running title: RNA polymerase backtracking and condensin buildup

Abstract
The mechanisms leading to the accumulation of the SMC complex condensin around specific transcription units in mitosis remain unclear. Observations made in bacteria suggested that RNA polymerases (RNAP) constitute an obstacle to SMC translocation, particularly when RNAP and SMC travel in opposite directions. Whether this also applies to eukaryotic condensin remains unclear. Here we show in fission yeast that condensin remains focused at the 3’ end of an RNAP2-transcribed gene after flipping its orientation, suggesting that gene termini harbor intrinsic condensin-positioning features whatever the orientation of transcription. Consistent with this, we provide evidence that transcription termination mechanisms position condensin whatever the RNAP involved. Moreover, to stabilize backtracked RNAP2 polymerases within gene bodies was sufficient to cancel the accumulation of condensin at gene termini and to redistribute it evenly within transcription units. Altogether, our results suggest that RNAP backtracking, which is frequent at gene termini, plays a key role in positioning condensin and strengthen the idea that dense arrays of proteins tightly-bound to DNA alter the distribution of condensin on mitotic chromosomes.

Keywords: backtracking, chromosomes, condensin, mitosis, Sen1
INTRODUCTION

Structural Maintenance of Chromosomes (SMC) complexes are essential for the organization and stability of chromosomes from bacteria to humans (Hassler et al., 2018; van Ruiten & Rowland, 2018; Uhlmann, 2016). The SMC complex condensin is particularly important for the compaction and the structuration of chromosomes throughout mitosis and for their faithful segregation to daughter cells (Hirano, 2016). Condensin is a ring-shaped DNA translocase that uses the energy of ATP-hydrolysis to organize mitotic chromosomes into large consecutive loops of chromatin anchored around a central protein scaffold (Naumova et al., 2013; Gibcus et al., 2018). It has been shown in vitro that purified condensin hydrolyses ATP to extrude loops of naked DNA (Kong et al., 2020; Ganji et al., 2018), but the structural details of the formation and enlargement of such loops remain misunderstood (Cutts & Vannini, 2020). Furthermore, whether such loop extrusion activity is the only way condensin organizes mitotic chromosomes is still under debate as computer models have suggested that condensin could also organize chromosomes by sequentially capturing two DNA molecules brought into proximity by Brownian motion, thereby producing a chromatin loop (diffusion capture model, (Cheng et al., 2015; Sakai et al., 2018)). The relative contributions of loop extrusion and diffusion capture to chromosome organization remain to be understood.

Another fundamental question is to understand how chromatin and large DNA-bound protein assemblies impact the loop extrusion activity of condensin in vivo. Loop extrusion on chromatin in vivo is predicted to be roughly 10 times slower than on naked DNA in vitro (Banigan & Mirny, 2020) and it was recently suggested that arrays of proteins tightly bound to DNA could hinder the loop extrusion activity of condensin, possibly by constituting a steric obstacle to the reeling of chromatin (Guérin et al., 2019). An inability to bypass obstacles might result in the formation of unlooped chromatin gaps within mitotic chromosomes (Banigan et al., 2020). Whether and how condensin bypasses chromatin-associated obstacles is currently unclear.

Gene transcription has been shown to influence the distribution of SMC complexes in several organisms. In Bacillus subtilis and Caulobacter crescentus, a single SMC complex juxtaposes the arms of a circular chromosome by translocating in a unidirectional fashion from a single loading site (Tran et al., 2017; Wang et al., 2015; Le et al., 2013; Sullivan et al., 2009; Gruber & Errington, 2009). A highly active transcription unit in the opposite direction (head-on orientation) was shown to slow down the translocation of SMC, which transiently accumulates towards the 3’ of the unit in a transcription-dependent manner (Brandão et al., 2019; Tran et al., 2017; Wang et al., 2017). It has been proposed that RNA polymerase (RNAP) molecules themselves constitute a directional albeit permeable barrier that impedes the translocation of SMC and each encounter with a RNAP molecule would force SMC to stall for a few seconds (Brandão et al., 2019). Great densities of RNAP (or other DNA-bound proteins) are therefore expected to impact the distribution of SMC along chromosome arms. Interestingly, specific mutations in B. subtilis SMC were shown to interfere with its ability to overcome transcription-dependent obstacles, suggesting that their bypass is an active process (Vazquez Nunez et al., 2019). Consistent with this, it was postulated that the bypass rate of different SMC complexes is a function of their intrinsic ATP hydrolysis rates (Brandão et al., 2019). In eukaryotes, transcription is also a positioning device for the SMC complex cohesin in interphase (Heinz et al., 2018; Busslinger et al., 2017; Bausch et al., 2007; Lengronne et al., 2004), suggesting that transcription is a conserved regulator of SMC occupancy. It is still unclear however whether the impact of transcription on the translocation of bacterial SMC complexes (Brandão et al., 2019) might also apply to SMC complexes in eukaryotes.

Transcription also impinges on the distribution of condensin complexes in eukaryotes, even in organisms where active transcription is strongly reduced in mitosis (Bernard & Vanoosthuyse, 2015). In mouse ES cells, the localisation of condensin II, which is nuclear throughout the cell cycle, correlates with RNAP2 occupancy (Dowen et al., 2013). In both chicken and human cells, condensin I, which only associates with predominantly transcriptionally-silent chromatin after nuclear envelope breakdown in mitosis, accumulates
towards the 5’ of RNAP2-transcribed genes that were highly transcribed in the previous G2 phase (Kim et al., 2013; Sutani et al., 2015). By contrast, RNAP1 transcription was proposed to antagonize the accumulation of condensin within the 35S transcription unit in budding yeast (Clemente-Blanco et al., 2009; Johzuka & Horiuchi, 2007). Fission yeast is a very good model to understand how transcription affects the function of condensin because transcription remains active during mitosis, when the activity of condensin is maximal. A number of studies have established that fission yeast condensin accumulates in a transcription-dependent manner in the vicinity of genes that are highly expressed in mitosis, whatever the RNA polymerase involved (RNAP1, RNAP2 or RNAP3) (Kim et al., 2016; Sutani et al., 2015; Nakazawa et al., 2015; Kim et al., 2014; Nakazawa et al., 2008). Moreover, the drug-induced inhibition of transcription partially rescued the loss of viability of condensin-defective mutants (Sutani et al., 2015) and it was recently proposed that active transcription interferes locally with the condensin-dependent resolution of sister chromatids (Nakazawa et al., 2019b). Taken together, these observations suggest that transcriptionally active RNA polymerases, and/or features associated with ongoing transcription, might challenge condensin function and the assembly of mitotic chromosomes in fission yeast.

We have previously proposed that condensin might load onto DNA at gene promoters depleted of nucleosomes (Toselli-Mollereau et al., 2016). In fission yeast, condensin would subsequently accumulate particularly towards the 3’ of genes actively transcribed by RNAP2 (Toselli-Mollereau et al., 2016; Sutani et al., 2015). Whether this is due to a head-on conflict between transcription and translocating condensin like in bacteria (see above) has not yet been investigated. On the other hand, there is evidence that the positioning of fission yeast condensin at the 3’ of RNAP2-transcribed genes could be functionally linked to the process of transcription termination. First, a number of positive and negative genetic interactions have been reported between mutants of the transcription termination machinery and mutants of condensin (Nakazawa et al., 2019a; Vanoosthuyse et al., 2014). As lack of condensin does not directly impact transcription termination in fission yeast (Nakazawa et al., 2019a; Hocquet et al., 2018), these genetic interactions suggest that RNAP2 transcription termination mechanisms might impinge on the function of condensin. Consistent with this interpretation, it was shown recently that to inactivate Xrm2^Dnp1, an enzyme that is key for RNAP2 transcription termination, was sufficient to displace condensin further downstream of transcription units (Nakazawa et al., 2019a), strengthening the possibility of interplay between transcription termination mechanisms, the 3’ edge of the RNAP2 domain and the positioning of condensin. To explain these observations, it was proposed that condensin is actively recruited at transcription termination regions because they accumulate single-stranded DNA (ssDNA) and/or chromatin-associated RNA molecules that interfere with the organization of mitotic chromosomes (Nakazawa et al., 2019a; Sutani et al., 2015). Condensin, thanks to its ability to re-anneal melted dsDNA molecules in vitro (Sutani et al., 2015; Akai et al., 2011; Sakai et al., 2003; Sutani & Yanagida, 1997), would suppress these structures, thereby allowing the formation of fully functional mitotic chromosomes. This hypothesis therefore posits that condensin plays a “clearing” role in the assembly of mitotic chromosomes (Yanagida, 2009) besides its role in the extrusion of chromatin loops. It remains unclear however how short chromosome regions that are rich in single-stranded DNA (ssDNA) and/or chromatin-associated RNA could interfere with the formation of segregation-competent mitotic chromosomes. Importantly, other models could also account for these observations: (i) the permeable moving barrier model could explain the accumulation of translocating condensin at the 3’ border of the RNAP2 domain or (ii) the transcription termination machinery could play a more direct role in the positioning of moving condensin. These models haven’t yet been tested experimentally.

Here we sought to better understand what features of transcription might influence the distribution of condensin in fission yeast mitosis. By switching the orientation of an RNAP2-transcribed gene expressed in mitosis, we tested whether or not gene transcription could be a directional barrier for condensin. Although these experiments neither confirmed nor infirmed
that transcription might be a directional barrier in fission yeast, they strongly reinforced the idea that the 3’ end of genes contain intrinsic condensin-positioning features. We then showed that to interfere with RNAP3 transcription termination also alters the distribution of condensin, suggesting that transcription termination defects impact the accumulation of condensin, whatever the RNA polymerase involved. This strengthened the idea that RNAP molecules rather than a specific transcription termination machinery could influence the positioning of condensin. Consistent with this, we provide evidence that to increase the stability of backtracked RNAP2 polymerases throughout the gene body was sufficient to shift condensin occupancy towards the 5’ end of transcribed genes. This strongly suggests that backtracked RNA polymerase molecules are themselves positioning devices for condensin. Taken together, our data clarify the role of transcription in the accumulation of condensin and are consistent with the idea that proteins that are tightly-bound to DNA impact the distribution of condensin along mitotic chromosomes.

RESULTS AND DISCUSSION
One prediction of the permeable moving barrier model is that the orientation of transcription impacts the distribution pattern of condensin (Brandão et al., 2019). To test this prediction in fission yeast, we changed the orientation of exg1, a gene that is transcribed by RNAP2 in mitosis and where condensin was shown previously to accumulate strongly towards the 3’ end (Kakui et al., 2017) (Fig 1A). Interestingly, it was shown previously that RNAP2 levels remain relatively constant throughout the gene (Sutani et al., 2015) (Fig 1A), arguing that the density of RNAP2 per se is unlikely to be sufficient to position condensin at the 3’ end of this gene. Importantly, the reversal of orientation did not affect RNAP2 levels around exg1 in mitotic cells (Fig 1B). Strikingly, the peak of condensin accumulation was moved symmetrically with the flipping of exg1 and coincided with the new genomic position of the 3’ end of the gene (Fig 1B). These observations could be interpreted in several ways: either (i) transcription is not a directional barrier for condensin in fission yeast, or (ii) transcription is a directional barrier for condensin but the chromatin around exg1 can be reeled by condensin from both directions with equal probability; alternatively, (iii) the transcription termination process itself or its machinery forces the accumulation of condensin in the 3’ end of transcribed genes.

To test the latter hypothesis, we wondered whether transcription termination at another class of genes could also modulate the distribution of condensin. Several ChIP-seq studies reported that fission yeast condensin accumulates at RNAP3-transcribed genes (Kakui et al., 2017; Kim et al., 2016; Sutani et al., 2015; Kim et al., 2014) and it was proposed that the B-box binding transcription factor TFIIIC and the TATA-binding protein Tbp1 were required for this accumulation by interacting directly with condensin (Iwasaki et al., 2015, 2010). Whether or not transcription termination at RNAP3-transcribed genes could impact the distribution of condensin was not investigated. We recently demonstrated that the conserved DNA&RNA helicase Sen1 is required for efficient transcription termination at RNAP3-transcribed genes in cis (Rivosecchi et al., 2019). In the absence of Sen1, RNAP3 strongly accumulates downstream of most of its target genes and we showed that this accumulation of read-through RNAP3 molecules downstream of gene ends could be suppressed by strengthening the endogenous terminators by the use of long polyT sequences (Rivosecchi et al., 2019). We tested whether the RNAP3 termination defects associated with lack of Sen1 could impact the distribution of condensin around RNAP3-transcribed genes. Strikingly, condensin levels increased significantly at a subset of RNAP3-transcribed genes in synchronized mitotic cells lacking Sen1 (Fig 2A). This accumulation was specific because lack of Sen1 had no impact on the association of the heterologous E. coli protein LacI (Fig 2A). Importantly, the accumulation of condensin in sen1Δ cells could not be caused by an accumulation of either TFIIIC or Tbp1, because their levels on chromatin remained largely unaffected in the absence of Sen1 (Fig 2B and Fig EV2). In the absence of Sen1, condensin did not accumulate either at COC sites (Fig 2A), which recruit TFIIIC but not RNAP3 (Noma et al., 2006), consistent with a transcription-mediated effect. To further determine whether the accumulation of condensin was mechanistically linked to the transcription termination defects observed in the absence of
that a number of tightly budding yeast transcribed genes in the absence of Sen1 the transcription rate.

density of this domain and prevented the accumulation of condensin. 

read (Rivosecchi et al, 2019) and in particular (Lemay et al, 2014). To test this hypothesis, we sought to prolong RNAP2 backtracking events by over-expressing a dominant-negative mutant of TFIIS (tfs1D274AE275A in fission yeast (Lemay et al, 2014), thereafter referred to as tfs1DN). This strategy was shown to interfere with transcription elongation throughout the gene in different organisms and to alter the distribution of RNAP2 (Zatreanu et al, 2019; Sheridan et al, 2019; Sigurdsson et al, 2010). Upon tfs1DN expression in mitotic fission yeast cells, the distribution of RNAP2 was reduced in the 3’ and shifted towards the 5’ of genes (Fig 3). Remarkably, the over-expression of tfs1DN had a similar impact on the distribution of condensin around RNAP2-transcribed genes in mitosis (Fig 3): the accumulation of condensin at the 3’ of genes was significantly reduced but its accumulation towards the 5’ increased significantly. Overall, condensin became evenly distributed throughout the gene body upon tfs1DN over-expression instead of being enriched at the transcription termination site. On the contrary, over-expression of tfs1DN had no impact on the association of condensin with the RNAP1-transcribed 18S (Fig EV3). Taken together, these observations are consistent with the idea that RNAP backtracking impacts the distribution of condensin within transcribed genes.

Our observations strongly suggest that the dynamics of elongating RNAP molecules and in particular RNAP backtracking impacts the positioning of condensin on chromosomes. Because backtracking is a prominent feature in the 3’ end of genes (Sheridan et al, 2019; Lemay et al, 2014), this might explain why condensin accumulates particularly over the 3’ of transcriptionally active genes in fission yeast (Toselli-Mollereau et al, 2016; Sutani et al, 2015). In the absence of Sen1, RNAP3 accumulates strongly over and downstream of class III genes (Rivosecchi et al, 2019). We speculate that those accumulated RNAP3 molecules are also often backtracked and that the increased levels of condensin downstream of class III genes in the absence of Sen1 depend on the size and the density of the domain occupied by these read-through polymerases. By introducing super-terminator sequences (Fig 2), we reduced the size of this domain and prevented the accumulation of condensin. Both the size and the density of this RNAP3-rich read-through domain would depend on the chromatin context and the transcription rate. This might explain why condensin did not accumulate at all RNAP3-transcribed genes in the absence of Sen1 (Fig 2). Our data are consistent with the observation that the number and density of DNA-bound Rap1 proteins influence condensin function in budding yeast (Guérin et al, 2019) and strengthen the idea that arrays of proteins that are tightly-bound to DNA could trigger the accumulation of condensin. Similarly, it is conceivable that a number of tightly-bound proteins (for example transcription factors or paused RNAP2
molecules) contribute to position condensin complexes in the 5’ of genes in mitosis in vertebrates (Sutani et al., 2015; Kim et al., 2013), even in the absence of significant transcriptional activity. It remains to be determined why tightly-bound proteins lead to the accumulation of condensin: is it that they oppose steric hindrance to the translocation of condensin or that they modify the local physical properties of the chromatin fibre or some properties of condensin itself in a way that would eventually challenge its translocation? Finally, our data confirm one prediction made by the permeable moving barrier model (Brandão et al., 2019), that to interfere with transcription elongation affects the distribution of condensin within genes. It would be interesting to determine experimentally by single molecule approaches whether SMC complexes pause for longer when facing backtracked RNAP molecules than when facing elongating, dynamic RNAP.

MATERIAL AND METHODS

Yeast strains. The strains used in this study are listed on Table EV1.

Cell synchronization. Two different methods were used to synchronize fission yeast cells in metaphase. The first method (Fig 1BC and Fig 2CD) used an analogue-sensitive version of the Cyclin-DK Cdc2 (cdc2-asM17, (Aoi et al, 2014)) which can be inhibited by 2 μM of 3-Br-PP1 (A602985, Toronto Research Chemicals). After 3 hours in the presence of the drug at 28°C in rich medium, 5.10⁷ cells were filtered, washed 3 times with warm medium and released in fresh medium without BrPP1. After 10 minutes, ~80% of cells were in mitosis, as judged by the localization of GFP tagged condensin (Cnd2) in the nucleus. The second synchronization method (Fig 2AB and Fig 3) relies on the inhibition of the expression of Slp1 (Petrova et al, 2013), a protein that is key to the metaphase to anaphase transition (Matsumoto, 1997). Cells expressing Slp1 under the control of the thiamine-repressible nmt41 promoter were grown in minimal medium at 32°C until mid-log phase, when 60 μM of thiamine was added to the culture for 3 hours. Cell synchrony in mitosis was checked as above by the presence of GFP tagged condensin (Cnd2) in the nucleus.

Exg1 inversion. ura4 was first integrated at the exg1 locus to generate the exg1Δ::ura4+ strain. PCR was then used to fuse the 3’ of exg1 to its 5’ domain and its 5’ to its 3’ domain using the primers exg1 qL2/exg1 RV3 and exg1 qR2/exg1 FW3 (see Table EV2 for a list of the primers used in this study). An overlapping PCR was then used to amplify the whole inverted locus. The resulting 2.8 kb PCR product was then transformed into the exg1Δ::ura4+ strain and stable integrants were selected by several rounds of FOA selection. The correct integration of the exg1 gene in the reverse orientation was confirmed by PCR and sequencing.

tfs1DN over-expression. The strains of interest were transformed with the pFB818 plasmid (a generous gift from François Bachand, University of Sherbrooke) that allows the inducible expression of tfs1-DN by addition of 7.5 μM of anhydrotetracycline hydrochloride (AhTET; Sigma-Aldrich, 94664), as described in (Lemay et al, 2014). AhTET was dissolved in DMSO. Cells were grown in PMG-Leu at 30°C until they reached a concentration of 5.10⁷ cells/mL. AhTET or DMSO was added for 3 hours, at which point 60 μM of thiamine was added to repress the expression of Slp1 as above.

Chromatin Immunoprecipitation (ChIP). ChIP was carried out as described previously (Rivosecchi et al, 2019), using the primers listed in Table EV2. GFP-tagged proteins were immuno-precipitated with the A11122 antibody (Thermo Fisher Scientific); Myc-tagged proteins were immuno-precipitated with the 9E10 antibody (Merck); Rpb1 was immunoprecipitated using the 8WG16 antibody (Merck).

DATA AVAILABILITY
This study includes no data deposited in external repositories.
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CONFLICT OF INTEREST.
The authors declare that they have no conflict of interest

AUTHOR CONTRIBUTION
Conceptualization: VV; Formal analysis: JR and VV; Funding acquisition: PB and VV; Investigation: JR, LV, FG and VV; Supervision: VV; Writing -original draft: VV; Writing -review&editing: JR, PB and VV.
FIGURE LEGENDS

Figure 1: Distribution of condensin and RNAP2 upon flipping of exg1. A. ChIP-seq profiles of condensin (left) and the RNAP subunit Rpb5 (right) around exg1 in mitotic cells. The ChIP-seq data are indicated by their SRA numbers and were generated in (Kakui et al., 2017) and (Sutani et al., 2015) respectively. B. Cells were synchronized in mitosis and ChIP-qPCR in two different biological replicates was used to determine the distribution of condensin (left) and Rpb1 (right) around exg1. C. Same as (B) when the orientation of exg1 has been flipped over. The scheme above shows the organization of the chromosome around exg1 in the wild-type (top) and in the reversed (bottom) orientations. Vertical dotted lines indicate the region of the chromosome that has been flipped over. Grey squares indicate the position of the exg1 transcription unit. The % IP were normalized using the values given at the site within the gene body indicated by the red vertical dotted line (exg1#1). The raw data are shown on Fig EV1.

Figure 2: RNAP3 transcription defects induced by lack of Sen1 trigger the accumulation of condensin. A. Cells were synchronized in metaphase and the association of condensin (Cnd2-GFP) or the heterologous LacI (lacI-GFP) at the indicated loci was investigated by ChIP-qPCR in the presence and in the absence of Sen1 (mean ± std of 4 biological replicates; p-values determined by the test of Wilcoxon Mann-Whitney are indicated above the graph). B. The association of the TFIIIC component Sfc6 at the indicated loci was investigated by ChIP-qPCR in cells synchronized in metaphase (mean ± std of 5 biological replicates). CD. Distribution of condensin (cnd2-GFP, top) and RNAP3 (rpc37-flag, bottom) around SPCTRNATHR.10 (C) and SPCTRNAARG.10 (D) in mitotic cells, in the presence or not of super-terminator sequences (thr10-20T and arg10-23T respectively) which correct the transcription termination defects in the absence of Sen1 (Rivosecchi et al., 2019) (compare the yellow and red curves). Results are presented as (mean ± std) of 3 (C) or 4 (D) biological replicates.

Figure 3: The over-expression of tfs1DN alters significantly the distribution of condensin around RNAP2-transcribed genes. Cells were synchronized in metaphase and the association of condensin (cnd2-GFP) at the indicated loci was investigated by ChIP-qPCR (mean ± std of 3 biological replicates). Cells carried a plasmid allowing the AhTET-induced over-expression of tfs1-DN, as described previously (Lemay et al., 2014). DMSO was used as control. For each locus investigated, the normal distribution of condensin and RNAP2 as determined by ChIP-seq is shown above, as published in (Kakui et al., 2017) and (Sutani et al., 2015) respectively. The data were normalized to the %IP at a site within the gene body indicated by the dotted line. The raw data are shown on Fig EV4.

Figure EV1: Raw data (not normalized) for the ChIP results presented on Fig 1.

Figure EV2: Lack of Sen1 does not significantly alter the association of Tbp1 at RNAP3-transcribed genes. Cells were synchronized in metaphase by depleting Slp1 (see Methods). The association of Tbp1 at the indicated loci was investigated by ChIP-qPCR (mean ± std of 4 biological replicates).

Figure EV3: The over-expression of tfs1DN does not interfere with the occupancy of condensin at the rDNA. The samples described in Fig 3 were used to monitor the occupancy of condensin within the RNAP1-transcribed 18S transcription unit.

Figure EV4: Raw data (not normalized) for the ChIP results presented on Fig 3.
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Rivosecchi. Figure 3.
Raw data for Figure 1

ChIP condensin

ChIP RNAP2

ChIP condensin

ChIP RNAP2
| Strain | Type | Genotypes | Notes |
|--------|------|-----------|-------|
| LY112  | h+   | leu1-32 ade6-210 ura4D18 ade6-210 |        |
| LY4232 | h    |           |        |
| LY4233 | h-   |           |        |
| LY4234 | h    |           |        |
| LY4237 | h+   |           |        |
| LY4339 | h-   | leu1-32 | cut3-GFP-ura4+ |
| LY4340 | h+   |           |        |
| LY4483 | h-   |           |        |
| LY4488 | h?   |           |        |
| LY4681 | h+   | leu1-32 ura4D18 | cdc2asM17 |
| LY4682 | h-   | leu1-32 ura4D18 | cdc2asM17 |
| LY4731 | h-   | leu1-32 | cdc2asM17 cnd2-GFP-LEU2 |
| LY4732 | h+   | leu1-32 | cdc2asM17 cnd2-GFP-LEU2 |
| LY4781 | h    |           | cdc2asM17 sen1Δ::KanR |
| LY4979 | h+   | leu1-32 ade6-704 | cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY4980 | h+   | leu1-32 ade6-704 | cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY4981 | h+   | leu1-32 ade6-704 | cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR sen1Δ::KanR |
| LY4982 | h+   | leu1-32 ade6-704 | cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR sen1Δ::KanR |
| LY5034 | h?   | leu1-32 ura4D ade6-704 | his7::LacI-GFP ura4-Pnmt41-slp1+ |
| LY5035 | h?   | leu1-32 ura4D ade6-704 | his7::LacI-GFP ura4-Pnmt41-slp1+ sen1Δ::KanR |
| LY5036 | h?   | leu1-32 ura4D ade6-704 | his7::LacI-GFP ura4-Pnmt41-slp1+ nda3-KM311 |
| LY5038 | h?   | leu1-32 ura4D ade6-704 | his7::LacI-GFP ura4-Pnmt41-slp1+ nda3-KM311 sen1Δ::KanR |
| LY5699 | h-   | leu1-32 | KanR-Pnmt41-slp1+ |
| LY5610 | h-   | leu1-32 | KanR-Pnmt41-slp1+ sen1Δ::HygroR |
| LY5615 | h+   | leu1-32 | KanR-Pnmt41-slp1+ sf6-13myc-KanR |
| LY5616 | h+   | leu1-32 | KanR-Pnmt41-slp1+ sf6-13myc-KanR |
| LY5617 | h+   | leu1-32 | KanR-Pnmt41-slp1+ sf6-13myc-KanR sen1Δ::HygroR |
| LY5618 | h+   | leu1-32 | KanR-Pnmt41-slp1+ sf6-13myc-KanR sen1Δ::HygroR |
| LY5764 | h+   | leu1-32 ura4D ade6-210/704? arg10-23T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY5765 | h-   | leu1-32 ura4D ade6-210/704? arg10-23T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY6201 | h-   | leu1-32 ura4D ade6-210/704 thr10-20T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY6203 | h+   | leu1-32 ura4D ade6-210/704 thr10-20T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY6215 | h-   | leu1-32 ura4D ade6-210/704 thr10-20T cnd2-GFP-LEU2 rpc37-3flag-NatR |
| LY6585 | h+   | leu1-32 ura4D ade6-210 inverted-exg1 cdc2asM17 cnd2-GFP-LEU2 |
| LY6586 | h+   | leu1-32 ura4D ade6-210 inverted-exg1 cdc2asM17 cnd2-GFP-LEU2 |
Table EV2: primers used in this study

| Primer   | Sequence                                     |
|----------|----------------------------------------------|
| exg1qL1  | GACGGTKAAATGAGCCTTTGG                       |
| exg1qR1  | AGCTGGAAGAGGATGACG                        |
| exg1qL2  | CGCGTGAACCACAATAAAC                      |
| exg1qR2  | CTGCTTGGATTTGGCGTACTG                     |
| exg1qL3  | CACATAGACCGACCACCTTTTGG                  |
| exg1qR3  | ATATGTCACCTGTGCGTACTG                    |
| Exg1qL4  | CGGATAAACCCCGGTTTTTGAC                    |
| Exg1qR4  | AATTTTCCAGCACCACACAG                    |
| Exg1qL5  | GGGAGCGTGTGGCTCTGTTTATC                   |
| Exg1qR5  | GACTTGCGCCACAGTTTTTACG                   |
| Exg1qL6  | GAGAAAGGTGACCGGAGAAATC                  |
| Exg1qR6  | CTAAAGTGTGCGTCTATGTG                  |
| Exg1qL7  | ATGGCTCCCAATGGAATATC                   |
| Exg1qR7  | AAGGTTTGGCAGCTAAGTG                      |
| Exg1qL8  | GCATCTGCTAGAGCGCTCGTGAG                  |
| Exg1qR8  | GGTGAAATGCTGTTTTTTAGATA                 |
| Exg1qL9  | ATGGCTCAGAGTACGCCGACTACCT                |
| Exg1qR9  | AAGGTTTGGCAGCTAAGTG                      |
| Exg1qL10 | GGTGAAATGCTGTTTTTTAGATA                 |
| Exg1qR10 | AAGGTTTGGCAGCTAAGTG                     |

Figure 1

| Primer   | Sequence                                     |
|----------|----------------------------------------------|
| Exg1_Del_FW3 | CTTGGTTAATTCAAGACGctactcagc                |
| Exg1_Del_RV3 | ATGATAAAATGCAAGGACtatcttgtttgc              |
| INV_EXG1_FW3  | ATTTAAATGTAATTTTTTAAAGT                   |
| INV_EXG1_RV3  | TTAGGTTTTCCGACATTTGAG                        |

Figure 2

| Primer   | Sequence                                     |
|----------|----------------------------------------------|
| 18sqL1  | TTTCTAGGACCGCCGTAATG                         |
| 18sqR1  | TGCCTTGCCAGTACTGCTGTC                       |
| rds1s-2qL1 | TCTGGCTCCTGTCATTGTTTTC                  |
| rds1s-2qR1 | TCATCGCTTCCCAACCTTAC                     |
| exg1qL1  | GACGGTKAAATGAGCCTTTGG                       |
| exg1qR1  | AGCTGGAAGAGGATGACG                        |
| gas1qL3  | AATACGATGTGAGGTGTGATTG                     |
| gas1qR3  | TGCATCAGCCGAAACCTTACC                     |
| ecm33qL4  | TGGGCAAGATGAGGACATAAGG                    |
| ecm33qR4  | AATAAACCGGTAGCTGCTGACAATC                 |
| Arg10 qL1 | GGTGTTAGCTAATGCTGTAG                        |
| Arg10 qR1  | GAGTGTCAGAGGACTCGAAC                    |
| Arg10 qL2  | GGAAGGGATCAATATCCACACAACG                  |
| Arg10 qR2  | GCTGTTATCCATCCACTTACCG                    |
Figure 3
| Gene   | Primer 1 | Primer 2 |
|--------|----------|----------|
| Exg1q5 | GGGAGCTGTTCGTCTGTTTATC | GACTTTGGTCCACCAGTTTATG |
| Exg1q5 | GACATCTGGTACAGATTTGAG | GACTTTGGTCCACCAGTTTATG |
| Exg1q6 | GAGGACATTTGACCCAGAAGGC | CTCAGGACTGTCGGATGAGAG |
| Exg1q6 | GAGGACATTTGACCCAGAAGGC | CTCAGGACTGTCGGATGAGAG |
| Exg1q7 | ATGGGCTGGGCTGCTGCTGCT | ATGGGCTGGGCTGCTGCTGCT |
| Exg1q8 | AGGTTTGGTCCCGCTCGCTAGTCA | AGGTTTGGTCCCGCTCGCTAGTCA |
| ecm33q1 | CAAATTTGCGAGAGGATGAC | CAAATTTGCGAGAGGATGAC |
| ecm33q2 | AAACCCGGGAGAGGATGAC | AAACCCGGGAGAGGATGAC |
| ecm33q3 | TCAGGACATTTGACCCAGAAGGC | TCAGGACATTTGACCCAGAAGGC |
| ecm33q4 | TCAGGACATTTGACCCAGAAGGC | TCAGGACATTTGACCCAGAAGGC |
| Ecm33q5 | TGTTTGGTCCCGCTCGCTAGTCA | TGTTTGGTCCCGCTCGCTAGTCA |
| Ecm33q6 | TGTTTGGTCCCGCTCGCTAGTCA | TGTTTGGTCCCGCTCGCTAGTCA |
| gas1q1 | ACGACAGAATTTGACCGTAC | ACGACAGAATTTGACCGTAC |
| gas1q2 | ACGACAGAATTTGACCGTAC | ACGACAGAATTTGACCGTAC |
| gas1q3 | ACGACAGAATTTGACCGTAC | ACGACAGAATTTGACCGTAC |
| gas1q4 | ACGACAGAATTTGACCGTAC | ACGACAGAATTTGACCGTAC |
| gas1q5 | ACGACAGAATTTGACCGTAC | ACGACAGAATTTGACCGTAC |
| eng1q1 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| eng1q2 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| eng1q3 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| eng1q4 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| eng1q5 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| Eng1q6 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| Eng1q7 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| Eng1q8 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| sod1q1 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| sod1q2 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |
| sod1q3 | GGGGATTTGGAAGGATGAC | GGGGATTTGGAAGGATGAC |