RESEARCH ARTICLE

Spo13 prevents premature cohesin cleavage during meiosis
[version 1; referees: 2 approved, 1 approved with reservations]

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Abstract

Background: Meiosis produces gametes through two successive nuclear divisions, meiosis I and meiosis II. In contrast to mitosis and meiosis II, where sister chromatids are segregated, during meiosis I, homologous chromosomes are segregated. This requires the monopolar attachment of sister kinetochores and the loss of cohesion from chromosome arms, but not centromeres, during meiosis I. The establishment of both sister kinetochore mono-orientation and cohesin protection rely on the budding yeast meiosis I-specific Spo13 protein, the functional homolog of fission yeast Moa1 and mouse MEIKIN.

Methods: Here we investigate the effects of loss of SPO13 on cohesion during meiosis I using a live-cell imaging approach.

Results: Unlike wild type, cells lacking SPO13 fail to maintain the meiosis-specific cohesin subunit, Rec8, at centromeres and segregate sister chromatids to opposite poles during anaphase I. We show that the cohesin-destabilizing factor, Wpl1, is not primarily responsible for the loss of cohesion during meiosis I. Instead, premature loss of centromeric cohesin during anaphase I in spo13Δ cells relies on separase-dependent cohesin cleavage. Further, cohesin loss in spo13Δ anaphase I cells is blocked by forcibly tethering the regulatory subunit of protein phosphatase 2A, Rts1, to Rec8.

Conclusions: Our findings indicate that separase-dependent cleavage of phosphorylated Rec8 causes premature cohesin loss in spo13Δ cells.

Keywords
Meiosis, cohesin, Spo13
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Author roles: Galander S: Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Barton RE: Investigation, Methodology, Writing – Review & Editing; Kelly DA: Software; Marston AL: Conceptualization, Funding Acquisition, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This research was funded by the Wellcome Trust through PhD studentships to SG [096994] and REB [102316], as well as a Senior Research Fellowship to ALM [107827] and core funding awarded to the Wellcome Centre for Cell Biology [203149]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Galander S, Barton RE, Kelly DA and Marston AL. Spo13 prevents premature cohesin cleavage during meiosis [version 1; referees: 2 approved, 1 approved with reservations] Wellcome Open Research 2019, 4:29 (https://doi.org/10.12688/wellcomeopenres.15066.1)

First published: 13 Feb 2019, 4:29 (https://doi.org/10.12688/wellcomeopenres.15066.1)
Introduction

Sexual reproduction relies on a cell division programme called meiosis. In humans, this is highly error-prone and may give rise to infertility, miscarriage or chromosomal abnormalities such as Down syndrome (reviewed by Hassold & Hunt, 2001). Meiosis consists of two consecutive divisions where homologous chromosome segregation in meiosis I is followed by mitosis-like sister chromatid segregation in meiosis II. Homologue segregation requires a number of adaptations to the chromosome segregation machinery (Marston & Amon, 2004), including recombination between homologues, mono-orientation of sister kinetochores and the protection of pericentric cohesin in meiosis I.

Cohesin is a multi-subunit protein complex made up of the core subunits Smc1, Smc3 and the kleisin α-Scc1 (Losada et al., 1998; Michaels et al., 1997) as well as the accessory subunits Scc3 (Tóth et al., 1999) and Pds5 (Hartman et al., 2000; Panizza et al., 2000). In mitosis, cohesin resists the spindle forces that pull sister chromatids towards opposite poles, likely by topologically linking sister chromatids (Gruber et al., 2003; Haering et al., 2002). Upon successful bi-orientation, securin (Pds1 in yeast) is ubiquitinated and destroyed by the proteasome, freeing separase (Esp1), which proteolytically cleaves Scc1 and thereby allows chromosome segregation.

Meiotic cohesin contains an alternative kleisin called Rec8 (Buonomo et al., 2000; Watanabe & Nurse, 1999). Rec8 supports a number of meiosis-specific functions of cohesin, particularly during recombination. Rec8 cleavage is dependent on its prior phosphorylation by three kinases—casein kinase 1δ (Hrr25), Dbf4-dependent kinase (DDK) Cdc7 (Katis et al., 2010) and Polo kinase (Cdc5) (Brar et al., 2006). However, it is currently unclear how these kinases contribute to cohesin removal, with the role of Cdc5 in cohesin cleavage coming under particular scrutiny (Argüello-Miranda et al., 2017; Attner et al., 2013; Brar et al., 2006; Katis et al., 2010). While cohesin phosphorylation occurs along the length of the chromosome, the pericentric adapter protein shugoshin (Sgo1) binds protein phosphatase 2A (PP2A) to dephosphorylate Rec8 in the pericentromere and prevent its cleavage (Katis et al., 2004a; Kitajima et al., 2004; Kitajima et al., 2006; Lee et al., 2008; Marston et al., 2004; Riedel et al., 2006; Tang et al., 2006). In meiosis II, Rec8 becomes deprotected by the action of Hrr25, which is thought to initiate Sgo1 degradation and phosphorylate Rec8 for cleavage (Argüello-Miranda et al., 2017; Jonak et al., 2017).

In mammalian and Drosophila mitosis, cohesin is also removed in two steps. First, during prophase, Wapl opens the cohesin ring at the Smc3-Scc1 interface to trigger sepaarse- and cleavage-independent cohesin removal (Buheitel & Stemmann, 2013; Sumara et al., 2000; Waizenegger et al., 2000; Warren et al., 2000). A subset of cohesin is resistant to Wapl due to its prior acetylation and association with sororin (Lafont et al., 2010; Nishiyama et al., 2010; Rankin et al., 2005; Rolef Ben-Shahar et al., 2008; Schmitz et al., 2007; Unal et al., 2008). Notably, pericentric cohesin is shielded from Wapl during mammalian mitosis by Sgo1-PP2A, which associates with, and dephosphorylates, both cohesin and sororin to prevent cohesin ring opening (Kitajima et al., 2006). Second, upon sister kinetochore bi-orientation, Sgo1 relocates from the kinetochore to the pericentric chromatin, and separase-dependent cohesin cleavage triggers anaphase onset (Liu et al., 2013a; Liu et al., 2013b).

While previous research has identified key mechanisms governing cohesin protection, a number of additional proteins have been implicated in this process, but their roles remain unclear. Amongst them is the meiosis I-specific Spo13 (Wang et al., 1987). Cells without SPO13 only undergo a single meiotic division and show a variety of meiotic defects, including failure to mono-orient sister kinetochores in meiosis I and inability to protect cohesin (Katis et al., 2004b; Klapolz & Esposito, 1980; Lee et al., 2004; Shonn et al., 2002). Spo13 is thought to have functional orthologs in both fission yeast (Moa1) and mouse (MEIKIN) (Kim et al., 2015). The unifying feature of these proteins is their interaction with Polo kinases, whose kinetochore recruitment by Moa1 and MEIKIN has been proposed to enable mono-orientation and cohesin protection (Kim et al., 2015; Matos et al., 2008; Miyazaki et al., 2017).

The exact role of Spo13 in cohesin protection is currently unclear. Interestingly, SPO13 overexpression blocks cohesin cleavage during mitosis (Lee et al., 2002; Shonn et al., 2002; Varela et al., 2010), suggesting that Spo13 may also influence cohesin cleavage in meiosis, but how it might do so remains unresolved. Although Spo13 was implicated in ensuring the proper pericentric localisation of Sgo1 (Kiburz et al., 2005), other studies have found no difference in chromosomally associated Sgo1 (Lee et al., 2004). In fact, it has been suggested that spo13Δ cells might retain residual pericentric cohesin in meiosis I (Katis et al., 2004b).

Here, we take a live cell imaging approach to re-evaluate the importance of Spo13 for cohesin protection. We show that both cohesin and sister chromatid cohesin are lost upon anaphase I onset in spo13Δ cells. Furthermore, we confirm that cohesin removal results from sepaarse-mediated cleavage rather than removal by the prophase pathway. We also provide evidence that cohesin phosphorylation is required for loss of cohesin in spo13Δ cells.

Results and discussion

Pericentric cohesin is prematurely lost in spo13Δ cells

Previous analyses of fixed cells found that centromeric Rec8 is undetectable or greatly diminished in spo13Δ anaphase I cells (Katis et al., 2004b; Klein et al., 1999; Lee et al., 2004). Moreover, inactivation of SPO13 allows mam1Δ cells (which lack sister kinetochore mono-orientation) to segregate sister chromatids during anaphase I (Katis et al., 2004b; Lee et al., 2004). Together, these findings provide evidence that centromeric cohesion is impaired in spo13Δ cells. However, it has been argued that residual centromeric cohesin persists after securin destruction in spo13Δ cells and prevents timely spindle elongation (Katis et al., 2004b). To clarify the importance of Spo13 in centromeric cohesion, we used live cell imaging of cells progressing through meiosis. We scored the percentage of cells where cohesin...
(Rec8-GFP) was retained at the pericentromere in anaphase I, as indicated by co-localisation with Mtw1 (Figure 1A, B). To ensure that observed effects in spo13Δ cells were not a consequence of mono-orientation loss, which partially impacts cohesion (Nerusheva et al., 2014), we simultaneously imaged mam1Δ cells for comparison. Quantification of pericentromeric Rec8 (Figure 1C) showed that, strikingly, deletion of SPO13 leads to complete loss of cohesin in anaphase I. This is not due to impaired cohesin loading in early meiosis, since prophase I-arrested spo13Δ cells have similar levels of Rec8 on centromeres compared to wild type (Figure 1D). We conclude that Spo13 is required for the retention of pericentromeric cohesin in anaphase I.

**spo13Δ** cells prematurely segregate sister chromatids

To assess sister chromatid cohesion in spo13Δ cells, we labelled one copy of chromosome V near the centromere with an array of tet operators (tetO), expressed GFP-tagged TetR repressor (Michaelis et al., 1997) and imaged CEN5-GFP foci in live meiotic cells. Upon anaphase I entry (as judged by degradation of Pds1 (Salah & Nasmyth, 2000)), three different phenotypes may be observed, depending on whether cells successfully mono-orient sister kinetochores and protect pericentromeric cohesin (Figure 2A). In wild-type cells, a single GFP focus segregates to one of the spindle poles (as marked by Spc42-tdTomato). Alternatively, in case of defective mono-orientation, split GFP foci stay in close proximity (>2 µm) because sister chromatids are cohered together by pericentromeric cohesin. Lastly, in cells lacking both mono-orientation and sister chromatid cohesion, GFP foci split over a greater distance (>2 µm). We subsequently scored the number of cells falling into either of these categories for each of the mutants analysed. This revealed that sister centromeres separate over large (>2 µm) distances in the half of spo13Δ anaphase I cells that bi-orient sister kinetochores (Figure 2B), consistent with all cohesion being lost. Note that although pericentromeric cohesion loss during anaphase I can only be readily observed where it is accompanied by sister kinetochore bi-orientation, the loss of cohesion in all spo13Δ cells withbi-oriented kinetochores, the near-complete absence of Rec8, and the fact that deletion of SPO13 permits efficient sister chromatid segregation in mam1Δ cells (Figure 2B) (Katis et al., 2004b; Lee et al., 2004) together confirm that pericentromeric cohesion is lost in spo13Δ anaphase I cells.

**Figure 1.** Cohesin is lost at anaphase I in the absence of SPO13. (A) Representative images of Rec8-GFP, Mtw1-tdTomato and Pds1-tdTomato in live sporulating wild-type (AM13716), spo13Δ (AM15133), mam1Δ (AM15134) and spo13Δ mam1Δ (AM15135) cells. Scale bars represent 1 µm. Arrows indicate pericentromeric cohesin. (B) The number of cells with pericentromeric Rec8-GFP in anaphase I is shown after scoring 50 cells from (A). (C) Rec8-GFP intensity was measured for 50 cells from (A) in the area occupied by the tdTomato-labeled kinetochore protein Mtw1. (D) Rec8 loading is unaffected by deletion of SPO13. Rec8-3HA association with the indicated sites was measured in prophase I in wild-type (AM4015), spo13Δ (AM15343), mam1Δ (AM15342) and spo13Δ mam1Δ (AM15344) cells carrying ndt80Δ and a no tag control (AM11633). Cells were arrested in prophase by harvesting 5 h after resuspension in sporulation medium and anti-Ha ChIP-qPCR performed. Error bars show standard error of the mean from three independent biological experiments.
Sister chromatid cohesion is restored by preventing cohesin cleavage

A cleavage-independent, Rad61/Wpl1-dependent, cohesin removal pathway, similar to that which occurs in mammalian mitosis, operates during prophase I of budding yeast meiosis (Challa et al., 2016; Challa et al., 2019; Yu & Koshland, 2005). We considered the possibility that cells lacking Spo13 lose cohesion, not due to its cleavage, but as a result of ectopic Rad61 activity. However, deletion of RAD61 did not restore cohesion to spo13Δ cells (Figure 3A), indicating that a failure to counteract cleavage-independent cohesin removal is not solely responsible for the cohesion defect of cells lacking Spo13.

Next, we assessed whether cohesin cleavage is required for cohesion loss during anaphase I in spo13Δ cells. First, we inactivated Esp1 (separase), using the temperature-sensitive esp1-2 mutant (Buonomo et al., 2000) and followed Rec8-GFP by live cell imaging (Figures 3B–D). As expected, cohesin remained on chromosomes even after anaphase I onset in both esp1-2 and esp1-2 spo13Δ cells and, consequently, sister chromatid segregation was largely prevented (Figure 3E).

Additionally, we prevented cohesin cleavage by mutating the separase cleavage site in Rec8 (Rec8-N) (Buonomo et al., 2000). We followed GFP-tagged versions of this Rec8 variant through meiosis in wild-type and spo13Δ cells (Figure 4A). Similar to esp1-2 mutants, rec8-N prevents cleavage of cohesin along the length of the chromosome in spo13Δ cells (Figure 4B) and pericentromeric cohesin intensity is drastically increased (Figure 4C). Furthermore, we find that Rec8-N prevented the segregation of sister chromatids in spo13Δ mutants (Figure 4D). We conclude that cohesin cleavage is required for sister chromatid segregation in spo13Δ cells.

PP2A is functional in the absence of Spo13

Rec8 cleavage during wild-type meiosis relies on its prior phosphorylation (Brar et al., 2006; Katis et al., 2010) which is reversed in the pericentromere by PP2A. We considered the possibility that PP2A function may be impaired in spo13Δ cells, rendering it unable to dephosphorylate, and therefore protect, cohesin. We assessed whether tethering PP2A directly to cohesin could prevent Rec8 cleavage in the absence of Spo13. We fused GFP-binding protein (GBP), a nanobody specifically
recognising GFP (Rothbauer et al., 2006), to the PP2A regulatory subunit Rts1 to irreversibly tether PP2A to GFP-tagged Rec8. This was sufficient to prevent cohesin removal, both in pCLB2-
spo13Δ and spo13ΔΔ cells (Figure 5A–C). To further confirm the full functionality of Rts1 in spo13ΔΔ cells, we utilised a separase biosensor (Yaakov et al., 2012) where a cleavable Rec8 moiety is fused to GFP and LacI, with the latter allowing targeting of the biosensor to a lacO array on chromosome arms (Figure 6A). In wild-type and spo13ΔΔ cells, this biosensor appears as a single GFP focus in meiosis I until separase is activated in anaphase I, causing biosensor cleavage and GFP focus dispersal (Figure 6B, C). Tethering of Rts1 to the biosensor, however, prevents biosensor cleavage (Figure 6B, C). Therefore, our results indicate that PP2A is functional and capable of dephosphorylating cohesin in spo13ΔΔ mutants.

**Conclusions**

The successful protection of pericentromeric cohesin is a key modification to the meiotic chromosomes segregation machinery as it ensures the fidelity of chromosome segregation in meiosis II. Key players in regulating cohesin cleavage are known. The kinases Hrr25 and Cdc7 (and possibly Cdc5) phosphorylate...
cohesin along the length of the chromosome to promote its cleavage by separase (Attner et al., 2013; Brar et al., 2006; Katis et al., 2010), while pericentromeric Sgo1 recruits the phosphatase PP2A to dephosphorylate Rec8 and thereby protect it (Katis et al., 2004; Kitajima et al., 2004; Lee et al., 2008; Marston et al., 2004; Riedel et al., 2006; Tang et al., 2006). However, the meiosis I-specific Spo13, is also required to retain pericentromeric cohesin in anaphase I (Katis et al., 2004b; Lee et al., 2004; Shonn et al., 2002) but its function is much less well understood. Our study demonstrates that pericentromeric cohesin is prematurely removed in spo13Δ cells in a manner that requires cohesin cleavage and phosphorylation. Future work should focus on elucidating how Spo13 elicits its protective function, and how this might be linked to its functions in both sister kinetochore mono-orientation and meiotic cell cycle control.

**Methods**

**Yeast strains and plasmids**

All strains are SK1-derivatives and are listed in Table 1. Plasmids generated in this study are listed in Table 2. Gene deletions, promoter replacements and gene tags were introduced using PCR-based methods (Gauss et al., 2005; Knop et al., 1999; Longtine et al., 1998; Moqtaderi & Struhl, 2008), pCLB2-CDC20 (Lee & Amon, 2003), REC8-GFP, PDS1-tdTomato (Matos et al., 2008), ndt80Δ (Vincenten et al., 2015), SPC42-tdTomato (Fox et al., 2017), REC8-3HA (Klein et al., 1999), CEN5-GFP dots, mam1Δ:TRP1 (Tóth et al., 2000) and REC8-N (Buonomo et al., 2000) were described previously. Separa biosensor constructs (Yaakov et al., 2012) were a kind gift from David Morgan (Departments of Physiology and Biochemistry and Biophysics, UCSF).
Figure 5. PP2A can prevent cohesin cleavage and sister chromatid segregation in spo13Δ cells. (A–C) Cohesin is retained on chromosomes when PP2A is tethered to Rec8. (A) Representative images of Rec8-GFP, Mtw1-tdTomato and Pds1-tdTomato in wild-type (AM13716), spo13Δ (AM20033), pCLB2-SGO1 (AM21315), RTS1-GBP (AM21316), spo13Δ pCLB2-SGO1 (AM21317), spo13Δ RTS1-GBP (AM21319), pCLB2-SGO1 RTS1-GBP (AM21318) and spo13Δ pCLB2-SGO1 RTS1-GBP (AM21320) cells undergoing meiosis. Scale bars represent 1 µm. Arrows indicate pericentromeric cohesin. (B) The number of cells with pericentromeric cohesin in anaphase I was scored for 50 cells per strain. (C) Rec8-GFP intensity in anaphase I was measured as described in Figure 2A.

Growth conditions
Cells were prepared for sporulation as described by Vincenten et al. (2015).

Chromatin immunoprecipitation
ChIP-qPCR was performed as previously described (Vincenten et al., 2015), using mouse anti-Ha (12CA5, Roche). All parameters and equipment are identical to those described previously, including qPCR mixes and thermocycling conditions. Primers for qPCR analysis are listed in Table 3.

Live cell imaging
Live cell imaging was performed on a DeltaVision Elite system (Applied Precision) connected to an inverted Olympus IX-71 microscope with a 100x UPlanSapo NA 1.4 oil lens. Images were taken using a Photometrics Cascade II EMCCD camera. The Deltavision system was controlled using SoftWoRx software, version 5.5.

Cells were imaged at 30°C (unless stated) on an ONIX microfluidic perfusion platform by CellASIC. Cells were pre-grown
Figure 6. Fusion of Rts1 to a separase biosensor prevents its cleavage in both wild-type and spo13Δ cells. (A) Schematic illustration of the separase biosensor and its Rts1 fusion. (B and C) Wild-type (AM21557) and spo13Δ cells (AM21558) carrying a wild-type separase biosensor (pCUP1-GFP-REC8(110-500)-LacI) or an Rts1 fused biosensor (pCUP1-GFP-REC8(110-500)-LacI-RTS1; wild type: AM21559, spo13Δ: AM21800) as well as lys2::lacOx256 and PDS1-tdTomato were sporulated in the presence of 100 nM CuSO4 for 2.5 h before imaging on a microfluidics plate. (B) Representative images are shown. Scale bars represent 1 µm. (C) Scoring of 50 cells per strain for the presence of GFP foci (uncleaved biosensor) or diffuse GFP signal (cleaved biosensor) within 30 min (two time points) of Pds1 degradation.

in culture flasks for ~3 h before transfer to microfluidics plates. Imaging began about 30 min later with images being acquired every 15 min for 12–15 h. Seven z-stacks were acquired with 0.85µm spacing. Image panels were assembled using Image-Pro Premier 3D, version 9.1 (Media Cybernetics). Images were analysed using ImageJ 1.48v (National Institutes of Health). Final image assembly was carried out using Adobe Photoshop CS5.1 and Adobe Illustrator CS5.1. Rec8-GFP intensities were measured using the DV_DotCounter custom plugin for ImageJ (Kelly, 2019). The plugin applied a Z projection to each colour channel and allowed the user to select a cell of interest. Kinetochores in

the red channel were identified by Yen Auto Threshold (Yen et al., 1995) and their XY central coordinates, mean intensity and area recorded. The coordinates were then used to measure mean intensity in the corresponding location in the green channel, equivalent to pericentromeric Rec8-GFP. In experiments where pericentromeric cohesin was likely to be found in between kinetochores (which is thought to occur in cells that bi-orient in meiosis I but retain cohesin), the XY coordinates in the red channel were used to generate a line profile between the 2 kinetochores in both colour channels over exactly the same pixels. The two brightest peaks in the line profile of the green channel were
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<td>spo13A::HphMX6/spo13A::HphMX6</td>
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<td>CEN6::tetOx224::HIS3/CEN6</td>
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</tbody>
</table>
Table 2. Plasmids generated in this study.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
<th>Purpose and notes</th>
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</thead>
<tbody>
<tr>
<td>AMp1317</td>
<td>YIplac128-REC8-GFP</td>
<td>LEU2 integration plasmid carrying REC8-GFP.</td>
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<tr>
<td>AMp1368</td>
<td>YIplac128-rec8-N-GFP</td>
<td>LEU2 integration plasmid carrying rec8-N-GFP.</td>
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</tbody>
</table>

Table 3. qPCR primers used in this study. For distances from centromeres, "-" indicates the location is upstream of the centromere, whereas "+" indicates the location is downstream of the centromere.

<table>
<thead>
<tr>
<th>Chr.</th>
<th>Location</th>
<th>Distance from centromere</th>
<th>Primer pair</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>III</td>
<td>Centromere</td>
<td>+0.25kb</td>
<td>1279</td>
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<tr>
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<td></td>
<td></td>
<td>1280</td>
<td>CTTCATGATTCGTCTAAATC</td>
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<tr>
<td>IV</td>
<td>Arm</td>
<td>-95kb</td>
<td>782</td>
<td>AGATGAAACTCAGGCTACCA</td>
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<td>783</td>
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<td>IV</td>
<td>Centromere</td>
<td>+0.15kb</td>
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<td></td>
<td></td>
<td></td>
<td>795</td>
<td>ACCGGAGGAAGAATAAGAA</td>
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</tbody>
</table>

calculated to give the maximum intensity value for each. Rec8-GFP intensity was measured in this manner for Figure 3C and Figure 4C.

The plug in used was the custom YeastLineProfiler for Image J (Kelly, 2019).

An earlier version of this article can be found on bioRxiv (DOI: https://doi.org/10.1101/488312).

Data availability
Raw data for scoring imaging experiments and ChIP-qPCR, arranged by figure, is available from OSF. DOI: https://doi.org/10.17605/OSF.IO/VBKU9 (Marston, 2019).

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

The file size of the raw microscopy movies precludes uploading them to OSF, but are available upon request from adele.marston@ed.ac.uk.

Software availability
Source code for DV_DotCounter is available from: https://github.com/dkelly604/DV_DotCounter.

Archived source code at time of publication: https://doi.org/10.5281/zenodo.2553081 (Kelly, 2019a).

Source code for YeastLineProfiler is available from: https://github.com/dkelly604/YeastLineProfiler.


License: MIT License.

Grant information
This research was funded by the Wellcome Trust through PhD studentships to SG [096994] and REB [102316], as well as a Senior Research Fellowship to ALM [107827] and core funding awarded to the Wellcome Centre for Cell Biology [203149].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We are very grateful to Robin Allshire, Angelika Amon, Kevin Hardwick, Heinrich Leonhardt, David Morgan and Ulrich Rothbauer for sharing plasmids. We also thank members of the Marston lab for helpful discussion.
References


Open Peer Review

Current Referee Status:  

[✓]  [✓]  [✓]

Version 1

Enrique Martinez-Perez
MRC London Institute of Medical Sciences, Imperial College London, London, UK

Accurate formation of haploid gametes during meiosis requires the step-wise removal of cohesin during the consecutive meiotic divisions. Two different pathways contribute to this process, one mediated by Wapl, which removes cohesin before the onset of the first meiotic division, and a second dependent on the protease separase, which cleaves the kleisin subunit (Rec8) of cohesin at the onset of the meiotic divisions. Phosphorylation of Rec8 by multiple kinases promotes cohesin release by Wapl and cleavage by separase, therefore the pool of cohesin bound to centromeric regions must be protected from phosphorylation during the first meiotic division to prevent premature loss of cohesion. Galander et al. use in vivo imaging to investigate cohesin protection in budding yeast, focusing on Spo13, which role in this process remains poorly understood.

The authors show convincingly that spo13 mutants display premature loss of Rec8 and sister chromatid cohesion during the first meiotic division, that this premature loss of cohesion requires separase but not Wapl, and that expression of a separase-resistant Rec8 rescues cohesin loss in spo13 mutants. These results demonstrate an important role for Spo13 in preventing separase-dependent Rec8 removal during meiosis I.

Specific comments:

Figure 4 shows that expression of Rec8-N (separase resistant) prevents loss of Rec8 from pericentromeric regions in spo13 mutants, but despite this, sister centromeres still show substantial separation in ~50% of the cells. How can sister centromeres achieve this level of separation despite extensive Rec8 binding?

The introduction doesn’t mention the Challa et al 2019 paper describing the role of Wapl in promoting Rec8 removal before anaphase I. Since distinguishing the contribution of the Wapl and separase pathways to the cohesion defects of spo13 mutants is a key aspect of the manuscript, mentioning the Challa et al 2019 in the introduction will help the reader.

Non-yeast experts will benefit from a more detailed description of some of the markers used in the study, such as Mtw1.

References

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Meiosis

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

---

Galandar and colleagues have revisited the role of Spo13 in protecting or regulating sister chromatid cohesion in meiosis I. Previous work has shown that Spo13 affects the disposition of cohesin at centromeres but the molecular basis for the modulation of centromeric cohesion by Spo13 has remained unclear. Recent work has shown that there are two pathways for cohesin removal in meiosis in budding yeast. Here the authors test which pathway is impacted by Spo13. The experiments have moved the field forward by using live cell imaging methods to address this question. The results demonstrate that Spo13, at least in part, protects Rec8 at the centromeres from cleavage by separase.

**Introduction**

Para. 3, line 7. Might be good to adjust the sentence saying Rec8 cleavage is dependent on Cdc5. The next sentence indicates the lack of clarity on this point.
Paragraph 4: It would be worth mentioning the two-step removal of cohesins in budding yeast meiosis here (Yu and Koshland, 2005; Challa et al., 2019), along with referencing the mammalian and fly two-step processes.

Results and Discussion

Page 4, second column, 4 lines from bottom: “withbi”

Fig. 3 E/Fig. 4 D – spo13 delete allows meiosis I sister centromere separation in esp1 and REC8-N mutants. This shows centromeres are more able to separate in spo13 mutants even without Rec8 cleavage. There are multiple possible explanations for these results. Is it because sister centromeres are more easily bi-oriented in spo13 mutants? Alternatively, could it be that Spo13 also promotes sister centromere cohesion also protects pericentromeric cohesion through a pathway that doesn’t involve cleavage? The manuscript would benefit from brief comments from the authors on the implications of these observations.

References


Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: meiotic chromosome biology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
Andreas Hochwagen
Department of Biology, New York University, New York City, NY, USA

This manuscript by Galander and colleagues investigates the function of the Spo13, a central but poorly understood regulator of meiotic chromosome segregation, using live-cell imaging in *S. cerevisiae*. Previous work had shown that Spo13 weakens the protection of centromeric cohesin during meiosis I, but the extent of this weakening had been questioned. The authors show an essentially complete loss of centromeric cohesin by fluorescence microscopy and support this data by monitoring the segregation of sister centromeres. In addition, they propose that this weakening occurs through increased separase activity because of reduced cohesin phosphorylation in the pericentromeric regions. The latter conclusion is complicated by the fact that the phosphorylation experiments do not distinguish between cohesin at centromeres and along chromosome arms.

I suggest the authors address the following points:

1. The data shown in panel 1C is central to the presented conclusions. As this bar graph relies on standard error, please provide statistical analysis including multiple hypothesis testing for this graph.
2. I am confused why there are cells that did not lose cohesion in the mam1 spo13 double mutants (panel 2B). Does this data not indicate that there may be some cohesion remaining in the absence of Spo13?
3. The Rts1-GBP construct will lead to ectopic protection also along chromosome arms. This increased signal is expected lead to an elevated Rec8 fluorescence intensity in the pericentromeric regions given the low spatial resolution of this assay. I think a ChIP experiment comparing Rec8 at arm and pericentromeric sites would be important to exclude the possibility that there is Rts1-independent Rec8 removal in the spo13 mutant. This issue is particularly relevant given a recent paper by Mehta et al. that came to the conclusion that Spo13 acts independently of Rts1.

Typo:
Page 4, second paragraph: withbi-oriented

References

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Chromosome biology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.