In mice oocytes, cell cycle kinase PLK1 is required for the removal of cohesin from chromosomes during meiosis prior to anaphase I.

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Introduction

- Meiosis is the specialized type of cellular division involving two rounds of division following a single round of DNA replication to create the sex cells. Female sex cells are called oocytes.
- Inaccurate segregation of the chromosomes within oocytes can cause miscarriage, infertility and birth defects such as Down's syndrome.

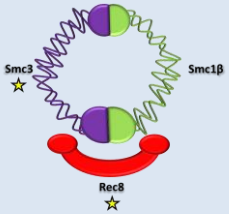

Figure 1. The meiotic **cohesin** protein complex, causes cohesion between sister chromatids by tethering them together. This is essential for accurate segregation of chromosomes in mitosis and meiosis. **Rec8** subunit is meiosis-specific. SMC3 subunit is non-specific.

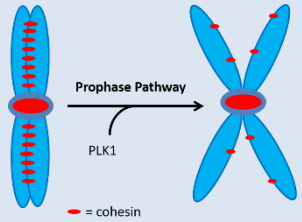

Figure 2. A simplified prophase pathway. This pathway is well-known in mitosis where the enzyme **PLK1** removes cohesin from the chromosomes prior to anaphase. It is yet to be confirmed if the prophase pathway exists in meiosis. The remaining cohesin is removed by the enzyme separase at a later stage.

Aims

1. **Investigate if and how PLK1 inhibition may affect cohesin as part of the prophase pathway.**
2. **Compare the differences between cohesin subunits Rec8 and SMC3.**

Methodology

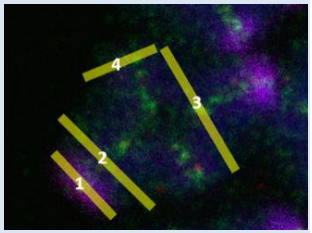

Figure 3. To inhibit PLK1, mice chromosomes were cultured in BI256. The controls were cultured in DMSO. Both were spread shortly before anaphase I. Line scan analysis was used on ImageJ to take an intensity value of the Rec8/SMC3/TopoII signal from chromosomal positions: 1) Centromere. 2) Distal Pericentromere. 3) Mid-arms. 4) Telomere. These values were then computed and analysed.

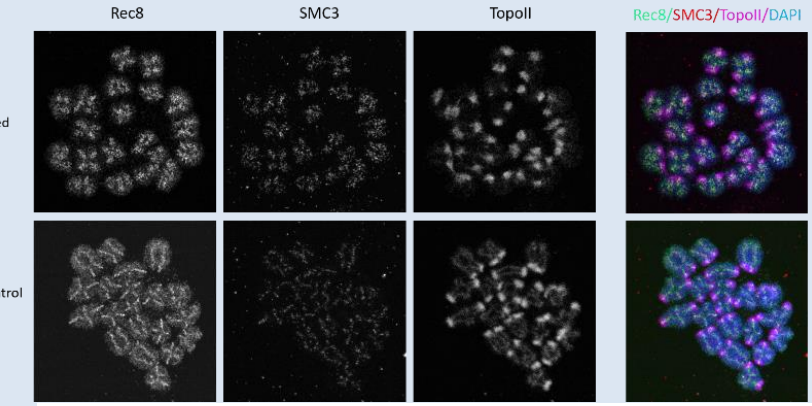

Figure 4. Fluorescent antibodies were tagged to SMC3, Rec8 and TopoII on BI2536 and DMSO (control) treated chromosome spreads. Images edited using ImageJ. Inhibition of PLK1 on the alters the architecture of the chromosomes.

Results

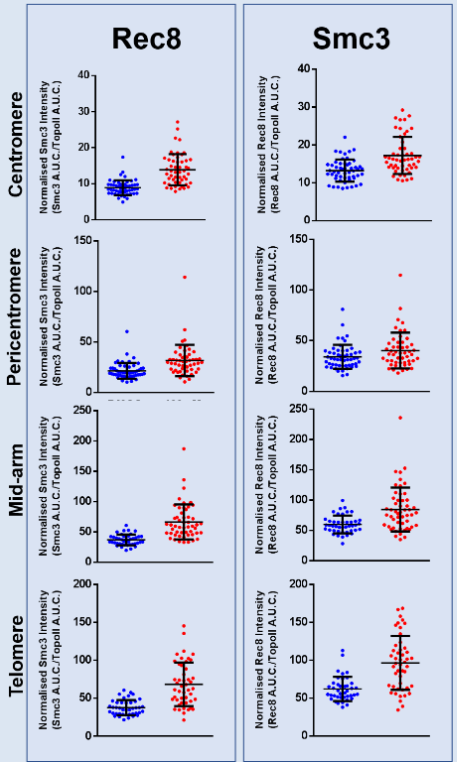

Figure 5. Cohesin subunits are retained on the chromosome following inhibition of Plk1. The line scan values were plotted in GraphPad and the area under the curve (AuC) was taken.
To normalize the AuC values, SMC3 and Rec8 were divided by TopoII. There was an increase in both Rec8 and SMC3 in the BI treated chromosomes.

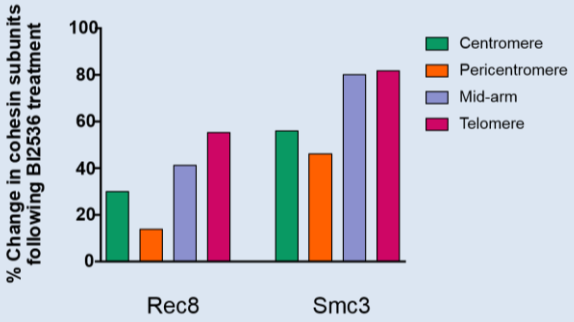

Figure 6. The increase in SMC3 is greater than in Rec8 following PLK1 inhibition. The % change was obtained by the mean BI/DMSO AuC values divided by the mean control (x100). Both cohesin subunits increase but SMC3 has a higher % change.

Conclusions

1. PLK1 inhibition generally **increased** the level of oocyte cohesin on the chromosomes.
   - When PLK1 was defective there was retention of cohesin on the oocyte chromosomes. It appears PLK1 removes some cohesin prior to anaphase I in the first division.
2. A higher % change of SMC3 indicates an increased retention of non-Rec8 cohesin.
   - SMC3 was retained at a higher proportion than Rec8.
   - PLK1 is non-discriminative in what type of cohesin it removes.

These conclusions indicate so far that the prophase pathway, or an analogous pathway is likely to exist in meiosis in mice.

Future Work

Both SMC3 and Rec8 are removed by PLK1, other cohesin subunits, meiotic and non-meiotic, need further investigation.

Acknowledgments

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