# Strategies to improve the robustness of acentrosomal spindle formation in female meiosis

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## INTRODUCTION

In meiosis, centrosomes organize microtubules and nucleation of the spindle for a correct chromosomal segregation in eukaryotics cells. Human female oocytes lack centrosomes, so microtubules must self-assembly, which can cause mistakes in the process and diseases to the embryo. In fission yeast *Saccharomyces pombe*, spindle pole bodies (SPBs), the equivalents of centrosomes, are sitting on the nuclear envelope (NE), which is dissasembled in each cell cycle during a process called nuclear envelope breakdown (NEBD) by activating proteins like Sad1 and Bqt1, that mediate chromosome-NE contacts (**Fig. 1**) [1].

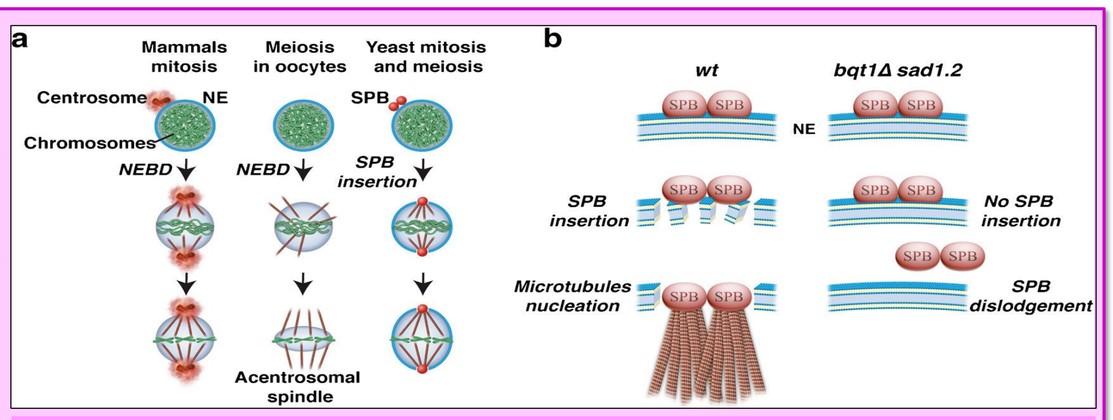


**Fig. 1. bqt1Δ sad1.2 meiotic cells do not show proper spindle formation. a** Comparison of microtubule organization in mammals mitosis and female meiosis, i.e., centrosomal and acentrosomal spindles, respectively. **b** In fission yeast, SPB insertion into the NE necessitates localized NE disassembly beneath the SPB which is regulated by previous chromosome-NE contacts. Loss of the chromosome-NE contacts by using sad1.2 allele together with the loss of the meiotic-specific protein Bqt1 fully abolishes NE disassembly and, consequently, the SPB insertion process in around 100% cells [1].

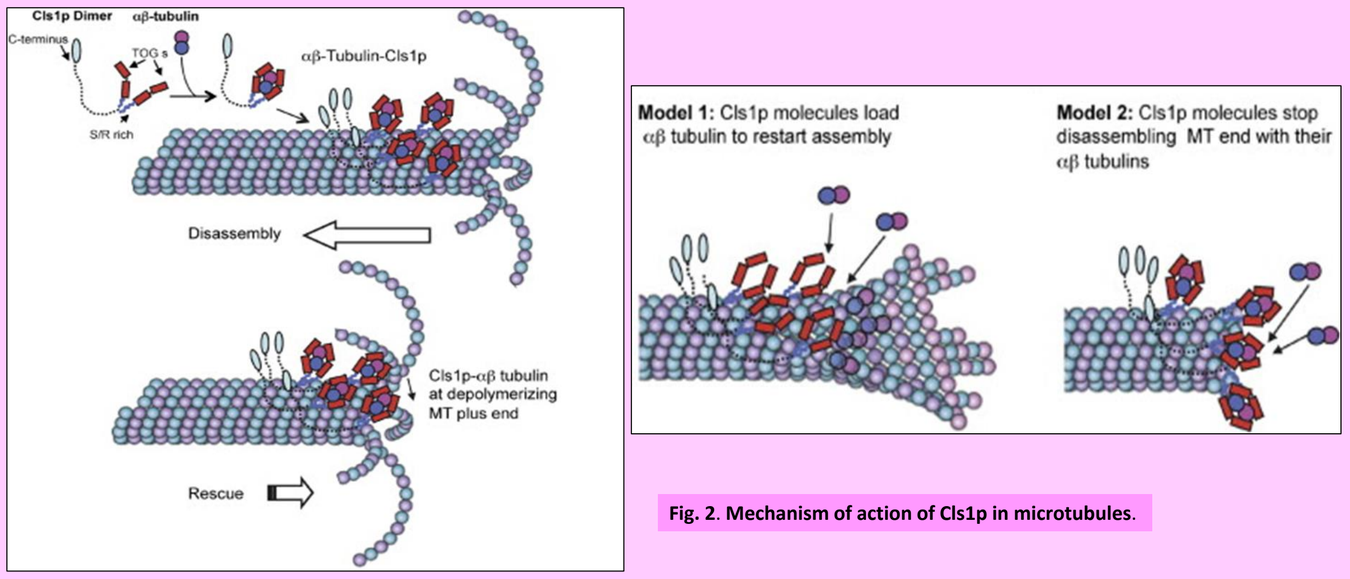





**Fig. 2. Mechanism of action of Cls1p in microtubules.**

Cls1p, a cytoplasmic linker associated protein (CLASP), stabilizes specific groups of MTs in *S. pombe* and has two homologous proteins in humans, CLASP1 and CLASP2. It contributes to the formation and maintenance of the spindle by promoting MT rescue events [2] (**Fig. 2**).

On the other hand, there are another important proteins in this process, like Klp6, a kind of kinesin-8, whose homologous proteins in humans are Kif18A, Kif18B, and Kif19. An in vivo study suggets that Klp6 binds to the tubulin triggering the birth of new MTs and promoting nucleation and catastrophe at the growing MT tip [3] (**Fig.3**). Deletion or knockdown of Klp6 leads to longer spindles and defects in its assembly and position in many cases [4], but we suggest that a longer acentrosomal spindle could also be stronger and more stable.

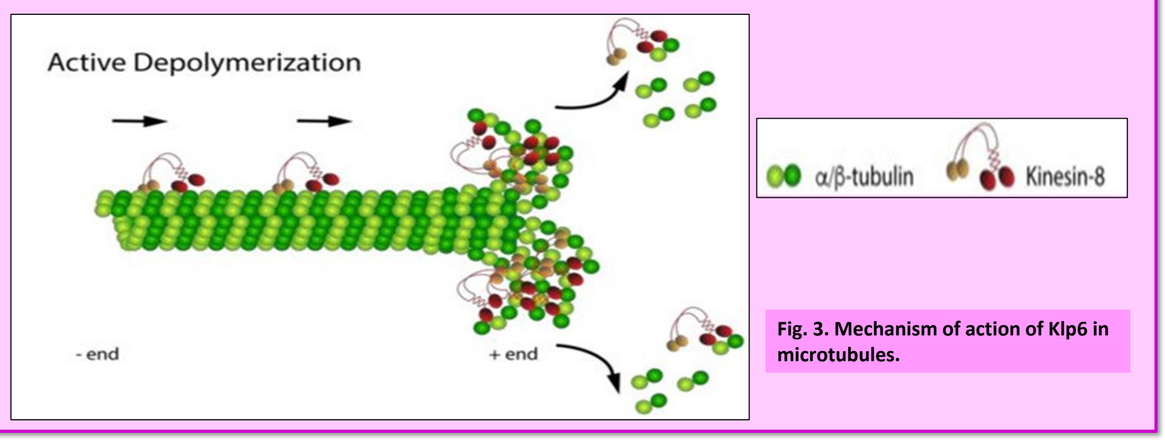


**Fig. 3. Mechanism of action of Klp6 in microtubules.**

## OBJECTIVES

Based on these findings, our aim is to optimize the spindle by deleting klp6 gene and overexpressing cls1 gene in order to try to minimize chromosomal segregation errors.

## METHODS

To study the molecular mechanisms supporting acentrosomal spindle, we are using *S. pombe* as model scenario. We pretend to analyze the effects of overexpression of cls1 and the impact of deletion of klp6 on the acentrosomal spindle behavior and chromosome movements. To perform that experiments, we have obtained two different mutants for klp6 and cls1 genes in a bqt1Δ sad1.2 background by crossing some strains with these characteristics and we are studying what happens in the cell nucleus by fluorescent microscopy, using a DeltaVision microscope.

## RESULTS & DISCUSSION

**Deletion of klp6 increases the robustness of the spindle in *bqt1Δ sad1.2* cells**

In *wt* cells of *S. pombe*, *horsetail* process occurs during prophase followed by meiosis I, where two masses of chromosomes separate, and meiosis II, in which four nuclei are formed. The spindle in this case is thick, stable and separates chromosomes correctly (**Fig. 4a**). *klp6Δ* mutant performs the process similarly, but has a longer spindle that divides chromosomes slightly worse (**Fig. 4c**). In contrast, the nucleus does not perform *horsetail* movement in *bqt1Δ sad1.2* meiotic cells and acentrosomal spindle is weak, so it is not efficient in chromosomal segregation (**Fig. 4b**). As we can see in this movie, masses of chromosomes of different sizes are separated in meiosis I and one of them do not get to do meiosis II. In some cases, the nuclei do not go through the process of meiosis I. This fact is improved in *bqt1Δ sad1.2 klp6Δ* mutants, where a longer and more robust acentrosomal spindle is formed, so most cells do meiosis II (**Fig. 4d**). Thus, spindle in this case is able to separate chromosomes more correctly during meiosis II.

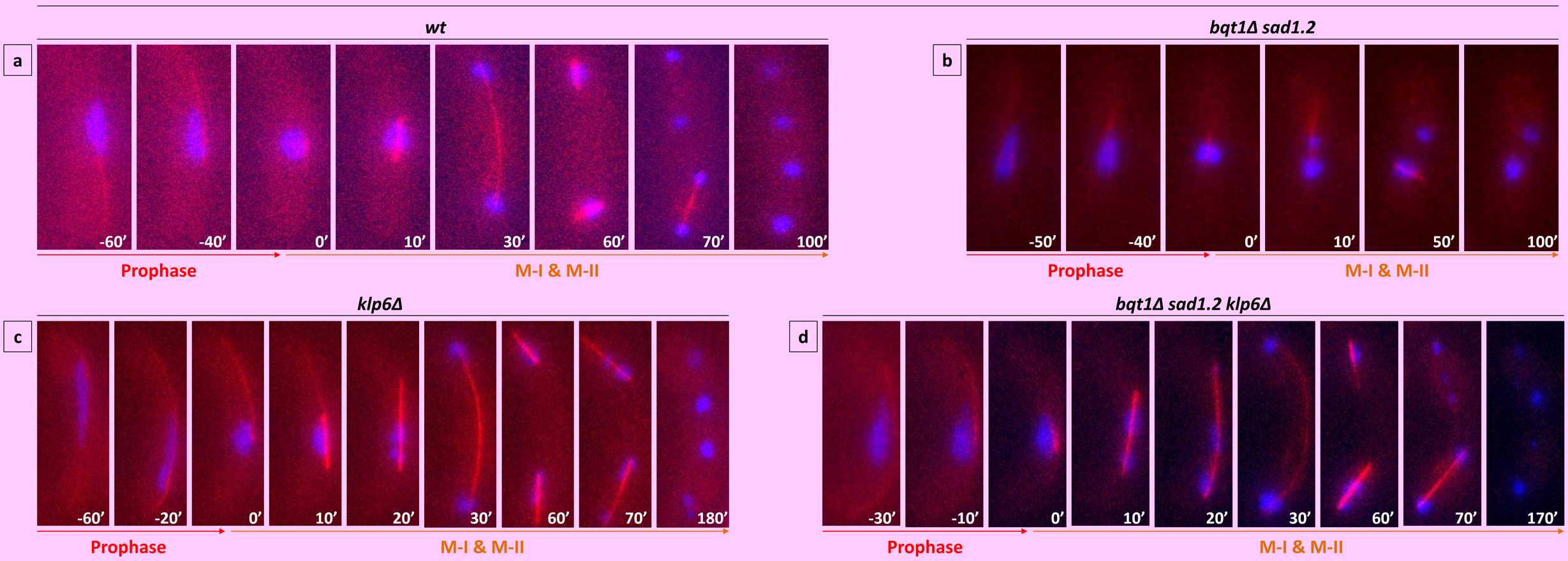


**Fig. 4. Images analyzed from movies of different cells: a) *wild type*, b) *bqt1Δ sad1.2*, c) *klp6Δ* and d) *bqt1Δ sad1.2 klp6Δ*. Tubulin and chromosomes are marked in red and blue, respectively. The experiment was performed at 28ºC, what is the optimal temperature for the meiotic process.**

## CONCLUSIONS

- Deletion of klp6 gene reinforces the spindle, making it longer and more robust.
- This spindle allows nuclei of most cells become divided by meiosis II.
- Acentrosomal spindle in *klp6Δ* mutants can reduce the number of errors in chromosomal segregation and therefore increase the efficiency of meiotic process with respect to *bqt1Δ sad1.2* mutants.
- Cls1 gene overexpression experiment is on going, but we hope to obtain similar results.

**References:**
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