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Prp4 kinase is required for proper segregation of chromosomes during meiosis in *Schizosaccharomyces pombe**

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Chromosome segregation during meiosis is a complex process, which leads to production of four haploid gametes from two precursor cells. Reversible phosphorylation of proteins plays a crucial role in this process. The *Schizosaccharomyces pombe* Prp4 is an essential serine/threonine protein kinase, which belongs to the Clk/Sty family. To study the role of Prp4 in meiosis, we analysed chromosome segregation in a strain carrying conditional analog-sensitive allele of Prp4 protein kinase (*prp4-as2*). Our data show, that Prp4 protein kinase plays important role in chromosome segregation during meiosis, as revealed by enhanced missegregation of chromosomes in *prp4-as2* mutant cells.

Key words: Prp4 protein kinase, *S. pombe*, meiosis, segregation, protein phosphorylation

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INTRODUCTION

The basic feature of all living organisms is cell division, which is required for growth and development. The cell undergoes various signal-transduction processes, to achieve its proper division. Protein phosphorylation by kinases is one of the key mechanisms required for regulation and maintenance of the normal cell cycle (Krebs, 1993). Ortholog of the essential *S. pombe* protein kinase Prp4 was previously described to play important role in chromosome alignment, and is involved in spindle assembly checkpoint (SAC) regulation. (*Montembault et al.*, 2007). Moreover, it is a serine/threonine kinase, which was shown to regulate pre-mRNA splicing in mammalian cells (Rosenberg *et al.*, 1991; Schneider *et al.*, 2010). However, its role in chromosome segregation during meiosis was not yet described. Therefore, we prepared strain carrying conditional analog-sensitive allele of Prp4 (*prp4-as2*(F238A)), where the gate-keeper residue within an ATP binding site of the Prp4 kinase (F238) was mutated to induce its affinity to the inhibitor (Cipak *et al.*, 2011; Gregan *et al.*, 2007). In this study, we analysed such strain in the presence or absence of the inhibitor, respectively, and compared it to wild type strain.

Our data revealed that genetic manipulations of the Prp4 kinase cause significant alterations in the process of chromosome segregation, even in the absence of the inhibitor as compared to wild type strain. The presence of the inhibitor enhanced the already existing defect, showing that the inhibitor blocked remaining, still functional protein kinase, and confirmed, that Prp4 protein kinase plays important role in the process of chromosome segregation.

MATERIALS AND METHODS

Yeast strains, media and growth conditions. *S. pombe* strains used to test sensitivity to ATP analog (3-BrB-PP1) were wild type (*h-*) and *prp4-as2* (*h- leu1-32 ura4-D18 ade6-M210 prp4::natMX4 prp4-as2*(F238A)*::h-phMX4*) strains. Sequence analysis of our *prp4-as2* strain revealed the presence of both wild-type *prp4*$^+$ and the *prp4-as2*(F238A) alleles, raising the possibility that this strain expresses a wild-type Prp4 protein in addition to Prp4-as2 (unpublished data). Yeast media and growth conditions were standard YE+5S or PMG-N media in the presence or absence of an ATP analog (Cipak *et al.*, 2011).

Immunostaining and microscopy. The immunofluorescence and microscopy techniques used to analyze chromosome segregation were as was previously described. Shortly, yeast strains with or without chromosome II labeled with GFP (*cen2(D107)::KanR-ura4+-lacO his7+::lacI-GFP*) were grown in YE+5S medium and meiosis was induced by crossing *h*+ and *h*– strains on PMG-N plates. After conjugation, strains were transferred to liquid PMG-N medium and inhibitor or DMSO were added. Cells were collected after 7 hours of incubation, fixed, and stained with primary TAT1 mouse monoclonal anti-tubulin and rabbit polyclonal anti-GFP antibodies, DNA was visualised using Hoechst 33342 (Rabitsch *et al.*, 2004; Rumpf *et al.*, 2010). Analyses were performed using regular fluorescent microscope equipped with digital camera.

RESULTS

To study the role of *S. pombe* essential Prp4 protein kinase during meiosis, we used *prp4-as2* strain and analysed its chromosome segregation during meiosis in the presence or absence of the inhibitor and compared it to the wild type (*wt*) strain.

Analysis of sensitivity of prp4-as2 to the inhibitor

The sensitivity of *wt* and *prp4-as2* strains to the inhibitor was tested on YES plates with or without addition of 30 μM of the inhibitor (3-BrB-PP1). Cells were

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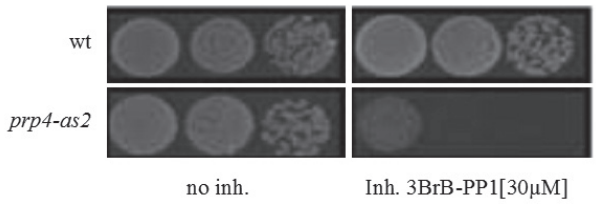
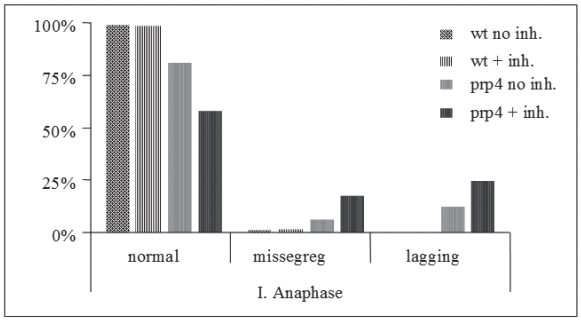


Figure1. Sensitivity of prp4-as2 cells to the inhibitor.
Prp4-as2 cells were able to grow on YES plates lacking the inhibitor, whereas in the presence of the inhibitor cell growth was inhibited. However, wt strains were able to grow under both conditions, pointing-out the effect of the inhibitor only to mutated strains (Cipak *et al*. 2011).

spotted on plates, incubated at 32ºC for 3–4 days, and their growth ability was determined. As shown on Fig. 1, *prp4-as2* cells grew on YES plates lacking the inhibitor, whereas in the presence of the inhibitor cell growth was inhibited. However, *wt* strain was able to grow under both conditions, pointing-out the effect of the inhibitor only to mutated strain (Cipak *et al.* 2011).

Chromosome segregation during meiosis is altered in prp4-as2 cells

To analyze chromosome segregation directly in anaphase I, cells with one copy of chromosome II marked by *cen2*-GFP were fixed and stained with antibodies against tubulin and GFP. DNA was stained with Hoechst 33342. Chromosome segregation was analysed in *wt* and *prp4-as2* cells incubated for 7 hours with the inhibitor or DMSO respectively using the fluorescent microscope. We observed large amount of lagging chromosomes in *prp4-as2* cells even in the absence of the inhibitor (12.5% of analysed cells), indicating, that already small manipulation with the ATP binding side of Prp4 protein kinase causes alterations in its function. Addition of the inhibitor doubled the observed defect suggesting, that remaining, still functional Prp4 protein kinase was blocked with the use of the inhibitor. Moreover, defects in segregation of sister chromatids (visualized by heterozygous *cen2*-GFP) during meiosis I in *prp4-as2* cells were also observed already in the absence of the inhibitor (6.25% of analysed cells showed missegregation), and the observed defect was enhanced when the inhibitor was added (17.6% of analysed cells). (Fig. 2) These results suggest that Prp4 protein kinase is involved in regulation of the first meiotic division, and thereby controls the process of correct chromosome segregation.



I. Anaphase	normal	missegreg	lagging
prp4 - inh.	81.25%	6.25%	12.50%
prp4 + inh.	57.90%	17.6%	25%
wt no inh.	98.75%	1.25%	0%
wt + inh.	98.50%	1.50%	0%

Figure 2. Prp4 is required for proper chromosome segregation during meiosis.
Missegregation in *prp4-as2* cells (prp4) with high amount of lagging chromosomes during anaphase I indicates defect in segregation of sister chromatids (only one copy of chromosome II was labelled with *cen2*-GFP).

DISCUSSION

Protein phosphorylation has been established as the major regulatory mechanism in the cell. Some protein kinases are already known to be involved in meiotic chromosome segregation, such as Hhp1/Hhp2, Bub1, Ark1, and Mph1 (Rabitsch *et al.*, 2004; Rumpf *et al.*, 2010; Kovacikova *et al.*, 2013). In order to identify novel protein kinases involved in regulation of chromosome segregation during meiosis, we screened a collection of our analog-sensitive essential protein kinases for their role in meiosis (Cipak *et al.*, 2011; *unpublished results*). It was previously shown that Prp4 is important for pre-mRNA splicing in human and yeast cells (Gross *et al.*, 1997; Schneider *et al.*, 2010), but it is also component of the phosphoproteome in mitotic spindle which contains kinetochore-associated proteins in human cells (Nousiainen *et al.*, 2006) and part of SAC (spindle assembly checkpoint) regulatory genes (Montembault *et al.*, 2007). However, direct analysis of the function of Prp4 protein kinase in chromosome segregation was not performed so far as it is an essential protein and lack of its function is lethal for the cell. Therefore, we used a conditional mutant form of Prp4 protein kinase (*prp4-as2*(F238A)) that is inhibited in the presence of ATP analog, to directly investigate its role in meiotic chromosome segregation. Strikingly, our analyses revealed, that inhibition of Prp4 protein kinase causes massive defects in chromosome segregation during meiosis. While missegregation of sister chromatids and presence of lagging chromosomes during meiosis I can be caused by various defects, such as defective sister-chromatid cohesion along chromosome arms (Gutierrez-Caballero *et al.*, 2012), it is very likely, that the observed defect in *prp4-as2* cells is probably due to alterations in spindle-kinetochore attachments in anaphase I. This is in agreement with previously published results, that Prp4 is kinetochore component required for functional SAC (Montembault *et al.*, 2007).

We identified this essential protein kinase as a novel protein kinase required for regulation of proper chromosome segregation during meiosis, as revealed by enhanced missegregation of sister chromatids, and enhanced occurrence of lagging chromosomes in *prp4-as2* mutant cells. However, further investigations are required to analyse the underlying mechanism of Prp4 function in this process.

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