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

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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.

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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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 (b+) and prp4-as2 (b⁻ leu1-32 ade6-M210 his7+::lacI-GFP prp4::natMX4 prp4-as2(F238A):hisMX4) 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alleles, raising the possibility that this strain expresses a wild-type Prp4 protein in addition to Prp4-as2 (unpublished data).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 I labeled with GFP (cen2(D107):KanR-met4::lacO bis7::lacI-GFP) were grown in YE+5S medium and meiosis was induced by crossing b+ and b− 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.
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.

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; Kovackova 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 spotted on plates, incubated at 32°C 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).

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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