CHROMOSOME SEGREGATION: NOVEL INSIGHTS INTO THE MECHANISM AND REGULATION

Miroslava Pozgajova*, Juraj Gregan2, Anna Trakovická

Address: 1Slovak University of Agriculture, Department of Genetics and Breeding Biology, Tr. A. Hlinku 2, 94976 Nitra, Slovakia.
2Max F. Perutz Laboratories, University of Vienna, Dr. Bohr-Gasse 1, 1030 Vienna, Austria.

*Corresponding author: mirka.pozgajova@gmail.com

ABSTRACT

A crucial feature of every healthy living organism is accurate segregation of chromosomes. Errors in this process may lead to aneuploidy, which is responsible for diverse genetic defects and diseases such as Down syndrome, miscarriages, cancer and others. Although, chromosome segregation has been studied intensively in the past, the exact mechanism of accurate chromosome segregation still remains unclear. Identification and characterization of proteins and protein complexes involved in this process is essential for understanding of processes that lead to chromosome missegregation. Basic molecular mechanism share common principles in animals, humans, plants and unicellular organisms; it is therefore possible to study these mechanisms in simple model organisms such as yeasts. The fission yeast Schizosaccharomyces pombe is an excellent model organism to study the function and regulation of chromosome segregation in both mitosis and meiosis.

Keywords: chromosome segregation, meiosis, mitosis, Schizosaccharomyces pombe
INTRODUCTION

The correct segregation of the genetic material into daughter cells during cell division is essential for the production of healthy progeny. Mistakes in the chromosome segregation may generate cells with incorrect chromosome number, which is associated with cancer, birth defects and infertility. In eukaryotic cells, there are two kinds of cell division, namely mitosis and meiosis. Mitosis is a cell division which results in the production of two daughter cells from a single parent cell. Daughter cells produced by mitosis are genetically identical to one another and to the original parent cell. While mitosis can occur in both diploid (2n) and haploid (n) cells, meiosis generates haploid cells (gametes) from a diploid precursor. Chromosome number during meiosis is reduced because a single round of DNA replication is followed by two rounds of chromosome segregation. These are called meiosis I and meiosis II. While the second meiotic division is similar to mitosis in that sister centromeres segregate to opposite poles, the first meiotic division is fundamentally different and ensures segregation of recombined homologous chromosomes.

There are three major steps required for the proper meiosis I segregation (Ishiguro et al., 2007; Kerr et al., 2012). The first step is meiotic recombination which ensures that homologous chromosomes cross over to form chiasmata and designates bivalents for disjunction. The second meiosis I-specific process is mono-orientation of sister kinetochores and the third one is the protection of centromeric cohesion (Ishiguro et al., 2007; Cromie et al., 2007). Disturbing any of these processes may lead to missegregation of chromosomes and aneuploidy. Although, the chromosome segregation has been extensively studied, we still do not fully understand how the cell ensures that each daughter cell receives a full set of chromosomes. The fission yeast Schizosaccharomyces pombe is an excellent model organism for the study of chromosome segregation, as it is amenable to both genetic and cell biology techniques. Moreover, its chromosomes have large complex centromere structure, similar to those of higher eukaryotes (Ding et al., 1993). Great number of studies have been performed with S. pombe to bring more light in the process of cell division including studies on S. pombe genes whose mRNAs were upregulated during meiosis, as well as deletion of various genes to identify new regulators of chromosome segregation (Ishiguro et al., 2007; Cromie et al., 2007; Dudas et al., 2011; Gregan et al., 2005). However, there are genes encoding essential proteins which can not be deleted in haploid cells. Therefore, new strategies of their inactivation or blocking have been developed, such as recently developed chemical-genetic strategy to conditionally inactivate protein kinases (Knihgt and Shokat, 2007; Gregan et al.,
Its aim is to mutate a single residue in the ATP-binding pocket, termed the gate-keeper residue, which confers sensitivity to small-molecule inhibitors (Figure 1). The specificity of the inhibitor is achieved by the fact that it only binds to the modified kinase, but not to any other wild type kinase. In most of the cases, the mutation confers sensitivity to the inhibitor, but does not interfere with kinase function in the absence of inhibitor (Cipak et al., 2011).

**Figure 1** Chemical-genetic strategy for sensitizing protein kinases to small-molecule inhibitors. Mutation of the gate-keeper residue in a protein kinase (kinase-as) creates a new pocket where the inhibitor (an ATP analog) binds. This binding leads to inactivation of the kinase (A). However, the kinase-as mutation is not interfering with the kinase function when the inhibitor is not present (B). The inhibitor does not bind to wild-type kinases, thus only mutant kinase is inactivated (C) (Cipak et al., 2011).

Another feature which makes small-molecule inhibitors a valuable tool for conditional inactivation is the possibility of their stepwise dosage which enables either total or only partial inhibition of the kinase. The cell-permeable nature of these inhibitors allows reversible inhibition of the target protein both *in vitro* and *in vivo*. Moreover, they do not influence the formation of protein complexes, and the expression levels of the target protein. Importantly, in order to exclude any possible off-target effects of the inhibitor, wild-type cells are treated with the inhibitor, in parallel with every experiment involving sensitized protein kinase and the inhibitor (Cipak et al., 2011). Small-molecule inhibitors can therefore reveal new biological functions of proteins that have already been studied genetically (Gregan et al., 2010).
To study the function of essential kinases of *S. pombe* on chromosome segregation, we created functional analog sensitive mutants of 13 (out of 17) essential kinases. Eight different inhibitors (ATP analogs) were created to test the sensitivity of the kinase mutants. Spot test showed that all mutants were sensitive to different concentrations of one or more of the tested inhibitors (Cipak et al., 2011). One of the *S. pombe* essential kinase, the Ksg1 kinase, is the homolog of the mammalian phosphoinositide-dependent protein kinase-1 (PDK1). Previous work established that ksg1-358 mutant cells have sporulation and mating defects at 30°C and they are temperature sensitive for growth at 35°C (Niederberger et al., 1999). Therefore, we decided to investigate the role of Ksg1 kinase in chromosome segregation during meiosis I and II. For this purposes, we used analog-sensitive allele of ksg1 (ksg1-as). Our recent in vivo studies revealed that ksg1-as mutant cells in the presence of the inhibitor show higher occurrence of missegregation during meiosis as compared to wild-type cells. We will verify these exciting preliminary results and extend the characterization of the Ksg1 kinase in our future studies.

**CONCLUSION**

One of the key conditions of animal development and reproduction is cell division. Therefore it is of great importance to study and understand the process of regulation and proceeding of cell cycle on molecular basis. The essential *S. pombe* kinase, Ksg1 seems to play a crucial role in the regulation of chromosome segregation. Thus, Ksg1 is strong target to further investigate its role in the process of chromosome segregation during cell division.

**Acknowledgments:** This work was supported by the Slovak Research and Development Agency under the Contract No. APVV-0636-11.

**REFERENCES**
