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Review

Schizosaccharomyces pombe as a model organism for studies of chromosome segregation

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Cell division is one of the key condition of development and reproduction of animals, plants, microorganisms and humans. Therefore, the study of the cell cycle has enormous relevance to the health, well-being, and biology of all living organisms, including growth and development of organisms, diseases such as cancer, to aging. Thus, it is of great importance to study and understand the process of regulation and implementation of the cell cycle on molecular basis. Two types of cell division evolved through evolution, namely mitosis and meiosis. Whereas mitotic events lead to generation of genetically identical cells, the main task of meiosis is to reduce the content of the genetic material by half, and thereby ensuring genetic variability and diversity. We study progress and regulation of chromosome segregation in meiosis using simple model organism *Schizosaccharomyces pombe* because basic molecular mechanism shares common principles in animals, humans, plants and unicellular organisms.

Keywords: *Schizosaccharomyces pombe*, cell cycle, meiosis

The aim of this review is to describe the progress in the studies of chromosome segregation in scientific laboratories with the use of single celled yeast *Schizosaccharomyces pombe*. One of the common features of all living organisms including microorganisms, plants, animals, or humans is their ability of reproduction, growth, and development. Necessary condition for normal life is therefore cell division, which must occur without errors to keep organism under healthy conditions. Although multicellular organisms are composed of millions of cells which play diverse functions in the organism, every cell possess the same genetic material and normally has the same number and structure of chromosomes. Thus, for studies on the process and regulation of chromosome segregation in eukaryotic cells, the simplest eukaryotic organism such as yeast is suitable as model system.

1. Cell cycle as a research target

Research focus of many scientific laboratories world-wide is the study of the cell cycle control (Nurse et al., 1981, Morgan 1995, Pozgajova et al. 2013, Kovacikova et al. 2013, Gregan et al., 2007; Kim et al., 2015 and others). The term cell cycle implies for organised events during which the content of the cell is accurately replicated and divided. It comprises of four phases: G1 (gap1), S (synthesis), G2 (gap2), and M (mitosis or meiosis). Cell cycle biologists target their research on studies of the cell cycle phases (Badodi et al., 2015; Bailis, Forsburg 2003) and their regulation (Nurse and Bissett 1981), as well as on the complex biochemical interactions that stop or start DNA replication (Krejci et al., 2012 and cell division (cytokinesis) (Kitagawa, Lee 2015).

Previous analysis revealed that many steps of the cell cycle regulation are conserved throughout eukaryotes, which allows scientists to study cell cycle events using simple eukaryotic organisms such as yeasts (Badodi et al., 2015).

1.2 Model organism *Schizosaccharomyces pombe*

Schizosaccharomyces pombe, also called fission yeast (Figure 1), is free living, single-celled eukaryotic organism, taxonomically belonging to archiascomycete fungi (Robbertse et al., 2006). Vegetative or mitotic cells are rod shaped and they divide by medial fission. Mitotic cycle is rather fast, with cell doubling of approximately 3 hours.

The genome of *S. pombe* was fully sequenced in 2002 by a consortium led by the Sanger Institute (Wood et al., 2002). Nowadays, because of its easy genetic manipulation and fast growth it is a frequently used model organism for the study of cell cycle control, mitosis, meiosis, DNA repair, and recombination. *S. pombe* was first described in the 1890s and has been extensively studied since the 1950s (Yarbrough, Clark 1957). Fission yeast comprises of three chromosomes and normally exists in haploid form. After mating and subsequent transfer of diploid cells to nutritionally rich media, it is possible to grow *S. pombe* also in diploid form.

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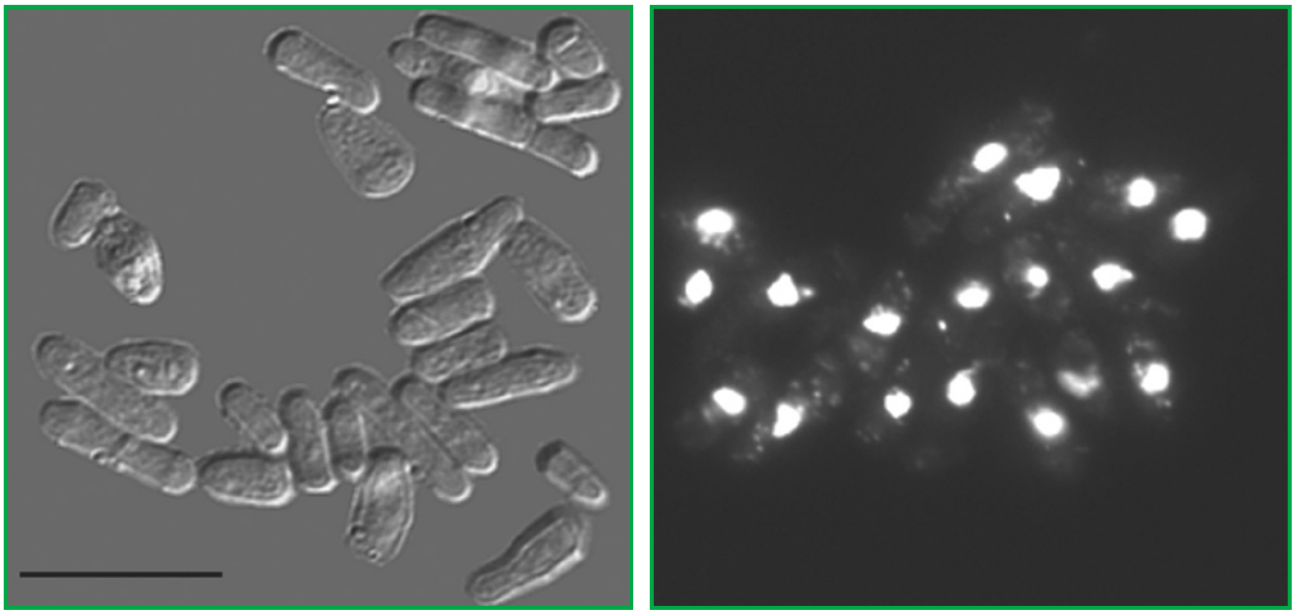

(A) **(B)**

Figure 1 Representative pictures of *Schizosaccharomyces pombe* cells. Cells shown in **(A)** are DIC pictures of vegetative wild type cells viewed under light microscope. The scale bar at the bottom indicates 10 μm. In **(B)** cell viewed under the fluorescence microscope are stained with DAPI, a fluorescent dye which stains the DNA in the nucleus and mitochondria
Source: Požgajová et al., 2015

1.3 Meiosis in *S. pombe*

Meiosis in *S. pombe*, similarly like in other eukaryots has the goal to maintain genetic diversity and generate gametes. During meiosis a single round of DNA duplication is followed by two rounds of nuclear division, called meiosis I and II.

For induction of meiosis in fission yeast it is necessary to prepare mixture of haploid *S. pombe* cells with opposite mating types, designated as *mat1-P* (or h^+) and *mat1-M* (or h^-) (Egel 1976) on nitrogen poor medium, as the yeast mate only upon starvation. Starvation-induced stress conditions activate transcription of *ste11*, which in turn induces expression of Mei2 which activates multiple meiotic events, and the heterozygous *mat1-P* and *mat1-M* genes (Davis, Smith 2001). Products of these genes induce expression of Mei3, which is an inhibitor of the critical protein kinase Pat1, as active Pat1 kinase in the absence of starvation and *mat1* heterozygosity prevents meiosis by inhibiting *Ste11* and Mei2 (Watanabe et al. 1997).

Upon conjugation of h^+ and h^- strains, cells undergo characteristic zig-zag (or banana) shape, and produce transient diploids (zygotes), which directly proceed into meiosis (Figure 2). At meiosis I homologous chromosomes segregate to opposite poles and subsequently at meiosis II, similar to mitosis, sister chromatids separate from each other. Proper chromosome segregation during meiosis requires several processes including:

a) physical connection of homologous chromosomes mediated by reciprocal recombination,
b) sister chromatid cohesion,
c) interaction between kinetochore, a proteinous complex in the centromeric region of the chromosome, and the spindle microtubules.

After DNA replication, programmed double-stranded breaks (DSBs) formation is catalysed by Spo11 (Rec12 in *S. pombe*) which subsequently initialises homologous recombination and crossing over (Sharif, 2002). This physical connection of homologous chromosomes, together with sister chromatid cohesion, is required for correct microtubule – kinetochore interaction, in the way that homologous chromosomes are connected to microtubules emanating from opposite spindle poles (Murata-Hori, and Wang, 2002). As a control mechanism, the spindle assembly checkpoint prevents metaphase/anaphase transition, until all homologous chromosomes biorient on the meiosis I spindle (Shandilya, Roberts 2015). Another important feature required for proper alignment of chromosomes on the spindle is sister chromatid cohesion. It is mediated by a specialized protein complex called cohesin, which is actively loaded to chromatids during late G1 phase and activated in S phase of the cell cycle (Michaelis et al., 1997). During the first meiotic division, meiosis specific component of cohesin, Rec8, is cleaved from chromosome arms by a protease called separase (Watanabe, and Nurse 1999), but remains protected by Sgo1 (shugoshin) at the centromere (Kim et al., 2015, Kitajima et al., 2004). This allows segregation of homologous chromosomes, but protects sister chromatids from segregation. During second meiotic

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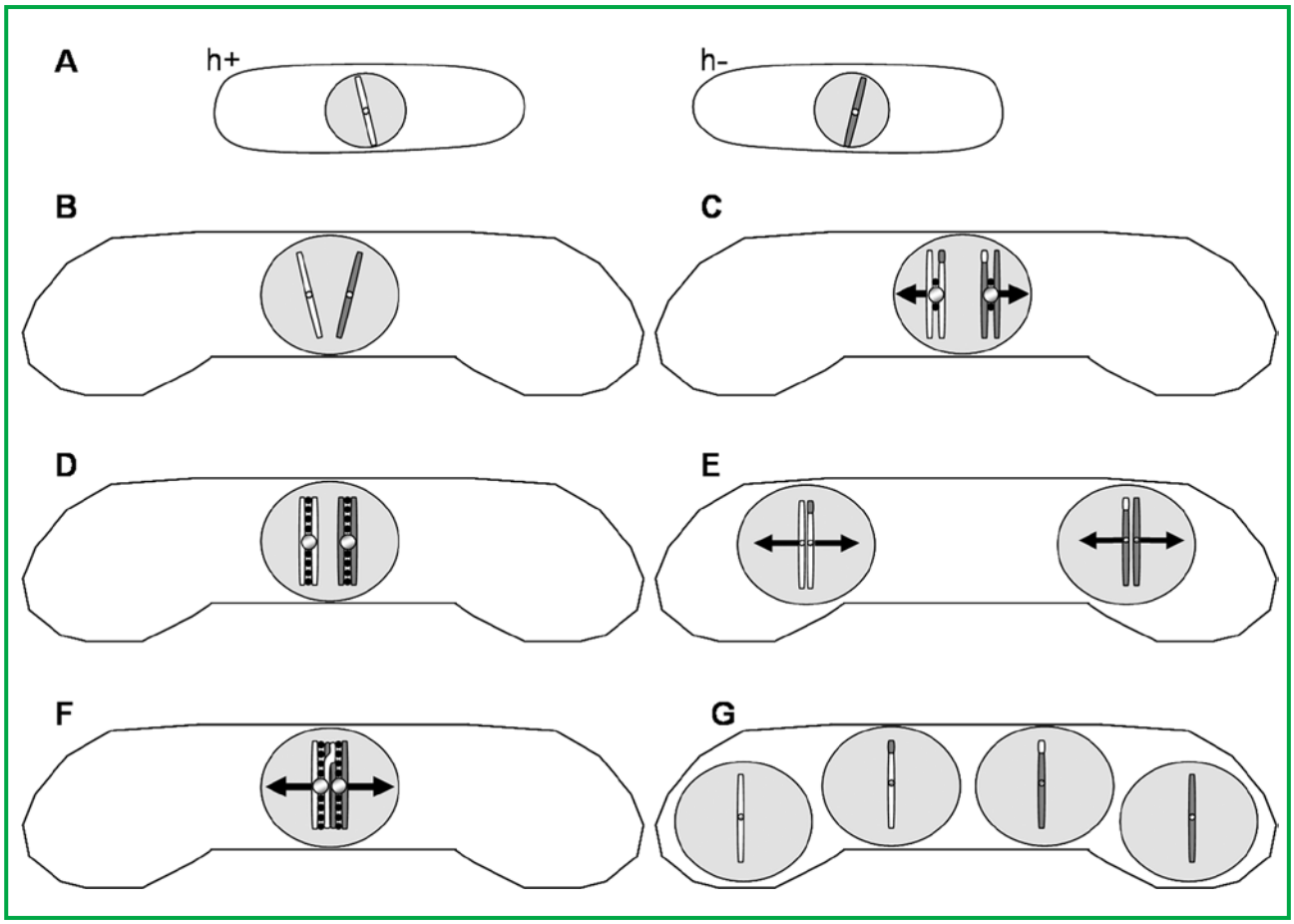

Figure 2 Chromosome segregation during meiosis in *S. pombe*. **A** – two haploid cells with opposite mating types; **B** – diploid cell (zygote) after conjugation; **C** – replication of DNA and establishment of sister chromatid cohesion; **D** – pairing of homologs, recombination, bipolar attachment of homologous chromosomes; **E** – release of cohesion from sister chromatid arms, protection of centromeric cohesion – first, reductional chromosome segregation; **F** – degradation of centromeric cohesion, segregation of sister chromatids – second, equational chromosome segregation; **G** – haploid progeny, spore formation
Source: Požgajová et al., 2015

division, cohesin is removed from centromeric region and sister chromatids segregate from each other (Kitajima et al., 2004). Last but not least, proper chromosome segregation during meiosis depends on regulatory processes controlled by reversible protein phosphorylation, which was experimentally confirmed in yeast strains with blocked function of protein kinases such as Prp4 (Pozgajova et al. 2014), Mph1 or Spo4 (Pozgajova et al., 2013, comment on: Kovacikova et al. 2013) which resulted in enhanced missegregation of chromosomes during meiosis.

The outcome of meiosis in sexually reproducing organisms is generation of gametes – eggs and sperm in animals, pollen in plants, or spores in fungi with reduced genome content by half. Proper ploidy is therefore maintained only upon fusion of two gametes. Correct chromosome segregation is thus crucial for the maintenance of healthy progeny, as missegregation of chromosomes during meiosis results in aneuploidy, the leading cause of birth defects and miscarriages.

2. Conclusions

Chromosome segregation in meiosis is a complex process, regulated throughout the cell cycle by large number of signalling pathways involving vast number of gene products that protect meiotic events from errors. Although the most significant progress in studies of the regulation of the cell cycle came with the demonstration that cyclins (Nurse, Bissett 1981) together with cyclin-dependent kinases (Morgan 1995) are the specific protein complexes critical for regulating the passage of cells through the cell cycle, there is still long way to go in understanding the complexity of interactions between already identified proteins and signalling pathways involved in cell cycle regulation. Thus, the main focus of our research is identification and characterization of novel proteins and protein complexes involved in the regulation of chromosome segregation during meiosis.

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