Studies on Regulation of the Cell Cycle in Fission Yeast

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Abstract
Essential for normal life progression and reproduction is correct chromosome segregation during mitosis and meiosis. Defects in the division program lead to aneuploidy, which in turn leads to birth defects, miscarriages or cancer. Although, researchers invented much about the regulation of the cell cycle, there is still long way to understand the complexity of the regulatory machineries that ensure proper segregation of chromosomes. In this paper we show the results of visualization of the cell cycle progression during meiosis and mitosis with the use of live cell imaging microscopy technique. As a model organism we use the fission yeast Schizosaccharomyces pombe.

Keywords: meiosis, mitosis, live cell imaging, Schizosaccharomyces pombe

1. Introduction

Cell is the basic element of all living species. Every time a cell divides it must completely duplicate its genome, which is subsequently segregated into daughter cells. During evolution, two types of cell division have developed namely mitosis and meiosis [1]. Mitosis results in production of two identical daughter cells with diploid chromosome number (2n) that arise from one mother cell. This process ensures growth of the organism. If a mistake occurs in this process, the resulting daughter cells will inherit either too many or too few chromosomes, a condition known as aneuploidy [2]. A wide number of research studies have shown that such abnormality is commonly present in tumor cells, suggesting that chromosome segregation defects play a significant role in tumor development and progression [3].

Special type of cell division, meiosis, is responsible for production of gametes that are essential for reproduction, as it reduces number of chromosomes and the result is production of four cells with haploid number of chromosomes (n). Correct segregation of the genome during gametogenesis is critical for the proliferation of sexually reproducing species. Errors in chromosome segregation during meiosis result in aneuploidy, the leading cause of birth defects and miscarriages in humans [4]. Thus, chromosome segregation is a research topic of many scientific laboratories [5, 6, 7, and others]. The exact mechanism of accurate chromosome segregation however, still remains unclear.

Chromosome segregation errors are highly frequent in mammalian female meiosis and their incidence gradually increases with maternal age. Identification and characterization of proteins and protein complexes involved in this process is essential for understanding of how and why chromosomes mis-segregate.

We use the fission yeast Schizosaccharomyces pombe as a model organism to study segregation of chromosomes. It is a unicellular organism widely used for studies in eukaryotic biology. S. pombe is a single-celled fungus which taxonomically belongs to class Archiascomycetes [8]. Its genome was fully sequenced in 2002 by a research partnership, led by the Sanger Institute. This sequencing revealed, that the 13,8-Mb genome of S. pombe distributed among 3
chromosomes, contains 4824 protein-coding genes, which makes it a free-living organism with the smallest amount of genes yet identified among eukaryotes [9]. The strength of the use of \textit{S. pombe} as model organism lies in its easy and inexpensive handling as well as rapid growth. Moreover, \textit{S. pombe} is harmless, and most importantly 172 \textit{S. pombe} proteins have similarity with proteins related to human diseases. Furthermore, the \textit{S. pombe} chromosome organisation is very similar to that of higher eukaryotes. This makes it a powerful tool for studying regulation, control and characterisation of biological processes in eukaryotic cells. To understand how biological processes, such as maintaining proper chromosome segregation function, it is necessary to perform detailed analysis on molecular basis with the help of microscopic visualisation. Modern visualisation techniques, such as live cell imaging, enable us to visually follow the whole process of cellular division [10]. Together with up-to-date molecular biology techniques, which are used to label, delete, or modify protein of interest, it is possible to analyse the function and role of the particular molecule in chromosome segregation. Therefore, we set up the live cell imaging system of cell visualisation, to observe the whole process of meiotic and mitotic division in higher eukaryotic cells. To follow the process of chromosome segregation, we used wild type yeast strains in which one copy of the chromosome II is labelled close to the centromere with GFP dots – heterozygous GFP. For tubulin visualisation m-cherry is expressed directly on the \(\alpha\)-tubulin, and the nuclei are visualised with Hoechst33342. The analyses were performed on the Olympus Cell R inverted epifluorescence microscope, equipped with Hamamatsu CCD camera, oil immersion objective 60x/1.42 PlanapoN, and a Cell-R software Olympus excellence 1.1. It also contains movable stage, where each position of the stage can be saved. This allows us to observe several positions with cells of interest during one experiment. Under such conditions we were able to analyse up to 30 cells per experiment.

2. Materials and methods

\textbf{Yeast strains}

The genotypes of the yeast strains used in this study are as follows:

- wild type: h\(^+\) ura4 mCherry-atb2-HygR
- wild type GFP: h\(^-\) mCherry-atb2-HygR leu1-32 lys1-131 ura4-D18 cen2(D107):KanR-ura4+-lacO his7+::lacI-GFP

\textit{Schizosaccharomyces pombe} strains were maintained and grown using standard conditions. [1, 17, 3].

\textbf{Time-lapse fluorescence microscopy}

Yeasts were grown on rich medium (YES) plates overnight at 32°C and for another 8 hours in liquid YES. Afterwards, for meiosis studies cells were crossed and plated on nitrogen low PMG-N plates for 12 h at 25°C to induce starvation, which is necessary condition for yeast to undergo meiosis. To study mitosis cells were grown on EMM (Edinburgh minimal medium) plates. Then, cells were resuspended in liquid PMG-N or EMM and transferred to a glass-bottom microwell dish (MatTek, Ashland) coated with 2 µl of 2 mg/ml lectin BS-1 (Sigma-Aldrich). Live cell imaging was performed using epifluorescence microscope Olympus Cell R system equipped with Olympus MT-20 150W mercury arc burner, Halogen Lamp 100W, Hamamatsu ORCA-ER CCD camera, 60x/1.42 PlanapoN oil immersion objective and standard filter sets: DAPI (excitation 381–392 nm, emission 420–460 nm), CY3 (excitation 547–572 nm, emission 569–623 nm), and FITC (excitation 475-495 nm, and emission 510-531 nm). All meiosis experiments were performed at 25°C, and mitosis experiments at 32°C. Images of cells were taken as time-lapse with five optical Z-sections, using 1 µm z distance, in 5 min intervals for eight hours. Image and data analyses were performed in ImageJ. The length of meiosis and mitosis duration was determined using the above described Olympus Cell R system.

3. Results and discussion

To follow the process of chromosome segregation, we used wild type yeast strains in which one copy of the chromosome II is labelled close to the centromere with GFP dots – heterozygous GFP. For tubulin visualisation m-cherry is expressed directly on the \(\alpha\)-tubulin, and the nuclei are visualised with Hoechst33342. The analyses were performed on the Olympus Cell R inverted epifluorescence microscope, equipped with Hamamatsu CCD camera, oil immersion objective 60x/1.42 PlanapoN, and a Cell-R software Olympus excellence 1.1. It also contains movable stage, where each position of the stage can be saved. This allows us to observe several positions with cells of interest during one experiment. Under such conditions we were able to analyse up to 30 cells per experiment.
With this system, it is possible to observe green dot (using the FITC filter) representing the chromosome, surrounded with blue-labelled DNA (using the DAPI filter) and attached to α-tubulin (using the Cy3 filter). For normal meiosis it is characteristic, that during first meiotic division GFP dot moves to one pole of the cell, and subsequently during second meiotic division sister chromatids separate (Fig. 1). All conditions diverse from described conditions are concerned as errors in chromosome segregation.

![Diagram of Meiosis](image)

**Figure 1.** Schematic pictures of chromosome segregation during meiosis. Proper segregation of chromosomes requires segregation of homologous chromosomes during first meiotic division and segregation of sister chromatids during second meiotic division.

Normally, there is very low amount, if any, of missegregating chromosomes in wild type cells. This was also observed in our system, in which we did not detect any missegregation of chromosomes in 70 visualised cells (Figure 2). Therefore, we can conclude, that the yeast strains with all labelling undergo normal meiosis suggesting, that the set up is suitable for analyses with reproducible results. Duration of the meiotic division was measured 12 hours after induction of mating on solid PMG –N plates. Afterwards, cells were resuspended in liquid PMG –N and after mild sonication, transferred on glass-bottom microwell dish coated with 2 µL of 2mg/ml lectin BS-1.

![Timeplot of Meiosis](image)

**Figure 2.** Representative pictures of meiotic cells. First anaphase starts at the time point 35 to 42 minutes and second meiosis starts at 77 to 84 minutes. Afterwards 4 nuclei are present, where two on the left side contain GFP labelled second chromosome and two do not contain GFP dots.
Almost the same procedure is used to study chromosome segregation in mitosis. Vegetative cells were grown in liquid nutritionally rich YES medium at 32°C for 6 hours. Exponentially growing cells were then transferred to minimal medium and visualised under the Olympus Cell R fluorescence microscope (Figure 3).

![Mitosis](image)

**Figure 3.** Schematic picture of chromosome segregation during mitosis.
In this schematic draw it is shown, how chromosomes segregate during mitotic anaphase. Blue balls represent DNA stained with Hoechst33342, green dot shows segregation of second chromosome, and the red line represents α-tubulin stained with m-cherry.

Wild type strains usually do not show chromosome missegregation under conditions which were used in our system. Indeed, no missegregation of the second chromosome was observed during the experiment in 70 analysed cells (Figure 4).

![Mitosis](image)

**Figure 4.** Representative pictures of mitotic cells. Cells underwent anaphase at the time point 63 to 70 minutes, this was followed by segregation of nuclei and subsequent cytokinesis.

Cell cycle is a complex process its progress is regulated by vast number of proteins and protein complexes [14]. Cell division may proceed either as mitosis or meiosis [1]. Mitosis, after DNA duplication and nuclei division ensures production of two identical daughter cells from one mother cell. During meiosis, a single round of DNA replication is followed by two rounds of chromosome segregation, called meiosis I and meiosis II [15]. Modern visualisation methods are a powerful tool that allows researches to follow and analyse the process of chromosome segregation both in meiosis and mitosis in fixed cells as well as in living cells. Microscopy
techniques helped to identify many key components of the cell cycle regulatory network such as Mde4/Pcs1 protein complex that protects merotely [16], or the role of Mph1 as well as Spo4 and Spo6 in the regulation of chromosome segregation and meiosis progression [7]. Live cell imaging, together with modern molecular biology techniques allow researchers to track single chromosome and study its architecture throughout meiosis or mitosis [17, 18], repair and dynamics of double-stranded breaks during meiotic recombination [19]. This state-of-the-art method might help to discover phenotypes, which could be overseen with other visualisation techniques.

4. Conclusion

Our experiments have shown that it is possible to visualize and observe the whole process of chromosome segregation during meiosis and mitosis, record it, and analyse it. The live cell imaging system we are using produces reproducible and comparable results, which makes it a suitable set-up for the analysis of chromosome segregation in meiosis and mitosis. This visualisation method together with modern molecular biology techniques will allow us to study the function of a single molecule during cell division.

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References

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