ASSAY OF CHROMOSOME MOVEMENT AND PAIRING DURING MEIOSIS IN SACCHAROMYCES CEREVISIAE USING LIVE CELL IMAGING

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Abstract

Meiosis is a specialized form of cell division, in which homologous chromosomes pair and form crossovers to ensure proper segregation. Improper segregation can lead to nondisjunction and diseases such as Down syndrome. It is not known if chromosome pairing is directed or stochastic, or whether chromosomes are held tightly together or allowed to dissociate. In this research, we assayed chromosome pairing in 3D space over time using Saccharomyces cerevisiae, with the goal of analyzing the role of actin and homology search with respect to chromosome motion and pairing during meiosis. Using homologous chromosomes tagged with Green Fluorescent Protein (GFP), we acquired images of yeast cells, and measured the distance between foci. We identified three classes of homolog interactions: always paired, never paired, and “kissing” where they pair and unpair. We calculated Mean Square Displacement (MSD) and the volume occupied by the foci to quantify chromosome motion. A shorter chromosome was tagged near the centromere, which exhibited active motion and a longer chromosome was tagged in the arm, which exhibited constrained diffusion.

Key Words: Meiosis, chromosomes, yeast, MSD, active motion, diffusion, GFP, tetO, tetR

Introduction

MEIOSIS is a specialized form of cell division in sexually reproducing organisms that results in haploid gametes. Errors occurring during meiosis can result in nondisjunction and aneuploidy, which can cause cell death and abortion, as well as birth defects and developmental disorders, including Down syndrome in humans. Two processes occurring during meiosis, recombination and segregation, are fairly well understood, but the events prior to these, including homologous chromosome pairing, are not.

Recombination occurs when the protein Spo11 forms double-strand breaks (DSB). In Saccharomyces cerevisiae, the conserved protein Spo11 has been knocked out (generating spo11Δ), which allows researchers to study the roles of Spo11. The protein Spo11 is not essential, and although spo11Δ proceeds through meiotic events and division, it does not produce viable spores. This is because Spo11 is needed in order for homologous recombination to occur, and homologous recombination is necessary for the proper segregation of chromosomes in yeast (7). Spo11 works with other proteins to generate DSB at specific loci in the genome. Then resection can occur, which results in single stranded 5' overhangs (4,7), which can invade the homologous chromosome in a process called homology search (Figure 1.A). Once the chromosome has found its homologous partner, it can undergo double-strand break repair (DSBR) and results in crossovers (CO) or non-crossovers (NCO) depending on the repair pathway (7). This process is believed to play a role in chromosome pairing, because one can imagine the base pairing during single-strand invasion helping to make chromosome-pairing possible by keeping the chromosomes in close proximity (1).

When chromosomes pair, the synaptonemal complex (SC) forms between homologous
chromosomes. The SC is comprised of proteins and forms along the entire length of the chromosomes (7). As mentioned above, homology search is associated with the movement of chromosomes to move them close together, which will allow the SC to form (1,7). Chromosome movement is driven by actin fibers outside of the nucleus through a SUN-KASH protein bridge, which crosses the nuclear envelope (1,3,6). The protein Ndj1 associates with the telomeres of chromosomes and, along with other proteins, interacts with Mps3, the SUN, located on the inner nuclear envelope. Mps3 interacts with Csm4, the putative KASH, located on the outer nuclear membrane. Csm4 and associated proteins then interact with actin, completing the “chain” linking actin fibers outside the nucleus to the chromosome telomeres (Figure 1.B). Actin polymerization can then exert forces on the chromosomes to result in movement of chromosomes (1,3,6). Researchers have previously shown in *C. elegans* that motion is required for the pairing of chromosomes, and that active motion facilitates pairing but is not required (8).

**Goals**

The goal of this paper is to analyze the role of actin and homology search with respect to chromosome motion and pairing during meiosis.

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**Figure 1.**

A) A model of double strand break formation and homology search.

B) A model of actin dynamics affecting chromosome movement through the SUN-KASH bridge.
Methods

Strain construction.

In order to assay the motion of chromosomes, an array has been developed that utilizes green fluorescent protein (GFP) marked loci that can be tracked through 3D space. This array consists of repeats of the tetracycline operator (tetO) integrated at certain loci in the yeast genome. In these experiments, the tetO repeats were integrated at the gene loci URA3 (near the centromere) and LYS2 (on the arm of the chromosome) in two different strains (Figure 2A). TetO binds its corresponding repressor, TetR, which is fused to GFP creating the TetR-GFP fusion protein. The many repeats of tetO concentrates GFP and GFP foci can be detected by light microscopy.

![Diagram](image1)

**Figure 2.**
A) A diagram depicting tetO repeats binding TetR-GFP at the LYS2 and URA3 loci.
B) An image displaying the GFP foci.

Results

4D images, or z-stacks captured over time, of cells containing the tetO repeats and TetR-GFP construct can be taken by light microscopy. A program written in the software MATLAB can then analyze these images. Each diploid cell should have two loci of tetO repeats, which results in one GFP focus when the chromosomes are paired and two GFP foci when the chromosomes are unpaired (Figure 2B). The MATLAB program detects the intensity of pixels...
and measures the distance between the brightest pixels. Thus if only one focus is found, the program returns a zero. Each image is then reviewed manually to insure the program has correctly identified one or two foci. Once the program generates the distance data, the mean squared displacement (MSD) can be calculated and graphed. MSD is the average change in distance for a given time step, which is the change in time from one capture to the next.

In this experiment, images were taken at one-second intervals and thirty-second intervals. The MSD can determine what types of motion are occurring: active motion, normal diffusion, or constrained diffusion (5) (Figure 3). The data consists of a field of view of cells, usually containing between 50-100 cells, and the MSD is averaged across all of the cells. For example, if the \( \Delta t \) is one, then all of the MSD for \( \Delta t=1 \) is taken for each cell and averaged, that is if a cell has \( t=50 \) then there will be 49 \( \Delta t=1 \) for a cell (1 to 2, 2 to 3, 3 to 4,...,49 to 50), and these will be averaged with other cells.

![Figure 3. Modeled Mean Squared Displacement (MSD) graph, showing normal diffusion as a linear relationship, confined diffusion as a logarithmic relationship, and directed (active) motion as an exponential relationship.](image)

![Figure 4. A) MSD graphs for 3 different trials of WT marked at URA3, with two out of three graphs showing active motion. Some time points are missing from different trials due to drift during image capture making it impossible to isolate and capture single cells from the field of view. B) The fraction one spot graphs, showing the percent pairing of cells over the time course. This is important since it shows the cells are able to pair after going through meiotic events, demonstrating the tetO inserts are not disrupting pairing. Data for all these figures were taken at thirty-second intervals, at 23°C.](image)
Figure 5. A) The MSD graph for cells marked at the LYS2 loci in WT. WT LYS2 marked cells differ from the WT at the URA3 loci because WT LYS2 cells lack the same active motion seen in WT URA3 cells. B) The MSD graph for spo11Δ marked at LYS2. It appears here that spo11Δ has a slight delay, which is unusual because spo11Δ normally progresses through meiosis faster due to bypassing the DSB repair pathway and its associated checkpoint. C) The fraction of one spot in WT LYS2 cells, demonstrating that after progressing through early meiosis the chromosomes do begin to pair. D) The fraction of one spot in spo11Δ LYS2 cells, demonstrating that these cells do not return to a paired state after prophase. Data for these figures were taken at thirty-second intervals and at 30°C.

Figure 6. The MSD graphs for both spo11 and WT cells marked at URA3, also showing cells treated with LatB and untreated cells (cells in DMSO). Images for this data were taken at one-second intervals and at 23°C. There is a small difference between spo11Δ and WT cells. There is a much larger difference between WT untreated cells and WT cells treated with LatB. The same is true of untreated spo11Δ cells and spo11Δ cells treated with LatB. The more constrained
diffusion exhibited in cells treated with LatB is likely a result of the telomeres attaching to the nuclear envelope and being unable to diffuse as much as a result of these fixed chromosome telomeres.

Figure 7. A) The MSD graph for spo11Δ URA3 taken at 23°C, showing that spo11Δ lacks the active motion of WT URA3 cells. B) The MSD graph for spo11Δ URA3 taken at 30°C, showing that although temperature seems to have an affect on how meiosis proceeds it does not affect the active motion which is still missing. C) The MSD graph for spo11Δ LYS2 at 30°C, showing that spo11Δ LYS2 and spo11Δ URA3 do not differ that much. D) The fraction of one spot for the corresponding MSD graphs showing spo11Δ cells don’t pair after progressing through meiosis.
Figure 8. The 3D pixel count of the unique locations that GFP foci occupied.

Figure 9. An interpretation of how chromosomes might be moving in the nucleus. Note that no strains have GFP foci on both the LYS2 (orange and red) and URA3 (light blue and darker blue), but for simplicity the two chromosomes are shown in the same nucleus. Both the LYS2 locus array and URA3 locus array exhibit constrained diffusion (gray arrow), but the URA3 locus array also exhibits active motion (black arrow). The LYS2 array occupies more volume, which can be inferred as motion (purple arrow).
Conclusion and Closing Remarks

It is unclear if the lack of active diffusion is limited to where on the chromosome we are observing and if spo11Δ affects it, or whether we are limited by photo-bleaching and by the stage we choose to image. However, it is worth investigating further.

Understanding how chromosomes move may increase our understanding of how chromosomes pair. Furthermore, the more constrained diffusion in LatB treated cells can also aid in our understanding of active motion, since actin generates the force that leads to active motion. We need to investigate mutants involved in the formation of the SUN-KASH bridge to understand if the more constrained motion is simply because the chromosome ends are fixed to the nuclear envelope, or because actin no longer pulls on the chromosomes. By looking at csm4Δ and ndj1Δ, one can deduce the effects of removing actin. One would expect the csm4Δ to look like latB treated WT cells, since the telomeres would still be attached to the nuclear envelope, but actin would be unable to move the chromosomes, since the SUN-KASH bridge will be disrupted. If one looked at the ndj1Δ, then one would expect to see a return to less constrained diffusion because the telomeres are no longer fixed to the nuclear envelope, but one would see the effects of removing actin as well, so one could deduce the effect of removing actin but not of fixing the chromosomes to the envelope. To assay if the active motion is occurring outside the period of imaging, we could try and image one field of view and then immediately image another field of view to try and capture some cells going through active motion at different times. Also, by analyzing a strain that is marked at two non-homologous loci, we should be able to identify what motion actually affects pairing versus motion that is random. Once these experiments have been performed, hopefully an understanding of chromosome movement and pairing will emerge that will lead to ways to address the errors resulting from improper segregation and nondisjunction pathways.

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Works Cited


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