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Real-time imaging of meiotic chromosomes in *S. cerevisiae*

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Abstract

Important information on cellular physiology can be obtained by directly observing living cells. The nucleus and the chromatin within are of particular interest to many researchers. Monitoring the behavior of specific DNA loci in the living cell is now commonly achieved through the insertion of binding sites for fluorescently tagged proteins at the sequence of interest (*e.g.* reference 1). However, visualizing the behavior of full length chromosomes can only be achieved when they constitute discrete, relatively well individualized units. During meiotic mid-prophase, chromosomes of budding yeast are well-organized structures that present such characteristics, making them remarkably suited for visualization. Here we describe the optimized protocols and techniques that allow monitoring of chromosome behavior during meiotic prophase in budding yeast.

Keywords

S. cerevisiae; chromosome dynamics; *in vivo*; meiosis; prophase

1. Introduction

A fundamental step of meiosis consists of the juxtaposition and interaction of homologous chromosomes all along their length. In the classical meiotic program, as happens in budding yeast, juxtaposition culminates with formation of the synaptonemal complex (SC) between homologous chromosomes (zygotene stage). At the pachytene stage, SC appears fully complete all along the axes, with regular arrays of chromatin loops emanating outward. Furthermore, telomeres of pachytene chromosomes are anchored to the nuclear envelope (NE) through telomere/NE complexes. Recent studies from our laboratory and others have exposed the dynamic behavior of mid-prophase chromosomes in *S. cerevisiae* (2; Koszul et al., submitted; Conrad and Dresser, personal communication). This chapter aims at providing a detailed protocol for optimal conditions to monitor chromosomes in real time during prophase.

2. Materials

2.1 Strains and Chromosome labeling

1. To visualize the chromatin, strains containing fluorescently tagged proteins are constructed using standard techniques for transformation of *S. cerevisiae*.

2.2 Meiotic time courses – SK1 strains (see Notes 1 and 2)

1. YEPG plates: 3% v/v glycerol, 2% w/v bactopectone, 1% w/v yeast extract, 2% w/v bactoagar. Autoclave on liquid cycle
2. YEPD liquid and plates: 2% w/v bactopectone, 1% w/v yeast extract. Add 2% w/v bactoagar for plates. Make a 40% w/v stock solution of glucose. Autoclave on liquid cycle. Add glucose to media to a final of 2%. (see Note 3).
3. SPS; 1% w/v potassium acetate, 1% w/v bactopectone, 0.5% w/v yeast extract, 0.17% w/v yeast nitrogen base with ammonium sulfate and without amino acids, 0.5% w/v ammonium sulfate, 0.05M potassium biphtalate, 2 drops per liter antifoam (Sigma). pH to 5.5 with 10 N KOH. Autoclave on liquid cycle
4. SPM: 1% w/v potassium acetate, 0.02% w/v raffinose, 2 drops per liter antifoam (Sigma). Autoclave on liquid cycle.
5. 40% ethanol, 0.1M sorbitol.

2.3 Cell preparation

1. DAPI (4',6-diamidino-2-phenylindole; excitation 340–370nm, emission 420–530nm): 2 mg/ml stock solution in water.
2. 25 x 75mm microscope slides
3. 22 x 22mm coverslips

2.4 Epifluorescence microscopy

1. Microscope: 100x objective, high resolution/high sensitivity camera, appropriate filters for the fluorescent probes used, and appropriate software for image acquisition (see Note 4).

¹If strain is a *trp1* or *ura3* auxotroph, add extra TRP/URA to plates and to liquid SPS media.

²We have tried many different waters and results seem random. House distilled seems to work as well or better than MilliQ filtered. Time courses during summer seem especially finicky. Some people swear by bottled water such as Poland Springs as being more reproducible year round.

³Glucose will caramelize if improperly prepared. Autoclave as a 40% stock solution at 110°C. The rest of the media components can be combined then autoclaved at 121°C.

⁴Any good epifluorescent microscope can be used. Important features are: high resolution and sensitivity of the camera to minimize exposure times, fast frame rates to allow minimal times between acquisition of images, a z-stepper capable of rapid, small steps, and an image acquisition program that can drive all hardware. We use an Axioplan 2IE motorized microscope (Zeiss, Gottingen, Germany) with a 100x objective (NA=1.45 Ph3 Plan-Apochromat or alpha-PlanFluar NA=1.4) and a filter sets appropriate to the fluorophores being imaged (GFP or DAPI in our case). Our EM-CCD camera is a Photometrics Cascade 512B (Roper Scientific, Tuscan, AZ). For three-dimensional analysis we use either the computerized z-motor of the Axioplan microscope's stage, or a piezoelectric translation

3. Methods

3.1 Chromosome Labeling

In vivo visualization of individual prophase chromosomes can be achieved by fluorescently labeling proteins involved in structural organization. Proteins that compose the chromosomal axes and the SC are targets of choice and, in principle, should allow illumination of individual chromatids and homologous pairs, respectively. It is important that the tagged protein provides a distinguishable signal without affecting meiotic progression as confirmed by FACS analysis, kinetics of divisions/sporulation, and spore viability. Visualization of the chromosomal axis has been achieved by fusing a GFP tag to the meiosis-specific cohesin, Rec8 (Conrad and Dresser personal communication). The SC can be illuminated using a GFP-tagged Zip1 protein (Koszul et al., submitted; 2). In the latter case, although homozygous strains exhibit a brighter signal, good resolution can nonetheless be achieved using a heterozygous allele.

Besides monitoring chromosomes directly labeled with fluorescent tags, a live cell DAPI-labeling method can be used to reveal the dynamic nature of chromosomes in any yeast strain during prophase. This technique presents the great advantage, beside its simplicity, that one can monitor chromosomes in any cell undergoing meiosis without having to modify the genome to introduce a tagged protein into each new strain of interest. Best results are achieved for the pachytene stage, where chromosomes are well individualized. Movement also can be observed at zygotene though it is difficult to clearly distinguish individual chromosomes. Furthermore, it is easy to combine imaging of DAPI-labeled chromosomes with one's favorite fluorescently tagged protein located on the chromosomes or elsewhere (e.g. Koszul et al., submitted).

3.2 Meiotic Time Courses

Proper synchronization of a meiotic culture is critical for virtually all studies. In order to analyze a population of cells at specific stages or, conversely, to monitor certain events as a function of stage, most of the cells must enter into the different prophase steps at approximately the same time. It is possible to observe cells undergoing meiosis using techniques such as immobilization onto agarose patches and use of flow cell observation chambers, these approaches result in slower progression through meiosis, probably largely due to aeration problems, which also dramatically affect chromosome dynamics (RK personal communication; 3). Thus far, only sporulation in liquid medium allows optimal synchrony of the population. The synchrony of meiotic progression can be monitored by FACS analysis to determine how synchronized are cells when they undergo meiotic replication (*see* Note 5) and by DAPI staining (*see* Note 6).

z-stepper driven by the amplifier E662 LVPTZ (Physik-Instrumente, Karlsruhe, Germany) mounted on the microscope objective. We use Metamorph software (MDS Analytical Technologies, Mississauga, ONT, Canada) for data acquisition.

⁵If the population is correctly synchronized, replication should be achieved within 3 hours, and pachytene reached by 5 hours, after transfer into sporulation medium.

⁶Meiotic progression can be monitored by DAPI staining: every hour after inoculation into SPM to initiate meiosis, spin down 1 ml of culture and resuspend in 40% ethanol plus 0.1M sorbitol. On a microscope slide add 2µl of ethanol permeabilized cell suspension to 2µl 1µg/ml DAPI. Cells that have passed through anaphase I or anaphase II will contain 2 or 4 DAPI staining bodies, respectively.

1. Day 1 - In the evening, patch cells from -80°C glycerol stock onto YEPG plates. This step ensures that cells emerging into growth will have mitochondria, which are readily lost in SK1 strains.
2. Day 2 - The next morning, streak for single colonies on YEPD plates. You want to make sure colonies are sparse so that they will grow large and healthy (*see* Note 7).
3. Day 4 - In the afternoon, pick a whole colony and put into 5ml YEPD liquid in a 10x13mm culture tube. Vortex and then grow overnight at 30°C on a roller drum.
4. Day 5 - Grow YEPD cultures for 24h, vortexing tubes a couple of times during the day. Set up at least 2 SPS cultures diluting the YEPD cultures 1/500 and 1/1000. For slow growing strains include a 1/250 dilution. Note that surface-to-volume ratios at this stage are important. Use 50 or 100 ml SPS in a 1 liter flask, or no more than 250 ml in a 2 liter flask.
5. Grow SPS cultures at 30°C , shaking at 325–350rpm, making the culture swirl up the edges of the flask to create the largest surface area possible.
6. Day 6 - SPS cultures should be grown for a total of 18h. Remove samples from cultures and assess synchrony (*see* Note 8).
7. Once culture(s) for analysis are selected, harvest cells by centrifugation at 3000 g for 3 minutes at room temperature.
8. Wash cells in an equal volume of 1% SPM (prewarmed to 30°C) and resuspend in 2 times the original volume of prewarmed SPM.
9. Take zero time point and place culture in incubator at 30°C , shaking as before (*see* Note 9).

⁷For mutants that have a mitotic growth defect (e.g. *rad51* and *rad52*) each growth step can be extended to allow for sufficient density of cells at the start of the time course. Also it is harder to distinguish haploid and diploids so more YPD cultures are started to insure an adequate selection. Often one must judge haploid and diploid under the microscope with haploids being smaller and budding apically while diploids are larger and bud from alternate poles.

⁸There are three criteria for judging the synchrony of a culture:

- a. Optical Density; On our spectrophotometer we take between 1.3 and 1.45 OD₆₀₀ measured on the undiluted culture (i.e. not a true OD because we do not dilute the cells into the range the spectrophotometer can measure in a linear fashion). Each spectrophotometer will give a different reading according to light path length. This OD corresponds to $\sim 2.1 \times 10^7$ cells per ml at OD₆₀₀=1.35. The lower densities seem to be consistently more synchronous and are best for cytological studies. We have found that if cultures are too dense diluting with SPM improves the synchrony of the subsequent time course.
- b. Percentage of G1/G0 Cells; Stain with cells DAPI by centrifuging a 500 μl sample for 10sec at 13,000x g. Resuspend in 40% ethanol, 0.1M sorbitol. On a microscope slide add 2 μl of ethanol permeabilized cell suspension to 2 μl 1 $\mu\text{g/ml}$ DAPI. Assess the percentage of cells in vegetative S-phase (small buds without DAPI bodies) and mitosis (large buds with DNA at, or stretched across the neck). A good culture will have 10% replicating and dividing cells. The absence mitotic figures *may* indicate that the culture has prematurely entered meiosis.
- c. Cell Morphology; Strings of 3–5 cells with daughter and mother cells of about the same size seem to correlate with synchronous cultures.

⁹At temperatures $>30^{\circ}\text{C}$, SK1 meiosis is sub-optimal; at 34°C meiosis is inhibited. Check the temperature of your incubator carefully – it may fluctuate throughout the day. Lower temperatures ($23\text{--}30^{\circ}\text{C}$) may be less problematic but you will have to be consistent. Meiotic DNA replication is particularly sensitive to high temperature. If it is necessary to perform a time course at higher temperature it is preferable to shift from 30°C after initiation of DNA replication ($\sim 2\text{h}$).

10. A 1ml sample is taken at hourly intervals to assess meiotic progression (MI and MII divisions) by DAPI staining. Samples are pelleted at 13,000xg for 10sec then resuspended in 40% ethanol/0.1M sorbitol (*see* Note 10).

3.3 Cell preparation

1. Aliquots of cells to be analyzed are taken from the sporulation medium culture at appropriate time points after initiation of meiosis. Because full aeration is essential to full chromosome dynamics during prophase, special care has to be brought at all steps of the protocol described below.
2. Harvest 500µl samples. If possible, proceed immediately to image acquisition. If one wants more concentrated samples let sit on ice for 20min then remove supernatant.
3. For DAPI-staining, add DAPI directly into two aliquots of the culture to a final concentration of 10 and 20µg/ml and incubate overnight in ice in a 4°C room (*see* Note 11).
4. Once the sample is ready for visualization, either immediately for visualization of GFP-labeled proteins or later for DAPI visualization, vortex for 7sec to reoxygenate the solution and then deposit 3µl of suspension onto the slide (*see* Note 12).
5. Add the coverslip (*see* Note 13). A few air bubbles must remain stuck under the coverslip: the cells located in their vicinity correspond to cells where aeration, thus dynamic movements, last the longer. Therefore it is better to deposit the sample at three or four positions, located within the total surface covered by a coverslip, in order to maximize the chance of trapping an air bubble once the coverslip is added. Repeat as needed, discarding slides with no trapped air bubbles until a correct one is obtained.
6. Proceed quickly to imaging. One slide can be used for ~7–8 min once ready before motion deteriorates.

¹⁰DAPI staining: On a microscope slide add 2µl of ethanol permeabilized cell suspension to 2µl 1µg/ml DAPI. Cells that have done anaphase I or anaphase II will have 2 or 4 DAPI staining bodies respectively.

¹¹Live cell DAPI staining: it is better to prepare duplicate cell aliquots because of sample to sample variations. In general, up to 40% of the cells treated with DAPI are dead and exhibit very strong DAPI signal with little movement. Some others show very little staining and should be discarded as well. However, between 10 to 30% of all the cells show a weak but distinguishable signal, which corresponds to the live cell-stained chromosomes. Note that mitochondrial DNA is labeled as well with this protocol, corresponding to the tubular structures and dots observed at the cell poles and equator, respectively.

¹²In order to increase the cell concentration and the number of cells in vicinity of trapped air bubbles, it is recommended to remove the supernatant of the tube (after cells have settled to the bottom overnight) before the 7 sec vortex. Avoid spinning. Deposit the 3 µl at 3 to 4 emplacements on the slide, so that the chance to trap an air bubble when adding the coverslip is maximized.

¹³Do not press on the coverslip. Let it fall down gently on the sample and wait ~1 min before positioning it under the objective. However, over longer periods of time and in the set-up described here, access to oxygen diminishes and motion may be attenuated.

3.4 Image acquisition

The microscope apparatus listed below (*see* Note 1) proved well-fitted for the monitoring of GFP labeled as well as DAPI-stained chromosomes. The combination of a highly sensitive CCD camera with the piezo Z-motor allows short exposure times in rapid succession as needed to help maintain cell viability.

3.4.1 Two-dimensional time-lapse recording—Because mid-prophase chromosomes in wild type budding yeast show dramatic movements, it is convenient to monitor them within a single focal plane. Useful measurements can be recovered from such 2D timelapse analysis (2; Koszul et al., submitted).

1. Nuclei are observed under appropriate excitation and emission wavelengths for the particular fluorophores being used.
2. Slides are viewed under phase contrast microscopy to locate the cells. For each timepoint, images of cells located in the vicinity of a trapped air bubble are recorded (ideally up to five cell diameters away from the air/water interface).
3. Conditions for capturing 2D time-lapse series: for both GFP- and DAPI-labeled chromosomes, 90–120 images can usually be recorded before movement diminishes, maybe as a result of photodamages, and/or photobleaching impedes satisfactory detection. Exposure of 800 ms is usually sufficient to achieve good quality pictures, although in homozygous Zip1-GFP strains it can be reduced down to ~350–500 ms. A series of exposures taken every one to three seconds present a good quality and are sufficient to reveal details of chromosome dynamics (Figure 1). Whereas smaller time intervals outline details of chromosome movements, larger ones underline more global trends such as large transitions and nucleus displacement. Eventually, longer time intervals can be used to achieve longer periods of observation (*see* Note 14).

3.4.2 Three-dimensional time-lapse recording—Three-dimensional analysis of chromosomes is achievable. However, little information about zygotene/pachytene chromosome disposition is directly extractable from such recordings in a wild type background due to the highly dynamic nature of the nucleus. However, mutations or conditions that disrupt motion can be analyzed using 3D time lapse. We recommend conditions as follow: 16 x 0.25 μ m or 10 x 0.4 μ m z-sections taken every 30 to 60 seconds, with 400 ms exposures.

References

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¹⁴Longer time intervals allow to monitor cells over longer periods of time, but may induce complications due to insufficient aeration, and/or stage drifting. However, significant results have nonetheless been achieved (5 sec time interval over 44 min, 2).

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Figure 1.

Two-dimensional time lapse of *S. cerevisiae* zygotene/pachytene nuclei with chromosomes co-labeled with Zip1-GFP and DAPI, 5hrs after transfer to sporulation medium. Upper panel: series of images showing the Zip1-GFP chromosomes, with 800 ms exposure and at 2 sec intervals. Lower panel: DAPI images of the same nuclei taken 2 seconds after the Zip1-GFP picture shown in the above panel (800 ms exposure). At some locations, DAPI signal reveals regions where chromosomes are not yet entirely synapsed and therefore where no GFP signal is already visible (white arrows). Scale bar = 2 μ m.