Methods to Map Meiotic Recombination Proteins in *Saccharomyces cerevisiae*

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Abstract

Meiotic recombination is triggered by programmed DNA double-strand breaks (DSBs), catalyzed by the type II topoisomerase-like Spo11 protein. Meiotic DSBs are repaired by homologous recombination, which produces either crossovers or non-crossovers, this decision being linked to the binding of proteins specific of each pathway. Mapping the binding of these proteins along chromosomes in wild type or mutant yeast background is extremely useful to understand how and at which step the decision to repair a DSB with a crossover is taken. It is now possible to obtain highly synchronous yeast meiotic populations, which, combined with appropriate negative controls, enables to detect by chromatin immunoprecipitation followed by sequencing (ChIP-Seq) the transient binding of diverse recombination proteins with high sensitivity and resolution.

Key words: Chromatin immunoprecipitation, high throughput sequencing, genome-wide maps, DNA sonication, meiotic proteins, recombination intermediates mapping

1. Introduction

Meiotic recombination in budding yeast is initiated via the programmed formation of DNA double strand breaks in gene promoters, and generally, in nucleosome-depleted regions (NDR) [1]. Among several hundreds of DSB formed per cell, only a subset of these will be repaired with a crossover, the other being repaired through non-crossover events [2]. The choice of repair pathway is controlled by many factors. At the level of single cells, it is governed by the phenomenon of interference, where the presence of a crossover-designated event lowers the probability of a nearby crossover occurring on the same chromosome [3]. In large cell populations, some studies have suggested that this decision could be also governed by specific chromosome features, such as the proximity to a centromere or a telomere [4-6]. One difficulty

to map proteins binding to recombination events in meiosis is that this event is by nature transient, and occurs at a given site in only a small fraction of the cells, at sites called hotspots. This makes it challenging to detect meiotic recombination proteins, especially those predicted to bind for a short time to recombination sites, in contrast with proteins such as transcription factors or chromosome structure proteins that have a longer residency on chromatin. In addition, nucleosome-depleted regions, where meiotic recombination occurs, due to their higher chromatin accessibility, are sometimes retrieved in different amounts than other regions upon purification, and therefore a particular attention should be paid to using the relevant controls, to reveal only the studied protein binding sites, without any artifact due to the nature of the region being analyzed. Indeed, it is very important to use as a control a sample that is processed in an identical way as the real sample, ideally from cell lysates sonicated the same way and immunoprecipitated using the same antibody as the experimental sample. This is particularly important for proteins giving a very low signal over background. For instance, input DNA would not be suitable here as a control. To circumvent these difficulties, we describe here a method to map meiotic recombination proteins by ChIP-Seq, using highly synchronized cells, and using as a negative control a recombination-deficient strain, which allows to reveal recombination-specific only binding sites of a given protein. The combined use of highly synchronized cells and of a recombination deficient strain (such as *spo11Δ*) as the negative control allowed us to map recombination proteins that are difficult to detect by chromatin immunoprecipitation, such as the Pif1 helicase and the Mlh3 crossover protein. To obtain a highly synchronized meiosis, we used the approach developed by Chia and van Werven [7], where the expression of the master regulator of meiosis, *IME1*, is put under the control of an inducible promoter, which allows all cells of the population to “prepare” for meiosis and reach the same stage upon meiosis induction. The ChIP-Seq technique presented here was previously used in our lab to map “ZMM” meiotic recombination proteins [8].

2. Materials

2.1. Media for yeast culture

1. YPD: 1% Bacto yeast extract, 2% Bacto peptone, 2% dextrose. Autoclave (20 min at 120°C) (see note 1).

2. “Semi-YPD”: 1% Bacto yeast extract, 2% Bacto peptone, 1% dextrose. Autoclave (20 min at 120°C) (*see Note 1*).
3. 1% potassium acetate (KAc). Autoclave.
4. 5 % raffinose. Filter-sterilize.
5. 1% poly propylene glycol 2000 (PPG) (Sigma)
6. Sporulation medium: add raffinose to 0.02% and PPG to 0.001% to autoclaved 1% KAc.
7. CuSO₄: 500 mM. Filter-sterilize

2.2. Cell crosslinking and lysis

1. FastPrep-24™ 5G Instrument (MPbio)
2. Thin tips (200 µl) (Dutscher)
3. 2-ml FastPrep screw cap tubes (MPbio).
4. Zirconia/silica beads BioSpec Products (Fisher Scientific)
5. 16% paraformaldehyde solution (Electron Microscopy Sciences)
6. Lysis Buffer: 50 mM Hepes-KOH pH 7.5, 140 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate. Prepare 250 ml. Filter-sterilize.
7. 1 mg/ml Aprotinin (Euromedex). 1 mg aprotinin is dissolved in 1 ml milliQ water.
8. cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail tablets (Roche)
9. 100 mM phenylmethylsulfonyl fluoride (PMSF) solution in isopropanol.

2.3. Immunoprecipitation

1. Bioruptor® Pico sonication device (Diagenode)
2. 15 ml Bioruptor® Pico Tubes & sonication beads (Diagenode)
3. DynaMag™-2 Magnet rack (Thermo Fisher Scientific)
4. Rotating wheel for use at 4°C.
5. 1.5 ml Eppendorf DNA LoBind tubes
6. 100 mg/ml BSA
7. 1 mg/ml c-Myc mouse monoclonal antibody (9E10)
8. Magnetic Dynabeads Pan mouse IgG (Thermo Fisher Scientific) (*see Note 2*)

9. Wash & Bind buffer (100 ml): 95.5 mM NaH₂PO₄, 4.5 mM NaH₂PO₄, 0.01% Tween 20 pH 8.2. Filter-sterilize.
10. Wash Buffer 2: 50 mM Hepes-KOH pH 7.5, 500 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate. Prepare 250 ml. Filter-sterilize.
11. Wash Buffer 3: 10 mM Tris-HCl pH 8.0, 250 mM LiCl, 5 mM EDTA, 0.5% sodium deoxycholate, 0.5% NP-40. Prepare 100 ml. Filter-sterilize.
12. TE Buffer : 10 mM Tris pH 7.5, 1 mM EDTA. Autoclave.
13. IPure kit v2 (Diagenode)
14. Isopropanol 100%

2.4. Samples quality and quantity control

1. Qubit 4 Fluorometer (ThermoFisher Scientific)
2. Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific)
3. Bioanalyzer instrument (Agilent)
4. Bioanalyzer High Sensitivity DNA Analysis (Agilent)

2.5. DNA library preparation and sequencing

1. Illumina® TruSeq®Chip Library Preparation Kit
2. Chloroforme/isoamyl alcohol (v/v, 24 :1)
3. 5PRIME Phase Lock Gel-Heavy tubes, 2 ml (Quantabio)
4. 70% and 100% Ethanol
5. 35 mg/ml glycogen (Support - MP Biomedicals)
6. Illumina HiSeq 2500 instrument

3. Methods

3.1. Highly synchronous meiotic cultures

This section is based on [7] with minor modifications. In this system, cells expressing the *IME1* gene under the *pCUP1* inducible promoter are grown in rich medium containing twice less glucose than the regular YPD (“semi-YPD”), transferred in sporulation medium, to allow all

cells to accumulate in the G1 phase (and perform the diauxic shift), and *IME1* is induced by the addition of copper after 2 h in sporulation medium. In these settings, premeiotic DNA replication should be over at 4 h [7]. When compared to the “classical” sporulation protocol following a pre-growth in SPS medium [9], we observed that DSB and crossover recombination occur in a shorter period of time and DSBs peak at a higher level, due to better cell population synchronization (Figure 1). In these conditions, DSB appear at 4 h and crossovers start appearing at 5 h 30, therefore, to map an “early” recombination protein (such as Pif1) we use the 5 h time-point, for a “middle” recombination protein such as a ZMM protein, we use the 5 h 15 time-point, and for a “late” recombination protein such as Mlh3, we use the 5h30 time-point.

1. Day -4: Streak out the diploid on a YPD plate to generate single colonies and incubate at 30°C for two days.
2. Day -2: At 6 pm, inoculate a single colony in 5 mL YPD and incubate overnight (ON), at 30°C under agitation (250 rpm).
3. Day -1: At 11.15 am, dilute saturated YPD pre-culture 100-fold into 20 ml fresh YPD. Then incubate at 30°C under agitation (250 rpm).
4. At 4.45 pm, cells will be in exponential phase ($OD_{600} < 2$) with an $OD_{600} = 1.7$. Inoculate at $OD_{600} = 0.05$ into 60 ml “semi-YPD”. Incubate ON at 30°C under agitation (250 rpm). Pre-warm the 500 ml autoclaved 1% KAc bottle at 30°C ON.
5. Day 0: At around 9-9.30 am, cells should be at $OD_{600} = 12-13$. Centrifuge 5 min at 5000 x g and discard the supernatant. (see Note 3)
6. Wash the pellet with one volume of prewarmed 1% KAc. Centrifuge 5 min at 5000 x g and discard the supernatant. Make the sporulation medium.
7. Resuspend in sporulation medium to an $OD_{600} = 2.5$. The 0 h time-point starts now. Incubate at 30°C under agitation (250 rpm).
8. After 2 h, add $CuSO_4$ to a 50 μM final concentration to induce the expression of *IME1* (20 μl from a 500 mM stock solution).
9. After 8h after putting in sporulation medium, check meiotic divisions by DAPI staining. A typical percentage of 95-100% binucleate plus tetranucleate cells is expected (Figure 1).

3.2. Cross-linking

The remaining of the protocol is written for one sample of one strain, where the protein to immunoprecipitated is tagged with a myc epitope. It is strongly recommended to process the test and control strain in parallels. We usually perform test and control experiments each in duplicates.

1. Cool PBS at 4°C

2. At the desired time point (for instance 5 h 30 for a late recombination protein), take $\approx 1.10^9$ cells (50 mL of the sporulation culture at $DO_{600}=2.5$) divide them into two 50 mL conical tubes. (*see Notes 4-5*)

3. Add 1.56 ml of 16% paraformaldehyde solution (final 1%) to each 25 ml culture and incubate at room temperature (RT) for 15 minutes on a rotating wheel (40 rpm).

4. Add 1.25 mL 2.5 M glycine (final 0.125 M) to quench the cross-linking reaction.

5. Incubate at RT for 5 minutes on a rotating wheel (40 rpm).

6. Centrifuge 5 min at 5000 x g and discard the supernatant under a fume hood in a suitable container for waste containing formaldehyde.

7. Resuspend pellet in one volume of cold PBS 1X. Centrifuge 5 min at 5000 x g and discard the supernatant.

8. Repeat step 7.

9. Resuspend each pellet in 2.5 mL PBS 1X. Pool the pellets (5 ml total) and distribute into 5 FastPrep tubes.

10. Centrifuge 1 min max speed at 4°C

11. Carefully discard supernatants, no liquid should remain in the tubes

12. Freeze pellets in liquid nitrogen and store at 80°C (or process immediately). (*see Note 6*).

3.3. Preincubation of the beads with antibody

1. Resuspend Pan mouse IgG magnetic beads by vortexing >30 seconds.

2. Transfer 250 µl of magnetic beads to a 2 ml Eppendorf tube

3. Place the tube on the magnet to separate the beads from the solution and remove the supernatant. (Use of the magnetic rack, *see Note 7*)

4. Wash 2 times with 1 ml Wash & Bind Buffer. Use the magnet between each wash to remove the supernatant.

5. Resuspend the beads in 1 ml Wash & Bind Buffer. Add 50 µl of 100 mg/ml BSA and incubate on a rotating wheel at 4°C for four hours. (*see Note 8*)
6. Remove the supernatant with the magnet.
7. Wash 2 times with 1 ml Wash & Bind Buffer. Use the magnet between each wash to remove the supernatant.
8. Resuspend the beads in 1 ml of Wash & Bind Buffer.
9. Add 40 µl 200 µg/ml c-Myc monoclonal antibody (20 µg total) and incubate on a wheel at 4°C for four hours.

3.4. Cell Lysis

1. Prepare 10 ml Lysis Buffer supplemented with protease inhibitors by adding 1 mM PMSF, 50 µg/ml aprotinin and 1X cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail and keep on ice (*see Note 9*).
2. Remove the 5 FastPrep tubes from the freezer from step 3.2.12 and thaw cells on ice.
3. Resuspend each pellet in 500 µl of cold Lysis Buffer supplemented (**Prepared in 1**).
4. Add an approximately equal amount of Zirconia/silica beads (a full 500 µl Eppendorf tube)
5. Process cell lysis in a FastPrep-24™ 5G Instrument with the following program to achieve 3 times: Intensity 6, 30 sec ON and 3 min pause on ice between each cycle
6. Collect the five lysates by pipetting with thin tips to avoid catching beads and pool them to a 15 ml conical tube. (*see Note 10*)
7. Adjust to a final volume of 2.5 ml with Lysis Buffer supplemented with protease inhibitors.

3.5. Sonication (*see Note 11*)

1. Add 900-1000 mg Bioruptor™ beads into a 15 ml Bioruptor™ Tubes graduation (equivalent to the fourth line of the 15 ml Bioruptor™ Tubes graduation scale). Wash beads by vortexing in 3 volumes of PBS 1X. Removed all PBS using thin tips.
2. Add the 2.5 ml lysate (from step 3.4.7).
3. Sonicate to an average fragment size of approximately 0.1-0.5 kb with the pre-cooled Bioruptor™ Pico instrument at 4°C with the following program: 45 cycles of 30 sec pulse, 30 sec pause (45 min total).

- 1 4. Collect the 2.5 ml sonicated lysate by pipetting with thin tips to avoid catching beads and
2 split it into five 1.5 ml Eppendorf tubes.
- 3 5. Clear the sonicated lysate by centrifugation for 5 min at 19000 x g and at 4°C. Transfer the
4 supernatant into new tubes.
- 5 6. Keep a 10 µl aliquot of this supernatant as input control, freeze in liquid nitrogen and store
6 at -20°C. (*see Note 12*)

3.6. Immunoprecipitation

- 10 1. Split 1 ml of magnetic beads plus antibody prepared in step 3.3.9. into five 1.5 ml Eppendorf
11 tube (200 µl each).
- 12 2. Wash 2 times with 1 ml Lysis Buffer. Use the magnet between each wash to remove the
13 supernatant.
- 14 3. Load the sonicated lysate from step 3.2.4.6. on the beads and incubate ON at 4°C on a
15 rotating wheel.
- 16 4. The next day, put tubes on the magnet and remove supernatant.
- 17 5. Perform the following washes each for 1 min on a rotating wheel at room temperature. Use
18 the magnet between each wash to remove the supernatant. (*see Note 13*)
 - 19 a. Wash 2 times with 1 ml Lysis Buffer
 - 20 b. Wash 2 times with 1 ml Wash Solution 2
 - 21 c. Wash 2 times with 1 ml Wash Solution 3
 - 22 d. Wash 1 times with 1 ml TE
- 23 6. Remove completely the supernatant. (*see Note 13*)

3.7. Elution and DNA purification

27 The IPure Diagenode kit is used with the following modifications to elute DNA (For more
28 details see the manufacturer's protocol):

- 29 1. Pre-warm buffer A at 25°C during 30 min before use.
- 30 2. Prepare the Elution Buffer by mixing 692.4 µl Buffer A and 27.6 µl B.
- 31 3. Thaw the 10 µl input sample on ice and add 120 µl of Elution Buffer
- 32 4. Add to the five tubes of IP (step 3.5.5) 120 µl of Elution Buffer and pool them in a new 1.5
33 ml Eppendorf DNA LoBind tube. From this stage use only Eppendorf DNA LoBind tube.

- 1 **5.** Incubate samples and input for 4 hours (or overnight) at 65°C on a thermomixer, with
- 2 continuous shaking.
- 3 **6.** Put IP samples and input tubes on the magnet and after 1 minute, transfer the supernatants
- 4 to a new Eppendorf DNA LoBind tube. Keep the samples on ice.
- 5 **7.** Add 2 µl or 10 µl of carrier to input or IP sample, respectively. Vortex briefly and do a short
- 6 spin.
- 7 **8.** Add 100 µl and 500 µl of 100% isopropanol to input and IP sample, respectively. Vortex
- 8 briefly and do a short spin.
- 9 **9.** Resuspend the Magnetic beads from the kit and add 10 µl to each input and IP sample.
- 10 **10.** Incubate samples for 50 min at room temperature on a rotating wheel (40 rpm).
- 11 **11.** Prepare the Wash buffer A by mixing 330 µl Wash buffer 1 w/o isopropanol and 330 µl
- 12 Isopropanol 100%.
- 13 **12.** Perform a short spin.
- 14 **13.** Put IP samples and input tubes on the magnet and after 1 minute, discard the supernatants
- 15 and add 100 µl Wash buffer A to each tube. From this moment, beads stick very strongly to
- 16 the tube's walls. Be careful to leave the liquid and the beads at the bottom of the tube. Mix
- 17 only by tapping the tubes 6 times.
- 18 **14.** Incubate 5 min at RT, mixing by tapping every 1 min.
- 19 **15.** Prepare the Wash buffer B by mixing 330 µl Wash buffer 2 w/o isopropanol and 330 µl
- 20 Isopropanol 100%.
- 21 **16.** Perform a short spin.
- 22 **17.** Put IP samples and input tubes on the magnet and after 1 minute, discard the supernatants
- 23 and add 100 µl Wash buffer B to each tube. Mix only by tapping the tubes 6 times.
- 24 **18.** Incubate 5 min at RT, mixing by tapping every 1 min.
- 25 **19.** Perform a short spin.
- 26 **20.** Put IP samples and input tubes on the magnet and after 1 minute, discard the supernatants
- 27 and add 40 µl Buffer C to each tube. Mix only by tapping the tubes 6 times.
- 28 **21.** Incubate 30 min at RT, mixing by tapping every 5 min.
- 29 **22.** Perform a short spin.
- 30 **23.** Put IP samples and input tubes on the magnet and after 1 minute, collect the supernatants
- 31 and transfer them to new Eppendorf DNA LoBind Tube.
- 32

33 **3.8. Samples quality and quantity control**

- 1 **1.** Quantify samples concentration. For precise quantification of DNA quantity, we strongly
2 recommend the use of the Qubit® instrument. Follow the manufacturer's protocol. A
3 typical yield of 0.8-2.5 ng/ul for Input and IP DNA respectively, is expected.
- 4 **2.** Test the efficiency of DNA fragmentation by analyzing only the Input DNA size using
5 Bioanalyzer instrument. Follow the manufacturer's protocol. A typical yield of 1-3 ng/ul
6 DNA is expected. The size of DNA fragments should be between 150-300 bp.
- 7 **3.** Freeze samples with liquid nitrogen and store at -80°C.

3.9. DNA library preparation and sequencing

The purpose of this part is to add adapter sequences onto the ends of DNA fragments to generate libraries for paired-end 50 bp reads sequencing. Many new sequencing machines can be used for the sequencing step (Illumina, Ion Torrent...), here we used Illumina HiSeq 2500 instrument. 10 ng of both immunoprecipitated (ChIP-DNA) and non-immunoprecipitated (input DNA) are used to prepare the DNA library for next generation sequencing. The Illumina® TruSeq®Chip Library Preparation Kit was used and involved the following steps (for more details see the manufacturer's protocol):

1. Repair of 3' and 5' ends.
2. Library size selection. Library size is selected using a 1.8X ratio of AMPure XP beads/sample.
3. Addition of adenylate 3' ends
4. Ligation of indexed paired end adapters
5. Library size selection. Library size is selected using two rounds of purification with 1X ratios of AMPure XP beads/sample.
6. Amplification via Polymerase Chain Reaction (PCR) of adaptor ligated DNA
7. Library size selection. Library size is selected using one round of purification with 1X ratios of AMPure XP beads/sample.
8. Libraries validation
9. Normalization and pooling libraries

3.10. Read Normalization and bioinformatic analyses

Our procedure for ChIP-Seq data analysis has been published previously and we summarize here its main steps. Sequencing data are analyzed using custom Bash and R scripts [8].

1. Reads are aligned to the sacCer2 version (SGD June 2008) of the *S. cerevisiae* S288C genome, using Bowtie, allowing for 2 mismatches. Reads that matched more than once in the genome or matching to mitochondrial or ribosomal DNA were eliminated from further analysis.
2. Aligned paired-end reads are extended and then normalized as follows: for the control, the top and bottom outliers of the distribution are removed and the average read number per position (coverage) of the remaining reads is then set to 1. Similarly, top and bottom outliers of the distribution are removed from the average read number in the experimental samples, and average coverage for the remaining reads is calculated, and its ratio compared to the control is used to normalize all reads of the experimental sample
3. Both scaled experimental and control samples are then converted to bigwig format, and the control bigwig file (usually from a *spo11Δ* mutant) is subtracted from the experimental file to generate a normalized, subtracted file that can be used to visualize the protein enrichment along chromosomes.

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4. Notes

1. Volvic™ water quality is constant and it is weakly mineralized so yeast cultures done in media prepared with Volvic™ water are much more reproducible. We recommend to use Volvic™ water but distilled water could also be used.
2. PanMouse IgG beads are only used when the primary antibody is a mouse antibody. For antibodies generated from others species, we use protein G magnetic beads.
3. Example: For one culture at $OD_{600} = 12$, take 41.7 ml culture. After washes in 1% KAc, resuspend the pellet in 200 ml Sporulation medium to obtain a $OD_{600} = 2.5$.
4. The time point for sample collection must be determined for each new protein. To determine the precise moment when the protein enrichment is maximal, it is recommended to make a classical ChIP experiment followed by qPCR analysis as described in [8]. For this, take 10 ml of a *pCUP1-IME1* synchronized culture every hour until 8h after transfer to sporulation medium and follow the protocol described in [8].
5. It is strongly recommended to take twice 50 ml. One sample is processed, and the second serves as a backup sample.

6. This is a freeze point. Flash-freezing of pellets must be done with liquid nitrogen and stored at -80°C until needed. Experiment can be stopped at this point.
7. Place the tube on the magnetic rack and wait 30 s until beads are separated from the solution. Invert the magnetic rack three times from top to bottom to recover all the beads contained in the tube cap. Wait again 30 s and remove all supernatant by aspirating with a needle connected to a vacuum pump.
8. Magnetic beads incubation with BSA before IP permit to decrease nonspecific background. The others option is to do a pre-clearing of your sample.
9. PMSF must be stored at -20°C, and it is recommended to make it fresh. The addition of PMSF to the Lysis Buffer is done just at the time of the experiment.
10. The lysate can also be collected by making a hole in the bottom of the FastPrep tube with a sterile needle. After removing its screw-cap, place quickly the tube into a 15 ml Falcon tube and spin 10 s. at 1000 x g to collect the lysate.
11. We recommend using the Bioruptor™ Pico, and the sonication conditions described are adapted to this instrument. If using another instrument, it is important to properly adapt the sonication conditions to obtain DNA fragmentation around 100-300 bp. Set the cooling device of the sonicator Bioruptor™ Pico at 4°C at least 30 minutes before use.
12. To decrease even more the non-specific background in the IP experiment, you can do a second pre-clearing of your lysate. To do this, incubate your sample with magnetic beads only (without antibody) on a wheel during four hours at 4°C. After four hours, collect the lysate cleared twice and proceed next step.
13. We recommended to use a needle and aspiration to completely remove all traces of supernatant remaining in the lid and the bottom of the tube.

References

1. Pan J, Sasaki M, Kniewel R, Murakami H, Blitzblau HG, Tischfield SE, Zhu X, Neale MJ, Jasin M, Socci ND, Hochwagen A, Keeney S (2011) A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. Cell 144 (5):719-731. doi:S0092-8674(11)00123-1 [pii] 10.1016/j.cell.2011.02.009
2. Hunter N (2015) Meiotic Recombination: The Essence of Heredity. In: Cold Spring Harb Perspect Biol. doi:10.1101/cshperspect.a016618

3. Muller HJ (1916) The mechanism of crossing over. *Am Nat* 50:193-434
4. Chen SY, Tsubouchi T, Rockmill B, Sandler JS, Richards DR, Vader G, Hochwagen A, Roeder GS, Fung JC (2008) Global analysis of the meiotic crossover landscape. *Developmental Cell* 15 (3):401-415. doi:S1534-5807(08)00285-2 [pii] 10.1016/j.devcel.2008.07.006
5. Serrentino ME, Borde V (2012) The spatial regulation of meiotic recombination hotspots: Are all DSB hotspots crossover hotspots? *Experimental Cell Research* 318 (12):1347-1352. doi:10.1016/j.yexcr.2012.03.025
6. Serrentino ME, Chaplais E, Sommermeyer V, Borde V (2013) Differential association of the conserved SUMO ligase Zip3 with meiotic double-strand break sites reveals regional variations in the outcome of meiotic recombination. *PloS Genetics* 9 (4):e1003416. doi:10.1371/journal.pgen.1003416
7. Chia M, van Werven FJ (2016) Temporal Expression of a Master Regulator Drives Synchronous Sporulation in Budding Yeast. *G3 (Bethesda)*. doi:10.1534/g3.116.034983
8. De Muyt A, Pyatnitskaya A, Andreani J, Ranjha L, Ramus C, Laureau R, Fernandez-Vega A, Holloch D, Girard E, Govin J, Margueron R, Coute Y, Cejka P, Guerois R, Borde V (2018) A meiotic XPF-ERCC1-like complex recognizes joint molecule recombination intermediates to promote crossover formation. *Genes & development* 32 (3-4):283-296. doi:10.1101/gad.308510.117
9. Murakami H, Borde V, Nicolas A, Keeney S (2009) Gel electrophoresis assays for analyzing DNA double-strand breaks in *Saccharomyces cerevisiae* at various spatial resolutions. *Methods in molecular biology* 557:117-142
10. Hunter N, Kleckner N (2001) The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. *Cell* 106 (1):59-70
11. Brachet E, Beneut C, Serrentino ME, Borde V (2015) The CAF-1 and Hir Histone Chaperones Associate with Sites of Meiotic Double-Strand Breaks in Budding Yeast. *PLoS One* 10:e0125965. doi:10.1371/journal.pone.0125965

Figure 1: Comparison of the timing of recombination events in “classical” SPS versus pCUP1-IME1-synchronized meiotic time-courses.

A: Southern blot monitoring the formation and repair of DNA DSBs at the *HIS4LEU2* hotspot, in a strain containing pCUP1-IME1 and induced to undergo meiosis by the addition of Cu⁺⁺ at 2 h after transfer to sporulation medium. P1 and P2: parental DNA fragments. CO1 and CO2:

1 bands corresponding to the crossover repair of *HIS4LEU2* DSB. DSB: DSB fragments from the
2 DSB formed at the *HIS4LEU2* hotspot [10] B: Quantification of DSB formation (DSB) and
3 repair as a crossover (%CO) from the experiment shown in A. C: Quantification of DSB
4 formation (DSB) and repair as a crossover (%CO) from a meiotic time-course using the
5 “classical” SPS meiotic time-course. Data are from [11].

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