

Chapter 2

Analysis of *Schizosaccharomyces pombe* Meiosis by Nuclear Spreading

Josef Loidl¹ and Alexander Lorenz²

¹Department of Chromosome Biology, University of Vienna, Dr. Bohr-Gasse 1, 1030 Vienna, Austria
(e-mail: josef.loidl@univie.ac.at)

²Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom
(e-mail: alexander.lorenz@bioch.ox.ac.uk)

Methods in Molecular Biology (2009) 558: 15-36
Scott Keeney (ed.) Meiosis, Volume 2: Cytological Methods

The final publication is available at link.springer.com
(http://dx.doi.org/10.1007/978-1-60761-103-5_2)

Abstract The fission yeast, *Schizosaccharomyces pombe*, much like the budding yeast, is a particularly well-suited model organism for genetic research. However, the miniscule size of both yeasts' nuclei has hindered their success as research models for cytologists. A solution to this problem is provided by the spreading of nuclei, which increases their volume and allows for a better spatial resolution of nuclear contents. Here we describe nuclear spreading in fission yeast. Spreading of meiotic nuclei is particularly helpful in exposing the linear elements (LinEs), which are the fission yeasts' rudimentary version of the synaptonemal complex. Although the LinEs' role is still not fully understood, they serve as important meiotic hallmarks and their presence and morphology can be used in characterizing meiotic mutants. We first describe methods to induce meiosis in liquid cell cultures, then outline a method to break down cell and nuclear membranes by detergent treatment to release chromatin on cytological slides, and finally provide a set of protocols for analyzing these nuclei by immunostaining and fluorescence in situ hybridization (FISH), and by electron microscopy.

Key words Linear element, meiosis, nuclear spreading, fluorescence in situ hybridization, immunocytochemistry, microscopy, Rad51, synaptonemal complex, chromosome, nucleus

1. Introduction

Instead of a canonical synaptonemal complex (SC), linear elements (LinEs) are present during the stage when meiotic pairing and recombination take place in *S. pombe* (1). They are biochemically related to the axial elements of SCs and are likely evolutionarily derived from them (2, reviewed in Ref. 3). At the same time, this stage is characterized by the presence of elongated nuclei, the so-called horsetails which seem to be the fission yeasts' functional equivalent to the bouquet (4,5). In these nuclei, the telomeres of all chromosomes are assembled at the spindle pole body, which in turn is in contact with microtubules. They confer an oscillating movement to the nuclei during which they are stretched into the horsetail shape and which contributes to lining up the equal sized homologs inside the nucleus which promotes the initiation of homologous pairing (6,7).

Horsetail nuclei are a good indicator of the pairing stage in a meiotic culture and can, in addition to flow cytometry (FACS analysis) of cell samples, be used to monitor the progress of meiosis, for example, for obtaining staged samples from a time course experiment for biochemical analyses. In addition to the staging of meiotic cultures, cytology is often performed in its own right for studies of individual cells. Several meiotic mutants are characterized by a lack of or by

abnormal development of LinEs, and the appearance of foci of recombination proteins can also be used for the characterization of mutants.

SCs and LinEs were originally detected in ultrathin sections prepared and contrasted for electron microscopy (see Refs. 1,8,9). In conventionally fixed cells, they were not visible by light microscopy. Only whole mount nuclear spreading methods, which liberate these structures from the surrounding chromatin made them amenable to detection after impregnation with silver. A variant of the spreading method using a detergent has found widespread application in animal and plants, as well as in the budding yeast (10). Later, it became possible to delineate LinEs by immunostaining of its various components (2), and while this may be feasible with conventionally prepared (unspread) nuclei in certain mutant backgrounds (11), some of the LinEs' characteristics can be analysed conveniently only when nuclei are spread (12). The advantage of spreading is the unfolding of the densely packed nuclear contents, which results in a gain in spatial resolution. It is, however, achieved at the cost of destroying the integrity and spatial organization of nuclei.

Here we describe a detailed protocol for the spreading of meiotic *S. pombe* nuclei and the staining of LinEs and other nuclear structures in these spreads by immunostaining techniques. Moreover, we describe how these spreads can also be used for FISH and how spread nuclei can be transferred to the electron microscope for high resolution analysis of silver-stained LinEs.

2. Materials

2.1. Cell Growth and Preparation

1. Rich medium, yeast extract medium (YE): 5 g/l yeast extract (Difco - marketed by Becton, Dickinson and Company, <http://www.bd.com/>), 30 g/l glucose in distilled water, autoclaved. (For solid medium 18 g/l agar is added.)
2. Presporulation medium, *pombe* minimal medium (PM): 5 g/l NH₄Cl, 3 g/l potassium hydrogen phthalate, 1.8 g/l Na₂HPO₄, 20 g/l glucose, 20 ml/l 50× salts stock (see below), 1 ml/l 1,000× vitamins stock (see below), 0.1 ml/l 10,000× minerals stock (see below) in distilled water, autoclaved. (For solid medium, 18 g/l agar is added.)
3. Sporulation medium, *pombe* minimal medium without a nitrogen source (PM-N): 3 g/l potassium hydrogen phthalate, 1.8 g/l Na₂HPO₄, 10 g/l glucose, 20 ml/l 50× salts stock (see below), 1 ml/l 1,000× vitamins stock (see below), 0.1 ml/l 10,000× minerals stock (see below) in distilled water, autoclaved.
4. Solid sporulation medium, malt extract agar medium (MEA): 30 g/l malt extract, 20 g/l agar in distilled water, pH adjusted to 5.4 with NaOH, autoclaved.
5. Stocks for minimal media:
 - 50× salts stock: 52.5 g/l (0.26 M) MgCl₂·6H₂O, 50 g/l (0.67 M) KCl, 2 g/l (14.1 mM) Na₂SO₄, 0.735 g/l (4.99 mM) CaCl₂·2H₂O in distilled water, autoclaved.
 - 1,000× vitamins stock: 10 g/l nicotinic acid, 10 g/l inositol, 1 g/l pantothenic acid, 0.01 g/l biotin in distilled water, filter sterilized.
 - 10,000× minerals stock: 10 g/l (47.6 mM) citric acid, 5 g/l (80.9 mM) boric acid, 4.5 g/l (23.7 mM) MnSO₄·H₂O, 4 g/l (13.9 mM) ZnSO₄·7H₂O, 1.5 g/l (7.4 mM) FeCl₂·4H₂O, 1 g/l (6.02 mM) KI, 0.4 g/l (1.6 mM) CuSO₄·5H₂O, 0.36 g/l (2.47 mM) molybdenum(VI)oxide in distilled water, filter sterilized. All stocks are stored at 4°C.
6. To ensure vigorous growth and sporulation of auxotrophic strains, the following supplements are added as required: adenine, uracil, lysine hydrochloride, leucine, histidine and/or arginine monohydrochloride to a final concentration of 225 mg/l in YE as well as MEA, 75 mg/l in PM and 10 mg/l in PM-N (see **Note 1**).
7. Stocks for enzyme cocktail for digesting the yeast cell wall: Prepare stock solutions of 10 mg/ml Zymolyase 100T (Seikagaku Co., Tokyo, Japan) in distilled water, 100 mg/ml lysing enzyme from *Rhizoctonia solani* (L8757, Sigma, St Louis, MO, USA) and 250 mg/ml lysing enzyme from *Trichoderma harzianum* (L1412, Sigma) in 0.65 M KCl (see **Note 2**).
8. Spheroplasting solution: 0.65 M KCl with 10 mM dithiothreitol (prepared from a frozen 0.5 M stock). Add 20 µl Zymolyase, 20 µl L8757 and 60 µl L1412 stock solutions per ml.
9. Protease inhibitor: 1 M phenylmethylsulfonyl fluoride (PMSF) in dimethyl sulfoxide (DMSO).
10. Stop solution: 0.1 M 2-(*N*-morpholino)ethane sulfonic acid (MES), 1mM EDTA, 0.5 mM MgCl₂, 1 M sorbitol in distilled water; adjust pH with NaOH to 6.4.
11. Detergent: Prepare a 1% solution of "Lipsol Detergent Concentrate" (now marketed by

Barloworld Scientific (<http://www.barloworld-scientific.com/>) in distilled water. The working solution can be stored for several months in the refrigerator (*see Note 3*).

12. Fixative: 4% paraformaldehyde supplemented with 3.6% sucrose (*see Note 4*).
13. 10× phosphate-buffered saline (PBS) stock: 80 g/l NaCl, 2 g/l KCl, 11.5 g/l Na₂HPO₄·7H₂O, 2 g/l anhydrous KH₂PO₄. Make up to 900 ml with distilled water, adjust pH to 7.5 using 1 M NaOH or 1 M HCl, add distilled water to 1,000 ml (*see Note 5*).

2.2. Immunostaining

1. 1× PBS + Triton X-100 (PBS-T): Add 0.5 ml Triton X-100 to 1,000 ml 1× PBS.
2. Humid chamber. Cover the bottom of an airtight plastic box or glass container which will provide space for several horizontally placed slides with filter paper or some other water-absorbent material and moisten with distilled water. Put slides inside the box on a tray to avoid contact with the water. The humid chamber will prevent the drying out of preparations which are incubated with small amounts of liquid under the cover slip.
3. Primary antibodies. Antibodies against various components of LinEs, recombination proteins, nucleoli, spindle pole bodies and other contents of meiotic nuclei are available upon request from non-commercial sources. Rabbit antibodies against Rec10 protein were created in the lab of Ramsay McFarlane (University of Wales at Bangor, UK) and a guinea pig antibody against Hop1 protein was made in the Loidl lab. A mouse monoclonal antibody against recombinant Rad51 protein (Clone 51RAD01, NeoMarkers, Fremont, CA, USA) works well with *S. pombe* Rad51. As an alternative to anti-Rec10 antibodies, an HA-tagged version of Rec10 constructed in the lab of Ramsay McFarlane can be used for the visualization of the LinEs. Strains expressing tagged versions of, for example, Rec7, Rec8, Rad32, Mek1 and Taz1 have been published (13-18). Most of these fusion proteins can be detected in spread nuclei (2,19 and unpublished observations). The authors can provide details of sources of various antibodies and strains carrying tagged proteins which can be detected in nuclear spreads.

4. Antibodies against GFP-, Myc- and HA-tags, and secondary antibodies: Purchased from various vendors.
5. DAPI (4'6-diamidino-2-phenylindole) as a DNA-specific counterstain. Prepare a 1 mg/ml stock solution in distilled water which can be stored at -20°C. DAPI is applied at final concentration of 1 µg/ml.
6. Antifade buffer to reduce bleaching: 245 mg diazabicyclo(2.2.2)octane + 200 µl 1 M NaHCO₃ (pH 8.0) + 800 µl distilled water + 9 ml glycerol. Alternatively, antifade buffers can be purchased under the tradenames Vectashield (Vector Laboratories Inc., Burlingame, CA, USA) or Slow Fade (Molecular Probes Inc., Eugene, OR, USA) (*see Note 6*).
7. Rubber cement (e.g. Fixogum, Marabuwerke GmbH, Tamm, Germany) or nail varnish for sealing slides.

2.3. FISH

1. Cosmids. Clones from the desired chromosomal loci can be selected from the *Schizosaccharomyces pombe* Gene Database (<http://www.genedb.org/genedb/pombe/>) and obtained from John Woodward at the Sanger Centre, Hinxton, UK (http://www.sanger.ac.uk/Projects/S_pombe/cloneres.shtml) (*see Note 7*).
2. Probes produced by PCR. Fragments of ca. 5 to 10 kb sizes are produced by long-range PCR (suitable kits are available from various companies: e.g. Expand™ Long Template PCR System (Roche Diagnostics, Basel, Switzerland); TaKaRa Ex Taq (TaKaRa Shuzo Co., Ltd., Otsu, Japan)) with appropriate primers from the *Schizosaccharomyces pombe* Gene Database (*see Note 7*).
3. 10× labeling buffer: 500 mM Tris, pH 8.0, 50 mM MgCl₂, 500 µg/ml BSA (Sigma, Chemical Co., St. Louis, MO, USA).
4. 1 mM dATP/dCTP/dGTP mixture.
5. Labeled nucleotides: e.g. Cy3-dUTP, Cy5-dUTP (Amersham Pharmacia Biotech, Uppsala, Sweden), fluorescein-dUTP, tetramethylrhodamin-dUTP, digoxigenin-dUTP or biotin-dUTP (Roche Diagnostics, Basel, Switzerland).
6. 280 mM β-mercaptoethanol.
7. DNase I (Roche Diagnostics, Basel, Switzerland).
8. *E. coli* DNA polymerase I (10U/µl, New England BioLabs, Beverly, MA, USA).

9. RNase, DNase free (Roche Diagnostics, Basel, Switzerland).
10. ST buffer: 4× SSC (0.6 M NaCl, 60 mM trisodium citrate, pH 7.0), 0.1% Tween-20.
11. 20× SSC: 3.0 M NaCl, 0.3 M trisodium citrate, pH 7.0.
12. 3 M Na-acetate pH 5.5.
13. Hybridization mixture: 4× SSC, 20 % dextran sulfate, 1 µg/µl sonicated salmon sperm DNA.
14. Rubber cement for sealing slides (as above).
15. Heating block or a thermocycler capable of holding slides (HYBAID Ltd., Ashford, England).
16. Blocking buffer: 3% BSA, 4× SSC.
17. Detection buffer: 1% BSA, 4× SSC, 0.1% Tween-20.
18. Detection reagents: e.g. Avidin-FITC conjugate, Extravidin®-FITC conjugate, Extravidin®-Cy3 conjugate; Biotin-conjugated anti-avidin monoclonal antibody (Sigma, Chemical Co., St. Louis, MO, USA); anti-digoxigenin-fluorescein, anti-digoxigenin-rhodamin, anti-digoxigenin-AMCA (Roche Diagnostics, Basel, Switzerland)
19. Anti-fading medium (as above).
20. DAPI (as above).

2.4. Silver Staining of Linear Elements

1. AgNO₃ solution: 5 g AgNO₃ in 10 ml ultrapure water (see **Note 8**). Caution: Corrosive; wear eye protection and gloves!
2. Polyamide cloth: e.g., Nybolt PA-100/31 (Swiss Silk Bolting Cloth Mfg. Co. Ltd., Zurich, Switzerland).

2.5. Spread Preparations for Electron Microscopy

1. N-hexane for removal of immersion oil.
2. 1% hydrofluoric acid. Prepared from 40% stock. Caution: Follow the safety regulations of your institution. Wear eye protection and gloves. Work under a chemical hood!
3. 1% solution of Formvar resin (polyvinyl formal) in chloroform.
4. EM grids (e.g. square 50 mesh, Agar Scientific Ltd., Stansted, England).
5. Benchkote paper (Schleicher & Schuell, Dassel, GER).

3. Methods

For culturing, recombination testing and other methods which are relevant to the study of meiosis we refer the reader to Ref. 20 and the references given therein, including the web resource

<http://www.pombe.net/> maintained by the Forsburg lab. All liquid cultures should be in flasks filled to only 1/10th of their volume and continuously agitated in a shaker.

3.1. Cell Growth and Sporulation

S. pombe is a haploid, i.e., when two starving haploid cells of opposite mating type (=sexes) mate to produce a diploid zygote, it will immediately undergo meiosis (=zygotic meiosis). However, the diploid state can be maintained in the lab if zygotes are quickly transferred to rich medium. The diploid state in fission yeast is relatively unstable and cells will sporulate at a low frequency even on rich medium. The most common method of stabilizing *S. pombe* diploids is to use heterozygotes for the intragenically complementing *ade6* alleles, *ade6-M210* and *ade6-M216*. While *ade6-M210* or *ade6-M216* haploid or aneuploid strains are *ade-* and thus cannot grow on selective PM plates lacking adenine, the heterozygous diploid *ade6-M210/ade6-M216* is *ade+* and able to grow on selective minimal plates (see **Note 9**). Azygotic meiosis in cultures of diploid cells can be induced more synchronously by transfer to nutrient-poor medium (PM-N) than the mating and zygotic meiosis by the mixing of haploid cultures. Therefore, if possible, it is recommended to work with diploid cells undergoing azygotic meiosis. *S. pombe* meiotic cytology is best done with cells which were sporulated in liquid medium but under certain circumstances cells can be scratched from sporulation plates. The following protocols describe a meiotic timecourse experiment of an *ade6-M210/ade6-M216* diploid (see **Note 10**).

3.1.1. Diploid *ade6-M210/ade6-M216* Azygotic Timecourse (Meticulous Protocol)

1. Streak strain for single colonies on solid YE medium (see **Note 11**), and grow for 4 days at 30°C.
2. Inoculate 3 tubes of 10 ml liquid YE with a single colony each and incubate shaking for 23 – 24 h at 30°C.
3. Transfer 50 – 100 µl of each of the above precultures to 10 ml fresh liquid YE and incubate shaking for another 23 – 24 h at 30°C. In addition, drop 30 – 50 µl of each of the precultures onto an MEA plate and incubate at 30°C overnight.
4. Test the three cultures on MEA for sporulation by transferring some material to a drop of

water on a slide and examining under the microscope. Continue the timecourse with the best-sporulating culture.

5. Dilute 2 ml and 4 ml of preculture into 200 ml presporulation medium (PM) each and incubate for 15 – 16 h (overnight) at 30°C (see **Note 12**).
6. Determine the cell density in the cultures; this should be between 1×10^7 and 2×10^7 cells/ml (see **Note 13**).
7. Harvest the culture with the right cell density by centrifugation (2,000 rpm, 4 min), wash once with 50 ml sterile distilled water, resuspend at 1×10^7 cells/ml in PM-N and start the timecourse.
8. Take 1 ml samples every hour from $t=0$ to $t=10$ h (for some mutant backgrounds it may be necessary to extend this period) to assess the progression of meiosis. For this purpose, spin the sample shortly (~10 sec) at full speed (13,000 rpm), discard the supernatant and resuspend the cells in 70% ice-cold ethanol (samples can be kept at -20°C for several weeks). For microscopic evaluation collect the cells by centrifugation (13,000 rpm, 10 sec) and resuspend in 20 μ l of distilled water. Mix 5 μ l of cell suspension with 5 μ l DAPI in anti-fading medium on a slide and examine under the microscope for uni-, bi- and tetranucleates as well as horsetail-stage cells (**Fig. 1**). There should be a high percentage of uninucleate cells (~80 %) at $t=0$ and $t=2$ h, and >50% binucleate cells (representing the end of premeiotic mitosis) at $t=1$ h. In poorly synchronized cultures, these figures deviate; if good synchrony is crucial for the experiment, it should be terminated at this point (see **Note 14**). Alternatively or additionally, the progress of the timecourse can be monitored by determining the DNA content of the cells via flow cytometry (FACS) (see Ref. 20).
9. Take 5 ml samples every hour from $t=3$ to $t=8$ h (this may vary for mutants) for spreading (**Section 3.2**).

3.1.2. Diploid *ade6-M210/ade6-M216* Azygotic Timecourse (Fast Track Protocol)

This protocol does not aim at a high sporulation frequency or good synchrony. It reduces the time from starting liquid cultures from a diploid strain on a plate to the harvest of meiotic cells from 4 to 2 days by dispensing with the selection of sporulation-competent colonies. This may produce

a culture of which only a subset of cells will sporulate, but for most cytological applications (where meiosis is studied at the level of individual cells) it may be sufficient if meioses occur in a reasonable proportion of cells.

1. In the morning, choose 3-5 clearly visible white (*ade+*) colonies from YE plates which should not be older than one week and resuspend them in 10 ml YE liquid. Incubate the tube in the shaker (200 rpm) at 30°C. The cells should reach a density of 0.5×10^7 to 1×10^7 cells/ml by the evening. (Doubling time is ~2.5 h)
2. In the evening, inoculate PM with the pellet from the YE culture and incubate the PM culture in the shaker (200 rpm) at 30°C. In PM, the generation time is about 3 h per doubling. Allowing for a lag time of 2-3 h (during which cells recover from handling and adjust to the new medium), a culture starting with 6×10^5 cells/ml should reach 1×10^7 cells/ml after 14-16 h (e.g., from 18:00 to 8:00)
3. In the morning, check the cell titer of the PM cultures with a counting chamber. It should be between 1×10^7 and 2×10^7 cells/ml (best is close to 1×10^7). If it is less than 1×10^7 , let the culture grow longer. If it is more than 2×10^7 , dilute to 0.5×10^7 cells/ml in fresh PM and allow growth to continue for 3 h.
4. Harvest the culture by centrifuging (2,000 rpm, 4 min), wash once with 50 ml sterile, distilled water, and resuspend at 1×10^7 cells/ml in sporulation medium (PM-N) and start the timecourse.
5. Take samples; most LinEs will be found in the 5, 6, and 7 h timepoints.

3.1.3. Azygotic *pat1-114* Timecourse

The *pat1-114* allele, which encodes for a temperature-sensitive version of the Ran1/Pat1 kinase, a key inhibitor of meiosis in *S. pombe* (see Ref. 5), is often used to induce meiosis in a highly synchronous fashion both in haploid as well as diploid cells. Meiosis in a *pat1-114* strain can be simply triggered by raising the temperature from the permissive 25°C to the restrictive 34°C. The cells will enter meiosis irrespective of their mating type, nutritional and cell cycle stage. It is essential that cells are well starved, otherwise a large proportion will enter meiosis from mitotic G₂ phase (**21**). In this case, chromosomes will have loaded mitotic cohesin and undergo meiotic missegregation. Also note that even diploid *pat1-*

114 meiosis deviates from wild-type meiosis in several respects (lower recombination rates, fewer Rad51-foci, aberrant LinE formation) (22, A. Baudrimont, J.L. and A.L., unpublished observations). Nevertheless, *pat1-114* strains can be useful for cytology, for example if a certain background is unstable as a diploid. The following protocol is modified after Ref. 23.

1. Inoculate 10 ml of liquid YE medium with a fresh *pat1-114* haploid or *ade6-M210/ade6-M216 pat1-114/pat1-114* diploid strain from a YE plate (see **Note 11**) and incubate at 25°C for ~20 h.
2. Inoculate 40 ml PM with 4 ml of preculture and let it grow for ~42 h at 25°C.
3. Determine cell density and resuspend in 200 ml PM at 2×10^6 cells/ml. Let this culture grow for ~6 h at 25°C and determine the cell density (which should be around 4×10^6 cells/ml). Centrifuge cells (2,000 rpm, 4 min) and wash once with 50 ml of sterile distilled water. Resuspend cells in 200 ml PM-N at 4×10^6 cells/ml and incubate at 25°C for 16 – 18 h.
4. Remove the culture from the incubator and raise temperature to 34°C. Check cell density; it should be between 0.8×10^7 and 2×10^7 cells/ml (see **Note 15**). Add 2 ml of 50 g/l NH₄Cl and adenine, uracil and lysine to a final concentration of 75 mg/l each. Add leucine, histidine and/or arginine to a 75 mg/l end concentration depending on the auxotrophies of the strains. Put the culture back into the incubator and start the time course.
5. Draw test samples and samples for spreading as described in **Section 3.1.1**, Steps 8 and 9, respectively: 1 ml-samples for DAPI-staining are drawn hourly from t=0 to t=6 h and a final one after 24 h (see **Note 14**) (**Fig. 1B**). 5 ml-samples for spreading (see **Section 3.2**) are drawn every 30 min from t=3 h to t=5 h (may vary with mutant backgrounds) (see **Note 16**).

3.2. Nuclear Spreading

The spreading protocol described here is a modification by Lorenz *et al.* 2006 (19) of the method by Bähler *et al.* 1993 (12) (see **Note 17**). While in the original paper a mixture of Zymolyase 100T and Novozym 234 was used for spheroplasting, the modified method uses an enzyme cocktail (19) (see **Sections 2.1.7** and **2.1.8**) (see **Note 18**).

1. Take 5 ml of a cell suspension obtained according to one of the procedures under **Section 3.1**.
2. Spin and resuspend the pellet in 1 ml spheroplasting solution (see **Section 2.1.8**) and add 2 µl protease inhibitor (see **Section 2.1.9**).
3. Spheroplast cells for 25-30 min at 30°C on the shaker.
4. Add 9 ml of stop solution (see **Section 2.1.10**), harvest the spheroplasts by centrifugation (2,000 rpm, 4 min) and resuspend them by carefully agitating with a pipette in 150 - 200 µl of stop solution (see **Note 19**). Add a further 1 µl of protease inhibitor.
5. Drop 20 µl of cell suspension onto a slide, add 40 µl fixative, 80 µl detergent, mix by slightly tilting the slide, wait for 30 sec, then add another 80 µl fixative (see **Notes 20, 21**).
6. Spread out the mixture with a glass rod and put slides in a chemical hood overnight (see **Note 22**).
7. Continue with one of the **Sections 3.3, 3.4.2, or 3.6**, or freeze slides at -20°C for later use.

3.3. Immunostaining of LinEs and other Structures in Spread Nuclei

Rec10 is the main protein of LinEs and Rec10 immunostaining is particularly useful for the detection of LinEs and the study of their development in the wild type and in mutants (**Fig. 2A-E**). Likewise, immunostaining of other LinE-associated proteins (such as Hop1 - **Fig. 3A**) and recombination proteins (such as Rad51 - **Fig. 3B**) can contribute to the characterization of meiotic mutants.

1. Wash slides that have been prepared according to **Section 3.2** three times for 15 min in PBS-T (**Section 2.2.1**) and drain excess liquid.
2. Incubate slides with a drop of primary antibody (diluted in 1× PBS; the appropriate dilution, usually 1:50 to 1:200, has to be tested empirically) under a coverslip in a humid chamber (**Section 2.2.2**) at room temperature overnight. Some of the background due to antibody cross-reaction can be removed by antibody preabsorption against total protein (acetone powder) extracted from an *S. pombe* strain that does not produce that specific protein (see **Note 23**).
3. Rinse coverslip away with 1× PBS, wash slides three times for 15 min in PBS-T and drain excessive liquid.

4. Incubate slides with a fluorochrome-conjugated secondary antibody (diluted in 1× PBS according to the instructions of the provider) under a coverslip for 90 min at room temperature.
5. Rinse coverslip away with 1× PBS, wash slides three times for 15 min in PBS-T and drain excessive liquid.
6. Mount preparations in anti-fading medium supplemented with DAPI.
7. Seal with rubber cement if the preparations should be kept for more than a few days. The slides may be stored in the refrigerator for about one week. For longer periods they should be frozen at -20°C.

3.4. (Multicolor) FISH

FISH is useful for assessing homologous pairing (**Fig. 3C**) and segregation during meiosis similar to the *lacO/LacI-GFP* system. One obvious disadvantage is that FISH does not work with live cells to examine chromosome movements during a meiotic time course. On the other hand, it is comparably easy to highlight different loci without the need for constructing strains with appropriate *lacO* inserts.

3.4.1. Labeling of the Probes

The probes are labeled by the incorporation of Cy3-dUTP, Cy5-dUTP, fluorescein-dUTP, tetramethylrhodamin-dUTP (direct labeling) or digoxigenin-dUTP or biotin-dUTP (indirect labeling), using standard nick translation procedures (*see Note 24*).

In the following, a typical nick translation labeling reaction is described:

1. Mix 2 µg probe DNA (*see Note 25*), 5 µl 10× labeling buffer, 1.5 µl 280 mM β-mercaptoethanol, 5 µl 0.5 mM dATP/dCTP/dGTP mixture, 1.5 µl 1 mM labeled dUTP, 2 µl DNase I (~1:1,000 from a 1 mg/ml stock; the optimal dilution must be empirically determined), 1 µl *E. coli* DNA polymerase I (10 U/µl), and bring the reaction volume to 50 µl with dH₂O (*see Note 26*).
2. Incubate the reaction mixture at 15°C for 105 - 120 min.
3. Stop the reaction by adding 2.5 µl 0.5 M EDTA and incubate for 15 min at 65°C.
4. Precipitate DNA by adding 1/20 volume of 3 M Na-acetate (pH 5.5) and 2.5 volumes of ice-cold pure ethanol over night at -20°C, centrifuge at full speed (13,000 rpm) for 30 min at 4°C, discard the supernatant and add 300 µl 70 % ethanol without disturbing the pellet, centrifuge again (13,000 rpm, 15 min, 4°C), pour off the ethanol, air dry the pellet and resuspend in 30 µl 1× TE or sterile distilled water.

3.4.2. Hybridization

For some applications, it is advantageous to inspect both immunostained structures and FISH signals in one and the same nucleus (e.g. if the spatial relationship of specific chromosomal loci to LinEs or recombination foci is of interest). While we did not succeed in immunostaining slides following the FISH procedure, FISH can be performed on immunostained slides. Some strong staining (such as by the anti-Rec10 antibody) is sometimes still weakly visible after subsequent FISH. If this is not the case, pictures have to be taken of immunostained nuclei and their coordinates on the slides be recorded. Following FISH, the nuclei are looked up again (*see Note 27*).

1. When starting with spread preparations (**Section 3.2**) that were not subject to prior immunostaining, incubate the preparations in distilled water until the sucrose-layer has dissolved (~5 - 10 min). Continue with step 3.
2. When starting with immunostained preparations (**Section 3.3**), remove immersion oil (if any) by slightly dabbing with filter paper and strip off the rubber sealing (if any). Rinse coverslip away with 1× PBS, wash with distilled water (~5 - 10 min) to remove anti-fading medium. Continue with Step 3.
3. Drain the slide and let it dry (*see Note 28*).
4. Apply 50 µl DNase-free RNase (100 µg/ml in 2× SSC) to each slide, cover with a coverslip, and incubate for 30 min at 37°C in a humid chamber (**Section 2.2.2**).
5. Incubate the slide in ST buffer (**Section 2.3.10**) at 37°C for 1 - 3 h.
6. To denature chromosomal DNA, place the slide in 70% formamide solution (in 2× SSC, pH 7.0) at 60°C for 2 min, then immediately immerse in ice-cold 70%, 90%, and 96% ethanol for 5 min each, and air dry.
7. Dry probe DNA in a vacuum concentrator. Use 1 µl (approx. 66 ng) per slide. If you apply two or three pooled probes to the same slide, mix 1 µl of each.
8. Resuspend the dried DNA in 3 µl 100% formamide by shaking vigorously for 30 min,

add 3 μ l of hybridization mix and shake for another 10 – 30 min.

9. Denature the probe DNA by heating to 95°C for 5 min and then chill on ice.
10. Apply 6 μ l of denatured probe mix onto each slide, place a coverslip (12 \times 12 mm) over the sample and seal with rubber cement.
11. Put slides on a thermocycler for slides and expose them for 10 min to 80°C (for co-denaturation of chromosomal DNA with probe mix) and then to 37°C for at least 36 h (for hybridization).
12. Peel off the rubber cement and gently rinse off the coverslip with 2 \times SSC.
13. Wash slides for 5 min in each of the following buffers: 50% formamide in 2 \times SSC (37°C), 2 \times SSC (37°C), and 1 \times SSC (room temperature).
14. After hybridization with directly labelled DNA, apply a drop of antifade medium with DAPI, and seal under a coverslip (see **Section 2.2**, agents 5, 6 and 7).
15. After hybridization with digoxigenin- or biotin-labelled DNA, go to **Section 3.4.3** (signal detection).

3.4.3. Detection of Digoxigenin- or Biotin-Labelled DNA

1. After washing the slides (**Section 3.4.2**, Step 13), put a large drop of blocking buffer (**Section 2.3.16**) under a coverslip and incubate slide for 1 h at 37°C in a humid chamber (**Section 2.2.2**).
2. To detect biotinylated probes, rinse coverslip away with detection buffer and incubate slides with 50 μ l FITC- or Cy3-conjugated (Extra-) avidin diluted in detection buffer under a coverslip for 1 h at 37°. Continue with Step 4 or 9.
3. To detect digoxigenin-labeled probes, apply FITC-, rhodamin- or AMCA- conjugated anti-digoxigenin antibody (see **Note 29**) and proceed as with applying secondary antibody in **Section 3.3** (immunostaining), Steps 3-6.
4. Weak signals of a biotin labeled probe can be amplified as follows:
5. Rinse coverslip away with ST buffer (**Section 2.3.10**), wash slides twice for 5 min in ST buffer and drain excess liquid.
6. Incubate preparation with a biotin-conjugated anti-avidin monoclonal antibody for 1 h at 37°C as above.

7. Rinse coverslip away with ST buffer, wash slides twice for 5 min in ST buffer and drain excess liquid.
8. Incubate preparation with FITC- or Cy3-conjugated (Extra-) avidin as in Step 2.
9. Rinse coverslip away with ST buffer, wash slides twice for 5 min in ST buffer and drain excess liquid.
10. Mount preparation in anti-fading medium supplemented with DAPI.

3.5. Microscopic Evaluation of Fluorescent Signals

For immunostaining and FISH, an epifluorescence microscope equipped with a mercury lamp and appropriate filter sets for the excitation and emission of fluorescence spectra characteristic for the fluorochromes used, is necessary to visualize signals. The optimal combinations of excitation filter, beam splitter and emission filter must be tested. Filter selection is a compromise between narrow band width (weaker signals) and wide spectrum (other fluorochrome may "leak through") (10). Images are best recorded with a cooled CCD camera with high sensitivity to a wide spectrum of wavelengths, including far-red (as emitted by Cy5).

3.6. Silver Staining of Linear Elements

1. Incubate slide (from **Section 3.2**) in distilled water until the sucrose-layer has dissolved.
2. Drain the slide and let it dry for several hours.
3. Apply several drops of AgNO₃ solution to the slide and cover with a piece of polyamide cloth, trimmed to the size of a coverslip (see **Note 30**).
4. Incubate slides in a humid chamber (**Section 2.2.2**) at 60°C for 40 min.
5. Rinse away the cloth with distilled water and allow the preparation to dry.
6. Inspect the slide (see **Note 31**) without embedding under a coverslip, as most microscopical mounting media will bleach or dissolve the silver deposit. For prolonged storage, immersion oil can be removed from the slide by 1-3 min incubation in hexane.

3.7. Transfer of Light-microscope Preparations to the Electron Microscope (EM)

Silver-impregnation produces highly contrasted structures which are suitable for inspection in the electron microscope (**Fig. 2F**). For this purpose, preparations on glass slides can be transferred to electron microscopy grids.

1. If required, remove immersion oil by incubating the slide for 1-3 min in hexane.
 2. Dip slide in Formvar solution and retract slowly such that a thin supporting film will deposit on the slide (*see Note 32*).
 3. Identify at low magnification (no immersion oil!) regions of interest and mark them with a water-resistant marker pen.
 4. Scratch the coating with a diamond glass-writer around the area with the markings. Put small drops of 1% hydrofluoric acid onto the slide; it will dissolve the glass surface and help to detach the Formvar film together with the cells from the slide. Caution: hydrofluoric acid and its vapours are corrosive and toxic. Follow the safety regulations of your institution. Work in a chemical hood and wear eye protection and gloves.
 5. Add water until the plastic film floats. Submerge the slide in a large bowl with water such that the coating will come off and float.
 6. Place EM grids on the marked regions of interest.
 7. Push the plastic film together with the grids under the surface with the edge of a piece of Benchkote paper such that the plastic film will attach to the smooth side of the paper and the EM grids will become sandwiched between the plastic film and the paper (*see Note 33*).
 8. After the film has dried, pick off the EM grids together with the adhering plastic film with fine tweezers; they are now ready for inspection in the EM.
3. Lipsol is a laboratory cleaning agent, a mixture of nonionic and anionic detergents plus a chelating agent and builders (information by the manufacturer). Several standard laboratory detergents (Nonidet, Triton X-100, sodium dodecyl sulfate, *N*-lauroyl sarcosine) were tested as alternatives but gave unsatisfactory results in our hands.
 4. 4 g paraformaldehyde is heated in 90 ml distilled water on a magnetic stirrer to 80°C (Caution: formaldehyde vapours! Work under a chemical hood!). After 20-30 min the solution should become clear. If it stays opaque, add 1 M NaOH until it becomes clear. Add 3.4 g sucrose to the solution after cooling. If NaOH has been added, the solution has to be titrated back to pH 8.5. Make up the volume to 100 ml. If the fixative is not completely clear, it can be filtered. It can be stored for several months in the refrigerator.
 5. There are almost as many recipes for PBS as there are labs. A simpler alternative for 10× PBS is: 90 g/l NaCl, 11.5 g/l anhydrous Na₂HPO₄, 2.3 g/l anhydrous NaH₂PO₄, make up to 1,000 ml with distilled water. Both worked in our hands.
 6. It is convenient to prepare antifade medium containing 1 µg/ml DAPI so that embedding and DAPI staining can be performed in a single step.
 7. FISH probes should be carefully selected and their sequence checked against the database to avoid the inclusion of repetitive genomic elements (e.g. transposons), which would result in unspecific speckled background staining.
 8. Working solution can be stored for several months in the refrigerator. If a black or brown deposit is formed, the solution should be filtered. Old AgNO₃ or used AgNO₃ solution should not be released into the environment. Check with a recycling service; it can be collected together with photographic fixer for the recycling of silver.
 9. If kept on PM for extended periods, diploid strains will sporulate. Therefore, they should be maintained on YE plates with no adenine supplement. While some haploid *ade*⁻ colonies will establish themselves on these plates, they can be easily discerned from the diploids because they are smaller and colored red (*ade6-M210*) or pink (*ade6-M216*) (20,24). Slowly growing strains can be kept on casein plates (1.7 g/l yeast nitrogen base without ammonium sulfate and amino acids (Difco), 3.7 g/l sodium

4. Notes

1. Supplements can be directly added in powder form to the YE and MEA before autoclaving; for use with PM and PM-N, it is more practical to prepare mixed supplement stocks of appropriate concentrations (e.g. 7.5 g/l) in distilled water and filter sterilize. Adenine and uracil tend to precipitate, so warm up the stocks before adding to the medium.
2. The stocks can be stored at -20°C for several months and repeatedly frozen and thawed. In the Zymolyase stock, the powder does not dissolve completely; therefore, after thawing a pellet is present which must be stirred up before use. Alternatively, the stock can be prepared in 10% (w/v) glucose instead of water.

- glutamate, 20 g/l glucose, 10 g/l casamino acids, 0.1 g/l uracil, 20 g/l agar).
10. If possible, the strain should not carry auxotrophic markers other than *ade6*, since they impair synchronous sporulation of the strain.
 11. Media in this experiment must not contain adenine supplement to avoid the untimely emergence of haploid cells.
 12. Two cultures with different cell densities are started to improve the chance that one of them will have the right density the next morning.
 13. If the cell density is much higher than 2×10^7 cells/ml, the culture should be discarded and the experiment started anew, since subsequent sporulation will be asynchronous.
 14. While the careful monitoring of meiotic progression may not be crucial for all experiments, it is advisable to take a sample after ~24 h to assess the sporulation efficiency of the culture.
 15. If the cell density is much higher you may prefer to abort the experiment since the frequency of cells entering meiosis will drop.
 16. After 4 h, the temperature should be decreased to 30°C, since the cells are already committed to meiosis and it is unnecessary to stress them unreasonably.
 17. This spreading protocol was originally developed for diploid strains. Spreading of haploid (e.g. *pat1-114*) strains seems to work better if the cells are killed by adding Na-azide (final concentration 0.1% from a 10% stock in water; ATTENTION: toxic!) to the cell suspension in **Section 3.2**, Step 1.
 18. Novozym 234 is not produced any more and the alternatively used lysing enzyme L2265 (Sigma) (25) is no longer available either.
 19. This suspension can be stored on ice for several hours.
 20. The presence of sucrose in the fixative has the additional advantage that the mixture is hygroscopic and does not dry out completely. Therefore, this kind of preparation can be used for immunostaining even after storage for several days in the refrigerator or several months in the freezer.
 21. Fixative is added to the slide before and after the detergent. A small amount of fixative present during detergent spreading prevents the disruption of spheroplasts but does not interfere too much with spreading. The relative amounts and order of application of nuclear suspension, detergent and fixative should be optimized by testing since the optimal spreading depends on the density of nuclei in the suspension, the degree of spheroplasting and the age of solutions. The process of spreading can be watched in the phase contrast microscope at low magnification without a coverslip. Spheroplasts should swell slowly, and turn continuously from white to black and then to grey. They should not "explode" instantly!
 22. Spheroplasts readily stick to the slides. It is, therefore, not necessary to coat slides with polylysine, but it may be a good idea to clean them with ethanol before use.
 23. To prepare acetone powder (26), collect 100 ml of a meiotically induced cell culture by centrifugation, wash once with a sterile 0.9% NaCl-solution and finally resuspend the cells in the same solution (1 ml of saline per 1 g of cells), keep on ice for 5 min. Add 4 ml acetone (-20°C) per 1 ml cell suspension and mix vigorously, incubate on ice for 30 min (mix every 5 min). Harvest by centrifugation (1,000×g, 10 min), discard the supernatant and resuspend precipitate in 100 % acetone (-20°C) by mixing vigorously and keep on ice for another 10 min. Collect precipitate again (1,000×g, 10 min), discard supernatant and spread the precipitate onto a clean sheet of filter paper, let the pellet air-dry at room temperature while dispersing it on the filter paper (avoid formation of large lumps). The dry acetone powder is moved to an airtight tube and can be stored at 4°C. For preabsorption, incubate diluted antiserum for 30 min at 4°C with the acetone powder at a final concentration of approximately 1% (w/v) in an Eppendorf tube, centrifuge for 10 min at full speed (~13,000 rpm) and transfer the supernatant to a fresh Eppendorf tube.
 24. Since nucleotides tagged with fluorochromes (Cy3-dUTP, Cy5-dUTP, fluorescein-dUTP) have become commercially available, direct probe labeling is becoming more and more common, and it is sufficient for most applications. However, if numerous different fluorochromes are needed for multicolor FISH or if fluorescence intensity of directly labelled probes is insufficient, it may be useful to work with indirectly labeled probes. Direct labeling of the DNA probe with the fluorescent tags Cy3, Cy5 or FITC usually produces a sufficiently

- strong signal to be readily detected. For small probes, indirect labeling is recommended.
25. We isolate cosmid DNA with the help of the QIAGEN Plasmid Midi Kit (Qiagen Inc.), following the instructions of the manufacturer.
 26. Labeling reactions should be optimized to give labeled products of 100 - 500 bp in length, by adjusting the DNase I concentration. The size of the product should be monitored on an agarose gel.
 27. Finding nuclei is facilitated if low-magnification overviews of DAPI-stained groups of nuclei are also photographed. These patterns are easily recognized in the microscope at low magnification. We have a success rate of close to 90% in re-locating nuclei.
 28. We noticed that slides which were left at this point to season for a couple of days worked better for FISH than fresh slides.
 29. AMCA is a blue fluorescent dye. It cannot be used in combination with DAPI as a DNA counterstain. Use propidium iodide (red) instead of DAPI.
 30. Polyamide cloth produces a homogeneous staining all over the coated area, whereas by using a coverslip instead, regions near the edges are stronger stained than the interior. A possible explanation for the enhancing effect, based on the chemical interaction of the polyamide with the staining reaction, is given by Herickhoff *et al.* 1992 (27).
 31. If the silver staining turns out too pale, modify the protocol by transferring slides to sodium tetraborate buffer (pH 9.2; Merck, Darmstadt, GER) for 30 sec after Step 1. Also, using different brands or batches of nylon cloth may influence staining intensity. Increasing the time or temperature of the incubation with silver nitrate solution is not recommended as it tends to enhance unspecific precipitation of silver.
 32. Work in a dry environment. Moist slides may cause holes in the plastic coating.
 33. Parafilm can be used instead of Benchkote.

Acknowledgments We are grateful to Eveline Doll and Katja Ludin (University of Berne), Harry Scherthan (Inst. Radiobiology Bw, Munich); Jörg Fuchs (Leibnitz Institute, Gatersleben) and Mario Spirek (University of Vienna) for technical hints and expert advice, and to Maria Siomos (Gregor Mendel Institute, Vienna) for correcting the manuscript. We thank Julia P. Cooper (Cancer Research UK), Jürg Kohli (University of Berne), Ramsay J. McFarlane (University of Wales, Bangor), Paul Nurse (The Rockefeller University), Pedro San-Segundo (University of Salamanca) and John Woodward (Sanger Centre, Hinxton) for kindly providing strains, antibodies and other materials mentioned in this article. Work in the lab of J.L. is supported by grant P18186 of the Austrian Science Fund (FWF). A.L. is an Erwin Schrödinger-Fellow of the FWF.

References

1. Olson L.W., Edén U., Mitani M.E., Egel R. (1978) Asynaptic meiosis in fission yeast? *Hereditas* **89**, 189-199
2. Lorenz A., Wells J.L., Pryce D.W., Novatchkova M., Eisenhaber F., McFarlane R.J., Loidl J. (2004) *S. pombe* meiotic linear elements contain proteins related to synaptonemal complex components. *J. Cell Sci.* **117**, 3343-3351
3. Loidl J. (2006) *S. pombe* linear elements: the modest cousins of synaptonemal complexes. *Chromosoma* **115**, 260-271
4. Chikashige Y., Ding D.-Q., Funabiki H., Haraguchi T., Mashiko S., Yanagida M., Hiraoka Y. (1994) Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270-273
5. Yamamoto M., Imai Y., Watanabe Y. (1997) Mating and sporulation in *Schizosaccharomyces pombe*. In: Pringle J.R., Broach J.R., Jones E.W. (eds) *The molecular and cellular biology of the yeast Saccharomyces*. Cell cycle and cell biology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp.1037-1106
6. Niwa O., Shimanuki M., Miki F. (2000) Telomere-led bouquet formation facilitates homologous chromosome pairing and restricts ectopic interaction in fission yeast meiosis. *EMBO J.* **19**, 3831-3840
7. Ding D.-Q., Yamamoto A., Haraguchi T., Hiraoka Y. (2004) Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. *Dev. Cell* **6**, 329-341
8. Loidl J. (2003) Chromosomes of the budding yeast, *Saccharomyces cerevisiae*. *Int. Rev. Cytol.* **222**, 141-196
9. Hirata A., Tanaka K. (1982) Nuclear behavior during conjugation and meiosis in the fission yeast *Schizosaccharomyces pombe*. *J. Gen. Appl. Microbiol.* **28**, 263-274
10. Loidl J., Klein F., Engebrecht J. (1998) Genetic and morphological approaches for the analysis of meiotic chromosomes in yeast. In: Berrios M. (ed) *Nuclear structure and function*. Academic Press, San Diego, pp.257-285
11. Ding D.-Q., Sakurai N., Katou Y., Itoh T., Shirahige K., Haraguchi T., Hiraoka Y. (2006) Meiotic cohesins modulate chromosome compaction during meiotic prophase in fission yeast. *J. Cell Biol.* **174**, 499-508
12. Bähler J., Wyler T., Loidl J., Kohli J. (1993) Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. *J. Cell Biol.* **121**, 241-256
13. Watanabe Y., Nurse P. (1999) Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* **400**, 461-464
14. Molnar M., Parisi S., Kakihara Y., Nojima H., Yamamoto A., Hiraoka Y., Bozsik A., Sipiczki M., Kohli J. (2001) Characterization of *rec7*, an early meiotic recombination gene in *Schizosaccharomyces pombe*. *Genetics* **157**, 519-532
15. Tuzon C.T., Borgstrom B., Weilguny D., Egel R., Cooper J.P., Nielsen O. (2004) The fission yeast heterochromatin protein Rik1 is required for telomere clustering during meiosis. *J. Cell Biol.* **165**, 759-765
16. Jin Y., Uzawa S., Cande W.Z. (2002) Fission yeast mutants affecting telomere clustering and meiosis-specific spindle pole body integrity. *Genetics* **160**, 861-876
17. Pérez-Hidalgo L., Moreno S., San-Segundo P.A. (2003) Regulation of meiotic progression by the meiosis-specific checkpoint kinase Mek1 in fission yeast. *J. Cell Sci.* **116**, 259-271
18. Nakamura T.M., Moser B.A., Russell P. (2002) Telomere binding of checkpoint sensor and DNA repair proteins contributes to maintenance of functional fission yeast telomeres. *Genetics* **161**, 1437-1452
19. Lorenz A., Estreicher A., Kohli J., Loidl J. (2006) Meiotic recombination proteins localize to linear elements in *Schizosaccharomyces pombe*. *Chromosoma* **115**, 330-340
20. Forsburg S.L., Rhind N. (2006) Basic methods for fission yeast. *Yeast* **23**, 173-183
21. Chikashige Y., Kurokawa R., Haraguchi T., Hiraoka Y. (2004) Meiosis induced by inactivation of Pat1 kinase proceeds with aberrant nuclear positioning of centromeres in the fission yeast *Schizosaccharomyces pombe*. *Genes Cells* **9**, 671-684
22. Bähler J., Schuchert P., Grimm C., Kohli J. (1991) Synchronized meiosis and recombination in fission yeast: observations with *pat1-114* diploid cells. *Curr. Genet.* **19**, 445-451
23. Cervantes M.D., Farah, J.A., Smith G.R. (2000) Meiotic DNA breaks associated with recombination in *S. pombe*. *Mol. Cell* **5**, 883-888
24. Moreno S., Klar A., Nurse P. (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Meth. Enzymol.* **194**, 795-823
25. Molnar M., Doll E., Yamamoto A., Hiraoka Y., Kohli J. (2003) Linear element formation and their role in meiotic sister chromatid cohesion and chromosome pairing. *J. Cell Sci.* **116**, 1719-1731
26. Harlow E., Lane, D. (1988) *Antibodies. A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp.1-726
27. Herickhoff L., Stack S., Robertson J. (1992) Staining plant cells with silver. III. The mechanism. *Biotech. Histochem.* **67**, 171-182

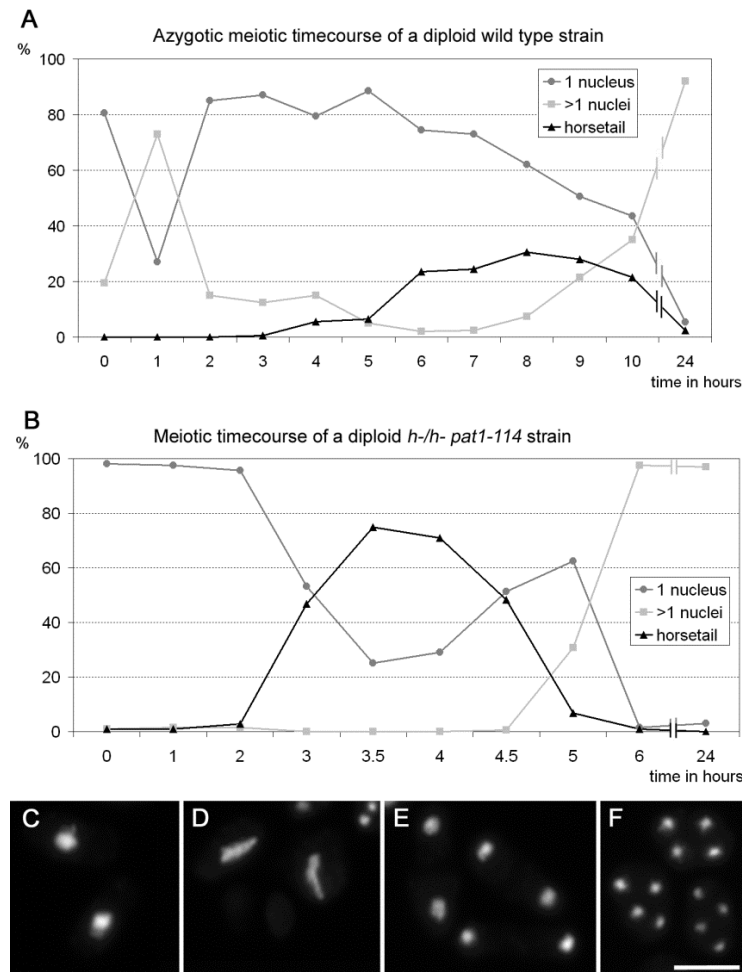


Figure 1. (A, B) Frequencies of different morphological cell stages during typical meiotic timecourse experiments (at least 200 nuclei were inspected for each timepoint) and (C-F) examples of ethanol-fixed and DAPI-stained cells from a wild-type timecourse. (A) Azygotic wild-type timecourse as described in **Section 3.1.1**. Several criteria are used to determine synchronicity and proper meiotic progression: At timpoint $t=1$ h binucleate cells should be more abundant than uninucleate cells. Horsetail stage cells should peak between $t=5$ h and $t=8$ h. At $t=24$ h more than 85% of cells should have produced asci with 4 spores. (B) A diploid *pat1-114* timecourse as described in **Section 3.1.3**. Horsetail nuclei should not be frequent before $t=3$ h and normally peak at $t=3.5$ h or $t=4$ h. At $t=6$ h most of the cells should have undergone meiosis I. After 24 h >85% of the cells should have formed asci. (C) The "1 nucleus"-stage. (D) Horsetail nuclei. (E) Binucleate cells which represent post-mitotic stages at early timepoints and the stage between the first and the second meiotic division at later timepoints. (F) Groups of four nuclei, representing ascospores. Binucleate cells and asci are pooled into the class ">1 nucleus" in the diagrams. Bar in F represents 5 μm.

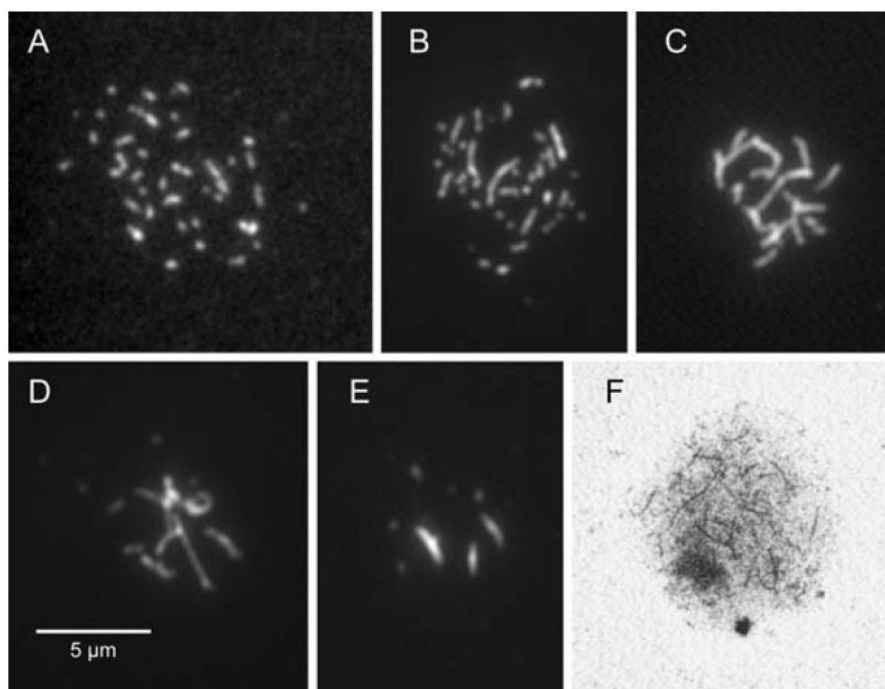


Figure 2. Visualization of spread LinEs in the light microscope and in the electron microscope. (A – E) Rec10 immunostaining of LinEs at different stages of development. (F) LinEs visualized in the electron microscope by silver contrasting.

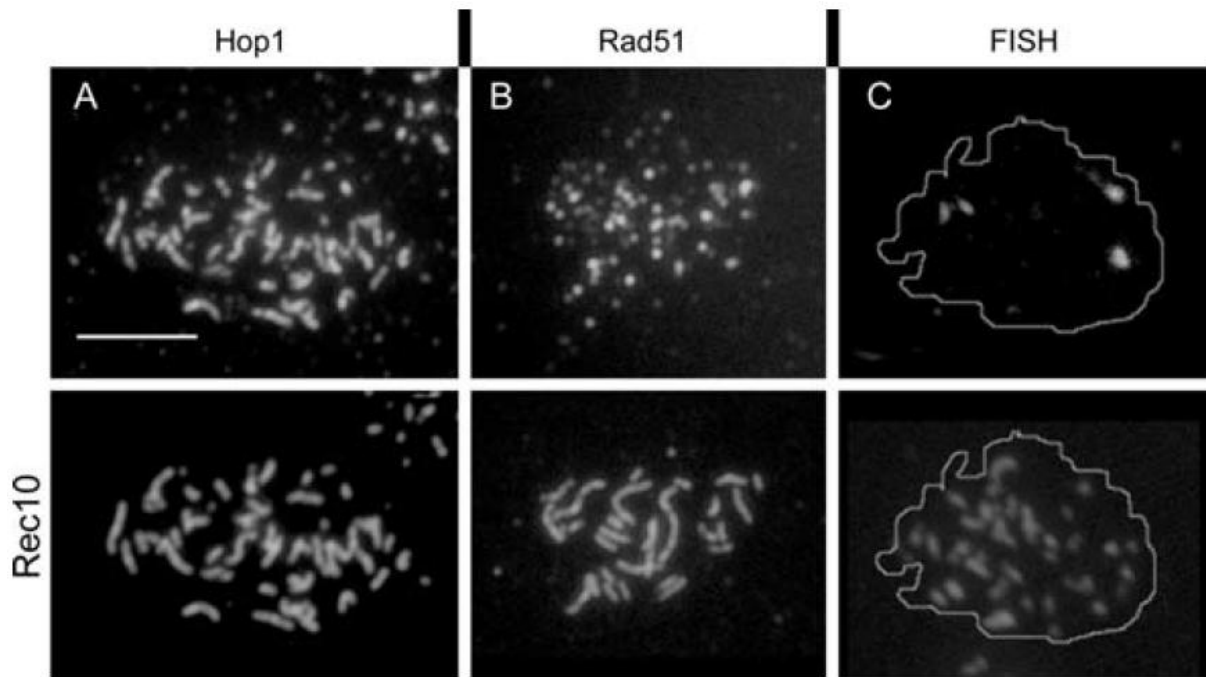


Figure 3. Localization of LinE-associated proteins by double immunostaining and monitoring of homologous chromosome pairing by FISH in spread nuclei. (A) Double immunostaining of Hop1 (top) and Rec10 (bottom). Hop1 localizes to most of the length of the LinEs. (B) Double immunostaining of Rad51 (top) and Rec10 (bottom). The recombination protein Rad51 forms foci on LinEs. (C) Simultaneous FISH with two different probes (top), cosmids SPAC550 (the two spots to the left, originally delineated in red) and SPAC922 (to the right, originally delineated in green). The corresponding loci are unpaired. LinEs are immunostained for Rec10 (bottom). Contours of the nuclei as seen by DAPI counterstaining are indicated. Bar: 5 μ m.