Dynamics of Chromosome Organization and Pairing during Meiotic Prophase in Fission Yeast

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Abstract. Interactions between homologous chromosomes (pairing, recombination) are of central importance for meiosis. We studied entire chromosomes and defined chromosomal subregions in synchronous meiotic cultures of *Schizosaccharomyces pombe* by fluorescence in situ hybridization. Probes of different complexity were applied to spread nuclei, to delineate whole chromosomes, to visualize repeated sequences of centromeres, telomeres, and ribosomal DNA, and to study unique sequences of different chromosomal regions.

In diploid nuclei, homologous chromosomes share a joint territory even before entry into meiosis. The centromeres of all chromosomes are clustered in vegetative and meiotic prophase cells, whereas the telomeres cluster near the nucleolus early in meiosis and maintain this configuration throughout meiotic prophase. Telomeres and centromeres appear to play crucial roles for chromosome organization and pairing, both in vegetative cells and during meiosis. Homologous pairing of unique sequences shows regional differences and is most frequent near centromeres and telomeres. Multiple homologous interactions are formed independently of each other. Pairing increases during meiosis, but not all chromosomal regions become closely paired in every meiosis. There is no detectable axial compaction of chromosomes in meiotic prophase. *S. pombe* does not form mature synaptonemal complexes, but axial element–like structures (linear elements), which were analyzed in parallel. Their appearance coincides with pairing of interstitial chromosomal regions. Axial elements may define minimal structures required for efficient pairing and recombination of meiotic chromosomes.

In sexually reproducing eukaryotes, two consecutive meiotic divisions are required to form gametes with new combinations of genetic information. Homologous chromosomes (homologues) specifically pair and recombine with high frequency during meiotic prophase I. These processes are crucial for their proper segregation from each other at the first meiotic division (reviewed by Giroux, 1988; Hawley, 1988). During prophase I, specific protein structures (axial elements) are formed along each pair of sister chromatids, and the process of chromosome pairing culminates in a close synapsis of the axial elements that become the lateral elements of the tripartite synaptonemal complex (SC)[1] (for review see von Wettstein et al., 1984; Giroux, 1988). The role of the SC in meiotic chromosome function is not understood, but there is increasing evidence that chromosome pairing and meiotic recombination can occur without SC formation (Roeder, 1990; Padmore et al., 1991; Hawley and Arbel, 1993; Loidl et al., 1994; Weiner and Kleckner, 1994). When, where, and how the homologues recognize each other and initiate pairing is still largely unknown (for discussion see Loidl, 1990; Moens, 1994).

Individual chromosomes occupy specific territories in the interphase nuclei of higher eukaryotes (Hilliker and Appels, 1989; Haaf and Schmid, 1991; Cremer et al., 1993). Such defined spatial arrangements of chromosomes may facilitate homologous interactions during meiosis. In *Drosophila*, it has been shown genetically (e.g., Henikoff and Dreesen, 1989; Pirrotta, 1990) and cytologically (Hiraoka et al., 1993) that the homologues are associated in somatic cells. Somatic chromosome associations might also be important for other eukaryotes (Tartof and Henikoff, 1991; Kleckner and Weiner, 1993 and references therein). Centromeres and telomeres contain special DNA sequences essential for the segregation and maintenance of eukaryotic chromosomes (Blackburn and Szostak, 1984). They may also contribute to the spatial organization of chromosomes in the nucleus (e.g., Gilson et al., 1993; Funabiki et al., 1993). Rabl (1885)

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1. *Abbreviations used in this paper*: DAPI, 4',6-diamino-2-phenylindole; FISH, fluorescence in situ hybridization; rDNA, ribosomal DNA; SC, synaptonemal complex; SPB, spindle pole body.

proposed that the chromosomes maintain the polarized telophase configuration throughout interphase, with centromeres clustered at one pole and telomeres localized at the nuclear periphery at the opposite pole. Since then, many organisms exhibiting a Rabl orientation of interphase chromosomes have been reported (Fussell, 1987; Funabiki et al., 1993, and references therein). During meiotic prophase of some organisms, all telomeres become clustered in an area near the centrosome, leading to a bouquet arrangement of meiotic chromosomes (reviewed by Fussell, 1987; Loidl, 1990). Several reports suggest that the telomeres represent important initiation sites for meiotic chromosome pairing (for review, see Loidl, 1990; Gilson et al., 1993; Dawe et al., 1994).

As a haplontic organism, the fission yeast *Schizosaccharomyces pombe* normally enters meiosis immediately after mating (zygotic meiosis). Diploid strains can be obtained (Egel, 1973) and shifted to nitrogen-free medium to induce an azygotic meiosis (Egel and Egel-Mitani, 1974; Bähler et al., 1991, 1993). Large quantities of synchronous meiotic cells can be analyzed in this way. *S. pombe* is proficient for meiotic recombination and shows an exceptionally high number of crossovers per bivalent (Munz et al., 1989; King and Mortimer, 1990). Similar to other eukaryotes (Baker et al., 1976), these crossovers seem to be crucial for proper chromosome segregation during meiosis I (Ponticelli and Smith, 1989). Interestingly, meiotic recombination and chromosome segregation are not accompanied by the formation of a mature SC in fission yeast (Olson et al., 1978; Hirata and Tanaka, 1982; Bähler et al., 1993). Spreading and sectioning of meiotic nuclei have revealed structures (linear elements) that are probably equivalent to unsynapsed axial cores of other eukaryotes. Functions of these linear elements in preparing the chromosomes for meiosis I segregation have been proposed (Bähler et al., 1993; Kohli and Bähler, 1994). Since linear elements do not synapse in fission yeast, it is of special interest to investigate whether and how chromosomes become paired during meiosis. In other organisms, the SC eventually leads to a close association along entire chromosomes, but it does not seem to function in early recognition and pairing of homologues (see above). Therefore, fission yeast also provides an opportunity to study chromosome pairing without the obscuring influence of SC formation.

S. pombe has a small genome consisting of only three chromosomes (Kohli et al., 1977) of about 5.7, 4.6, and 3.5 Mbp for chromosomes I, II, and III, respectively (Fan et al., 1989). This makes it an ideal organism to study aspects of chromosome organization and pairing. Recently, fluorescence in situ hybridization (FISH) has been applied to yeasts as a powerful technique to localize specific DNA sequences along chromosomes (Scherthan et al., 1992*a*; Uzawa and Yanagida, 1992; Funabiki et al., 1993; Bähler et al., 1994; Guacci et al., 1994; Loidl et al., 1994; Weiner and Kleckner, 1994).

We present a quantitative analysis of chromosome organization and pairing before and during azygotic meiotic prophase of fission yeast. Whole chromosomes and subregions were visualized by FISH with DNA probes of different complexity. Painting of chromosomes with composite probes indicated that chromosomes are organized into nuclear domains (territories), and that the homologues occupy joint territories in the diploid nucleus. Probes recognizing centromeres and telomeres of all chromosomes revealed specific clustering behavior of these loci. Single-copy cosmid probes, hybridizing to centromeric, telomeric, and interstitial regions of chromosome II allowed to study and compare pairing of defined chromosomal subregions. We could demonstrate regional differences in the extent of homologous interactions before and during meiotic prophase. In any given meiosis, only a subset of chromosomal regions forms interhomologue connections. Pairing interactions are formed most frequently in late meiotic prophase in the absence of any detectable chromosome condensation. Centromeres and telomeres appear to be key structures in the nuclear organization and pairing of chromosomes. In parallel, the formation of linear elements was studied and temporally compared to chromosomal behavior. These linear elements may represent essential structures required to prepare the chromosomes for segregation during meiosis I.

Materials and Methods

Strains, Culture Conditions, and Preparation of Whole-mount Spread Nuclei

The diploid standard strain JB8 (*h*$^+$/*h*$^-$ *ade6-M216/ade6-149*) and the diploid *mei4* mutant strain JB9 (*h*$^+$/*h*$^-$ *ade6-M216/ade6-149 mei4-B2/mei4-B2*) were used (Bähler et al., 1993). The cells were cultured and shifted to meiosis-inducing medium as described by Bähler et al. (1993). At different times after the shift to meiotic conditions, cells were protoplasted and nuclei were spread as described (Loidl et al., 1991; Bähler et al., 1993). The spread nuclei were either processed for in situ hybridization (see below) or were silver stained and examined by electron microscopy (Bähler et al., 1993).

DNA Probes and Labeling

The single-copy cosmid clones were kindly provided by E. Maier and H. Lehrach (Imperial Cancer Research Fund, London, United Kingdom) (Maier et al., 1992; Hoheisel et al., 1993). For painting of chromosome I, 105 cosmids covering almost the entire chromosome were used. They were organized in 3 probe pools of 35 cosmids each. Chromosome II was painted with 15 pooled cosmids from 5 contigs mapping to both chromosome ends, the centromere region, and two interstitial regions in the short and long arm, respectively. As single-copy probes of chromosome II, we used the cosmids 16F7, 11C10, 19C2, 15D4, and 16A3 for chromosomal regions 1–5, respectively (Hoheisel et al., 1993; see Fig. 4). As a probe for all fission yeast, centromeres we used the plasmid pKH-K, which contains a complete K repeat (Clarke et al., 1986) as a 6.4-kbp Cla I fragment, cloned into the ClaI site of KS$^+$ Bluescript (Baum, M., K. Hahnenberger, and L. Clarke, personal communication). The K repeat is present in ~2, 3, and 12 copies on chromosomes I, II, and III, respectively (Steiner et al., 1993). The terminal telomeric repeats common to all chromosomes of fission yeast are too small to be visualized by FISH (~300 bp: Matsumoto et al., 1987; Allshire et al., 1988). Therefore, the plasmid pNSU21 was used as a probe against telomere-adjacent sequences. This plasmid contains ~7.9 kbp of telomere-associated repeated sequences that are found at chromosomes I and II of fission yeast (Sugawara, N., personal communication). To detect the nucleolar organizer regions, which are located at the ends of chromosome III, we used a conserved ribosomal DNA (rDNA) probe from the fungus *Phanerochaete chrysosporium* (Scherthan et al., 1992*a*).

Transformed *Escherichia coli* cells were grown in LB medium, and DNA was isolated with a purification kit according to the instructions of the manufacturer (Qiagen Inc., Chatsworth, CA). 1 μg of purified probe DNA was labeled with biotin-14-dATP (Life Technologies, Inc., Gaithersburg, MD) or digoxigenin-11-dUTP (Boehringer Mannheim GmbH, Mannheim, Germany) using a nick translation kit (Life Technologies, Inc.). In the case of the rDNA probe, ratio labeling was achieved by nick translating the probe in the presence of dig-11-dUTP and bio-11-dATP in a molar ratio of 1:1. After ethanol precipitation the labeled DNA probes were resuspended at

20 ng/μl in hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate, 1 μg/μl sheared salmon DNA), and were stored at −20°C. For details of the labeling procedure, see Scherthan et al. (1992*b*).

Fluorescence In situ Hybridization

FISH was performed as described (Scherthan et al., 1992*a*, 1993) with minor modifications. Briefly, preparations were incubated for 8 h in 4× SSC/0.1% Tween 20, 1 μg/ml RNase A at 37°C. Preparations were then rinsed in distilled H_2O and air dried. DNA probes were applied to the slide and sealed under a coverslip with rubber cement. Denaturation was performed by placing the slide with the probe for 10 min on a hot plate at 72°C. Slides were incubated for hybridization at 37°C for 36 h. After three washes in 0.03× SSC at 40°C, they were transferred into BT/0.5%BSA for 5 min (BT = 0.15 M $NaHCO_3$, 0.1% Tween 20, pH 8.3). Solution containing avidin-FITC (1:250 in BT; Sigma Immunochemicals, St. Louis, MO) and a mouse antidigoxigenin monoclonal antibody (1:500 in BT; Boehringer Mannheim GmbH) was applied to the slides followed by incubation at 37°C for 1 h. Subsequently, the preparations were washed 3× 3 min in BT, and the avidin was further amplified by incubation with a biotinylated anti-avidin antibody (1:200 in BT; Vector Labs, Inc., Burlingame, CA) and a second round of avidin-FITC (Pinkel et al., 1986). Simultaneously, secondary and tertiary TRITC-conjugated antibodies (1:500 in BT; Sigma Immunochemicals) were bound to the first antibody (Scherthan et al., 1992*b*). After a final wash in BT buffer, slides were drained and mounted in antifading solution (Vectashield; Vector Labs, Inc.) supplemented with 0.5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) to counterstain for chromatin.

Microscopy and Evaluation of Data

For the evaluation and photography of the FISH experiments, an epifluorescence microscope (Axioskop; Carl Zeiss, Inc., Frankfurt, Germany) equipped with single- and dual-band pass filters for fluorescein, rhodamine, and DAPI was used (Chroma Technology Corp., Brattleboro, VT). Nuclei were analyzed directly in the microscope. Pictures were recorded on color slide film (Ektachrome 400; Eastman Kodak Co., Rochester, NY). Distance measurements were obtained from slides projected with a standardized magnification.

The portion of accidental homologous associations induced by the spreading procedure was estimated from the observed heterologous associations. In a nucleus hybridized with two cosmids, a signal has a two- to fourfold higher probability to get randomly associated with a heterologous signal than with the homologous signal. The exact probability is influenced by several parameters, such as the number of homologous and heterologous interactions, the chromosomal positions of the two regions, and the arrangement of the homologues relative to each other. The fraction of nuclei showing accidental homologous associations was estimated as 50% of the fraction showing heterologous contacts. Subtraction of these random associations from the observed homologous associations yielded an estimate of truly homologous interactions.

Results

FISH was applied to synchronized meiotic nuclei of fission yeast to analyze the organization and pairing behavior of chromosomes. Different combinations of probes delineated either whole chromosomes or specific chromosomal subregions. The analysis was performed with nuclei spread on glass slides (Loidl et al., 1991; Bähler et al., 1993), preserving nuclear structures and resulting in an area of chromatin with a diameter of 4–10 μm as visualized by DAPI staining. Diploid cells were cultured to a titer of 1×10^7 cells/ml (late logarithmic phase). These vegetative cells, which are still growing mitotically, were then induced to undergo an azygotic meiosis by a shift to nitrogen-free medium. Nuclei were spread and processed for FISH analysis immediately before and at 2-h intervals after induction of meiosis. The applied protocol results in quite a good synchrony, with 80–90% of cells going through meiosis within 12 h (Bähler et al., 1993). Such time course experiments were quantified in a diploid standard strain ("wild-type") and a diploid *mei4-B2* mutant strain (Materials and Methods). The *mei4-B2* mutants (Bresch et al., 1968) initiate a normal meiosis but are then arrested at a late stage of meiotic prophase (Olson et al., 1978; Bähler et al., 1993). We exploited this arrest to specifically enrich and study meiotic prophase nuclei. Meiotic division stages, which are difficult to interpret in spread nuclei, are absent in this mutant strain and, therefore, do not interfere with the FISH analysis. Moreover, the mutant strain allows to analyze late meiotic prophase independently of synchrony.

Homologous Chromosomes Occupy Joint Territories in the Nucleus

Individual chromosomes were delineated with composite probes (chromosome painting: Lichter et al., 1988; Pinkel et al., 1988; Materials and Methods), which were obtained from an ordered cosmid library (Hoheisel et al., 1993). Painting of chromosome I, which represents 41% of the genome, revealed that the two homologues were generally not spread over the whole nuclear area outlined by DAPI staining. They occupied a single limited territory covering about half the size of the spread nucleus. The two chromosomal copies could not be distinguished within this compact territory. The frequencies of diploid nuclei that showed such a joint territory for both homologues of chromosome I are indicated in Table I. Both copies occupied the same territory in >90% of the nuclei, even in vegetative cells immediately before induction of meiosis (0 h). In fact, the homologues of chromosome I might become only separated during anaphase. This is consistent with the finding that most nuclei with separate territories for chromosome I appear at 0 and 2 h (in both strains), and at 8 h (in the standard strain). At these timepoints, mitotic and meiotic I anaphases, respectively, are observed by DAPI staining of whole cells (Bähler et al., 1993).

Chromosomes I and II were then visualized simultaneously by two-color painting in green and red, respectively. Both the green and red signals were restricted to single distinct nuclear areas in >90% of the nuclei (Fig. 1, *a* and *b*). The residual nuclei showed separated territories for each homologue (Fig. 1 *a*, *inset*). Nonrandom distribution of signals were observed in more than two hundred nuclei at each time point in both the standard and the *mei4-B2* strain. Overlapping signals from different chromosomes were often found at the border of different territories (Fig. 1, *a* and *b*, *yellow*). This can be expected as a consequence of spreading, which projects three-dimensional nuclei onto a surface. The

Table I. Frequencies of Nuclei with Both Chromosomes I Occupying a Joint Territory

	Time after meiotic induction:				
Strain	0 h	2 h	4 h	6 h	8 h
	Percent of nuclei with a single territory for both chromosomes I				
Standard	95	94	98	99	95
mei4-B2	96	95	99	99	98

Time course experiments were performed with the diploid standard strain and the diploid *mei4-B2* mutant strain. Spread nuclei were hybridized to delineate the entire length of chromosome I. At all time points, 150–280 nuclei were analyzed.

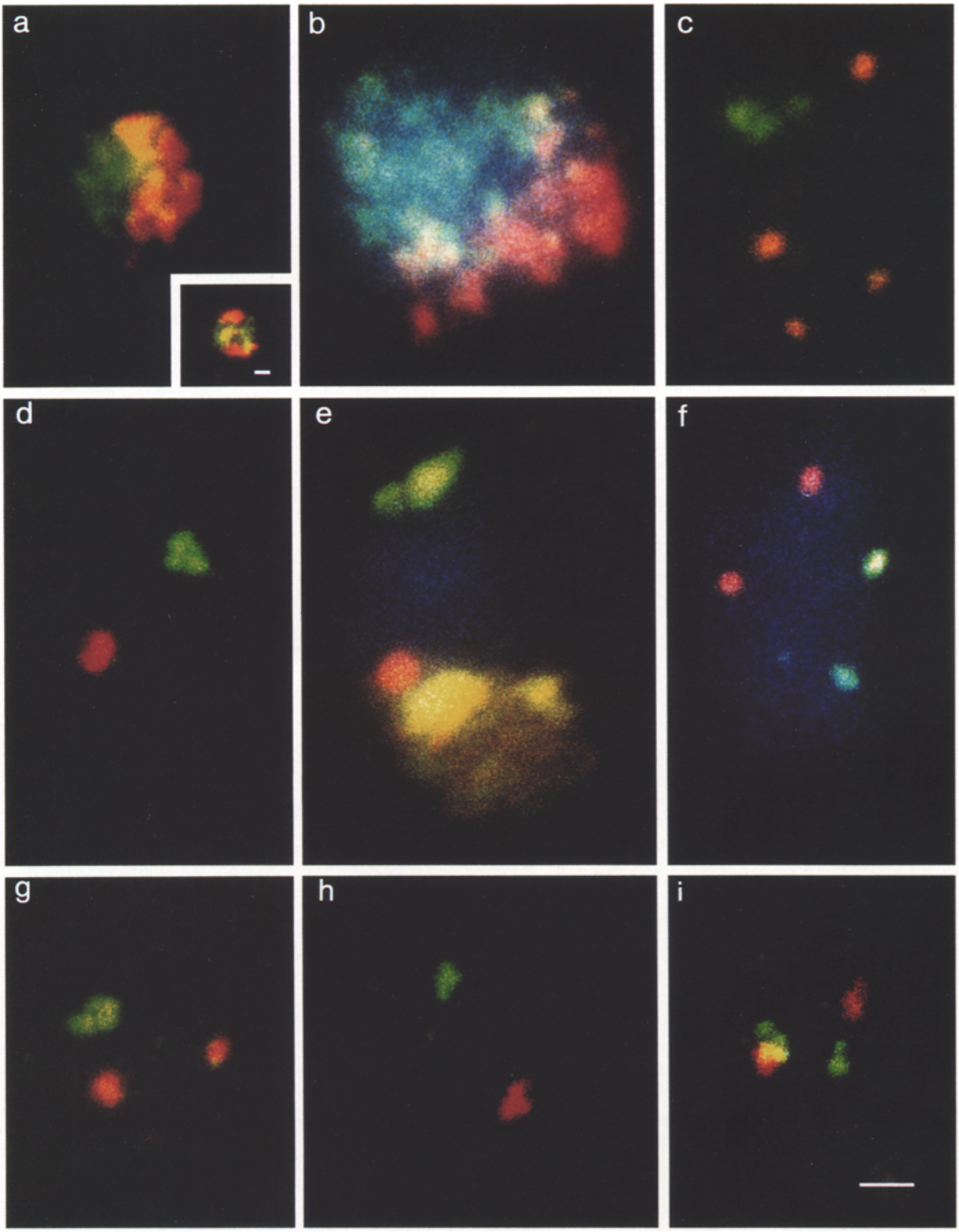

Figure 1. FISH analysis on spread diploid nuclei of *S. pombe.* Specific probes to whole chromosomes and defined chromosomal subregions were hybridized, followed by detection with fluorescent antibodies. The signals were evaluated and photographed directly in the fluorescence microscope. (*a* and *b*) Two-color painting of chromosomes I (*green*, FITC) and II (*red*, TRITC) with composite cosmid probes. The pictures are from vegetative nuclei before induction of meiosis (0 h). The arrangement is identical in meiotic cells. (*a*) A weakly spread nucleus exhibiting two distinct territories for chromosomes I and II. Signal overlap results in yellow fluorescence. (*Inset*) Nucleus with homologues of chromosomes I and II separated in distinct territories. Bar, 1 μm. (*b*) Chromosome painting as in *a* in a nucleus that is more spread out. The faint blue counterstain is created by double exposure with DAPI-stained chromatin. Chromatin that is not painted by the probes (at the top of the nucleus) probably belongs to chromosome III. (*c–e*) Two- and three-color FISH with plasmid probes for conserved repeated sequences of centromeres (*green*, FITC), telomeres (*red*, TRITC), and rDNA (*yellow*, FITC+TRITC). (*c*) Diploid

territorial arrangement of chromosomes I and II was also evident in whole nuclei by applying FISH to spheroblasted cells (not shown). Thus, the chromosomes seem to occupy distinct nuclear territories with homologues sharing common territories in diploid nuclei. Even after spreading, the ordered nuclear organization in chromosomal territories is still evident.

Specific Clustering of Centromeres, Telomeres, and Nucleolar Organizers

Centromeres and telomeres are thought to be important elements for ordered chromosome arrangement in the nucleus (see Introduction). Therefore, all centromeres and telomere-adjacent regions of *S. pombe* were simultaneously visualized by two-color FISH with DNA probes specific for repeated sequences at these chromosomal loci (Materials and Methods). In most vegetative nuclei (0 h), the centromeres of all chromosomes were not randomly positioned relative to each other, but all clustered together in a small area (Figs. 1 *c* and 2 *a*; see also Funabiki et al., 1993). This clustering was largely maintained into and throughout prophase of azygotic meiosis (Figs. 1 *d*, 2 *a*, and 3 *a*). In the great majority of nuclei, only three or less centromere signals could be distinguished (Fig. 2 *a*). Since diploid cells of *S. pombe* have six chromosomes, this finding raises the possibility that homologous centromeres are normally tightly associated. A control hybridization to spread nuclei of tetraploid cells resulted in nuclei showing up to six distinct centromere signals (not shown).

Unlike the centromeres, the telomere regions showed mostly a dispersed distribution in vegetative nuclei with widely spaced signals (Figs. 1 *c* and 2 *b*). The ends of chromosome III contain rDNA repeats instead of telomere-associated sequences (Fan et al., 1991; Hoheisel et al., 1993). Thus, we expected to detect only the telomere regions of chromosomes I and II with the subtelomeric probe, in accordance with the observation that nearly all diploid nuclei contained eight distinct telomere signals or less (Fig. 2 *b*). To study possible interactions of the chromosome III ends with the other chromosome ends, the nucleolus organizer regions were illuminated with a conserved rDNA probe (Materials and Methods). The nucleolar organizers appeared as a large signal area that was split into two distinct signals in some nuclei, probably reflecting the rDNA repeats from both ends of chromosome III (Fig. 1 *e*). In vegetative cells (0 h), the telomere signals were randomly distributed relative to the nucleolar organizer (not shown).

Telomeres tightly clustered early in meiosis, i.e., immediately before or at the very beginning of meiotic prophase, and they retained this clustering throughout meiotic prophase (Figs. 1 *d* and 3 *a*). The *mei4-B2* mutants, which

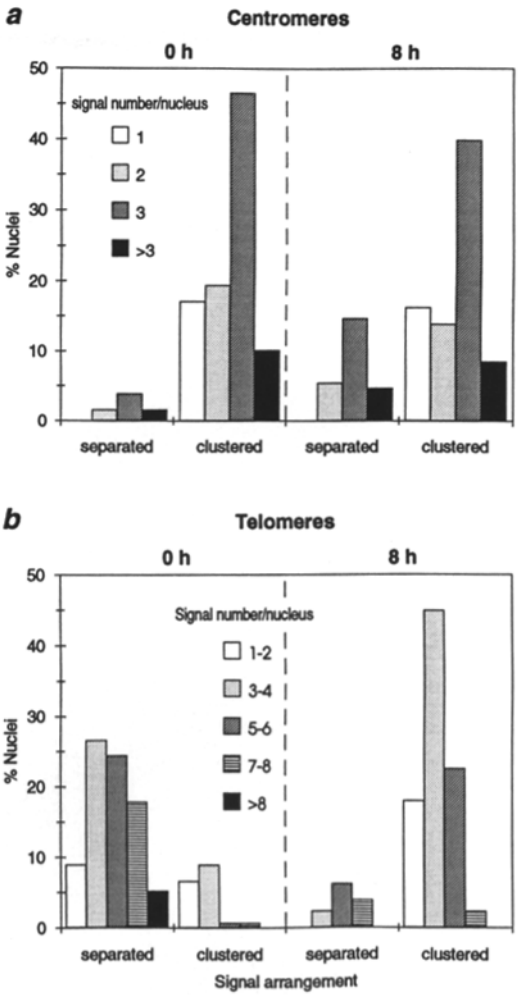

Figure 2. Number of centromere and telomere signals in spread nuclei. The signals were counted in the diploid *mei4-B2* strain before induction of meiosis (0 h, vegetative cells) and at a time when most cells are arrested in meiotic prophase (8 h). Separated and clustered signals are indicated separately. Signals were defined as separated if two or more signals have a distance of at least two signal diameters (1.6 μm) as exemplified by the red signals in Fig. 1 *c*. Clustered means that all signals are aggregated in a single limited region with the centers of neighboring signals separated by less than two signal diameters (Fig. 1 *c*, *green signals*). Associations of centromeres and telomeres are indicated by the often less than expected numbers of separated and clustered signals. (*a*) The percentages of different numbers of centromere signals per nucleus are shown. Diploid nuclei contain six centromeres (not including sister centromeres). 129 and 130 nuclei were analyzed at 0 and 8 h, respectively. (*b*) The percentages of different numbers of telomere signals per nucleus are shown. The probe applied should detect eight telomeres in diploid nuclei assuming tight association of sister chromatids. 135 and 129 nuclei were analyzed at 0 and 8 h, respectively.

vegetative nucleus with three clustered centromeres and dispersed telomeres. (*d*) Meiotic prophase nucleus with both centromeres and telomeres clustered in different nuclear areas. (*e*) Meiotic prophase nucleus with telomeres clustered near the nucleolus organizer regions. Centromeres are clustered distant from the nucleolus. The intense yellow signals correspond to rDNA, whereas the diffuse yellowish area results from rRNA that was not digested by RNase. (*Blue*) chromatin stained by DAPI. (*f–i*) FISH with single-copy cosmid probes to study the pairing behavior of defined subregions of chromosome II. Representative distributions of signal pairs for region 1 (telomeric; *red*, TRITC) and region 3 (centromeric; *green*, FITC) are shown. (*f*) Regions 1 and 3 both separated (not paired). The blue background results from DAPI staining of nuclear DNA, and it was recorded in this picture by double exposure to show the nuclear boundaries. (*g*) Region 1 (*red*) separated, region 3 (*green*) paired. (*h*) Regions 1 and 3 both paired. (*i*) Homologous regions are separated and show a heterologous association. Note the split signals of region 3 (*green*), reflecting the two sister chromatids. Bar, 1 μm.

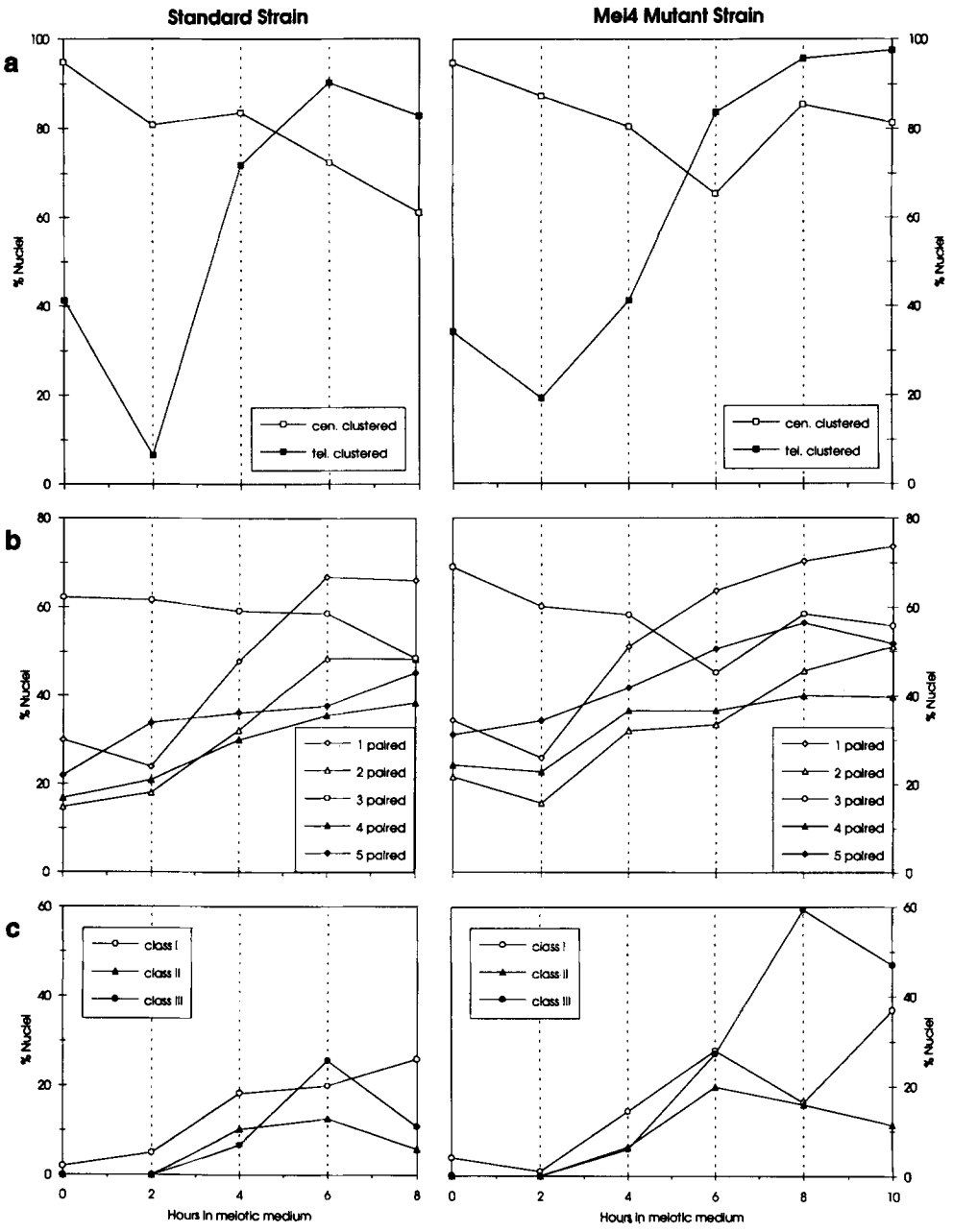

Figure 3. Time course experiments revealing the chromosomal behavior during meiotic prophase. Time points were taken before induction of meiosis (0 h, vegetative cells) and every 2 h thereafter. Nuclei were spread and quantified by FISH and by analyzing the linear elements. The graphs on the left and right side represent a single time course experiment each, one with the diploid standard strain (wild type) and one with the diploid *mei4-B2* mutant strain, respectively. Most cells perform a mitotic division after the shift to meiotic medium before they can enter meiosis from the G1 phase (Egel and Egel-Mitani, 1974). This division is quite synchronous, and 2 h after meiotic induction, most cells initiate premeiotic DNA replication (Bähler et al., 1993). In the standard strain, 10% of cells had sporulated 8 h after meiotic induction. This fraction of cells cannot be evaluated in the light and electron microscope and was taken into account by multiplying all data of the 8 h timepoint with the factor 0.9. At 10 h, >60% of the cells from the standard strain have initiated meiotic divisions, making it impossible to obtain reliable data. In total, 88% of the standard cells performed meiosis. The *mei4* mutant strain is blocked in late meiotic prophase. (*a*) The centromeres and telomeres of all chromosomes were detected with probes recognizing repeated sequences. The percentages of nuclei with clustered signals (Fig. 1, *c–e*) are shown for both loci. The decrease in telomere clustering at 2 h may be caused by mitosis and/or premeiotic DNA replication. At least 100 nuclei were examined at each time point. (*b*) The subregions 1–5 of chromosome II were detected with specific cosmid probes (Fig. 4). The percentages of nuclei with paired signals (two signals in contact or fused, Fig. 1, *g* and *h*) are shown for each region. The regions were evaluated in different pairwise combinations. A total of 300–450 nuclei were examined per time point and region. (*c*) The portions of spread and silver-stained nuclei with linear elements are shown. Class I, II, and III nuclei represent successive stages of meiotic prophase with short elements (first and last stage), interconnected elements, and long single elements, respectively (Bähler et al., 1993). At least 150 nuclei were examined at each time point.

arrest shortly before the first meiotic division (Bähler et al., 1993), showed clustered telomeres in the arrested nuclei (Figs. 2 *b* and 3 *a*). In contrast to vegetative cells, the clustered telomeres of meiotic cells (4–8 h) were tightly associated with the nucleolar organizer in >80% of all nuclei in the standard and the *mei4-B2* strain (Fig. 1 *e*). Thus, early in meiosis, the telomeres of chromosomes I and II specifically assemble near the nucleolus, which is itself organized by the ends of chromosome III. During entire meiotic prophase, both centromeres and telomeres are clustered in different regions of the nucleus (Fig. 1, *d* and *e*). This bouquet configuration is maintained until the end of meiotic prophase.

Homologous Pairing Behavior of Defined Chromosomal Regions

Chromosome pairing in vegetative cells and during meiosis was then studied by FISH with single-copy cosmid probes

mapping to five subregions of chromosome II (see Materials and Methods; Hoheisel et al., 1993). Fig. 4 shows the selected cosmids that recognize unique sequences near the left and right ends of chromosome II (regions 1 and 5, respectively), near the centromere (region 3), and in two interstitial regions (2 and 4). The centromere- and telomere-associated locations of cosmids 1, 3, and 5 were confirmed by double hybridizations with the centromere or telomere plasmid probes (not shown). The cosmid probes yielded highly reproducible signals with an average diameter of 0.8 μm, representing single-copy sequences of ~35 kbp. They allowed us to study and compare the pairing behavior of the selected subregions of chromosome II.

Spread nuclei were hybridized simultaneously with various pairwise combinations of cosmids from two chromosomal regions (Fig. 1, *f–i*). Hybridization of a particular cosmid is detected as a signal pair of the same color, which represents the corresponding chromosomal regions on the two homologues. Signal pairs that are detected as two separated spots indicate that the corresponding regions are unpaired (Fig. 1, *f* and *g*). A close contact or even fusion of the two signals suggests that the corresponding regions are associated (Fig. 1, *g* and *h*). In the following, we will refer to a chromosomal region as being "paired" if the distance between the signal centers of a signal pair is equal or less than the diameter of the signals (~0.8 μm), i.e., if the signals touch or overlap each other. This interpretation for pairing is supported by data from Weiner and Kleckner (1994). Thus, the fraction of nuclei showing associated signals of equal color yield an estimate for the percentage of nuclei with homologous pairing. Under the assumption that there is relatively little axial compaction along the chromosome (see below), associated signals reflect a homologous contact in, or close to, the probed region. It is noteworthy that each cosmid probe generally resulted in only two signals, which indicates that the two sister chromatids are closely associated. In a portion of nuclei (~40%), sister chromatids could be distinguished as closely spaced signals (Fig. 1 *i*, *green signals*). In budding yeast, only 5% of the nuclei show distinct signals for sister chromatids (Weiner and Kleckner, 1994), which might reflect a difference in chromatin organization between the two yeasts.

Table II shows the frequencies of nuclei being paired in different pairwise combinations of chromosomal regions. Vegetative cells (0 h) and cells arrested in meiotic prophase (8 h) of the diploid *mei4-B2* strain are compared. The percentages of nuclei showing pairing at both, only one, and neither region(s) are given separately. These data are compared to those expected for independent pairing of the two regions. Experiments with the standard strain show that 80–90% of the cells perform meiosis in our protocol (Bähler et al., 1993; unpublished observations). In accordance with these observations, our data show a much better fit between observed and expected values if it is assumed that only 85% of all cells are active for chromosome pairing (Table II). With this correction for active cells, all regions seem to pair independently of each other, except regions 1 and 5, which are localized near the ends of chromosome II (Fig. 4). Nuclei with both chromosome ends paired were significantly overrepresented, and nuclei with only one end paired were fewer than expected. Thus, the ends of chromosome II tend to pair coincidently, both at 0 and 8 h. Table II also shows that pairing is generally more frequent at 8 h than at 0 h, with the exception of region 3 (near the centromere), which is frequently paired in vegetative cells.

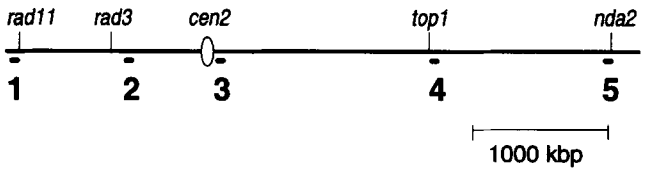

Figure 4. Physical map of chromosome II. Cosmid probes of ~35 kbp of selected unique positions on chromosome II (numbered 1–5) were used for the FISH analysis of chromosome pairing. Chromosome II has a length of 4.6 Mbp. Some markers near the regions of interest are indicated. The centromere is marked with an ellipse. The positions of the probes and markers are indicated approximately according to the map presented by Hoheisel et al. (1993).

Table II indicates that there are striking differences in the relative amounts of pairing at the different chromosomal regions. These differences and the timing of pairing were studied in more detail by analyzing the homologous pairing behavior of the five selected chromosomal regions in spread nuclei of the same time course experiments used to study centromere and telomere clustering (Fig. 3). The five cosmid probes from chromosome II were hybridized and evaluated in the six pairwise combinations indicated in Table II. At each timepoint, the percentage of nuclei showing pairing of a specific region was calculated as the mean value of the two or three hybridizations with the corresponding probe. The sizes of both the nuclei and the signals may influence the frequency of associations between signals in spread preparations. To take these variations into consideration and to account for accidental associations of two homologous signals, we also determined the fraction of nuclei showing heterologous contacts of a red and a green signal (Fig. 1 *i*), which allows to estimate the portion of fake homologous contacts (Materials and Methods). These heterologous associations represent an internal control. Their frequencies were <20% for all time points and did not increase during meiosis (data not shown). Subtraction of this unspecific background did not affect the conclusions from Table II (not shown).

Fig. 3 *b* shows the meiotic time-course of homologous pairing at the five chromosomal regions, with all data corrected for accidental homologous contacts. The centromeric region (3) was frequently associated in vegetative nuclei of diploid cells (0 h) and remained highly paired throughout azygotic meiotic prophase. The other regions showed basal levels of homologous associations in vegetative nuclei, but the portions of nuclei with paired signals increased during meiotic prophase. However, this meiosis-specific increase in pairing was rather modest (up to threefold). The two interstitial regions seem to pair less efficiently than the telomeric regions. Region 1 showed the highest portion of paired signals, followed by regions 5, 2, and 4. The pairing behavior of these chromosomal regions was similar in the standard and the *mei4-B2* mutant strain. The arrested *mei4-B2* cells maintain pairing late in meiosis (10 h), but they did not show higher portions of paired nuclei than the standard cells.

To get an idea on chromosome condensation and the localization of chromosomal regions relative to each other, we measured distances between signals. Table III shows average distances between homologous and heterologous regions in

Table II. Frequencies of Nuclei with Pairing at Two, One, or No Chromosomal Region(s)

Combinations of regions analyzed*	Time‡	Fraction of nuclei paired at region(s):§				Number of nuclei analyzed	χ^2 test: *P* values
		Both (*a* and *b*)	*a* only	*b* only	Neither *a* nor *b*		
a/*b*	*h*	Percent observed (percent expected $A = 1.0$/percent expected $A = 0.85$)‖					$A = 1.0/A = 0.85$¶
1/2	0	13 (10/12)	30 (30/28)	12 (15/13)	45 (45/46)	122	0.19/0.55
	8	27 (26/31)	36 (37/32)	14 (15/11)	23 (22/26)	133	0.66/0.06
1/3	0	31 (25/30)	12 (17/13)	29 (34/30)	28 (23/28)	134	**0.01**/0.70
	8	47 (38/45)	31 (40/33)	2 (11/4)	20 (11/18)	108	**<0.01**/0.16
1/5	0	25 (16/19)	11 (20/18)	19 (28/25)	45 (36/39)	135	**<0.01/<0.01**
	8	51 (40/46)	19 (31/24)	5 (17/10)	25 (13/20)	114	**<0.01/0.03**
2/3	0	20 (16/19)	7 (10/8)	41 (45/42)	32 (29/32)	131	0.06/0.73
	8	31 (25/29)	15 (21/17)	23 (30/25)	31 (25/29)	120	<0.01/0.48
2/4	0	14 (11/12)	25 (28/27)	14 (17/15)	48 (45/47)	126	0.12/0.57
	8	27 (21/24)	24 (31/27)	13 (20/16)	35 (29/33)	144	**<0.01**/0.18
4/5	0	14 (10/12)	13 (18/16)	22 (26/25)	50 (46/48)	157	**0.02**/0.18
	8	34 (31/36)	12 (15/10)	33 (36/30)	21 (18/23)	147	0.10/0.22

* Six pairwise combinations of chromosomal regions were analyzed. The regions are numbered as indicated in Fig. 4.
‡ The data are from the diploid *mei4-B2* strain before induction of meiosis (0 h) and when arrested in meiotic prophase (8 h).
§ *a* refers to the left and *b* to the right chromosomal region as indicated in the first column.
‖ Corrections for active cells were performed according to Weiner and Kleckner (1994). With the observed total fractions of nuclei paired at region *a* (a^p) and *b* (b^p), the expected fractions of nuclei paired at *a* and *b* (ab_{exp}), at *a* only (a_{exp}), at *b* only (b_{exp}), or at neither region (n_{exp}) can be calculated under the following two assumptions: regions *a* and *b* pair independently of each other, and in the population of cells, a major fraction (A) is active for pairing while the residual fraction ($1\text{-}A$) is unable to pair. The expected fractions are calculated as follows: $ab_{exp} = (a^p/A)(b^p/A)(A)$; $a_{exp} = (a^p/A)(1\text{-}b^p/A)(A)$; $b_{exp} = (b^p/A)(1\text{-}a^p/A)(A)$; $n_{exp} = (1\text{-}a^p/A)(1\text{-}b^p/A)(A)+(1\text{-}A)$. The observed and the expected percentages (for 100% and 85% of active cells) are indicated.
¶ The *P* values of the χ^2 test of independence (degree of freedom = 1) are indicated if 100% or 85% of active cells are assumed, respectively.
P values that show a significant deviation from independence at the 5% level of significance are in boldface.

all possible combinations at 0 and 8 h in the *mei4-B2* strain. The analysis of distances is complicated by chromosome looping that results from telomere clustering. This is most evident for the subtelomeric regions 1 and 5. At 8 h, the distance between these regions is smaller than at 0 h, and it does not differ significantly from the corresponding homologous distances. Distances between adjacent chromosomal regions are less affected by chromosome bending than distances between nonadjacent regions. Interestingly, the three distances between the adjacent regions 1-2, 2-3, and 4-5 become significantly longer at 8 h compared to 0 h, while 3-4 does not differ significantly (Table III).

The average heterologous distances in Table III represent a mixture of distances within chromosome II and between the homologues. This might interfere with the analysis of distances between adjacent regions, especially if the homologues are not closely aligned. However, qualitatively the same results were obtained when only nuclei with pairing at one or both homologous region(s) were considered for evaluation of heterologous distances (see also Weiner and Kleckner, 1994). The corresponding average distances in μm between regions 1-2, 2-3, 3-4, and 4-5 (0/8 h) were 1.9/2.2, 1.6/2.2, 2.4/2.1, and 1.7/2.1, respectively. These distances can be directly related to chromosome length. Because the distances within chromosome II do not become shorter at 8 h, we conclude that chromosomes of fission yeast do not undergo detectable axial compaction in meiotic prophase.

The analysis of Table III also represents an independent approach to further characterize chromosome pairing. Unlike the distances between adjacent regions, the average distances between the homologous regions 1, 2, and 5 are significantly smaller at 8 h compared to 0 h, whereas the corresponding distance of region 3 shows an increase at 8 h, and the one of region 4 does not differ significantly between 0 and 8 h. This confirms the data in Fig. 3 *b*. Both at 0 and 8 h, most distances between homologous regions are significantly shorter than the corresponding heterologous distances. Thus, vegetative diploid nuclei show already a significant degree of chromosome alignment within their joint territory, as also reflected by the observed chromosome pairing in vegetative nuclei (Fig. 3 *b*).

Temporal Comparison of Linear Element Morphology With Chromosomal Events

Fission yeast does not develop a mature SC. Instead, there appear linear elements during meiotic prophase that probably correspond to axial cores (SC precursors) of other eukaryotes (see Introduction). In parallel to the FISH analysis, the formation of different classes of linear elements was determined in the same time course experiments (Fig. 3 *c*). The classes were distinguished as described before (Bähler et al., 1993). Class I nuclei contain short pieces of linear elements, representing an early stage of formation and a late stage of degradation of the elements. Class II nuclei show long elements being in contact with each other and forming networks and tangles. The third stage of meiotic prophase is represented by class III nuclei with single long elements that are ordered longitudinally in the elongated prophase nuclei. As shown before (Bähler et al., 1993), the *mei4* mutants become highly enriched in class III nuclei. The linear elements appeared coincidently with progressive pairing. Maximal abundance of nuclei with linear elements coincided with maximal pairing at late prophase stages. However, no specific stage of linear elements was especially correlated with high portions of paired signals (Fig. 3, *b* vs. *c*).

Discussion

Synchronous meiotic cells of fission yeast were quantitatively studied by FISH to gain direct insight into the arrange-

Table III. Distances between Signals of Homologous and Heterologous Chromosomal Regions

Region*	Time‡	Distance to region:§ 1	2	3	4	5	N‖
	h			*μm*			
1	0	**1.6 ± 1.3 (0.2–7.0)**	2.1 ± 1.0 (0.2–4.7)	2.5 ± 1.1 (0.5–4.7)	1.7 ± 0.9 (0.3–5.3)	2.5 ± 1.6 (0.3–7.0)	81
	8	**1.1 ± 0.7 (−) (0.3–4.0)**	2.4 ± 0.9 (+) (0.8–4.8)	3.3 ± 1.4 (+) (0.4–7.2)	3.2 ± 1.8 (+) (0.7–9.0)	1.3 ± 0.6 (−) (0.3–4.0)	79
2	0		**1.5 ± 0.8 (0.2–3.0)**	1.7 ± 0.7 (0.5–4.0)	1.9 ± 0.8 (0.5–4.5)	1.5 ± 0.8 (0.3–4.0)	78
	8		**1.3 ± 0.7 (−) (0.2–4.0)**	2.2 ± 0.9 (+) (0.2–3.0)	2.1 ± 1.1 (=) (0.1–5.0)	2.0 ± 0.9 (+) (0.4–4.8)	82
3	0			**0.8 ± 0.5 (0.2–3.4)**	2.4 ±1.0 (0.3–4.7)	2.7 ± 1.2 (0.4–5.7)	81
	8			**1.2 ± 1.0 (+) (0.2–4.5)**	2.5 ± 1.3 (=) (0.7–6.4)	2.1 ± 1.0 (−) (0.5–4.1)	83
4	0				**1.6 ± 1.0 (0.2–5.3)**	1.6 ± 0.8 (0.3–3.9)	84
	8				**1.8 ± 1.2 (=) (0.2–5.3)**	2.3 ± 1.0 (+) (0.4–5.6)	75
5	0					**1.7 ± 1.5 (0.2–7.0)**	70
	8					**1.2 ± 1.0 (−) (0.3–6.5)**	99
	8						

* The chromosomal regions are numbered as indicated in Fig. 4.
‡ The data are from the diploid *mei4-B2* strain before induction of meiosis (0 h) and when arrested in meiotic prophase (8 h).
§ The average distances between the regions in the first column and the corresponding homologous regions (in bold type), as well as all possible combinations of heterologous regions, are indicated together with the standard deviation. The extreme values are given in parentheses. The distances were measured from the centers of the signals. Data of the 8-h timepoint are marked with (+) or (−) if the average values are significantly larger or smaller, respectively, than the corresponding 0 h values. The symbol (=) indicates that values do not differ significantly between 0 and 8 h (z test, 5% level).
‖ Number of nuclei studied to determine the average homologous distances. This number is the sum of the nuclei analyzed in the four possible pairwise combinations within heterologous regions. About 20 nuclei from each combination were analyzed to determine the average heterologous distances. In each nucleus, all four heterologous distances were measured (from both homologous signals to each of the two heterologous signals).

ment and pairing behavior of chromosomes in vegetative cells and during meiotic prophase. We used probes of different complexity to delineate whole chromosomes, and to visualize repeated sequences of centromeres, telomeres, and rDNA, as well as single-copy sequences of chromosome II. Linear elements appearing during meiotic prophase were analyzed in the same experiments for comparison. The study was restricted to azygotic meiosis because this system is more synchronous. Furthermore, the evaluation is not confused by haploid nuclei, which cannot be distinguished from diploid nuclei in spread preparations.

Chromosome Territories and Pairing in Vegetative Nuclei

The arrangement of chromosomes in specific nuclear domains is well documented in higher eukaryotes (see Introduction), but the homologues often occupy separate domains in vegetative nuclei (Hilliker and Appels, 1989; Loidl, 1990; Haaf and Schmid, 1991). In fission yeast, the homologues colocalize in a joint territory that is distinct from the territories of the other chromosomes, as revealed by painting of whole chromosomes. The ordered organization of the nucleus into chromosomal compartments remains detectable even after spreading nuclei of both vegetative and meiotic cells (Fig. 1, *a* and *b*). Diploid cells of *S. pombe* have six chromosomes with their centromeres clustered. If the centromeres are considered as the central point from where the chromosome arms radiate, then the probability that a given chromosome has its homologous partner adjacent by chance alone is 2/5 (five potential positions for the homologue with two of them being adjacent). The observed frequencies of nuclei showing joint territories for chromosomes I are clearly higher than the frequency of ~40% that can be expected for a random arrangement of the six chromosomes in the nucleus (Table I). Since karyogamy is normally succeeded by zygotic meiosis, we suggest that the joining of the homologues into common territories occurs immediately after karyogamy as a first step towards chromosome pairing, and is then maintained in vegetatively growing diploid cells. The homologues may be brought into a joint domain by chromosome-specific anchoring sites in the nuclear lamina (Hubert and Bourgeois, 1986) or by unstable homologous interactions (Kleckner and Weiner, 1993).

In budding yeast cells about to enter meiosis the homologues are frequently associated at several regions (Loidl et al., 1994; Weiner and Kleckner, 1994), but no homologue pairing has been observed in vegetatively growing cells (Guacci et al., 1994). In fission yeast, ~65, 30, and 20% of the centromeric, telomeric, and interstitial regions, respectively, showed pairing before induction of meiosis (Fig. 3 *b*). It therefore appears that the homologues are nonrandomly positioned relative to each other in their joint territory already in vegetative diploid cells. This conclusion is further corroborated by the finding that in nuclei at 0 h, most average distances between homologous regions are significantly shorter than the average distances between heterolo-

gous regions (Table III). However, our results also suggest that the centromere is the main region that becomes stably associated in vegetative cells (Figs. 2 *a* and 3 *b*). This is consistent with genetic data showing that mitotic recombination frequencies are much higher in centromeric regions than elsewhere (Minet et al., 1980). Besides the centromeres, the telomeres may be other important sites of homologous interactions in vegetative cells (see below).

Dynamic Clustering of Centromeres and Telomeres in Defined Nuclear Areas

Funabiki et al. (1993) have reported that the centromeres of all chromosomes are clustered near the spindle pole body (SPB) in haploid vegetative nuclei; this configuration is only disrupted during mitosis. The present study shows also that in diploid vegetative nuclei, all centromeres are assembled in a single cluster. This cluster is retained into and throughout prophase of an azygotic meiosis (Fig. 3 *a*). In zygotic meiosis, on the other hand, the centromere clusters of the two parental cells join only in the course of meiotic prophase (Chikashige et al., 1994).

It has been demonstrated that the telomeres of chromosome I and II are aggregated at the nuclear periphery of vegetative interphase cells (Funabiki et al., 1993). Associations of telomeres have also been reported from other organisms (reviewed by Gilson et al., 1993; Palladino et al., 1993; Blackburn, 1994). In accordance with these results, we find that there are often fewer telomere signals than expected, but telomeres of vegetative cells are normally not clustered in a single area (Fig. 2 *b*). In 0-h nuclei, the average distance between the ends of chromosome II (regions 1 and 5) is significantly longer than the average homologous distances of regions 1 and 5 (Table III). Therefore, it is unlikely that the ends of the chromosomes are associated before meiosis. The observed telomere aggregations may reflect associations of homologous telomeres and/or of telomeres from different chromosomes, which may interact on the surfaces of the chromosome territories. The finding that the ends of chromosome II do not pair independently of each other in vegetative cells (Table II) indicates that pairing of homologous telomeres is coordinated during the cell cycle.

Early in azygotic meiosis, the telomeres of chromosomes I and II become strongly clustered near the nucleolar organizers, which correspond to the ends of chromosome III (Figs. 1 *e* and 3 *a*). This meiosis-specific clustering is also reflected by the average homologous and heterologous distances of the subtelomeric regions 1 and 5 (Table III). Recently, Hiraoka and colleagues have reported that telomere clustering coincides with a shift of the telomeres to the SPB (Chikashige et al., 1994). Thus, the telomeres take up the position that had previously been occupied by the centromeres. In zygotic meiosis, the telomere shift takes place before karyogamy, and the telomere clusters of the two sets of homologues are then brought together by the fusing SPBs during karyogamy (Chikashige et al., 1994; Kohli and Bähler, 1994).

The association of telomeres with the SPB is consistent with the finding that the nucleolus, which is organized by the ends of chromosome III, is adjacent to the SPB during meiotic prophase (Bähler et al., 1993). Interactions of the chromosome ends with cytoplasmic microtubules embracing the nucleus might lead the telomeres to the SPB (Loidl, 1990, and references therein). Microtubules running along the outer nuclear membrane have been observed in fission yeast (Olson et al., 1978; Bähler et al., 1993). This telomere shift does not seem to disrupt the centromere cluster (Fig. 3 *a*), which may be maintained directly by interactions of repeated DNA sequences (Takahashi et al., 1992).

Maintenance of a Bouquet Configuration and No Detectable Chromosome Condensation in Meiotic Prophase

Interestingly, the clustered telomeres in fission yeast do not represent a transient stage of meiotic prophase as in other eukaryotes, where a bouquet configuration is only observed in leptotene/zygotene, whereas in pachytene, the telomeres are dispersed again (Fussell, 1987). Both telomere and centromere clustering are retained in *mei4-B2* mutants arrested shortly before the first meiotic division (Fig. 3 *a*). The telomere clustering in *S. pombe* seems to be more tight than in a classical bouquet configuration. It is not clear at the present time how the meiotic chromosome configuration of fission yeast is related to those of other organisms.

Unlike spread pachytene chromosomes of *Saccharomyces cerevisiae* (Scherthan et al., 1992*a*; Bähler et al., 1994; Weiner and Kleckner, 1994), the chromosomes of *S. pombe* do not show visible chromatin condensation by DAPI staining. Moreover, the distances between adjacent regions on chromosome II are extended rather than shortened in meiotic prophase (Table III). Thus, there is no detectable axial compaction of chromosomes during meiotic prophase. These findings, together with the lack of mature SCs, indicate that fission yeast skips events of late prophase that are cytologically prominent in other eukaryotes.

Multiple and Independent Homologous Interactions Increase in Meiotic Prophase

We studied chromosome pairing with cosmid probes derived from defined unique regions of chromosome II (Fig. 4; Hoheisel et al., 1993). The data on chromosome pairing suggest that interhomologue connections are formed at multiple unspecific sites. Regions between these pairing sites may not be brought into close contact because chromosomes of fission yeast are not condensed and do not synapse. With the exception of the region near the centromere (see below), there is a meiosis-specific 1.5–3-fold increase in nuclei with paired signals (Fig. 3 *b*) and a decrease in the average distances between homologous regions (Table III). Pairing is maximal in late meiotic prophase. Homologous interactions are formed independently of each other (Table II). This increased pairing during meiotic prophase is less pronounced than one would expect under the assumption that each region pairs in every meiotic cell. The frequencies of nuclei with paired signals remain below those with clustered centromeres and telomeres (Fig. 3, *a* and *b*). Also in *mei4-B2* cells, which are arrested in meiotic prophase, there are no higher frequencies of nuclei with paired signals. Moreover, in cells arrested during meiotic prophase, there are still many nuclei that are paired in only one region in every pairwise combination analyzed (Table II). Thus, only a subset of homologous regions gets paired in any given meiosis.

Homologous regions might show a dynamic pairing/unpairing behavior. It has been proposed that interactions of ho-

mologous sequences are generally unstable during an early stage of pairing (Kleckner and Weiner, 1993). Transient and/or unstable contacts would allow ectopic interactions (recombination between homologous sequences at nonallelic positions: Munz et al., 1982; Amstutz et al., 1985; Haber et al., 1991 and references therein) and their subsequent replacement by interactions between homologues. Ectopic interactions might be restricted to the surfaces of chromosome territories in nuclei with territorial organization. In fission yeast, where pairing is never stabilized along whole chromosomes by SC formation, the sites of crossovers may be the only chromosomal regions that remain stably paired throughout meiotic prophase. Crossovers affect different sites in each different meiosis, and they are expected to result in an association of neighboring regions (besides the sequence actually involved in the exchange), which would then be detected as "pairing" in our assay. The molecular nature of pairing before chromosomes are connected by mature crossovers is not known. Direct interactions between intact DNA duplexes may be involved (Camerini-Otero and Hsieh, 1993; Schwacha and Kleckner, 1994; Collins and Newlon, 1994).

The differences in the observed amounts of pairing of regions 1–5 (Table II and Fig. 3 *b*) most probably reflect variations in the probability of a specific region to become paired during meiosis. Region 1 shows clearly the highest levels of pairing, possibly resulting from its proximity to the telomere of the short arm (Fig. 4). The larger physical distance of region 5 from the chromosome end might be the reason that it pairs less efficiently than region 1. The constant association of centromeres seems to lead to correspondingly high pairing levels of region 3 at all time points, although there is some relaxation of pairing during meiotic prophase (Fig. 3, *a* and *b*; Table II). Region 4, which is more distant from the telomere and the centromere than region 2 (Fig. 4), shows the lowest pairing. These regional differences in pairing are also reflected in the average distances between homologous and heterologous regions (Table III).

Functions of the Bouquet and Linear Elements in Meiotic Chromosome Pairing

There is no evident regional difference in the initiation time of meiosis-specific pairing, although the subtelomeric regions tend to pair somewhat earlier than the interstitial regions (Fig. 3 *b*). Pairing of interstitial regions does not seem to be directly dependent on previous pairing of subtelomeric regions, as can be concluded from their independent pairing behavior (Table II). Nevertheless, it is likely that the clustering of centromeres and telomeres in defined nuclear areas facilitates the recognition of homologues. Chromosome pairing within clustered regions is suggested by the numbers of centromere signals within their cluster (Figs. 1 *c* and 2 *a*), and by the finding that the centromeric region 3 is more frequently paired than other regions (Table II and Fig. 3 *b*). Furthermore, telomeres seem to be paired within their cluster (Figs. 1, *d* and *e*, and Fig. 2 *b*), and the chromosome ends initiate pairing coincidently with or slightly after the telomeres become clustered (Fig. 3, *a* and *b*). Clustering of the telomeres is observed in zygotic and azygotic meiosis (Chikashige et al., 1994, and this study). It is, therefore, a meiotic event and not specific to mating and karyogamy. This makes it likely that the telomere clustering has a function in meiosis.

We suggest that the homologues of *S. pombe* recognize each other at the telomeres or telomere-adjacent regions (as a consequence of the bouquet formation), and initiate pairing at the chromosome ends. Alternatively, telomeres (and centromeres) might be predisposed to efficient pairing interactions. This would mean that clustering is a consequence of pairing rather than vice versa. However, at least for telomere clustering, which occurs at a defined time and location (see above), the latter hypothesis seems to be less attractive. Interlocks with other chromosomes that are trapped during the pairing process could be resolved by relative movements of the clustered telomeres around each other, providing another attractive function of the bouquet configuration. This would require that an early stage of meiotic chromosome pairing is unstable and reversible (Kleckner and Weiner, 1993).

It has been suggested before (Bähler et al., 1993) and recently demonstrated that the elongated meiotic prophase nuclei show striking movements, including U-turns at the cell ends, with the SPB and the associated telomere cluster at the leading end (Chikashige et al., 1994). A stretching and/or stiffening of chromosome arms in the prophase nuclei, which become elongated during these movements, could explain that average distances between adjacent regions within chromosome II increase in meiotic prophase (Table III). The only exception is the distance between regions 3 and 4 in the central part of the chromosome (Fig. 4), which is expected to be bent by telomere traction. The nuclear movements may provide the mechanical force to get interstitial chromosomal regions close enough for pairing interactions and recombination.

Interestingly, the interstitial regions start to show enhanced pairing at about the same time as the first linear elements appear (Fig. 3, *b* and *c*). These linear elements may provide structural support for the chromosomes in the dynamic prophase nuclei and facilitate homologous interactions (for possible mechanisms see Bähler et al., 1993). This is consistent with the finding that a *rec8* mutant of *S. pombe* forms aberrant linear element structures (Molnar, M., and J. Bähler, manuscript in preparation), and strongly reduces recombination frequencies near the middle of chromosome III, but less so towards the chromosome ends (DeVeaux and Smith, 1994). An analysis of pairing by FISH in this mutant is in progress. Moreover, *pat1-114* mutants show reduced meiotic recombination frequencies (Iino and Yamamoto, 1985; Bähler et al., 1991), and they seem to form shorter and less abundant linear elements (Bähler, J., unpublished results). The linear elements may also function in connecting the sister chromatids, which act as a unit after premeiotic DNA replication (Fig. 1, *f–h*).

Whereas our data are consistent with a role of the linear elements in the process of chromosome pairing, the maintenance of pairing does not seem to depend on linear elements. High levels of pairing are retained when the elements have been degraded. Moreover, the accumulation of class III nuclei in the *mei4* mutant strain at 8 h does not lead to a similar accumulation of nuclei with paired signals (Fig. 3, *b* and *c*). It might, therefore, be that class II nuclei, which are not accumulated in *mei4* mutants, represent the meiotic prophase stage that is active for pairing initiation. The elements

of this stage contact each other at several regions (Bähler et al., 1993) which might reflect a search for homology. The high levels of pairing late in meiotic prophase might then be realized and maintained by those sites that have succeeded to interact with their homologous partner at the DNA level.

Axial Cores as Elementary Structures Required for Efficient Meiotic Chromosome Pairing and Recombination?

The formation of a complete SC is not required for meiotic pairing and recombination in fission yeast. This conclusion is in accordance with recent findings in budding yeast (see Introduction). The SC may be responsible for the nonrandom distribution of crossover events leading to interference, which is not observed in the asynaptic fungi *S. pombe* and *Aspergillus nidulans* (reviewed by Kohli and Bähler, 1994; Munz, 1994). The linear elements, which probably correspond to axial cores of other eukaryotes, may represent a minimal structural requirement for efficient pairing and recombination along entire chromosomes. Consistent with this view, asynaptic mutants of *S. cerevisiae* that still pair their homologues and/or show high levels of meiotic recombination, form at least fragments of axial elements (axial cores) (Roeder, 1990; Hawley and Arbel, 1993; Sym et al., 1993; Loidl et al., 1994). Thus far, no mutant has been found in *S. cerevisiae* that completely lacks axial cores, yet retains significant levels of meiotic recombination. However, until now, no axial core–like structures have been found in *A. nidulans* (Egel-Mitani et al., 1982).

Conclusions

FISH is an efficient method to analyze meiotic chromosomes because direct insights into the behavior and organization of whole chromosomes and their subregions can be obtained. Spread nuclei cover a large area, which facilitates a high resolution analysis of signal distributions by standard fluorescence microscopy. Moreover, associations of signals that resist the spreading forces provide a good argument for true physical association of the corresponding chromosomal regions. However, to gain insight into the three-dimensional nuclear arrangements of chromosomes, it will be useful to complement the present study with a spatial analysis of whole nuclei.

In diploid nuclei of fission yeast, the homologues occupy joint territories, and they are partially aligned already before entry into meiosis. Fission yeast shows a typical Rabl orientation of chromosomes in vegetative nuclei, whereas meiotic chromosomes form a bouquet by additionally clustering all telomeres. This configuration is maintained throughout meiotic prophase. Because of the defined and fixed positions of centromeres and telomeres in the nucleus and their clustering behavior, they probably play crucial roles in nuclear organization such as territorial arrangement of chromosomes. Furthermore, centromeres and telomeres appear to be important for homologous chromosome pairing, as reflected by regional differences in homologous interactions. Chromosomes pair by multiple and independent homologous interactions, which become more frequent during meiotic prophase. Not all regions are paired during meiosis, which probably reflects the lack of axial chromosome compaction, as well as SC formation.

It is not known whether the pairing level influences the recombination level or whether pairing and recombination are two manifestations of the same basic mechanism. More comparative data of physical and genetical genome maps are required to conclude whether the observed regional differences in pairing efficiency are reflected by regional recombination frequencies. If there is a direct correlation between pairing and recombination efficiency, our data would predict meiotic recombination to be more frequent in regions adjacent to centromeres and telomeres. A FISH analysis of the many known meiotic recombination mutants of fission yeast (Ponticelli and Smith, 1989; DeVeaux et al., 1992) might give insight into the relationship between pairing and recombination, and the importance of special chromosome configurations for these meiotic events.

The present study confirms that meiotic chromosome functions do not necessarily require the formation of a mature SC. Axial cores, however, may represent minimal requirements for chromosome structure and function during meiotic prophase. Fission yeast shows both parallels and deviations in chromosome behavior compared to other eukaryotes. It is, therefore, a valuable model organism to contribute to the understanding of chromosome pairing, recombination, and segregation during meiosis I.

We are grateful to Elmar Maier, Hans Lehrach, Mary Baum, Louise Clarke, and Neal Sugawara for kindly providing cosmids and plasmids, which were essential for this study. We thank Yasushi Hiraoka, Nancy Kleckner, and Beth Weiner for communication of results before publication, Edgar Hartsuiker, Wolf-Dieter Heyer, and Peter Munz for critical reading of the manuscript, and Daniel Schümperli for use of the EM facilities. Nancy Kleckner was an exceptionally constructive reviewer of the manuscript and gave many insightful comments.

This work was supported by the Swiss National Science Foundation.

Received for publication 16 May 1994, and in revised form 14 July 1994.

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