

Organization and pairing of meiotic chromosomes in the ciliate *Tetrahymena thermophila*

Josef Loidl^{1,*} and Harry Scherthan²

¹Institute of Botany, University of Vienna, Rennweg 14, 1030 Vienna, Austria

²Institute for Radiation Biology Bw, Neuherbergstrasse 11, 80937 Munich, Germany

*Author for correspondence (e-mail: josef.loidl@univie.ac.at)

Accepted 19 August 2004

Journal of Cell Science 117, 5791-5801 Published by The Company of Biologists 2004

doi:10.1242/jcs.01504

Summary

During meiotic prophase in the ciliate *Tetrahymena thermophila* micronuclei dramatically elongate and form thread-like crescents. The arrangement of the chromosomes within the crescent as well as the timing of chromosome pairing and recombination with respect to the elongation process have been subjects of ongoing debate. Here, we addressed these issues by means of fluorescence in situ hybridization, labeling of individual chromosomes by BrdU (BrdU-painting) and by immunostaining of the recombination protein, Rad51. BrdU-painting indicated that chromosomes are arranged as parallel bundles within the crescent, and telomere-directed fluorescent in situ hybridization (FISH) revealed that most if not all telomeres are assembled near one end of the developing crescent. Prior to full crescent formation, Rad51 localizes to chromatin as numerous foci. Locus-specific FISH demonstrated that close pairing of homologues only occurs in the full crescent. Meiotic DNA double-strand break

formation and the initiation of recombination thus seem to precede close pairing. A synaptonemal complex was not detected. We conclude that the chromosomes adopt a polarized arrangement within the crescent, probably resembling the classical bouquet arrangement. Furthermore, we propose that the elongated shape of meiotic micronuclei promotes the parallel arrangement of chromosomes and supports the juxtaposition of homologous regions in the absence of a synaptonemal complex. Several pieces of evidence indicate the presence of one to four chiasmata per bivalent, which would call for crossover interference to explain regular bivalent formation in spite of this low mean number. *Tetrahymena* might, therefore, pose a case of interference in the absence of a synaptonemal complex.

Key words: Chromosome pairing, Meiosis, Recombination, Rad51, Protists

Introduction

The two homologous chromosome sets of diploid cells must separate prior to sexual reproduction in order to allow cells with a single set of chromosomes to fuse and to restore the diploid complement in the zygote. The separation of homologues during meiosis requires their pairing and, in most cases, the reciprocal exchange of chromosomal portions in a process called crossing over. Crossing over results in the formation of physical bridges, known as chiasmata, between homologues, which ensure their faithful segregation. In addition, it causes the rearrangement of alleles within chromosomes and contributes to the genetic diversity of offspring.

One of the mysteries in meiosis is how homologous chromosomes recognize each other and move towards each other to form pairs. Meiotic chromosome pairing probably employs sophisticated mechanisms for an efficient chromosome-wide homology screen, which excludes ectopic homologies from subsequent recombination. In most organisms homologous contacts first occur during meiotic prophase and it is possible that different organisms employ different strategies to facilitate these contacts (see Loidl, 1990; Loidl, 1994; Dorninger et al., 1995). In many organisms, all of the chromosome ends assemble within a limited area at the

nuclear periphery. This leads to the so-called bouquet arrangement of chromosomes. The physical proximity of corresponding chromosome regions within the bouquet or the dynamics involved in bouquet formation could promote homologous contacts (for review, see Zickler and Kleckner, 1998; Cowan et al., 2001; Scherthan, 2001; Bass, 2003). A very similar phenomenon was observed in the fission yeast, *Schizosaccharomyces pombe*. During meiotic prophase, the nucleus assumes an oblong shape ('horsetail') and performs oscillatory movements within the cell. The telomeres of all six chromosomes are attached to the spindle pole body in the nuclear membrane, which directs the movement. In the course of the movements, chromosomes are arranged in parallel and homologous pairs are sorted according to their lengths, which promotes contact between homologous loci (Chikashige et al., 1994; Chikashige et al., 1997; Ding et al., 1998; Yamamoto and Hiraoka, 2001; Chikashige and Hiraoka, 2001).

The freshwater ciliate *Tetrahymena thermophila* [formerly known as variety 1 or syngen 1 of *T. pyriformis* (Nanney and McCoy, 1976)], possesses a polyploid macronucleus (MAC) and a micronucleus (MIC), which represent the soma and the germline, respectively. For sexual reproduction, starving cells of different mating types conjugate and the MICs of the conjugating partners undergo meiosis. Haploid products of

meiosis fuse to form zygote nuclei and these divide and differentiate into new MACs and MICs, while the old MACs are eliminated (see Martindale et al., 1982; Cole et al., 1997; Karrer, 2000).

The MIC undergoes a remarkable change in shape during meiotic prophase (Ray, 1956; Sugai and Hiwatashi, 1974; Wolfe et al., 1976; Martindale et al., 1982) and six stages of meiotic prophase development of the MIC (Fig. 1) have been discriminated (Sugai and Hiwatashi, 1974; Martindale et al., 1982). Soon after the pairing of cells of different mating types, the MIC migrates away from the pocket in the side of the macronucleus (MAC), where it resides during interphase. In the process, it changes from spherical (stage I) to drop- or egg-shaped (early stage II). Next, it assumes the shape of a spindle (late stage II) and later of a torch (stage III). During stages I to III Sugai and Hiwatashi (Sugai and Hiwatashi, 1974) noted that the distribution of chromatin within the MIC is polarized. There is a dense mass near one end of the elongating MIC, from which thin chromonemata extend towards the opposite pole. In stage III nuclei the region on the side of the dense chromatin is elongated and narrowed (the 'neck') with a thickening at its end (the 'head'), whereas the 'trunk' contains less densely packed parallel chromatin threads (Fig. 1a). This stretching of the MIC is followed by further, extreme elongation to form the thread-like so-called crescent (stage IV). MIC elongation is promoted by intranuclear microtubules

(Davidson and LaFountain, 1975; Wolfe et al., 1976; Gaertig and Fleury, 1992). During stages V and VI, the MIC shortens again, which finally leads to the formation of five bivalents at diakinesis/metaphase I.

Establishing a temporal correlation between the stages of MIC development and the key events of meiosis, namely chromosome pairing and crossing over, is not easy as the chromosomes within the MIC cannot be clearly resolved. In addition, a synaptonemal complex (the meiotic pairing structure) has not been detected in *Tetrahymena* (Wolfe et al., 1976). Early interpretations of stage I MICs as corresponding to the pairing stage were inspired by the observation in related ciliate species of a 'parachute' stage [see p. 200 of Raikov (Raikov, 1982)] in which the chromatin arrangement resembled the bouquet, commonly believed to correspond to zygotene. The parachute/presumptive bouquet precedes the elongation of the MIC in these species and hence, it was inferred that the formation of the elongated MIC/crescent followed upon homologous pairing.

There are, however, several lines of evidence suggesting that recombination takes place in crescents prior to their maximal elongation. First, labeling RNA with [³H]uridine only produces grains on MICs from stages II to IV, suggesting the occurrence of RNA synthesis related to chromosome pairing activity during these stages (Sugai and Hiwatashi, 1974; Martindale et al., 1985). Second, DNA synthesis in micronuclei, which could

be interpreted as repair synthesis occurring in the course of meiotic recombination, does take place prior to full crescent formation (Allis et al., 1987). Third, the thickness of Giemsa-stained chromatin threads in stage II and III MICs led Sugai and Hiwatashi (Sugai and Hiwatashi, 1974) to conclude that the former corresponds to leptotene and the latter to zygotene. The interpretation that the critical stages of chromosome pairing do occur prior to full crescent formation is supported by the observation that the microtubule poison nocodazole prevents bivalent formation at metaphase I when it is applied after the initiation of but prior to full MIC elongation (Kaczanowski et al., 1985).

Ray (Ray, 1956) and Sugai and Hiwatashi (Sugai and Hiwatashi, 1974) proposed that the crescent contains chromosomes or bivalents in a tandem arrangement, rather than bundles of chromosomes in parallel arrangement. This interpretation was based on the observation that long crescents sometimes appear longitudinally split into two

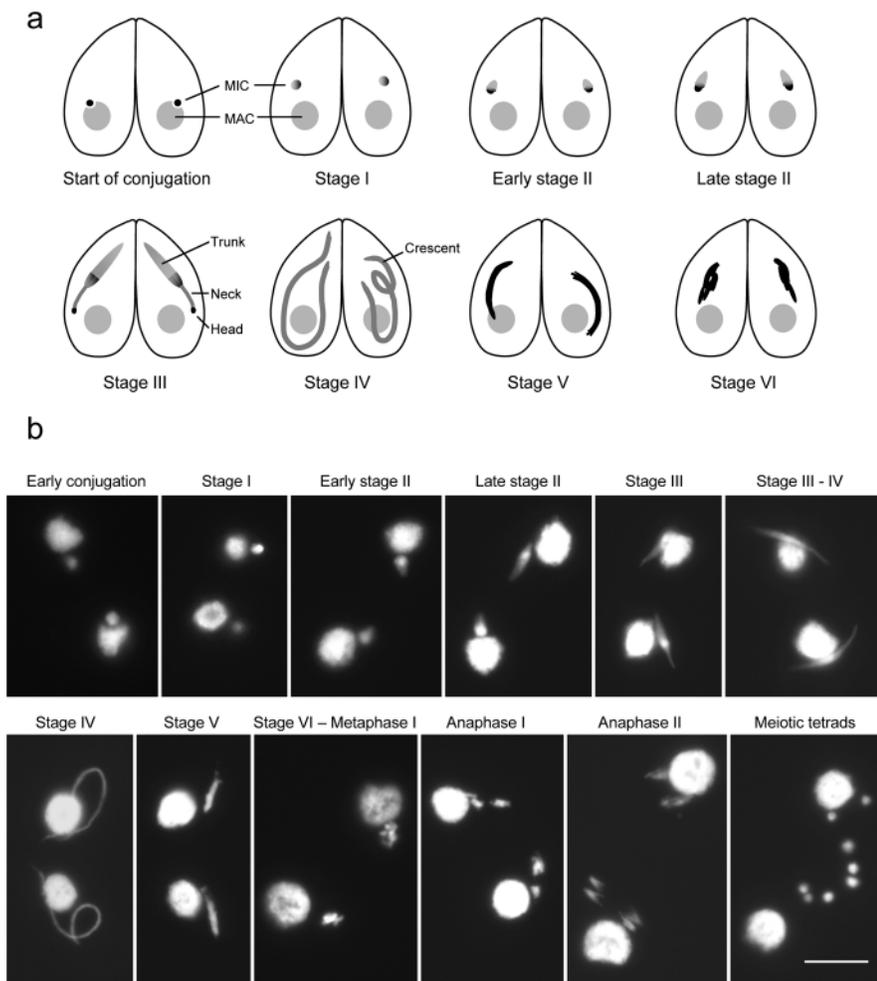


Fig. 1. (a) Schematic diagram of MIC development according to Sugai and Hiwatashi (Sugai and Hiwatashi, 1974), and Martindale et al. (Martindale et al., 1982). (b) Changing morphology and divisions of the MIC throughout conjugation as visualized by DAPI staining. Bar, 10 μ m.

subunits which could be either chromatids or chromosomes. By contrast, no evidence for such subdivision was found by Wolfe et al. (Wolfe et al., 1976) in electron microscope sections of MICs, but instead, only a single chromatin mass was present. However, in pictures of nuclei published by Davidson and LaFountain (Davidson and LaFountain, 1975) and by Karrer (Karrer, 1985), several strands of chromatin can be discriminated. Moreover, Bruns et al. (Bruns et al., 1982) found in nullisomic strains, in which some chromosomes are missing from the MICs, that crescents were fainter but not shorter. It is, therefore, conceivable that chromosomes are arranged side-by-side within the crescent and that the pairing of chromosome arms of similar lengths is promoted by the spatial constraints within the crescent in a similar manner to *S. pombe* horsetails.

We have examined which stages of meiotic prophase correspond to the various conformations of the MIC and how the chromosomes are arranged within it. Fluorescence in situ hybridization (FISH) was used to determine the stage at which chromosomes pair, individual chromosomes with incorporated BrdU were traced inside the MIC, and the temporal pattern of the localization of the recombination protein, Rad51, was studied by immunostaining. Finally, we discuss how the observed disposition of chromosomes can help to bring homologues into juxtaposition.

Materials and Methods

Strains and culture conditions

Strains B2086.1 (wild type) mating type II and CU428.2 *mpr1-1/mpr1-1* (6-methylpurine resistant) MAC mp sensitive mating type VII were kindly provided by Arno Tiedke (University of Münster, Germany). Culture was performed according to the method of Orias et al. (Orias et al., 2000). Cells were grown in proteose peptone medium (0.25% proteose peptone, 0.25% Difco yeast extract, 0.5% glucose) supplemented with 0.033 mM FeCl₃ at 30°C. Cells were starved in 10 mM Tris (pH 7.4). The cultures were kept in tall culture dishes filled with liquid to a level of less than 10 mm to provide sufficient aeration without shaking.

For mating, strains were cultured under the above conditions up to a concentration of 2×10^5 cells/ml, centrifuged, washed in 10 mM Tris (pH 7.4) and kept in this medium at 2×10^5 cells/ml for 16–24 hours. After starvation, cultures of different mating types were mixed and samples were taken at appropriate times.

Preparation and immunostaining

The preparation protocol was modified from Cole et al. (Cole et al., 1997). In short, a suspension of conjugating cells was fixed by the addition of formaldehyde and Triton X-100 (final concentrations of 4% and of 0.5%, respectively). After careful mixing the cells were left for 30 minutes at room temperature, then centrifuged and the pellet resuspended in 1:10 volume of 4% formaldehyde + 3.4% sucrose. Eighty microliters of this mixture were spread on a clean slide and air-dried. These slides can be used for immunostaining right away or stored for several days (up to several weeks in the freezer). The sucrose forms a viscous layer that prevents the denaturation of antigens.

For immunostaining, slides were incubated in 1× phosphate buffered saline (PBS) and 1× PBS + 0.05% Triton X-100 for 10 minutes each. Primary antibodies (1:50 mouse monoclonal antibody against recombinant Rad51 protein, Clone 51RAD01, NeoMarkers, Fremont, CA or 1:500 rabbit anti-phosphorylated H3(Ser10), Upstate Biotechnology, Charlottesville, VA, USA) were applied separately or together under a coverslip for 3 hours to overnight at room temperature. The coverslip was removed and the preparations were

rinsed with 1× PBS, postfixed with ice-cold 96% ethanol for 30 seconds and incubated in 1× PBS + 0.05% Triton X-100 for 10 minutes. FITC-conjugated anti-mouse antibody and/or Cy3-conjugated anti-rabbit antibody was applied under a coverslip for ~2 hours at room temperature. Finally, the slides were incubated twice for 10 minutes in 1× PBS and mounted under a coverslip in Vectashield anti-fading agent (Vector Laboratories Inc., Burlingame, CA, USA) supplemented with 1 µg/ml DAPI (4',6-diamidino-2-phenylindole) as a DNA-specific counterstain.

BrdU incorporation and preparation for BrdU staining

5-Bromo-2'-deoxyuridine (BrdU) was added to a final concentration of 2×10^{-4} M to exponentially growing cultures of vegetative cells. All manipulations with cultures exposed to BrdU were performed under red darkroom illumination. After 75 minutes exposure, unincorporated BrdU was removed by washing the cells twice with an excess of growth medium. The cells were then appropriately diluted in fresh medium and grown for 4–5 mitotic cycles to a density of $\sim 2 \times 10^5$ cells/ml. At a generation time of ~2.5 hours this density was reached after 10–13 hours. Cells were then starved and cultures of different mating types were mixed as described above. Those MICs that have not completed S phase in rich medium will do so after the transfer to starvation medium, such that by the initiation of meiosis most nuclei have gone through five replication cycles in the absence of BrdU and contain on average less than one BrdU-labeled chromosome (see below). Cells were harvested and prepared for cytology at 2.5 hours, 3.5 hours, 4.5 hours and 5.5 hours after mixing.

Five milliliters of conjugating cells were spun for 4 minutes at 1500 rpm, the supernatant was gently removed and the pellet was resuspended in 500 µl of Carnoy's fixative (methanol-chloroform-acetic acid, 6:3:2). Fixation was performed for 1 hour at room temperature; for longer storage, the cells were transferred to -20°C. Prior to preparation, the Carnoy's fixative was replaced with 100 µl of 70% ethanol. A 10 µl drop of cell suspension in ethanol was put on a slide and most of the liquid allowed to evaporate. A drop of 45% acetic acid was added and the material was squashed under a coverslip. The coverslip was then removed in liquid nitrogen and the preparation air-dried.

For the immunodetection of incorporated BrdU, slides were incubated in 70% formamide for 2 minutes at 65°C to induce DNA denaturation and to expose the bases to the antibody. Abcam (Cambridge, UK) ab6326 rat anti-BrdU antibody (1:40) was used as primary antibody and FITC- or Cy3-conjugated anti-rat antibody as secondary antibody. The staining regimen was the same as described above for Rad51 and phosH3 immunostaining.

Calculation of the frequency of occurrence of a single BrdU-painted chromosome per nucleus

Assuming that intact sister chromatids segregate independently into daughter cells, the probability P_0 that all chromosomes are unlabeled after a given number of generations (g) post BrdU pulse is $P_{0(g,x)} = (1 - 1/2^g)^x$, where x is the diploid chromosome number (i.e. 10 in *Tetrahymena*). The probability P_1 that exactly one chromosome is labeled after g generations is $P_{1,g} = x [1/2^g (1 - 1/2^g)^{x-1}]$. From the observed fraction of unlabeled nuclei (P_0), the mean number of replication cycles undergone after withdrawal of BrdU can be determined. The expected frequency $P_{>1}$ of nuclei with more than one labeled chromosome is $P_{>1} = 1 - P_0 - P_1$.

Fluorescence in situ hybridization (FISH)

Two single-copy DNA probes, *Teth1* and *Teth2* were amplified from *Tetrahymena* genomic DNA using the following primers: T1.3 forward CATCATCCATATATCCAGGAC and T1.2 reverse ACTC-TGAGACATCATCAGGG (*Teth1*) and: T2.3 fwd CGTTCATTA-

TGCTATAGCCA and T2.2 rv AAAGGAATGCTTGAAGGCTC (*Teth2*). The primers were designed from the *Tetrahymena* MAC genomic sequence available from The Institute for Genomic Research website at <http://www.tigr.org>. Each reaction yielded a PCR product of approx. 4 kb. *Teth1* and *Teth2* are located on the same contig and separated by 167 kb of sequence. The PCR products were labeled with digoxigenin or biotin using the respective Nick Translation kit according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany).

A compound probe against the telomere ($(G_4T_2)_n$ repeat (approximately 2.0–3.4 kb per average telomere) and the subtelomere ($(G_4T_3)_n$ repeat (corresponding to 0.6–1.0 kb per average telomere) (Kirk and Blackburn, 1995) was used. 5' biotinylated 24-mer oligonucleotides homologous to the telomere and subtelomere sequences were synthesized (Invitrogen GmbH, Karlsruhe, Germany). To enhance the telomere repeat signal oligos were 3'-tailed with biotin-11-dUTP as described previously (Scherthan, 2002).

FISH was performed on slides prepared either by formaldehyde fixation (as for immunostaining) or by Carnoy fixation (as for BrdU detection) as described above. Preparations were hydrated in H_2O followed by treatment with 1 M sodium thiocyanate at 90°C for 15 minutes. Denaturation was performed in 70% formamide, $2\times$ SSC, pH 7.1 for 2 minutes at 68°C. The slides were then rinsed in H_2O and air-dried. DNA probes were dissolved in hybridization solution (50% formamide, 10% dextran sulphate, $2\times$ SSC) at 50 ng/ μ l and unlabeled *Tetrahymena* DNA was added to 125 ng/ μ l to suppress unspecific binding of the probe. Telomere probes, either G-strand probes only or a mixture of all oligonucleotides, were diluted to 0.5 ng/ μ l in hybridization solution containing 1 μ g/ μ l of *E. coli* carrier DNA. The hybridization mixtures were denatured at 95°C for 3 minutes. The PCR product-containing mixture was incubated at 37°C for 25 minutes. Probes were sealed with rubber cement (Marabu, Hamm, Germany) on the denatured slides under a coverslip. Hybridization was allowed to proceed for 48 hours at 37°C in a moist incubator. After hybridization, slides were washed three times in $0.05\times$ SSC at 37°C and biotin was detected using avidin-FITC (Sigma) as described previously (Scherthan, 2002). Hybrid molecules were visualized by rhodamine-conjugated anti-digoxigenin Fab fragments (Roche) and/or by Extravidin-FITC (Sigma) for biotin molecules (for details, see Scherthan, 2002). Finally, slides were embedded in Vectashield supplemented with DAPI.

Microscopy and evaluation

Preparations were evaluated using an epifluorescence microscope equipped with single-band-pass filters for the excitation of blue (DAPI), green (FITC) and red (rhodamine and Cy3) fluorescence. Pictures were taken with a cooled CCD camera of sufficiently flattened cells or, in some instances, a series of focal planes was recorded, deconvolved and projected in two dimensions using Zeiss Axiovision and ImageJ (Wayne Rasband, N.I.H.; <http://rsb.info.nih.gov/ij/>) software.

Whole-mount spreading and silver staining of nuclei

The method for the detection of silver-positive structures within nuclei was similar to protocols that have been used previously for visualizing SCs of a variety of plant, fungal and animal species (e.g. Loidl et al., 1998). For the spreading of nuclei, 20 μ l of a dense suspension of conjugating cells (4×10^6 cells/ml) in 10 mM Tris (pH 7.4) were mixed with 50 μ l detergent (0.5% Lipsol laboratory cleaning agent) on a slide, which caused the cells to swell and burst. After a few seconds, 80 μ l of fixative (4% paraformaldehyde + 3.4% sucrose in distilled water) was added. The slides were air-dried in a chemical hood overnight. Staining with silver nitrate (a 50% solution of $AgNO_3$ in distilled water) and transfer of stained material to electron microscopic grids was performed as described previously (Loidl et al., 1998).

Results

The recombination protein Rad51 is abundant in meiotic micronuclei

Conjugating cells were immunostained for the conserved Rad51 protein. Rad51 is a recombinase that is involved in the homologous DNA pairing and strand exchange reaction in the course of recombinational DNA repair, and it is generally believed to associate with meiotic double-strand DNA breaks (DSBs) (Shinohara et al., 1992) (for reviews, see Paques and Haber, 1999; Sung et al., 2003). MICs up to early stage II contained no or only traces of Rad51 (Fig. 2a,b). Homogenous Rad51 labeling was observed in spindle-shaped MICs in late stage II (Fig. 2c). To establish a temporal correlation between incipient chromosome condensation in MICs and the deposition of Rad51, double immunolabeling with Rad51 and phosphorylated histone H3 (phosH3) was performed (Fig. 2e–l). Phosphorylation of H3 has been found to correlate with mitotic and meiotic chromosome condensation in *Tetrahymena* (Wei et al., 1998).

Crescents in stages III to V showed strong Rad51 labeling along their entire lengths (Fig. 2d–h). High magnification and image deblurring revealed that the label consisted of numerous individual spots (Fig. 2d). From a few particularly well-flattened crescents it was estimated that they contain over 100 spots. To rule out the possibility that the grainy appearance of the Rad51 staining merely reflects the overall staining of inhomogeneously distributed chromatin, parallel immunostaining of phosH3 was performed. Rad51 and phosH3 spots did not colocalize (Fig. 2f), thus validating the genuineness of the Rad51 foci. That Rad51 was most abundant in the crescent stage agrees with the report that *RAD51* transcription is strongest around 4 hours after conjugation (Marsh et al., 2000). Rad51 was still present in stage V nuclei (Fig. 2h), but much of the staining fell outside the DAPI-positive areas and hence probably represents Rad51 that has detached from chromatin. Only during metaphase I and anaphase I, did Rad51 staining become progressively weaker and disappeared completely during the second meiotic division (Fig. 2i–l). In post-meiotic MICs, Rad51 reappeared, probably in conjunction with DNA replication (data not shown).

Rad51 foci are present in macronuclei

As can be seen in many of the figures showing Rad51 immunostaining (Fig. 2), the MACs also often display granular or filamentous Rad51 signals. In fact, all of the 200 MACs in conjugating cells that were specifically inspected for this feature, exhibited Rad51 signals. To determine if this was specific to nuclei in conjugating cells, both exponentially growing and starving cells (that had been kept for 72 hours on 10 mM Tris) were scored. In the former, 77% ($n=300$) of non-dividing MACs, and in the latter all 300 MACs scored showed Rad51 signals. Similarly, detectable *RAD51* message has been found to be present in cells at 0 hours of mating (Marsh et al., 2000). Notably, Rad51 signals were absent in amitotically dividing MACs and in the new macronuclear precursors (anlagen) after fertilization. Thus, while it is clear from these preliminary observations that the occurrence of Rad51 spots in MACs is not limited to meiotic cells, their nature has to remain the subject of future studies.

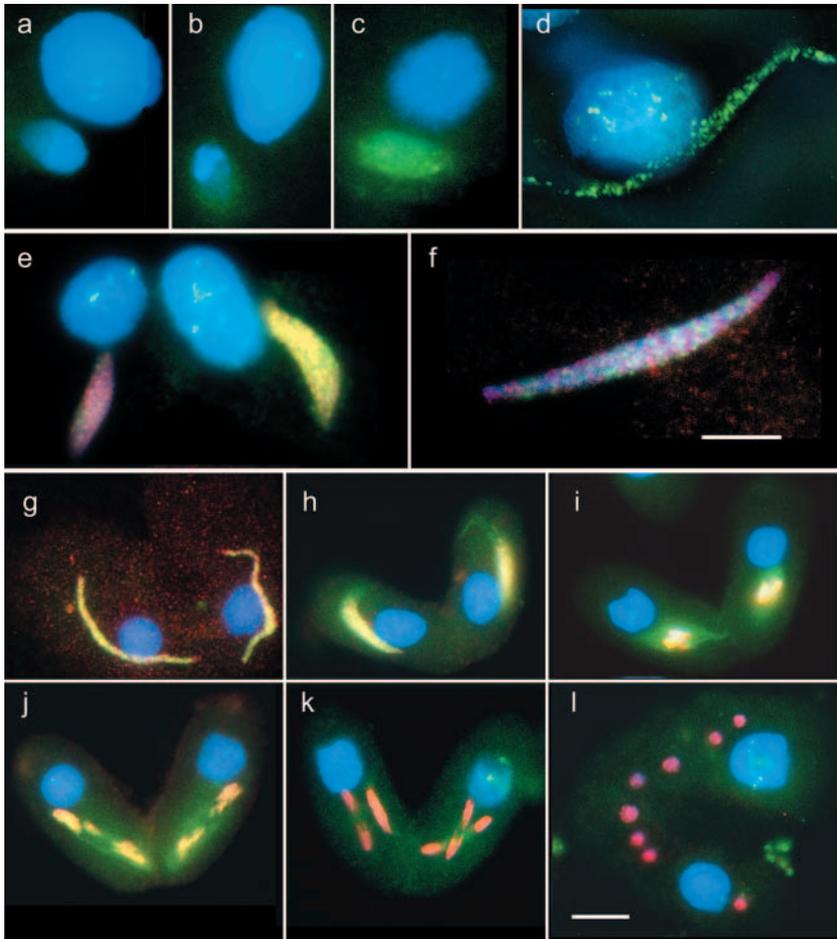


Fig. 2. (a-d) Immunostaining of Rad51 (green). No Rad51 (a) or very little (b) was detected in the early stage II MIC. (c) A large amount of Rad51 was detected in the somewhat later stage II MIC. (d) A stage IV MIC with granular staining of Rad51 (projection of a deconvolved Z stack of images). (e-l) Immunostaining of Rad51 (green) and phosphorylated histone H3 (phosH3) (red). phosH3 acts as a marker for chromatin condensation. (e) MACs and MICs in a conjugated pair of cells with slightly asynchronous meiosis. In the smaller (earlier) MIC (left), the red phosH3 staining predominates because there is only a small amount of Rad51 present. In the later MIC (right) the strong Rad51 and the phosH3 staining mix to produce a yellow color. (f) In this stage III MIC, both phosH3 and Rad51 produce granular immunostaining patterns but the spots do not overlap. (g-l) Stages IV (g), V (h), metaphase I (i), anaphase I (j), anaphase II (k) and the tetrad stage (l). Some Rad51 remains on chromatin (overlapping with phosH3) until anaphase I, but much of it is shed from the chromosomes by metaphase I (note the green staining of the spindle in i), and it has virtually disappeared by anaphase II. Note that the MACs contain Rad51-positive spots (see text). The chromatin is stained with DAPI (blue). Bars, 5 μm (in f for a-f) and 10 μm (in l for g-l).

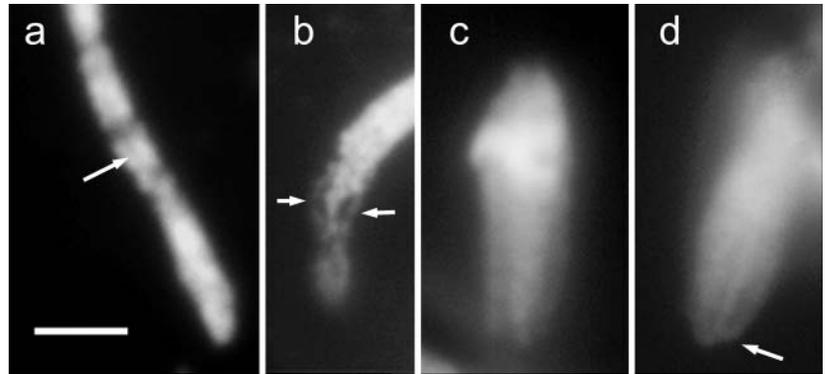
Chromosomes span much of the length of the crescent
Two reports in the past (Ray, 1956; Sugai and Hiwatashi, 1974) have proposed that chromosomes are arranged in tandem within the crescent. The basis for this interpretation was that the chromatin in the crescents sometimes appears segmented and, in addition, is longitudinally split in half. While this observation (Fig. 3a) may appear to imply the tandem arrangement of bipartite structures, either replicated chromosomes or bivalents, this interpretation cannot be reconciled with the occasional observation of crescents that seem to contain bundles of DAPI-positive chromatin fibers (Fig. 3b) [see also Fig. 1 of Davidson and LaFountain (Davidson and LaFountain, 1975) and Fig. 1b of Karrer (Karrer, 1985)].

To study the organization of chromosomes within the crescent in more detail, we applied the method of replication labeling (see Zink et al., 1998). With this method, individual chromosomes are differentially stained through the incorporation of BrdU instead of thymidine during S phase. At the end of S phase in the presence of BrdU, both chromatids of every chromosome have incorporated BrdU in one half-strand and daughter cells obtain BrdU chromatids. During subsequent S phases in the absence of BrdU, the newly synthesized half-strand will incorporate thymidine. During the following mitosis, chromatids are segregated randomly, such that only some (on average 50%) of the chromosomes of a daughter nucleus are BrdU labeled. During each subsequent

cell cycle following the withdrawal of BrdU, the number of BrdU-labeled chromosomes of a nucleus is further reduced, and after a certain number of divisions (which depends on the number of chromosomes), on average, only a single chromosome will be labeled with BrdU. For *Tetrahymena* MICs in which $2n=10$, the probability of a nucleus carrying a single labeled chromosome after five generations post BrdU pulse is $\sim 24\%$. The probability that two or more chromosomes are labeled is $\sim 3\%$; i.e., in seven out of eight nuclei carrying a signal, it stems from a single chromosome (see Materials and Methods). If MICs are then induced to perform meiosis and BrdU is detected by immunostaining, the spatial organization of individual chromosomes can be studied.

After the described pre-treatment, MICs at stages II, III and IV always contained elongated BrdU signals, which extended for most of or the entire length of the nuclei (Fig. 4). If chromosomes were arranged in tandem, labeling would be expected only in a sector of the MIC. Although we cannot exclude that a minority of the nuclei contained two or more BrdU-stained chromosomes, the sole presence of thread-shaped signals strongly suggests that the chromosomes within the crescents are arranged in parallel and that they extend from one end of an elongated MIC to the other. BrdU threads occasionally formed a U-turn in stage II and III MICs (Fig. 4b), which is consistent with the presence of DAPI-stained chromatin loops in the trunk (Fig. 3d). BrdU-labeled chromosomes were often observed not to extend along the entire length of crescents (Fig. 4e), which could be explained by sister chromatid exchanges (SCEs) between labeled and unlabeled chromatids during one of the mitotic S phases prior to conjugation. We have obtained circumstantial evidence for the existence of such SCEs (data not shown).

Fig. 3. The substructure of meiotic MICs as seen by DAPI staining. (a) Stage IV crescents sometimes appear split into two longitudinal subunits that are separated by a gap (arrow) and have dark cross bands, which gives them the misleading appearance of being composed of tandemly arranged bipartite units (either replicated chromosomes or bivalents). (b) A broken stage IV crescent, which reveals its true composition of multiple strands (arrows). (c) A stage II MIC with a DNA-rich region (brightly stained with DAPI) near one pole, striated as if consisting of individual strands, and less dense chromatin towards the opposite pole. (d) In this stage II MIC the chromatin strands seem to form loops (arrow). Bar, 5 μm .



While most nuclei exhibited a single BrdU-positive thread, some contained double threads (Fig. 4d,e). The latter could represent nuclei containing more than one BrdU-labeled chromosome or chromosome portion. Alternatively, double threads could result from loop formation by a single BrdU-painted chromosome, which is consistent with a bouquet arrangement of chromosomes. In contrast to stage II to IV MICs, late stage V and stage VI nuclei showed one or several thick spots of BrdU (Fig. 4f-h), which suggests that the chromosomes that initially fill the space from one end of the MIC to the other, shorten prior to diplotene.

Most if not all telomeres assemble near one pole of the early crescent

Sugai and Hiwatashi (Sugai and Hiwatashi, 1974) observed

chromatin threads projecting from a densely-packed chromatin mass near one pole of the MIC. Our DAPI staining (Fig. 3d) and BrdU-painting (Fig. 4b) suggest that these chromatin threads may form loops within the trunk, at least in stage II and stage III MICs. To decide whether chromosomes within crescents are arranged in such a way that one end points to one end of the crescent and the other end to the opposite end, or if chromosomes form loops, we performed FISH to highlight chromosome ends. While FISH signals were too weak to be detected on individual telomeres, possibly owing to the small size of the target sequences (no signals were observed in diakinesis bivalents or on anaphase I chromosomes), the chromatin-dense pole of stage II MICs and the head of stage III MICs regularly carried a signal, which presumably resulted from the assembly of several if not all of the telomeres in this region (Fig. 5a-c). Furthermore, in the strongly elongated stage IV crescents, the former head end (as identified by the adjacent DAPI-dull neck) carried strong telomere FISH signals (Fig. 5d). This suggests that at the beginning of MIC elongation, telomeres congregate near the head end of the MIC and that during MIC elongation the arrangement of chromosomes resembles the bouquet (Scherthan, 2001; Bass, 2003). Condensed chromosomes in late stage V and stage VI can assume positions anywhere along the spindle-shaped MIC (see Fig. 4f-h), which implies that telomeres have detached by these stages.

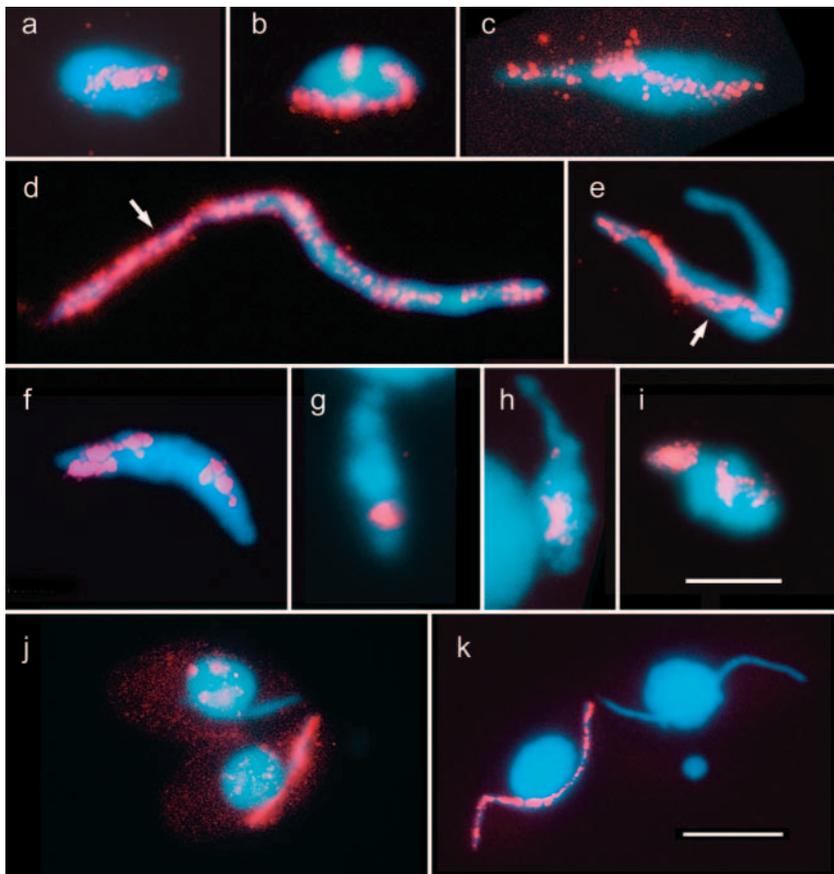


Fig. 4. Chromosome painting by BrdU incorporation. BrdU-labeled chromosomes (red) extend along their entire length of the MICs in stage II (a,b), stage III (c) and stage IV (d). This observation is compatible both with a pole-to-pole and a narrow hairpin-loop arrangement, but not with a tandem arrangement of chromosomes within the crescents. In stage V (e,f) and stage VI (g,h) MICs, and also in diplotene-diakinesis (j), the chromosomes occupy an increasingly compact area. In d and e, some regions of the BrdU-positive threads appear double (arrows). The nuclei shown in f and i seem to contain two labeled chromosomes. (j,k) Conjugating cells, one of which has a labeled crescent. In addition both MACs in j carry labeled DNA. In a-d, the head pole is on the left. The chromatin is stained with DAPI (blue). Bars, 5 μm (in i for a-i) and 10 μm (in k for j,k).

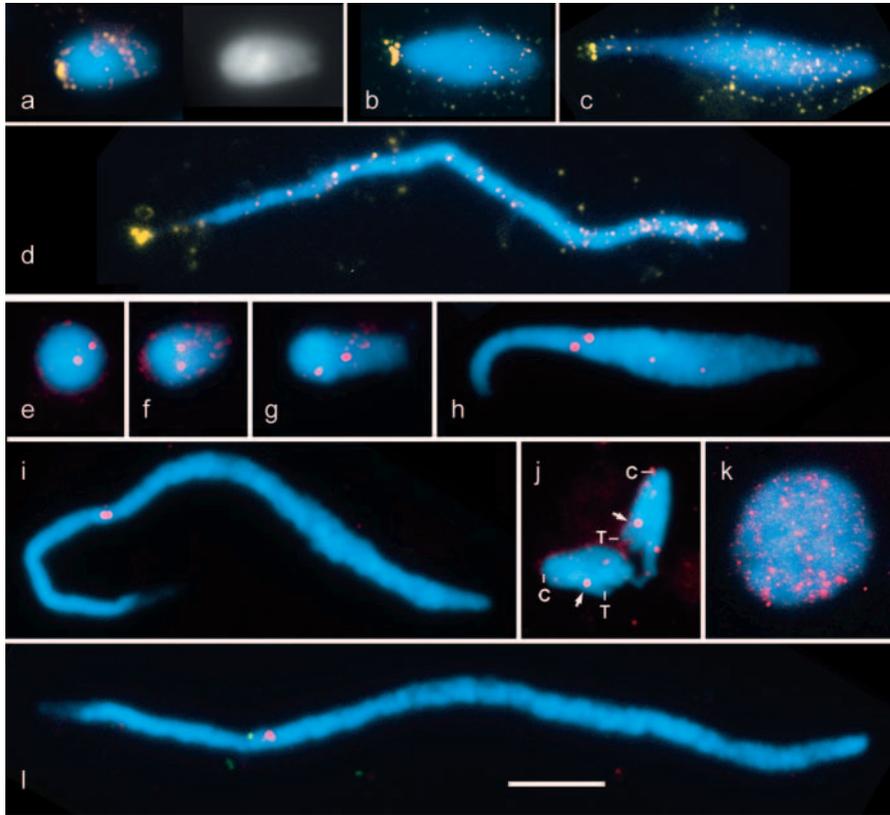


Fig. 5. FISH of meiotic MICs. (a-d) Telomere FISH (yellow) signals are most prevalent at the DAPI-bright, DNA-rich pole of stage II nuclei (a,b). The black and white image in (a) shows the DAPI distribution within the nucleus shown on the left. (c) In stage III nuclei, telomere FISH delineates the head region, which is separated from the trunk by the DAPI-dull neck (for designation see Fig. 1a). (d) The telomeres in stage IV MICs are clustered in the region corresponding to the former head as can be seen by the adjacent DAPI-dull area. (e-l) FISH with locus-specific probes. A pair of *Teth2* loci (red) on homologous chromosomes appear mostly separate in stages I to III nuclei (e-h) and associated or fused as a single dot in stage IV (i). The position of the FISH signal in anaphase I (j) configurations confirms that the probed locus occupies a region in the distal third of a chromosome arm [arrows between C (centromere) and T (telomere)]. (k) A macronucleus (at half the magnification) shows multiple copies of the locus highlighted by the *Teth2* probe. (l) A second locus (*Teth1* – green) on the same chromosome and 167 kb away from *Teth2* (red) is always located closer to the neck region (DAPI-dull to the left) of the crescents. All MICs are oriented with the head pole to the left. Bar, 5 μ m.

Table 1. Pairing of homologous loci at different meiotic stages as indicated by the presence of separate, closely associated (double) and fused (single) FISH signals

	Stage I	Early stage II	Late stage II	Stage III	Stage IV
Separate dots	83%	84%	86%	77%	16%
Double dots	9%	11%	6%	17%	38%
Single dot	8%	5%	8%	6%	46%

100 nuclei from each stage were scored.

Homologous loci become paired in stage IV micronuclei

To determine the onset and progress of meiotic pairing, we performed FISH with a 4 kb probe (*Teth2* – see Materials and Methods) which was derived from the random sequences of the approx. 270 macronuclear ‘chromosomes’ (<http://www.tigr.org>). Since these ‘scaffolds’ have not been anchored to micronuclear chromosomes, the precise position of this probe remains to be elucidated. However, the probe created two distinct signal spots, thus providing us with the means of monitoring the pairing of homologous loci in the MIC.

FISH signals were mostly clearly separated in stage I to stage III MICs (Fig. 5e-h) and appeared as single or as associated (touching) double dots in stage IV crescents (Fig. 5i). There was little evidence of progressive alignment prior to stage IV (Table 1). From the point at which two opposite poles (DAPI-bright and DAPI-dull) could be discriminated in stage I nuclei, signals were found to localize to the DAPI-bright area (next to what is later to become the ‘neck’) (Fig. 5e-h). In stage IV crescents whose two poles could be discriminated, the position of *Teth2* FISH signals was determined. In the 30 nuclei scored, the average position of the signal was at $28 \pm 5\%$ of the distance from the head (=telomere pole) to the opposite end.

In another series of experiments, two chromosomal loci (*Teth1* and *Teth2*) separated by 167 kb were labeled with different colors (Fig. 5l). In all 20 stage IV crescents where the two colors could be detected simultaneously, the *Teth1* probe was closer to the head. This indicates that the orientation of the chromosomes within the crescent is straight and not meandering. The fact that the probed chromosomal locus virtually always occupied the head-proximal portion of the crescent, coupled to the fact that the order of red and green signals was always the same, indicates that the probe-tagged chromosome arm was positionally fixed and suggests that it is anchored with its telomere in the head region, which carries the telomere cluster. If this is the case for one chromosome, it is reasonable to assume that all of the chromosome arms behave similarly and hence, that chromosomes are arranged in a bouquet configuration.

In accordance with the localization of the *Teth2* locus in the vicinity of the head, which also accommodates the telomeres, FISH on diakinesis (not shown) and anaphase I chromosomes (Fig. 5j) confirmed its position in the subtelomeric region of a chromosome arm. In MACs multiple copies of the locus were highlighted with the *Teth2* probe (Fig. 5k).

The search for a synaptonemal complex (SC)

The SC is a proteinaceous structure, which has been described in most organisms that perform canonical meiosis and is of similar appearance across phylogenetic groups. The SC has been implicated both in the stabilization of homologous pairing and in the conversion of crossovers into chiasmata (see Zickler and Kleckner, 1999). While previously it had also been attributed a role in crossover interference (the phenomenon in which only a subset of recombination intermediates is transformed into crossovers and that there is a reduced probability of neighboring intermediates becoming crossovers), this has been challenged recently (see Discussion).

As Wolfe et al. (Wolfe et al., 1976) failed to detect an SC by electron microscopy of thin-sectioned crescents, we applied a spreading method in an attempt to observe SCs in silver-stained whole-mount nuclei. Neither structures resembling SCs or SC precursors nor even SC-related structures, such as the linear elements of *S. pombe* (Bähler et al., 1993), were observed (data not shown). Moreover, a preliminary bioinformatic search of the *Tetrahymena* genome has not recovered sequences with an obvious homology to genes for SC structural proteins.

The probable absence of an SC raises the question of whether its lack in *Tetrahymena* is an ancient property of meiosis, because the ciliates are phylogenetically widely separated from animals, plants and fungi (Frankel, 2000; Stechmann and Cavalier-Smith, 2003) in which SC have been found. However, the likely occurrence of an SC in other members of the ciliates [see pp. 199-201 in Raikov (Raikov, 1982)] suggests that, like the fungi *S. pombe* (Olson et al., 1978), *Aspergillus nidulans* (Egel-Mitani et al., 1982) and possibly also *Ustilago maydis* (Fletcher, 1981), *Tetrahymena* has lost the SC late in its evolution.

Discussion

Rad51-dependent recombination initiates in stage II MICs

Since an SC has not been detected in *Tetrahymena*, correlating the stages of MIC development to the classical meiotic prophase stages of leptotene, zygotene and pachytene is difficult, as these stages are normally defined by SC development and the progression of synapsis. In most organisms studied to date, the Rad51 protein, which plays a role in the recombinational repair of meiotic DSBs (Paques and Haber, 1999; Brush, 2002), first appears as foci around leptotene/early zygotene and disappears soon after the initiation of SC formation, at which time the intimate pairing of homologues is accomplished (Alpi et al., 2003; Colaiácovo et al., 2003) (also references cited in Alpi et al.). Accordingly, the period between early and late stage II of *Tetrahymena* meiosis, might roughly correspond to zygotene.

It was proposed that Rad51 plays a role in the homology search in addition to its role in recombination (Pawlowski et al., 2003). In *Tetrahymena*, Rad51 is still abundantly present in stage IV crescents, which might suggest that the processing of DSBs (and homology search) continues up to this stage. In fact, close pairing as visualized by the association of homologous FISH signals, only occurs in stage IV. However, considerable amounts of Rad51 persist until metaphase I (when the presence of chiasmata indicates the successful completion of crossing over) and beyond, and it is conceivable that Rad51 may be retained within nuclei in an inactive state.

In addition to Rad51 foci in meiotic MICs, we also detected

Rad51 foci in the MAC precursors (anlagen) of exconjugants (but not of conjugants!), in MACs of exponentially growing cells except during amitosis, as well as in MACs of conjugating and starving cells. The probable explanation for the presence of Rad51 in Anlagen and in the MACs of dividing cells is the involvement of Rad51 in repair processes during replication (Marsh et al., 2000) as well as in chromosome fragmentation, sequence elimination, telomere addition and recombination processes during macronuclear development (Deak and Doerder, 1998). Furthermore, transcription-associated recombination may take place and may require the activity of Rad51 (García-Rubio et al., 2003). A possible explanation for the presence of Rad51 in starving cells could be its involvement in apoptosis-like DNA degradation in starved cells (Maercker et al., 1999).

A polarized chromosome arrangement prevails in meiotic MICs

The organization of chromosomes in the crescent stage of *Tetrahymena* has so far remained elusive (see Karrer, 1985). We have now shown that after BrdU-painting, individual labeled chromosomes in stage II to stages IV-V MICs occupy most of or the entire length of the crescent. Moreover, telomere FISH revealed that most if not all chromosome ends are clustered near one pole of the nucleus, and finally, chromosomal loci highlighted by FISH always occupied a fixed position within the polarized MIC. This combined evidence suggests that chromosomes are arranged either in a classical bouquet or in a one-sided bouquet [the polarization of only one telomere per chromosome – cf. fig. 1c in Scherthan (Scherthan, 2001)] in stage II to stage IV MICs.

The bouquet is a widely conserved arrangement of chromosomes in meiotic prophase which is characterized by the congression of telomeres at the inner surface of the nuclear membrane (Zickler and Kleckner, 1998; Scherthan, 2001). It is not yet clear if and how the bouquet supports the mutual recognition and alignment of homologues. Recently, it was shown that homologous chromosomes in *Sordaria* are presynaptically aligned before the bouquet is formed (Storz et al., 2003). It is possible that the contact of chromosomes with a specialized region of the nuclear periphery facilitates SC initiation (Moens et al., 1989; Alsheimer et al., 1999). However, simultaneous pairing and initiation of synapsis of chromosomes in the bouquet was convincingly demonstrated by Scherthan et al. (Scherthan et al., 1996) and Bass et al. (Bass et al., 2000).

In *Tetrahymena*, it is not yet possible to say with certainty whether the chromosomes adopt a classical bouquet arrangement or a 'one-sided bouquet' arrangement, with only one end of each chromosome anchored to the head pole of the MIC and the other pointing towards the opposite pole (trunk). In late stage II nuclei, DAPI-positive chromatin strands and BrdU-painted chromosomes sometimes exhibit loops (Fig. 3d, Fig. 4b), which suggests a classical bouquet arrangement with U-shaped chromosomes. This arrangement is not, however, necessarily maintained until stage IV. The observation of telomere clustering at the head-pole does not help in discerning between a classical bouquet and a one-sided bouquet, as the free ends of chromosomes, if scattered across the crescent, might escape detection. The fixed position of loci (as detected by FISH) near the head region, strongly suggests that the telomere of the corresponding chromosome arm is invariably anchored in the

head region. Therefore, unless it is assumed that a strong preference exists for one particular telomere of each chromosome to be included in the cluster, the assembly of all telomeres and hence the presence of a classical bouquet remains the more likely explanation.

The crescent shape of the MIC may promote the juxtaposition of homologous loci

The change in shape of the MIC invites the interpretation that spatial constraints are imposed on the chromosomes, which promote and stabilize the alignment of homologues. This could be analogous to the situation in the so-called horsetail nuclei of *S. pombe* where the nucleus becomes elongated and moves within the cell. This is believed to facilitate homologous chromosome pairing by aligning homologous telomere-bundled chromosomes (e.g. Yamamoto and Hiraoka, 2001).

In organisms that develop an SC, the bouquet is transient and the SC helps to stabilize the physical contact of homologous regions beyond the bouquet stage even when chromosomes disperse within a normally spherical nucleus. In *S. pombe*, which does not form an SC, the bouquet is maintained until crossing over is achieved (Ding et al., 2004). In *Tetrahymena*, in which an SC has not yet been detected (Wolfe et al., 1976) (this study), chromosomes might be kept in register until completion of the crossing over process by the prolonged maintenance of telomere attachment and their confinement within the narrow tube of the crescent.

Does chiasma interference occur in the absence of an SC?

Chiasma/crossover interference is defined as the mutual suppression of chiasmata or crossovers in adjacent regions of chromosomes (for review see Roeder, 1997). Another expression of chiasma interference is that the distribution of chiasma number per bivalent is narrower than the Poisson distribution predicts (Haldane, 1931; Jones, 1984). As organisms lacking an SC do not exhibit interference (Egel-Mitani et al., 1982; Maguire, 1988; Kohli and Bähler, 1994) and in mutants incapable of synapsis, interference is reduced or missing (Sym and Roeder, 1994), it has been proposed that the SC mediates crossover interference by transporting a suppressor signal (Chua and Roeder, 1997; Tung and Roeder, 1998; Novak et al., 2001).

The failure of our and previous attempts (Wolfe et al., 1976) to cytologically detect an SC in *Tetrahymena* prompts the question of the existence of crossover/chiasma interference in this organism. Limited cytological evidence suggests that *Tetrahymena* features morphologically regular bivalents with one or two chiasmata [see figures of bivalents in Ray (Ray, 1956) and Karrer (Karrer, 2000)]. With a genome size of ~210 megabases (Karrer, 2000), a recombination frequency of 200 kb/cM [estimated from RAPD polymorphisms (Wickert and Orias, 2000)] would translate into ~21 chiasmata per chromosome complement and meiosis or ~4 chiasmata per bivalent. The presence of such a low number of chiasmata (estimated to be 1-2 or ~4 from cytological and genetic assays, respectively) would suggest that chiasma formation is under some form of control. If random chiasma formation resulted in an average of one or two chiasmata per bivalent, a high percentage of bivalents would run the risk of not receiving a

single chiasma. According to the Poisson distribution $P_{(0)}=e^{-\lambda}$ (where $P_{(0)}$ is the probability of receiving zero chiasmata, $e=2.718$, and λ is the average number of chiasmata for a pair of homologues), homologous pairs with an average number of 2 or 4 chiasmata would remain univalent in over 10% or ~2% of cases, respectively. For *Tetrahymena* with five pairs of homologues, this would mean that ~50% or ~9% of metaphase I cells, respectively, would contain at least one pair of univalents prone to nondisjunction. (In order to reduce the risk for a pair of homologues receiving no chiasma to less than 0.1%, an average seven crossovers per bivalent would have to occur. In this case, 99.5% of cells would form only bivalents.) In the presence of interference, a large number of recombination events would be initiated (as is in fact suggested by the presence of numerous Rad51 spots) but not result in crossovers except for the 2-4 chiasmata per bivalent that are believed to occur.

An alternative possibility, which remains to be tested, is that a minimum number of chiasmata is ensured by the existence of strong crossover hotspots, leading to localized chiasmata. It is also possible that *Tetrahymena* has an SC whose components are uncommon, in that they are refractory to the conventionally used contrasting and staining procedures. Future searches of the sequenced *Tetrahymena* genome will reveal whether orthologues of genes encoding known SC components are present in *Tetrahymena*.

Evidence is, however, accumulating that the SC is dispensable for crossover interference. Page and Hawley (Page and Hawley, 2001) showed in *Drosophila* mutants that complete SC formation in oocytes is not required for crossover interference. In budding yeast, Fung et al. (Fung et al., 2004) observed interference between Zip3 foci, which are considered to mark the sites of crossing over and appear prior to or in the absence of the SC. However, these authors did not preclude the existence of an additional interference mechanism that depends on the SC. Similarly, Börner et al. (Börner et al., 2004) compared the timing of DNA events of recombination and SC formation in yeast and showed that the crossover/non-crossover (gene conversion) decision is made before or during the appearance of single-end invasions, which is long before the appearance of the SC. In the light of these recent findings, it is possible that *Tetrahymena* presents yet another example of crossover/chiasma interference in the absence of an SC.

We are grateful to Arno Tiedtke for strains, to Nils Hartmann for technical assistance, Franz Klein for helping with the mathematics, and to Kathleen Karrer and Maria Siomos for valuable comments on the manuscript. Preliminary sequence data was obtained from The Institute for Genomic Research website at <http://www.tigr.org>. This work was supported in part by research grant P16282 from the Austrian Science Fund.

References

- Allis, C. D., Colavito-Shepanski, M. and Gorovsky, M. A. (1987). Scheduled and unscheduled DNA synthesis during development in conjugating *Tetrahymena*. *Dev. Biol.* **124**, 469-480.
- Alpi, A., Pasierbek, P., Gartner, A. and Loidl, J. (2003). Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. *Chromosoma* **112**, 6-16.
- Alsheimer, M., von Glasenapp, E., Hock, R. and Benavente, R. (1999). Architecture of the nuclear periphery of rat pachytene spermatocytes: distribution of nuclear envelope proteins in relation to synaptonemal complex attachment sites. *Mol. Biol. Cell* **10**, 1235-1245.

- Bass, H. W. (2003). Telomere dynamics unique to meiotic prophase: formation and significance of the bouquet. *Cell Mol. Life Sci.* **60**, 2319-2324.
- Bass, H. W., Riera-Lizarazu, O., Ananiev, E. V., Bordoli, S. J., Rines, H. W., Phillips, R. L., Sedat, J. W., Agard, D. A. and Cande, W. Z. (2000). Evidence for the coincident initiation of homolog pairing and synapsis during the telomere-clustering (bouquet) stage of meiotic prophase. *J. Cell Sci.* **113**, 1033-1042.
- Bähler, J., Wyler, T., Loidl, J. and Kohli, J. (1993). Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. *J. Cell Biol.* **121**, 241-256.
- Börner, G. V., Kleckner, N. and Hunter, N. (2004). Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. *Cell* **117**, 29-45.
- Bruns, P. J., Brussard, T. B. and Merriam, E. V. (1982). In vitro genetic engineering in *Tetrahymena*. In *Progress in Protozoology* (Proc. VI. Int. Congr. Protozool.), Acta Protozool. special congress volume, Part I (ed. S. Dryl, S. L. Kazubski, L. Kuznicki and J. Ploszai), pp. 31-44. Stuttgart, Germany: Fischer Verlag.
- Brush, G. S. (2002). Recombination functions of replication protein A. *Curr. Org. Chem.* **6**, 795-813.
- Chikashige, Y. and Hiraoka, Y. (2001). Telomere binding of the Rap1 protein is required for meiosis in fission yeast. *Curr. Biol.* **11**, 1618-1623.
- Chikashige, Y., Ding, D.-Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M. and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270-273.
- Chikashige, Y., Ding, D.-Q., Imai, Y., Yamamoto, M., Haraguchi, T. and Hiraoka, Y. (1997). Meiotic nuclear reorganization: switching the position of centromeres and telomeres in the fission yeast *Schizosaccharomyces pombe*. *EMBO J.* **16**, 193-202.
- Chua, P. R. and Roeder, G. S. (1997). Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference. *Genes Dev.* **11**, 1786-1800.
- Colaiácovo, M. P., MacQueen, A. J., Martinez-Perez, E., McDonald, K., Adamo, A., la Volpe, A. and Villeneuve, A. M. (2003). Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev. Cell* **5**, 463-474.
- Cole, E. S., Cassidy-Hanley, D., Hemish, J., Tuan, J. and Bruns, P. J. (1997). A mutational analysis of conjugation in *Tetrahymena thermophila*. 1. Phenotypes affecting early development: meiosis to nuclear selection. *Dev. Biol.* **189**, 215-232.
- Cowan, C. R., Carlton, P. M. and Cande, W. Z. (2001). The polar arrangement of telomeres in interphase and meiosis. Rab1 organization and the bouquet. *Plant Physiol.* **125**, 532-538.
- Davidson, L. and LaFountain, J. R., Jr (1975). Mitosis and early meiosis in *Tetrahymena pyriformis* and the evolution of mitosis in the phylum Ciliophora. *BioSystems* **7**, 326-336.
- Deak, J. C. and Doerder, F. P. (1998). High frequency intragenic recombination during macronuclear development in *Tetrahymena thermophila* restores the wild-type SerH1 gene. *Genetics* **148**, 1109-1115.
- Ding, D.-Q., Chikashige, Y., Haraguchi, T. and Hiraoka, Y. (1998). Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. *J. Cell Sci.* **111**, 701-712.
- Ding, D.-Q., Yamamoto, A., Haraguchi, T. and Hiraoka, Y. (2004). Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. *Dev. Cell* **6**, 329-341.
- Dorninger, D., Karigl, G. and Loidl, J. (1995). Simulation of chromosomal homolog searching in meiotic pairing. *J. Theor. Biol.* **176**, 247-260.
- Egel-Mitani, M., Olson, L. W. and Egel, R. (1982). Meiosis in *Aspergillus nidulans*: another example for lacking synaptonemal complexes in the absence of crossover interference. *Hereditas* **97**, 179-187.
- Fletcher, H. L. (1981). A search for synaptonemal complexes in *Ustilago maydis*. *J. Cell Sci.* **50**, 171-180.
- Frankel, J. (2000). Cell biology of *Tetrahymena thermophila*. In *Tetrahymena thermophila* (ed. D. J. Asai and J. D. Forney), pp. 27-125. San Diego, CA: Academic Press.
- Fung, J. C., Rockmill, B., Odell, M. and Roeder, G. S. (2004). Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. *Cell* **116**, 795-802.
- Gaertig, J. and Fleury, A. (1992). Spatio-temporal reorganization of intracytoplasmic microtubules is associated with nuclear selection and differentiation during the developmental process in the ciliate *Tetrahymena thermophila*. *Protoplasma* **167**, 74-87.
- García-Rubio, M., Huertas, P., González-Barrera, S. and Aguilera, A. (2003). Recombinogenic effects of DNA-damaging agents are synergistically increased by transcription in *Saccharomyces cerevisiae*: new insights into transcription-associated recombination. *Genetics* **165**, 457-466.
- Haldane, J. B. S. (1931). The cytological basis of genetical interference. *Cytologia* **3**, 54-65.
- Jones, G. H. (1984). The control of chiasma distribution. In *Controlling events in meiosis* (ed. C. W. Evans and H. G. Dickinson), pp. 293-320. Cambridge: Company of Biologists.
- Kaczanowski, A., Gaertig, J. and Kubiak, J. (1985). Effect of the antitubulin drug nocodazole on meiosis and postmeiotic development in *Tetrahymena thermophila*. Induction of achiasmatic meiosis. *Exp. Cell Res.* **158**, 244-256.
- Karrer, K. M. (1985). Nonrandom localization of DNA sequences in the crescent micronucleus of *Tetrahymena*. *J. Protozool.* **32**, 555-557.
- Karrer, K. M. (2000). *Tetrahymena* genetics: two nuclei are better than one. In *Tetrahymena thermophila* (ed. D. J. Asai and J. D. Forney), pp. 127-186. San Diego: Academic Press.
- Kirk, K. E. and Blackburn, E. H. (1995). An unusual sequence arrangement in the telomeres of the germ-line micronucleus in *Tetrahymena thermophila*. *Genes Dev.* **9**, 59-71.
- Kohli, J. and Bähler, J. (1994). Homologous recombination in fission yeast – absence of crossover interference and synaptonemal complex. *Experientia* **50**, 295-306.
- Loidl, J. (1990). The initiation of meiotic chromosome pairing: the cytological view. *Genome* **33**, 759-778.
- Loidl, J. (1994). Cytological aspects of meiotic recombination. *Experientia* **50**, 285-294.
- Loidl, J., Klein, F. and Engebrecht, J. (1998). Genetic and morphological approaches for the analysis of meiotic chromosomes in yeast. In *Nuclear structure and function* (ed. M. Berrios), pp. 257-285. San Diego, CA: Academic Press.
- Maercker, C., Kortwig, H., Nikiforov, M. A., Allis, C. D. and Lipps, H. J. (1999). A nuclear protein involved in apoptotic-like DNA degradation in *Styloynchia*: implications for similar mechanisms in differentiating and starved cells. *Mol. Biol. Cell* **10**, 3003-3014.
- Maguire, M. P. (1988). Crossover site determination and interference. *J. Theor. Biol.* **134**, 565-570.
- Marsh, T. C., Cole, E. S., Stuart, K. R., Campbell, C. and Romero, D. P. (2000). *RAD51* is required for propagation of the germinal nucleus in *Tetrahymena thermophila*. *Genetics* **154**, 1587-1596.
- Martindale, D. W., Allis, C. D. and Bruns, P. J. (1982). Conjugation in *Tetrahymena thermophila*. A temporal analysis of cytological stages. *Exp. Cell Res.* **140**, 227-236.
- Martindale, D. W., Allis, C. D. and Bruns, P. J. (1985). RNA and protein synthesis during meiotic prophase in *Tetrahymena thermophila*. *J. Protozool.* **32**, 644-649.
- Moens, P. B., Bernelot-Moens, C. and Spyropoulos, B. (1989). Chromosome core attachment to the meiotic nuclear envelope regulates synapsis in *Chloalettis* (Orthoptera). *Genome* **32**, 601-610.
- Nanney, D. L. and McCoy, J. W. (1976). Characterization of the species of the *Tetrahymena pyriformis* complex. *Trans. Am. Microsc. Soc.* **95**, 664-682.
- Novak, J. E., Ross-Macdonald, P. B. and Roeder, G. S. (2001). The budding yeast Msh4 protein functions in chromosome synapsis and the regulation of crossover distribution. *Genetics* **158**, 1013-1025.
- Olson, L. W., Edén, U., Mitani, M. E. and Egel, R. (1978). Asynaptic meiosis in fission yeast? *Hereditas* **89**, 189-199.
- Orias, E., Hamilton, E. P. and Orias, J. D. (2000). *Tetrahymena* as a laboratory organism: useful strains, cell culture, and cell line maintenance. In *Tetrahymena thermophila* (ed. D. J. Asai and J. D. Forney), pp. 189-211. San Diego: Academic Press.
- Page, S. L. and Hawley, R. S. (2001). *c(3)G* encodes a *Drosophila* synaptonemal complex protein. *Genes Dev.* **15**, 3130-3143.
- Paques, F. and Haber, J. E. (1999). Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **63**, 349-404.
- Pawlowski, W. P., Golubovskaya, I. N. and Cande, W. Z. (2003). Altered nuclear distribution of recombination protein RAD51 in maize mutants suggests the involvement of RAD51 in meiotic homology recognition. *Plant Cell* **15**, 1807-1816.
- Raikov, I. B. (1982). *The Protozoan Nucleus. Morphology and Evolution*, pp. 1-474. New York, NY: Springer-Verlag.
- Ray, C., Jr (1956). Meiosis and nuclear behaviour in *Tetrahymena pyriformis*. *J. Protozool.* **3**, 88-96.

- Roeder, G. S.** (1997). Meiotic chromosomes: it takes two to tango. *Genes Dev.* **11**, 2600-2621.
- Scherthan, H.** (2001). A bouquet makes ends meet. *Nat. Rev. Mol. Cell Biol.* **2**, 621-627.
- Scherthan, H.** (2002). Detection of chromosome ends by telomere FISH. *Methods Mol. Biol.* **191**, 13-31.
- Scherthan, H., Weich, S., Schwegler, H., Härle, M., Heyting, C. and Cremer, T.** (1996). Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing. *J. Cell Biol.* **134**, 1109-1125.
- Shinohara, A., Ogawa, H. and Ogawa, T.** (1992). RAD51 Protein involved in repair and recombination in *Saccharomyces cerevisiae* is a RECA-like protein. *Cell* **69**, 457-470.
- Stechmann, A. and Cavalier-Smith, T.** (2003). The root of the eukaryote tree pinpointed. *Curr. Biol.* **13**, R665-R666.
- Storlazzi, A., Tesse, S., Gargano, S., James, F., Kleckner, N. and Zickler, D.** (2003). Meiotic double-strand breaks at the interface of chromosome movement, chromosome remodeling, and reductional division. *Genes Dev.* **17**, 2675-2687.
- Sugai, T. and Hiwatashi, K.** (1974). Cytologic and autoradiographic studies of the micronucleus at meiotic prophase in *Tetrahymena pyriformis*. *J. Protozool.* **21**, 542-548.
- Sung, P., Krejci, L., van Komen, S. and Sehorn, M. G.** (2003). Rad51 recombinase and recombination mediators. *J. Biol. Chem.* **278**, 42729-42732.
- Sym, M. and Roeder, G. S.** (1994). Crossover interference is abolished in the absence of a synaptonemal complex protein. *Cell* **79**, 283-292.
- Tung, K.-S. and Roeder, G. S.** (1998). Meiotic chromosome morphology and behavior in *zip1* mutants of *Saccharomyces cerevisiae*. *Genetics* **149**, 817-832.
- Wei, Y., Mizzen, C. A., Cook, R. G., Gorovsky, M. A. and Allis, C. D.** (1998). Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in *Tetrahymena*. *Proc. Natl. Acad. Sci. USA* **95**, 7480-7484.
- Wickert, S. and Orias, E.** (2000). *Tetrahymena* micronuclear genome mapping: a high-resolution meiotic map of chromosome 1L. *Genetics* **154**, 1141-1153.
- Wolfe, J., Hunter, B. and Adair, W. S.** (1976). A cytological study of micronuclear elongation during conjugation in *Tetrahymena*. *Chromosoma* **55**, 289-308.
- Yamamoto, A. and Hiraoka, Y.** (2001). How do meiotic chromosomes meet their homologous partners?: lessons from fission yeast. *BioEssays* **23**, 526-533.
- Zickler, D. and Kleckner, N.** (1998). The leptotene-zygotene transition of meiosis. *Annu. Rev. Genet.* **32**, 619-697.
- Zickler, D. and Kleckner, N.** (1999). Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* **33**, 603-754.
- Zink, D., Cremer, T., Saffrich, R., Fischer, R., Trendelenburg, M. F., Ansoorge, W. and Stelzer, E. H. K.** (1998). Structure and dynamics of human interphase chromosome territories in vivo. *Hum. Genet.* **102**, 241-251.