0014-4754/94/030295-12$1.50 + 0.20

Homologous recombination in fission yeast: Absence of crossover interference and synaptonemal complex

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Abstract. The study of homologous recombination in the fission yeast *Schizosaccharomyces pombe* has recently been extended to the cytological analysis of meiotic prophase. Unlike in most eukaryotes no tripartite SC structure is detectable, but linear elements resembling axial cores of other eukaryotes are retained. They may be indispensable for meiotic recombination and proper chromosome segregation in meiosis I. In addition fission yeast shows interesting features of chromosome organization in vegetative and meiotic cells: Centromeres and telomeres cluster and associate with the spindle pole body. The special properties of fission yeast meiosis correlate with the absence of crossover interference in meiotic recombination. These findings are discussed. In addition homologous recombination in fission yeast is reviewed briefly.
Key words. Chromosome structure; DNA repair; fission yeast; homologous recombination; meiosis cytology; *Schizosaccharomyces pombe.*

This article is dedicated to Urs Leupold, the founder of fission yeast genetics.

Introduction

The fission yeast *Schizosaccharomyces pombe* was introduced to genetic analysis by Urs Leupold[55,57]. He developed the basic methodology of fission yeast genetics, isolated and characterized a large number of mutants, and presented the first data on intergenic recombination (genetic chromosome mapping), the analysis of intragenic recombination (fine-structure mapping and occurrence of non-reciprocal events), and the special features of recombination at the mating-type locus[56]. In the meantime fission yeast has become the most thoroughly studied unicellular eukaryote besides the budding yeast *Saccharomyces cerevisiae*. Both yeasts owe their popularity as eukaryotic model organisms to the simplicity of their handling and the highly developed classical and molecular genetics tools that allow rapid construction of designed mutations in vivo and the quick analysis of the resulting phenotypes. The parallel study of both yeasts is justified despite their taxonomic closeness (ascomycetous fungi). The two yeasts show many profound differences (the example of meiosis is discussed below) that have been compiled recently by Eriksson et al.[17] who propose that the newly defined orders *Saccharomycetales* and *Schizosaccharomycetales* represent a very early split in the subdivision *Ascomycotina*. This split might have occurred not too long after the split of the lines of descent that has led to fungi and metazoans (animals) which occurred after splitting off plants and other eukaryotes[104]. This may explain the originally surprising, and now more and more common finding that those biological functions (genes) that have been conserved between the two yeasts (despite their pronounced differences in many details) are also conserved in animals and plants. This has first been exploited by researchers in the field of cell cycle analysis by cloning of a human homologue of the *cdc2* gene coding for the $p34^{cdc2}$ kinase by direct complementation of a fission yeast *cdc2* mutation with human cDNA[54]. It is likely that many DNA recombination and repair functions (genes) are also conserved in all eukaryotes and can be identified in higher eukaryotes after comparison of functionally homologous genes in the two yeasts (evidence is given below and in the articles of Bezzubova and Buerstedde, and Heyer in this issue). This article describes the differences between budding yeast (*S. cerevisiae*) and fission yeast (*S. pombe*) that are relevant for the study of DNA recombination and shortly reviews the knowledge that has been gained so far on homologous recombination in fission yeast. More emphasis is then put on genetic interference and the cytology of fission yeast meiosis.

The life cycle of fission yeast with respect to the analysis of homologous recombination

Unlike budding yeast *S. pombe* is a predominantly haploid organism (fig. 1, for general reviews see 28, 74). It may be an adaptation to this circumstance that fission yeast cells (unlike budding yeast cells) have a long G2 and a short G1 phase[22]. The advantage would be that mitotic recombination between sister chromatids can then contribute more to the repair of DNA damage. *S. pombe* is more resistant to UV and ionizing radiation than budding yeast (for review see 95). The molecular study of DNA repair in fission yeast is a developing field of research[63]. Upon DNA damage inflicted by radiation or chemicals cells are arrested in G2 by intri-

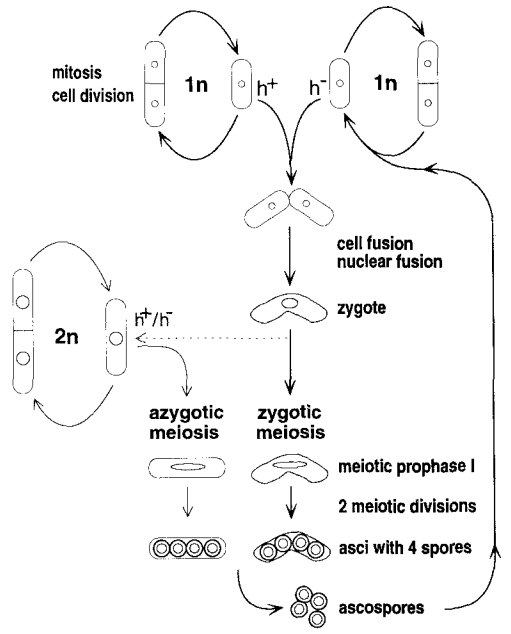

Figure 1. The life cycle of the fission yeast *Schizosaccharomyces pombe*. The haploid ascospores germinate into vegetatively growing cells that are able to mate upon starvation. Usually the resulting zygote undergoes meiosis immediately (zygotic meiosis). When zygotes are transferred to rich medium they may enter a diploid vegetative phase (dotted arrow). Upon starvation the diploid cells undergo azygotic meiosis. In haploid and diploid vegetative cells mating-type switching occurs continuously, if the mating-type genes on chromosome(s) II are in the configuration corresponding to homothallism. Mitotic recombination is best studied in diploid cells that cannot switch mating-type and are homozygous for h^- or h^+. In general meiotic recombination is analyzed in zygotic meiosis. For further explanations see text.

cate check point mechanisms until repair has occurred (for review see 91).

All strains used in *S. pombe* research are isogenic (derived from a single isolate) and mostly haploids are used with the mating types h^{90} (homothallic), h^- or h^+ (both heterothallic). Usually the diploid phase is confined to zygotes that form by mating of haploid gametes that differentiate from vegetative cells upon nutrient (predominantly nitrogen) starvation (fig. 1). Without delay the zygotes undergo meiosis and form zygotic asci with four haploid spores. On rich medium the spores germinate and enter the haploid vegetative phase even when the spore clones are homothallic (that is they show active mating-type switching, see below). Zygotes are committed to meiotic divisions and meiotic levels of recombination at the stage of premeiotic DNA synthesis (for review see 3). Thus the late return to mitotic division (from pachytene) after commitment to meiotic levels of recombination is not possible in fission yeast in contrast to budding yeast (for review see 80). For the study of meiotic recombination the specific aspects of its life cycle have important consequences. An advantage is that recombinant genotypes studied in spore clones are derived from meiotic recombination only. There are no mitotic divisions of the diploid zygote that can give rise to mitotic recombination events before meiosis is induced, a problem that has to be kept in mind for the study of meiotic recombination in budding yeast. One consequence of the easy use of homothallic strains was the establishment of a very successful scheme for the isolation of recessive meiotic recombination deficient mutations[10,81]. Another advantage derives from the properties of *S. pombe* asci and spores. The ascus walls decay spontaneously. The spores are easily separated from each other by vigorous mixing of suspensions, and from vegetative cells (that have not mated in the crossing mixture) by lysis of the vegetative cells with glusulase (for the most recent assessment of these methods see 86). Thus study of recombination by random spore analysis is a very efficient and reliable procedure in fission yeast (for a recent example see 87). In addition *S. pombe* forms linear, ordered tetrads (unlike *S. cerevisiae*) that allow direct determination of first and second division segregation. However, unordered tetrads are analyzed usually which has been sufficient for the identification of most of the known centromere markers[49]. An important boost for the study of fission yeast chromosomes is the recent establishment or ordered collections of DNA clones (cosmids, P1 phages, and YACs) covering the whole genome[39,62,68].

When zygotes are transferred to rich medium they develop with low frequency to vegetatively dividing diploid clones that undergo azygotic meiosis as soon as they are starved for nitrogen (fig. 1). Obviously synchronization of meiosis for the study of cytological structures and recombination intermediates is achieved more easily for azygotic than for zygotic meiosis. Because it is difficult to maintain the sporulating h^+/h^- diploids in the vegetative cycle over long periods of time, methods have been developed for the isolation of stable (non-sporulating) diploids. They are homozygous for mating types (h^+/h^+ or h^-/h^-) and derived from h^+/h^- diploids by mitotic crossover between centromere and mating-type locus. An easier method for synthesis of stable diploids is based on mating of an h^- haploid with an h^{90} haploid carrying the *mat2-B102* mutation[49]. Such diploids are unable to sporulate and retain heterozygosity of the whole genome. Stable diploids obtained by either method can be used for the study of mitotic recombination.

Thus meiotic recombination and cytological structures can be studied in either zygotic or azygotic meiosis. Most of the recombination data derive from work with zygotic meiosis. But it has been noted that recombination frequencies in azygotic meiosis may be lower than in zygotic meiosis[3]. The recent cytological work has been carried out in zygotic and azygotic meiosis (see below).

Homologous recombination in fission yeast

A special kind of mitotic recombination in both fission and budding yeast is mating-type switching. The actively

expressed mating-type loci code for regulatory proteins that determine the cell type: a or α in *S. cerevisiae* and P (plus) and M (minus) in *S. pombe*. In addition to the expression locus two silent gene loci exist on the same chromosome. Each silent locus carries one type of mating-type information that is transferred to the expression locus by directed gene conversion when the cell switches its mating type. This information transfer is initiated by a double-strand break of the chromosome at one end of the expression locus. In both yeasts the mechanisms of mating-type switching are studied in detail, and it turns out that they are fundamentally different despite the overall similarity of mating-type gene organization just described. This work provides insights into the processing of recombination intermediates and to some extent serves as a model system for the analysis of homologous recombination in general (for review see 14, 34, 46, 47).

Fine-structure maps of many fission yeast genes have been constructed in U. Leupold's laboratory by crossing of mutants and frequency determination of wild-type recombinants among random spores. Although many maps have never been published, they serve as a treasure of unexplained phenomena that are now amenable to molecular analysis. Most use has been made from the work on *ade6*[27] and the suppressor tRNA genes[38,72]. This was the case for the description of map expansion[40], the study of ectopic recombination between tRNA genes (see below), and the analysis of marker effects. Marker effect mutations distinguish themselves from the majority of mutations in a gene by causing site-assignment problems when a fine-structure map is constructed based on intragenic recombination frequencies. One type of marker effect turned out to be related to mismatch repair (see below). Another marker effect mutation is *ade6-M26* that creates a hotspot of recombination initiation (see G. Smith in this issue). A noticeable result deriving from the comparative analysis of many *S. pombe* genes is that aberrant segregation (conversion) occurs with rather low frequencies (typically not more than 1% of tetrads)[29]. This is different in *S. cerevisiae*[80] and hampers the physical analysis of meiotic recombination intermediates due to their low abundance. This situation may be changed by the description of the *ura4-aim* gene (artificially inserted marker upstream of *ade6*) as a polaron that yields aberrant segregation frequencies between 16% (5′ end) and 5% (3′ end) (M. Baur, E. Hartsuiker, E. Lehmann and J. Kohli, manuscript in preparation).

Fine-structure maps of genes can also be constructed by induced mitotic recombination[38]. Spontaneous intragenic and intergenic recombination has been studied in a number of genes and intervals[25,30,66]. Interesting in relation with homologous chromosome pairing is the observation that intragenic mitotic recombination occurs more frequently in genes close to centromeres than in genes far from centromeres[66]. In addition simultaneous intragenic events in unlinked genes occur with higher frequency than expected. It is concluded that a subpopulation is more competent for recombination than the majority of the cells. The subpopulation of competent cells is increased in a *rad2* mutant[25,66]. Thus, it is not possible to assume that all cells of a population recombine with the same probability without testing.

Observations made during fine-structure mapping of suppressor tRNA genes led to the discovery that DNA sequence information can be transferred from one gene to other related genes that are located elsewhere on the same or on different chromosomes[71]. The unilateral transfer of DNA sequences (conversion) between three different $tRNA^{Ser}$ genes was studied in detail[1,35,73] and also reviewed[50,51]. These information transfers ensure the homogeneity of the sequences of all members of a gene family that code for gene products performing the same function, and are thus under identical selection pressure. This mechanism assuring concerted sequence evolution of genes has been named ectopic recombination (for review see 79). Ectopic conversion between $tRNA^{Ser}$ genes in fission yeast occurs in meiosis and with lower frequency also in vegetative cells. It is only rarely associated with crossover leading to reciprocal translocations[98]. Junker et al.[42] have demonstrated that ectopic recombination occurs by DNA-DNA contact and is not RNA-mediated. In addition they have shown that the sequence of the donor gene is not altered when it transfers information to the recipient gene, and that this transfer occurs after premeiotic DNA replication. Ectopic recombination between the two copies of the artificially duplicated *ade6* gene has been studied in connection with the *ade6-M26* hot spot[90].

Strains with specific mutations altering recombination frequencies are an important tool for the mechanistic analysis of recombination. Most of the long known UV-sensitive *rad* mutations have little effect on recombination frequency[26]. The influence of a temperature-sensitive mutation in DNA ligase on recombination has been studied[92]. A few mutations have been isolated that alter the frequency of ectopic recombination[102]. More recently mutations have been characterized that affect ectopic and classical recombination in mitotic cells. They have no effect on meiotic recombination[30]. Mutations in 16 different genes have been characterized with an assay involving plasmid-chromosome recombination[10,81]. They reduce the frequency of meiotic recombination to varying degrees, some by three orders of magnitude (down to the level of mitotic recombination frequency). The screening system has selected against mutations that block meiosis, but the spores resulting from crosses homozygous for the strong rec^- mutations show markedly reduced germination frequencies. This indicates that proper meiotic chromosome segregation is impaired in the absence of recombination, as in other

eukaryotes[6]. Thus, these types of mutations might only be obtained because fission yeast has a low chromosome number (n = 3) and viable spores might be formed even after random segregation of chromosomes. Aneuploids are not tolerated in fission yeast strains except disomes for the smallest chromosome III which can only be maintained by selection[77]. First attempts to study chromosome segregation have been made[7,69]. Four of the genes identified by mutation to recombination deficiency have been isolated and sequenced[58,59]. They show meiosis-specific transcription and no homology to other genes in data banks. Mutations in three genes reduce recombination frequencies in chromosome-region specific manner[11]. It is surprising that the known recombination genes are specific for either meiotic or mitotic recombination. Only the *swi5* mutation affects mating-type switching as well as mitotic and meiotic intragenic and intergenic recombination[10,89]. Obviously many more recombination genes in fission yeast remain to be characterized, especially those that lead to a block in meiosis.

Pronounced marker effect mutations in the fine-structure maps of $tRNA^{Ser}$ genes turned out to be G to C transversions[35,38]. Two-factor crosses of the marker effect mutations with other mutations in the same gene yield increased frequencies of prototrophic recombinants (up to 40-fold) when compared with control crosses involving a normal mutation mapping very closely to the marker effect mutation. The overall frequency of non-Mendelian segregation of the marker effect mutations (tetrad analysis) is not increased significantly in comparison with other mutations. However, they display increased frequency of postmeiotic segregation (PMS)[70,101]. PMS results when a base mismatch in hybrid DNA is not repaired. The resulting spore forms a sectored colony. One sector contains wild-type cells and the other the mutant cells corresponding to the different genetic informations carried by the single strands of hybrid DNA. A thorough genetic analysis of a G to C transversion in the *ade6* gene has recently been published[86,87]. Based on these results a model has been proposed that assumes at least two different pathways for mismatch repair in fission yeast. One corresponds to the MutH/L/S pathway that has been well characterized in prokaryotes (see Fox, Radicella and Yamamoto, in this issue). It also exists in eukaryotes as concluded from the phenotype of mutations in *S. cerevisiae* genes homologous to *mutS*[82] and *mutL*[52,53]. This phenotype includes enhanced frequencies of spontaneous mutations in vegetative cells and high levels of PMS in meiosis. A gene homologous to *mutS* has also been found in fission yeast (*swi4*). It is involved in mating-type switching[19,20], but does not increase PMS frequencies in meiosis (P. Schär, pers. commun.). Recently the *mutL* homolog *mlh1* of *S. pombe* has been sequenced and a disruption mutation shows mitotic mutator phenotype as well as enhanced PMS in meiosis (P. Schär, M. Baur and J. Kohli, manuscript in preparation).

In *E. coli* neither the MutH/L/S nor other pathways are able to repair C-C mismatches (see Fox, Radicella and Yamamoto, in this issue). In contrast C-C mismatches in fission yeast are repaired with an efficiency of 70%[86]. It is proposed that a second repair pathway is responsible for CC repair and also participates in the repair of other mismatches. Genetic data indicate that the MutH/L/S type pathway described above can repair all mismatches except C-C with higher efficiency than the novel pathway that is able to repair C-C. While the novel pathway is a short patch repair system (approximately 10 nucleotides unidirectionally), the MutH/L/S type pathway produces longer patches (approximately 100 nucleotides unidirectionally). The newly proposed pathway may also occur in other eukaryotes as concluded from the existing data on mismatch repair in *S. cerevisiae*[87]. Recently an activity has bene found in fission yeast cell extracts that binds to C-C and all other C-containing mismatches: Double-stranded DNA molecules with specific mismatches are retarded on gels by protein from the extracts. This band shift is different from the MutS-type band shift[67] that has also been observed in fission yeast extracts with most types of mismatches, but not with C-C (O. Fleck, P. Schär and J. Kohli, manuscript in preparation). In addition a recently described meiotic exonuclease of fission yeast[99] may be involved in the C-C mismatch repair pathway: A disruption mutation of the corresponding *exo1* gene shows both mutator phenotype and reduction of wild-type recombinant frequencies in two-factor crosses involving G to C transversions (P. Szankasi, pers. commun.).

Deviations from random distribution of homologous recombination events and the absence of interference in fission yeast

Recombination events are not randomly distributed along the chromosomes of eukaryotic organisms. This has been observed for conversion and crossover. For the study of the different types of deviations, it is important to keep in mind the basis for the description of the distribution of recombination events. The basis can be physical chromosome length in nucleotide numbers, the cytologically observed length of more or less condensed chromosomes, or the genetically determined length of linkage groups in centiMorgans.

Only recenly large scale cloning and sequencing has begun to provide exact physical lengths of whole chromosomes or larger parts of them. Symington and collaborators have studied a 22kb region on chromosome III of *S. cerevisiae* in detail, and they have documented the non-random distribution of crossover and conversion events in this region[96,97]. Hotspots and cold regions

for recombination events have been observed before in many organisms (see G. Smith, in this issue). If one focuses on specific genes and their flanking regions, it is a common observation in fungi that conversion events are arranged in a frequency gradient from one end of the gene to the other (see A. Nicolas and T. Petes, in this issue). After the discovery that double-strand breaks are likely to be involved in initiation of meiotic recombination in *S. cerevisiae*, their distribution along chromosome III was investigated (see 106 and citations therein). Recently a thorough comparative analysis of double-strand breaks, crossover distribution and chromatin structure has shown that meiotic double-strand breaks occur in the promoter regions of most genes and that their location correlates with DNase I hypersensitive sites. The extent of double-strand breakage varies widely at the different sites and correlates well with the frequency of crossovers in the corresponding intervals[105].

Another phenomenon is the biased distribution of crossovers between chromosomes as reported for *S. cerevisiae*[43] and *N. crassa*[65]. In both organisms short chromosomes have a higher number of crossovers than long chromosomes per physical length. This has been substantiated by splitting and fusion experiments for chromosome I of budding yeast[44]. These observations are interpreted as evidence for a mechanism that ensures proper segregation of small chromosomes at the first meiotic division.

While the phenomena on uneven distribution of recombination discussed above are assessed on the basis of DNA molecule length, the distribution of cytological structures thought to be associated with recombination events (chiasmata, recombination nodules) is studied with reference to cytological chromosome length (for review see, 8, 32, 41, and the contribution of J. Loidl in this issue). It has been proposed that the early (randomly distributed) recombination nodules observed in many eukaryotes correspond to conversion events, while the late (non-randomly distributed) nodules correspond to crossover events. Late nodules show a distribution similar to the one of chiasmata (also non-random). The correlation between the genetically observed crossovers and the cytologically observed chiasmata is well accepted, although there are still open questions[76]. It is generally assumed that common mechanisms are responsible for the non-random distribution of late nodules, chiasmata and crossovers. For this reason the terms chiasma interference and crossover interference are often used interchangeably[64]. This can lead to confusion because the distribution of chiasmata is determined cytologically (basis is chromosome length) while the distribution of crossovers is determined genetically (basis is linkage group length).

The phenomenon of positive crossover interference is observed when coincident crossovers in two genetic intervals occur with a lower frequency than expected from random crossover distribution. The two intervals are defined by genetic markers and the frequencies of crossovers in each of them serve for the calculation of the expected frequency of coincident crossovers in both intervals. Crossover frequencies between genetic markers are used for the construction of linkage maps. The phenomena described above (hotspots, cold regions, chromosome size dependence of crossover frequency) are not detectable in genetic maps, while crossover interference is studied solely on the basis of genetic mapping. Thus crossover interference must be based on mechanisms that are different from those involved in the generation of hotspots and cold regions. Crossover interference has been described first in *Drosophila melanogaster*[75]. It has been stated as recently as 1991: 'There is no convincing molecular model for chiasma interference in any organism'[80]. Recently two models have been published[23,45]. Both models assume recombination intermediates that are randomly distributed over the bivalents at an early stage of meiosis. When not processed to crossovers, these intermediates may result in gene conversions. This is in agreement with the observation that there is no interference between conversion events in *S. cerevisiae*[21]. At a later stage non-random processing of some of the intermediates to crossover results in the observed patterns of crossover interference, as substantiated by computer simulations. King and Mortimer[45] propose that polymers are formed along the bivalents starting from those intermediates that make the transition to become crossovers. The transition of other intermediates is repressed by such polymers. In this way a crossover represses additional crossovers in the neighbourhood in agreement with the observation that interference is not observed between intervals that are far apart. Foss et al.[23] propose a counting mechanism that, once an intermediate is maturing to a crossover, represses the processing of a fixed number of adjacent intermediates to crossovers. While the polymer formation mechanism proposed by King and Mortimer[45] is biochemically attractive, it seems not so easy to devise a counting mechanism for intermediates at the molecular level. King and Mortimer[45] propose a biological function for crossover interference by assuming that the early intermediates are formed by enzyme complexes (early recombination nodules) that after dislocation from a bivalent are able to reload on other bivalents. Small chromosomes that have not obtained a recombination nodule in the first round of random nodule distribution are then recognized by the displaced nodules assuring proper segregation in meiosis I.

It has been published that fission yeast does not show crossover interference based on limited results and evaluation of two-point crosses[13,74,93]. Recently a detailed study of crossover interference was carried out by

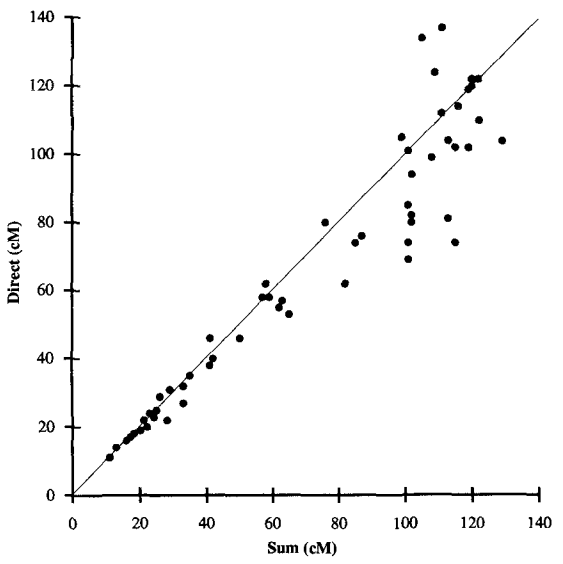

Figure 2. Absence of interference in *S. pombe*. The evaluation of three-point crosses shows additivity of genetic lengths (in centi-Morgans) for adjacent intervals in comparison with the directly measured distance between the outer markers. The abscissa gives the sum of genetic distances in the two intervals, and the ordinate the direct distance between the outer markers. Each point in the plot corresponds to one three-factor cross. The oblique line is 'direct = sum'. Genetic distances (d) were calculated with the formulas based on the Poisson distribution: $d = -50 \log \{(a_1 - a_2)/n\}$ for tetrad data, and $d = -50 \log \{1 - 2(a_3/n)\}$ for random spore data. a_1 = observed number of parental ditype tetrads, a_2 = observed number of nonparental ditype tetrads, a_3 = observed number of recombinant spores, n = sample size of tetrads or random spores.

P. Munz (pers. commun.). Many crosses with multiple markers in several regions of all three chromosomes were studied by random spore and tetrad analysis. At first the data were checked with respect to chromatid interference (non-random involvement of the four chromatids of a bivalent in multiple crossover events). No chromatid interference could be detected. This is in agreement with results from other organisms. Without crossover interference the events should be distributed according to Poisson[31]. Thus, the *S. pombe* data were checked for compatibility with the Poisson distribution. The genetic distances were calculated for every three-point cross based on formulas derived from the Poisson distribution (see legend of fig. 2). The sum of genetic distances of adjacent intervals should be equal to the directly determined distance between the outer markers, when crossovers are Poisson distributed. The data points in figure 2 are very close to expectation for the shorter intervals. This is the most reliable indication for absence of crossover interference in *S. pombe* (see ref. 69a). The data points corresponding to long intervals scatter around the oblique line with a slight trend towards higher values for the sums. If this is due to interference at all, it would represent negative crossover interference. Alternatively, this slight deviation from the Poisson distribution may be due to subpopulations of zygotes with different competence for crossover formation[85]. The analysis of the data on the long intervals with new mathematical methods is in progress.

Another organism with absence of crossover interference is the mycelial fungus *Aspergillus nidulans*[9,94]. No synaptonemal complex could be found in meiocytes of *A. nidulans*[16]. Interestingly this is also the case for *S. pombe*[4,36,78]. The parallel absence of synaptonemal complex and crossover interference in these two organisms has led Egel[13] to propose that the formation of a synaptonemal complex is required for the manifestation of crossover interference. The mechanistic model and the biological function for crossover interference proposed by King and Mortimer[45] is in agreement with the observations on fission yeast. If redirection of crossovers to the smallest chromosomes is the true biological function of crossover interference, then it is not required in *S. pombe*. In meiosis on average 19, 15 and 11 crossovers occur on the three bivalents of fission yeast (deduced from published linkage map lengths[74]). Thus, the probability that the smallest bivalent gets no crossover is extremely low (2×10^{-5}), when crossovers are randomly distributed. Exceptionally high frequencies of exchanges per bivalent are also observed in *A. nidulans*[9]. For comparison with other organisms see King and Mortimer[45].

Nuclear structures in fission yeast meiosis and absence of synaptonemal complex

The early work on cytology of fission yeast meiosis has been reviewed[14,83]. Serial sectioning of meiotic nuclei did reveal the presence of 25–30 'linear elements' that are in parallel array at a stage when the nucleus assumes an elongated shape ('horse-tail nuclei'). In difference to other eukaryotes no synaptonemal complex (SC) could be detected in *S. pombe*[36,78]. Similarly no SC was detected in *S. octosporus*[2]. In the meantime the spreading technique for meiotic nuclei had been applied to budding yeast by two groups independently[12,61]. This approach greatly accelerates and facilitates the analysis of nuclear structures such as SCs. The contents of many nuclei can be studied in time-course experiments and combined with immunofluorescence techniques to localize protein components of nuclear organelles. In addition the hybridization of DNA probes to specific regions on the chromosomes (FISH = fluorescence in situ hybridization) can be used to study chromosome pairing[5,37,88]. FISH can be combined with immunofluorescence to study the localization of specific chromosomal sites (e.g. centromeres and telomeres) with respect to nuclear organelles[24].

A systematic analysis of meiotic nuclei by the spreading technique was carried out by Bähler et al.[4] with a h^-/h^+ diploid induced to undergo azygotic meiosis. In these time-course experiments the electron microscopy of

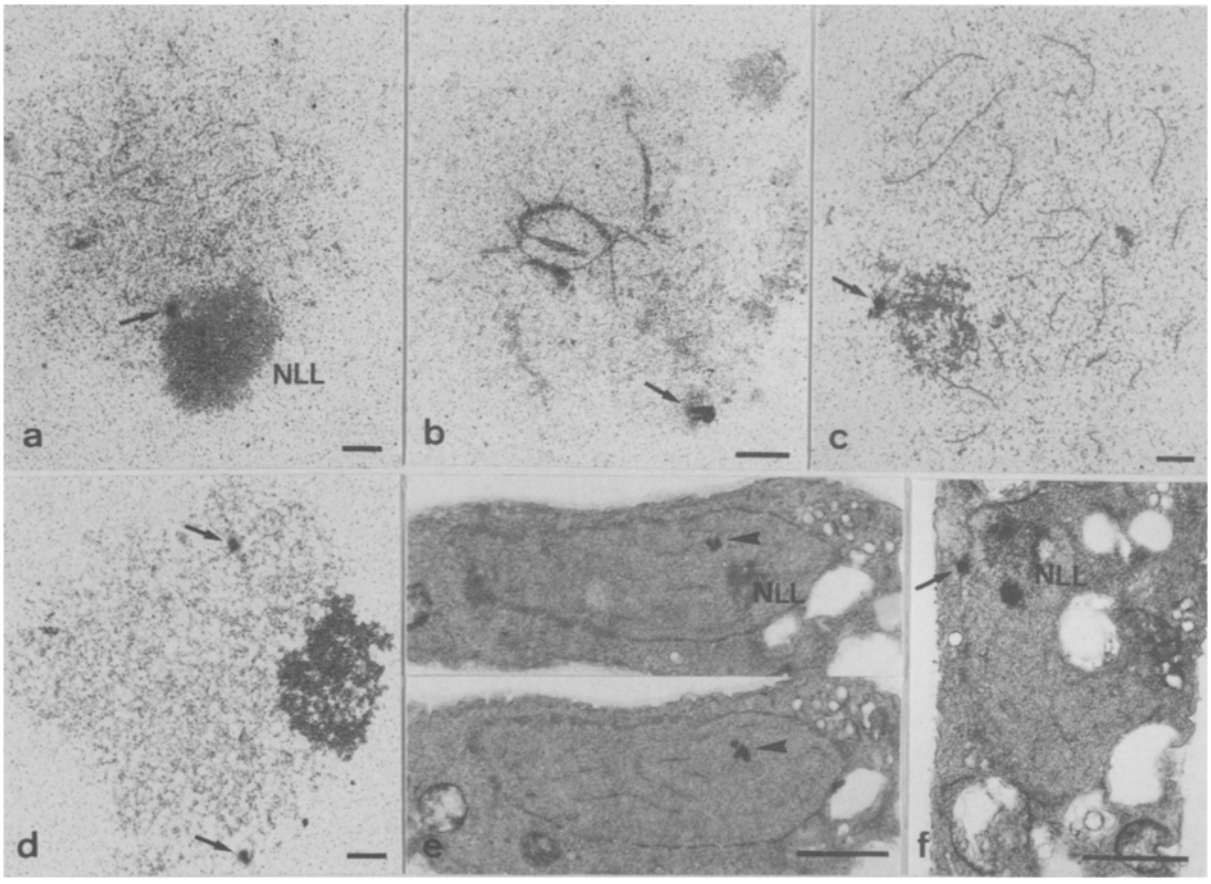

Figure 3. Nuclear structures in fission yeast meiosis. Meiotic nuclei are spread, stained with silver nitrate, and visualized by electron microscopy (*a*–*d*). The nucleolus (NLL), the spindle pole body (SPB, arrows) and the linear elements (filamentous structures) stain strongly, while the chromatin is stained only diffusely. In early prophase (stage 1) short linear elements become visible (*a*). Then the linear elements elongate and make extensive contacts, so that bundles and networks are visible (stage 2, *b*). Later (stage 3) the elements are spread out individually, and they reach maximal length (*c*). Then the linear elements disappear, the SPB divides, and the nucleus prepares for the first meiotic division. Metaphase I (nucleolus still undivided) is shown in (*d*). For sectioning meiotic nuclei were fixed with glutaraldehyde and osmium tetroxide and contrasted with uranyl acetate and lead citrate. Two consecutive slices of an elongated meiotic nucleus corresponding to stage 3 (horse-tail stage) display parallel arrangement of the linear elements and the 'black bodies' (arrowheads) that are always found in the neighbourhood of the nucleolus (*e*). Nucleolus and black bodies are always close to the SPB as shown in a section of a stage 2 nucleus (*f*). At this stage the linear elements touch frequently and they run in different directions. Bars represent 1 μm.

spread nuclei was combined with the analysis of premeiotic DNA synthesis and nuclear morphology (DNA stained with DAPI). No classical tripartite structure corresponding to the SC could be detected in hundreds of spread nuclei from all time points. The linear elements were also observed in spread nuclei and they closely resemble axial cores (= SC precursors) of other organisms. They appear well after premeiotic DNA replication and are first visible as short filaments (stage 1, fig. 3a) on the background of the faintly stained chromatin. Nucleolus and spindle pole body (SPB) are strongly stained by silver. The linear elements grow in length and appear as bundles and networks at stage 2 (fig. 3b). A similar stage was observed in serial sections with linear elements touching each other and running in different directions (fig. 3f). Stage 3 is characterized by single linear elements of maximal length (fig. 3c) and may correspond to the classical horse-tail nuclei with regular arrangement of the elements parallel to the long axis of the elongated nuclei (fig. 3e). But the data did not allow a clear assignment of all strongly elongated nuclei to stage 3 versus stage 2. Finally the linear elements are shortened (stage 4) before they disappear. The spindle pole body then duplicates and separates to form the spindle for meiosis I (fig. 3d). Three-dimensional reconstruction of serial sections revealed a constant triangular arrangement at one end of the nucleus for the SPB, the nucleolus, and a newly discovered structure named 'black bodies' (fig. 3e, f). The same structures and sequences of events were also observed in matings of homothallic and heterothallic cells (zygotic meiosis). The mutation *mei4-B2* blocks meiosis after S-phase and before meiosis I[15]. This block in prophase was confirmed by nuclear spreading. The mutant behaves like wild-type up to stage 3 that is greatly extended before (unspecific?) degradation of linear

elements takes place. The number and length of linear elements was quantified in nuclear spreads (stage 3) in *mei4* mutant strains of different ploidy. Six linear elements would be expected in diploid meiosis, if they formed continuously along the homologous chromosomes, whereas about 24 have been counted. Individual element lengths vary continuously between nuclei of a given ploidy and between nuclei of different ploidy. The mean lengths (and correspondingly mean numbers) of elements per nucleus are not constant for a given ploidy. The total length of elements per nucleus is the parameter that correlates best with ploidy: haploids 19 μm, diploids 34 μm, tetraploids 67 μm. The results indicate that there is no fixed pattern of non-continuous linear element formation along the homologs. Absence of a tripartite SC and presence of linear elements have also been observed in *S. japonicus var. versatilis* (A. Svoboda and J. Bähler, unpubl. observ.).

Thus, *S. pombe* manages to recombine and segregate its chromosomes without a tripartite SC. Based on the data just reviewed a model for the course of meiotic prophase in fission yeast has been proposed[4]. A revised version of this model is shown in figure 4. Since meiosis normally occurs immediately after conjugation and karyogamy in fission yeast (zygotic meiosis, fig. 1), the model includes karyogamy. The behaviour of the SPB, and of centromeres and telomeres in vegetative cells has been described recently[24,100,103]. The centromeres of the three chromosomes are clustered throughout G2 in vegetative cells. This explains increased mitotic recombination levels in genes close to centromeres[66]. Moreover, the centromere cluster is associated with the SPB which is located at the surface of the nucleus in contact with cytoplasmic microtubules. The telomeres show some aggregation in G2 at the nuclear surface, and are placed randomly with respect to the SPB. It is known that karyogamy begins by fusion of the two SPBs of the haploid nuclei in the zygote[2,36]. Our initial model[4] proposed that immediately after SPB fusion the clustered centromeres also fuse and then provide a starting point for chromosome pairing. The black bodies observed in serial sections close to the SPB would then correspond to the clustered centromeres. However, in the meantime the group of Y. Hiraoka has found that before karyogamy a switch occurs and that the telomeres become clustered and associated with the SPB instead of the centromeres. This is consistent with our finding that the nucleolus is adjacent to the SPB in meiotic prophase, since both ends of chromosome III contain nucleolar organizers[18,39]. The telomeres maintain this configuration throughout meiotic prophase both in zygotic and azygotic meiosis. As suggested before[4] pronounced movements of meiotic prophase nuclei from one end of the cell to the other occurs with the SPB at the leading end (Y. Hiraoko, pers. commun.). It seems that the nuclei move along the cytoplasmic microtubules. Cytoplasmic microtubules outside the nuclear membrane of prophase nuclei have been observed in serial sections of cells[4,78]. This movement of meiotic nuclei has also been studied in *S. japonicus var. versatilis* (A. Svoboda, Brno, pers. commun.) and can explain the variable nuclear shapes during meiotic prophase[4].

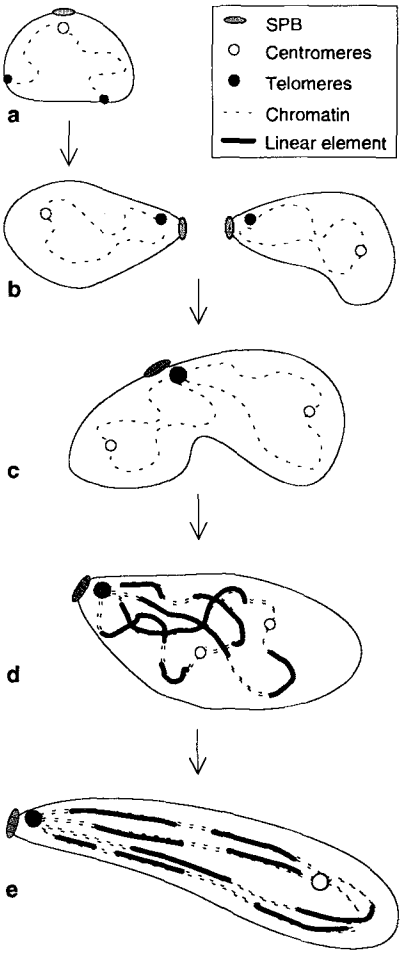

Figure 4. A revised scheme for prophase in zygotic meiosis of *S. pombe*. Only one of the three chromosomes is shown schematically. The centromeres of all chromosomes are clustered throughout interphase of vegetatively growing cells. They are attached to the spindle pole body (SPB) (*a*). Before karyogamy the telomeres become clustered near the SPB with concomitant release of the centromere cluster (*b*). Karyogamy is initiated by the SPBs, leading to fusion of the two telomere clusters. This leads to initiation of chromosome pairing from homologous telomeres (*c*). After DNA replication the linear elements become visible in the nuclei. The centromere clusters approach each other and chromosome pairing proceeds (*d*). The homologous chromosomes are paired and arranged in a 'bouquet'. This leads to parallel alignment of the linear elements. Telomere and centromere clusters are at opposing ends of the nucleus (*e*). Throughout karyogamy and meiotic prophase the nuclei move ceaselessly from one cell end to the other with the SPB at the leading end.

In the revised model (fig. 4) we propose that the black bodies observed close to the SPB in serial sections represent the clustered telomeres. After fusion of the SPBs and the telomere clusters of the two haploid nuclei (fig. 4c) premeiotic DNA replication occurs, followed by pairing of the homologous chromosomes (fig. 4d).

The linear elements may be required for chromosome pairing and consequently also for initiation of recombination. Consistent with this view, asynaptic mutants of *S. cerevisiae* that still form axial elements, pair their homologs to some extent (J. Loidl, F. Klein, and H. Scherthan, pers. commun.). In addition all asynaptic mutants of *S. cerevisiae* that still show meiotic recombination, are forming axial elements[33,84]. The linear elements may be necessary for chromatin condensation and/or provide a scaffold facilitating the pairing process as well as the resolution of interlocks (for a detailed discussion see Bähler et al.[4]). As soon as the two centromere clusters meet, the homologous centromeres can recognize each other, and then may provide a second initiation point for chromosome pairing. Figure 4e represents a horse-tail nucleus in stage 3 of prophase, with the clustered telomeres associated with the SPB and the nucleolus (not shown in the figure) at one end. At the opposite end of the elongated nucleus the centromeres are also clustered. This stage corresponds to the bouquet configuration described in other organisms (for review see Loidl[60]). In fission yeast this bouquet arrangement together with the linear elements are obviously sufficient for recombination and preparation of chromosomes for ordered segregation during meiosis I. They do not proceed to the tripartite SC stage (pachytene) that is accompanied by dissolution of the bouquet structure. Moreover, strong chromatin condensation (bivalents as 'sausages') is not observed during prophase (H. Scherthan and J. Bähler, unpublished), in contrast to budding yeast pachytene[5,88]. Thus fission yeast seems to skip late meiotic prophase stages seen in other organisms (dissolution of bouquet, chromosome condensation, SC formation). At this stage, it is a matter of speculation why fission yeasts do not need SC formation. The correlation with absence of crossover interference has been discussed above. It may be that the properties that are described (bouquet configuration, linear elements, clustering of telomeres and centromeres, nuclear movement, organization of homologous chromosomes into domains), and that are summarized by our model, are sufficient for the support of recombination and chromosome segregation. All these properties are not unique to fission yeasts, although some of them seem to be emphasized more than in other organisms. As far as they are present, they are thought to contribute to ordered meiosis in other eukaroytes as well. The following three properties of *S. pombe* may be important for the origin of its unusual meiotic features: low chromosome number ($n = 3$), small genome size (14 Mb), and higher average crossover frequency per bivalent than in any other organism studied[11,15,19].

Obviously the presented model has to be substantiated and refined by future experiments. Important data have been obtained by in situ hybridization with different DNA probes on spread nuclei (H. Scherthan, J. Bähler and J. Kohli, manuscript submitted). In diploid vegetative cells the homologous chromosomes seem to share common domains. This was concluded from chromosome painting experiments: Integral labelling of chromosomes I and II with different fluorescence dyes results in little overlap of the signals in spread nuclei. Thus, the two homologous chromosomes share a common territory distinct from the other chromosomal territories in a diploid nucleus. Quantitative evaluation of FISH experiments with probes specific for centromeric repeat sequences showed that the centromeres of all chromosomes are clustered throughout prophase of azygotic meiosis. This differs from zygotic meiosis (data from Y. Hiraoka and collaborators cited above and shown in the model in fig. 4). When meiosis is induced in vegetative diploids, the switch of SPB association from centromeres to telomeres does not seem to disrupt the centromere cluster whereas in zygotic meiosis the two centromere clusters can join only after nuclear fusion. The FISH analysis on azygotic meiosis with telomeric repeat sequences as probes shows clustering of telomeres early in prophase. Possibly, the homologous telomeres recognize each other within the cluster and initiate pairing. When probes for interstitial regions in the arms of chromosome II are simultaneously hybridized with a single copy probe adjacent to the centromere, it is found that the centromeric region is closely associated in vegetative cells already. When the same interstitial probes are combined with unique probes close to the telomeres, it is observed that pairing occurs first at the telomeres, and only afterwards in the interstitial regions. Pairing of interstitial regions is observed coincidently with the appearance of linear elements. None of the stages with linear elements (see above) seems to be especially correlated with highly paired chromosomes. This is in accordance with dynamic pairing/unpairing behaviour of homologous chromosomes throughout meiotic prophase in fission yeasts. Ectopic interactions throughout prophase would be facilitated by 'breathing' of the pairs. The strong movement of the nuclei in meiotic prophase, including U-turns at the cell ends (see above), is proposed to provide the mechanical force for movement of the chromatin during dynamic pairing. Such dynamic behaviour during chromosome pairing in early prophase has also been suggested for organisms with an SC, but it is then stopped (or at least strongly reduced) by SC formation[48]. If our scheme for dynamic fission yeast meiosis is correct, maintenance of the bouquet structure by firmly anchoring the telomere cluster to the leading end of the elongated horse-tail nucleus must be vital. In fact, FISH experiments with triple labelling of telomeres, centromeres and nucleolar DNA confirm that the bouquet structure shown in figure 4 survives the spreading of nuclei. Avoidance of general disorder in the nuclei deformed by the movements may be achieved

by the confinement of homologous chromosomes to domains that are also stable enough to withstand disintegration by the spreading forces.

The detailed analysis of chromosome behaviour in prophase of zygotic and azygotic meiosis will be the subject of future work involving FISH and immunofluorescence studies. More data on the location and aggregation of centromeres and telomeres in the G1 phase of growing and nitrogen-starved vegetative cells are needed for a better understanding of the switch of SPB association from centromeres to telomeres. Preliminary experiments show that topoisomerase II is not involved in prophase, but is required for chromosome segregation (J. Bähler and E. Hartsuiker, unpubl. observ.). The study of mutants blocked in meiosis and mutants deficient in recombination will contribute greatly to the understanding of mechanisms. DeVeaux and Smith[11] describe centromere-region specific reduction of recombination frequencies on chromosome III in strains carrying *rec8*, *rec10* or *rec11* mutations. Interestingly, the *rec8* mutation shows aberrant linear elements in meiotic prophase (M. Molnar and J. Bähler, unpubl. observ.). In the context of our model these data can be interpreted as lack of chromosome pairing in the centromeric regions, when pairing initiated at the telomeres can reach the centromeric regions only with help of intact linear elements. Alternatively the linear elements may be required for recombination mediated from pairing initiated at the centromeres, while another pairing mechanism provides for recombination in the distal regions of the chromosomes. The cytological data presented on meiotic prophase are a frame for the detailed study of recombination intermediates and enzymes in *S. pombe*.

In conclusion, the study of fission yeast with a combination of genetical, cytological and biochemical methods is providing novel insights into the mechanisms of homologous recombination, DNA repair, chromosome segregation and the functions of nuclear organelles. The specific advantage of *S. pombe*, as well as the study of its unusual properties, are expected to reveal mechanisms that are also present in other eukaryotes, but less accessible to analysis. The comparison with budding yeast and other organisms will contribute to the identification of the basic traits of eukaryotic cellular organization and function.

Acknowledgements. We apologize for not fully citing all the work on recombination and cytology in fission yeast and other organisms in this short review. We are indebted to M. Baur, A. Carr, O. Fleck, E. Hartsuiker, Y. Hiraoka, N. Kleckner, F. Klein, M. Lichten, J. Loidl, M. Molnar, P. Munz, P. Schär, H. Scherthan, G. Smith, A. Svoboda and P. Szankasi for communication of data prior to publication. We thank P. Munz and O. Fleck for discussions and critical reading of the manuscript. Work in our laboratory was supported by grants from the Swiss National Science Foundation.

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