Increased ploidy and *KAR3* and *SIR3* disruption alter the dynamics of meiotic chromosomes and telomeres

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Summary

We investigated the sequence of chromosomal events during meiotic prophase in haploid, diploid and autotetraploid SK1 strains of *Saccharomyces cerevisiae*. Using molecular cytology, we found that meiosis-specific nuclear topology (i.e. dissolution of centromere clustering, bouquet formation and meiotic divisions) are significantly delayed in polyploid SK1 meiosis. Thus, and in contrast to the situation in plants, an increase in ploidy extends prophase I in budding yeast. Moreover, we found that bouquet formation also occurs in haploid and diploid SK1 meiosis deficient in the telomeric heterochromatin protein Sir3p. Diploid s*ir3Δ* SK1 meiosis showed pleiotropic defects such as delayed centromere cluster resolution in a proportion of cells and impeded downstream events (i.e. bouquet formation, homologue pairing and meiotic divisions). Meiotic telomere clustering occurred in diploid and haploid *sir3Δ* strains. Using the haploid system, we further show that a bouquet forms at the *kar3Δ* SPB. Comparison of the expression of meiosis-specific Ndj1p-HA and Zip1p in haploid control and *kar3Δ* time courses revealed that fewer cells enter the meiotic cycle in absence of Kar3p. Elevated frequencies of bouquets in *kar3Δ* haploid meiosis suggest a role for Kar3p in regulation of telomere dynamics.

Key words: Bouquet, Haploid, *Kar3*, Meiosis, Polyploid, *Sir3*, Telomeres, Yeast

Introduction

Meiosis is the central mechanism by which the somatic chromosome number is halved and haploid germ cells (or spores) are formed. Two subsequent divisions without an intervening DNA replication achieve the bisection of the parental genome. Prior to this chromosome reduction, homologous chromosomes recombine and exchange genetic material during the lengthy meiotic prophase I (reviewed by Keeney, 2001; Pâques and Haber, 1999). Meiotic chromosome pairing, recombination and segregation have been subject of many genetic and cytological studies (Loidl, 1990; Roeder, 1997). At the chromosomal level, it has been recognized that faithful chromosome segregation depends on connections between homologous chromosomes that are laid down during prophase I (for reviews, see Walker and Hawley, 2000; Zickler and Kleckner, 1998).

In preparation for homologue pairing, dramatic changes in nuclear and chromosome architecture occur: the premeiotic centromere and chromosome distribution and organization are resolved, chromosomes elongate and reposition within the nucleus, and the chromosome ends transiently form a cluster at a limited region of the nuclear envelope (bouquet formation) (for review, see Scherthan, 2001; Zickler and Kleckner, 1998).

Genetic and cytological analyses suggest that the formation of a chromosomal bouquet in the synaptic meiosis of budding yeast occurs independently of homologous recombination and synapsis (Dresser and Giruox, 1988; Rockmill and Roeder, 1998; Trelles-Sticken et al., 1999). In the vegetative (premeiotic) yeast nucleus, telomeres associate in a few aggregates at the nuclear periphery (Gotta et al., 1996; Klein et al., 1992) and centromeres form a cluster near the spindle pole body (SPB; the fungal microtubule-organizing centre) (Goh and Kilmartin, 1993; Hayashi et al., 1999; Jin et al., 2000). Telomeres and centromeres depart from this vegetative positioning when cells are starved for nitrogen and fermentable carbon sources, and enter sporulation. Soon after the induction of meiosis, centromeres disperse from the SPB (Hayashi et al., 1999; Jin et al., 2000) and telomeres redistribute to cluster transiently at this location (Trelles-Sticken et al., 2000). Thus, the bouquet stage of budding yeast resembles the classical bouquet arrangement that occurs in a conserved manner during prophase I of most, if not all, eukaryotes (Zickler and Kleckner, 1998; Yamamoto and Hiraoka, 2001; Scherthan, 2001).

The meiosis-specific telomer protein Ndj1p is required for ordered cross-over distribution (interference), sister chromatid cohesion at meiotic telomeres and segregation of small chromosomes in budding yeast meiosis (Chua and Roeder, 1997; Conrad et al., 1997). It has previously been shown that Ndj1p is essential for bouquet formation and effective homologue pairing (Rockmill and Roeder, 1998; Trelles-Sticken et al., 2000).

In addition to its occurrence in synaptic and asynaptic organisms, meiosis can also be induced in auto- and allopolyploids, and even in the absence of homologues, as in haploid plants, in which heterosynapsis (synapsis between non-homologous chromosomes) is observed (Gillies, 1974; Levan, 1942; Santos et al., 1994). Similarly, haploid yeast strains that express both mating-type loci can be induced to undergo

meiosis, and these meiocytes also display heterosynapsis and foldback pairing (Loidl et al., 1991). The occurrence of heterosynapsis in haploid meiosis and the association of non-homologous chromosomes in disomic meiosis (Loidl et al., 1994) suggests the existence of a homology-independent 'chromosome mover' mechanism – a task that has long been ascribed to meiosis-specific telomere clustering (for reviews, see Loidl, 1990; Zickler and Kleckner, 1998; Scherthan, 2001).

Chromosome movements and bouquet formation at meiosis might involve motor proteins that move along microtubules, because microtubule-disrupting drugs have been shown to interfere with chromosome movement, pairing and bouquet formation (Cowan and Cande, 2002; Loidl, 1990; Svoboda et al., 1995). Observations of the asynaptic meiosis of *Schizosaccharomyces pombe* have shown that bouquet formation and nuclear movements depend on the integrity of the SPB, telomeres and the cytoplasmic dynein heavy chain motor protein (reviewed in Yamamoto and Hiraoka, 2001). In *S. cerevisiae*, the absence of the kinesin-like microtubule motor Kar3p confers a defect in nuclear congression during mating (Meluh and Rose, 1990; Rose, 1996) and alters microtubule dynamics, leading to longer, more abundant cytoplasmic microtubules (Saunders et al., 1997). Because Kar3p has been found to interfere with synapsis and recombination, it has been assumed to play a role in chromosome and telomere movements during prophase I (Bascom-Slack and Dawson, 1997).

Finally, telomere clustering has also been observed to occur in polyploid insects (e.g. Rasmussen and Holm, 1980) and plants (Martinez-Perez et al., 1999; Schwarzacher, 1997). In plants, the bouquet forms during prophase I, whereas the sorting of homologous centromeres takes place premeiotically (Martinez-Perez et al., 2001; Martinez-Perez et al., 1999). It is assumed that this feature simplifies the homologue-alignment process and leads to more rapid progress through prophase I (Moore, 2002). In agreement with this assumption, polyploid plants (which often display a Rabl orientation in somatic cells; Cowan et al., 2001; Dong and Jiang, 1998) undergo meiosis faster than their diploid progenitors (Bennett and Smith, 1972; Martinez-Perez et al., 2000).

Here, we have determined the timing of prophase progression and bouquet formation in haploid and autotetraploid yeast meiosis, and in strains deficient for the motor protein Kar3p and Sir3p, the latter being a component of silent telomeric heterochromatin (for reviews, see Grunstein et al., 1998; Shore, 2001). The experiments, which all were done in the SK1 strain background, revealed that an increase in ploidy delays prophase I progression, as assayed by occurrence of meiotic divisions, centromere cluster resolution and bouquet formation. Delayed prophase I progression was also noted in diploid and haploid *sir3Δ* meiosis. Our data show that meiotic telomere clustering occurs in the absence of Sir3p and Kar3p, with the latter possibly being involved in control of telomere dynamics.

Materials and Methods

Yeast strains

To exploit the relatively synchronous progress of meiosis in the strain SK1 of budding yeast (Padmore et al., 1991; Trelles-Sticken et al., 1999), all described gene disruptions and substitutions were performed in the SK1 background (Kane and Roth, 1974). The genotypes of the strains used are listed in Table 1. The autotetraploid SK1 strain used was described previously and is a derivative of the diploid SK1 strain used (Loidl, 1995). Tetraploidy, which was apparent by an increased nuclear diameter compared with the wild type, was verified by the occurrence of up to four cosmid signals in vegetative nuclei after single cosmid fluorescent in situ hybridization (FISH) experiments (not shown) and an increased nuclear diameter (Fig. 2). Its diploid parent strain was used as a control.

Table 1. Genotypes of the strains used in this study

SK1 strains	Genotype
Diploid wild type	MAT**a**/*MATα, HO/HO*
Haploid	MAT**a**, *ho::LYS2, lys2, leu2::hisG, his4X, ura3, trp1::hisG, spo13::hisG, sir3::LEU2*
Tetraploid (FKY76)	MAT**a**, *ho::LYS2, lys2, leu2::hisG, his4, ura3* MAT**a**, *ho::LYS2, lys2, leu2::hisG, his4, ura3* MAT*α*, *ho::LYS2, lys2, leu2::hisG, his4, ura3* MAT*α*, *ho::LYS2, lys2, leu2::hisG, his4, ura3*
Haploid *kar3Δ* (SLY1178)	MAT**a**, *ho::LYS2, lys2, leu2::hisG, his4X, ura3, trp1::hisG, spo13::hisG, sir3::LEU2, kar3::KanMX*
Diploid *sir3Δ* (SLY1131)	MAT**a**/*MATα, ho::LYS2/ho::LYS2, his4B/his4X, ura3/ura3, sir3::LEU2/sir3::LEU2*

To test haploid yeasts for the ability to form bouquets, we used a haploid strain with *SIR3* and *SPO13* deleted (Table 1) (Loidl and Nairz, 1997) that undergoes meiosis because of *sir3Δ*-induced mating-type heterozygosity (Rine and Herskowitz, 1987), whereas the *spo13* mutation induces the cell to skip the meiosis I division, leading to the formation of two viable spores (Klapholz and Esposito, 1980; Wagstaff et al., 1982). A *kar3Δ* derivative of this strain (Table 1) was constructed by replacing the *KAR3* gene with a KanMX cassette using a one-step PCR-mediated technique (Wach et al., 1994). Furthermore, in both the *KAR3* and the *kar3Δ* haploid strain, the native *NDJ1* gene was substituted by a hemagglutinin (HA)-tagged version of the gene for improved immunodetection of Ndj1p (Conrad et al., 1997; Trelles-Sticken et al., 2000).

As a control, we also used a diploid SK1 strain with a homozygous deletion for *SIR3*. This strain was constructed by mating haploid strains that had the chromosomal *SIR3* loci disrupted and Sir3p expressed from plasmid pJL276 (Loidl et al., 1998). These plasmids, which carried the *URA3* marker, were eventually removed by selection on FOA.

Cell culture and preparation

For time-course analysis, cultures were grown in presporulation medium to a density of 2×10^7 cells ml^{-1} followed by transfer to sporulation medium (2% KAc) at a density of 4×10^7 cells ml^{-1} (Roth and Halvorson, 1969). Briefly, aliquots from the sporulating cultures were obtained during time-course experiments at induction of meiosis (transfer to sporulation medium = 0 minutes) and from 180 minutes to 420 minutes at 10 minute or 20 minute intervals, or from 30 minutes up to 420 minutes at 30 minute intervals. In another set of experiments, aliquots were collected at 0 minutes and from 160 minutes to 300 minutes at 10 minute intervals. Aliquots were immediately transferred to tubes on ice containing a tenth of a volume of acid-free 37% formaldehyde (Merck). After 30 minutes, cells were removed from fixative, washed with 1× SSC and spheroplasted with Zymolyase 100T (100 μg ml^{-1}, Seikagaku) in 0.8 M sorbitol, 2% KAc, 10 mM dithiothreitol. Adding 10 volumes of ice-cold 1 M sorbitol terminated spheroplasting. Limited nuclear spreading was used to enhance cytological resolution as described previously (Scherthan and Trelles-Sticken, 2002; Trelles-Sticken et al., 2000).

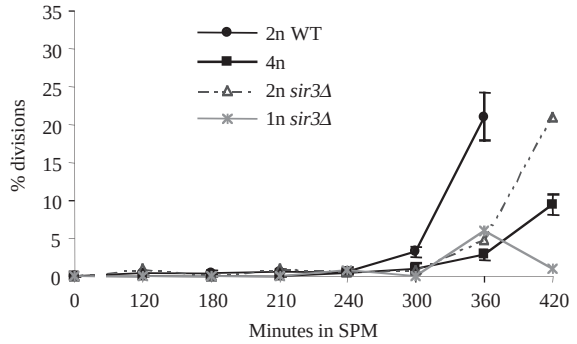

Fig. 1. Occurrence of meiotic divisions after induction of sporulation in diploid wild-type (2*n* WT), tetraploid (4*n*) and *sir3Δ* diploid (2*n* (*sir3Δ*)), and in *sir3Δ spo13Δ* haploid (1n (*sir3Δ*)) SK1 strains. Bi- and tetranucleated cells (100-200 per time point) were scored as divisions at the indicated time points in sporulation medium (SPM). The diploid and tetraploid time courses are given as the mean (±S.D.) of three experiments. Meiosis in diploid wild-type SK1 progresses significantly faster than in the tetraploid strain. Divisions in the *sir3Δ spo13Δ* haploid and *sir3Δ* diploid occur at similar times after induction of meiosis and are delayed with respect to diploid wild-type meiosis.

Meiotic time courses with diploid and tetraploid strains were only analysed when sporulation rates were >80%.

DNA probes and labelling

A composite pan-centromeric DNA probe was used to delineate all yeast centromeres by FISH (cen-FISH) and a subtelomeric XY′ plasmid probe that delineates all telomeres in the SK1 strain (2*n*=32) investigated was used to tag yeast telomeres (telo-FISH) (Jin et al., 1998; Trelles-Sticken et al., 2000). Cosmid *h* (pEKG011) (Thierry et al., 1995) was used to visualize a defined locus on the left arm of chromosome *XI*. Probes were labelled either with dig-11-dUTP (Roche Biochem) or with biotin-14-dCTP (Invitrogen) using a nick translation kit according to the instructions of the supplier (Invitrogen).

FISH

All preparations were subjected to two colour FISH as described previously (Scherthan and Trelles-Sticken, 2002). The hybridization solution contained two differently labelled probes: the pan-telomere XY′ probe and either the pan-centromeric DNA probe or the cosmid probe mapping near the centromere on the left arm of chromosome *XI* (*h*; Trelles-Sticken et al., 2000). Immunofluorescent detection of hybrid molecules was carried out with Avidin-FITC (Sigma) and rhodamine-conjugated sheep anti-dig Fab fragments (Roche Biochemicals) (for details, see Scherthan and Trelles-Sticken, 2002). Prior to microscopic inspection, preparations were embedded in antifade medium (Vector Labs, Burlingame) containing 0.5 μg ml^{-1} DAPI (4′-6-diamidino-2-phenylindole) as DNA-specific counterstain.

Immunostaining

A polyclonal antiserum against *S. cerevisiae* SPB component (Tub4; Marschall et al., 1996) was used to stain the SPB in conjunction with telomeres (for details, see Trelles-Sticken et al., 1999). A rabbit antiserum against Zip1p transverse filament protein (Sym et al., 1993) of the yeast synaptonemal complex was applied to identify nuclei at all stages of synapsis. Ndj1p was stained in freshly prepared, mildly spread nuclei obtained from the strains that express HA-tagged Ndj1p using a monoclonal anti-HA-tag antibody (Biotec Santa Cruz) and secondary anti-mouse Cy3-conjugated antibodies (Dianova).

Microscopic evaluation

Signal patterns in spread nuclei were investigated using a Zeiss Axioskop I epifluorescence microscope equipped with a double-band-pass filter for simultaneous excitation of red and green fluorescence, and single-band-pass filters for excitation of red, green and blue (Chroma Technologies). Digital images were obtained using a cooled greyscale CCD camera (Hamamatsu) controlled by the ISIS fluorescence image analysis system (MetaSystems). More than 110 nuclei were scored for each time point and probe combination directly in the microscope in randomized preparations. Fluorescence signal patterns were analysed in nuclei with an undisrupted homogeneous appearance in the DAPI image.

Results

Increased ploidy prolongs prophase I progression in yeast

Based on the comparison of prophase I progression in meiosis of diploid and polyploid plants, it has been suggested that an increase in ploidy shortens meiotic prophase (Bennett and Smith, 1972; Martinez-Perez et al., 2000; Moore, 2002). To address the question of whether accelerated prophase I progression in response to increased ploidy is an evolutionarily conserved feature, we investigated the timing of meiotic divisions and chromosomal prophase events in closely related SK1 strains of different ploidy of the budding yeast *S. cerevisiae* (Loidl et al., 1991; Loidl, 1995). First, we induced the diploid and tetraploid strain to undergo sporulation and sampled aliquots of the cultures at 0 minutes and several subsequent time points during the following 5 or 7 hours (see Materials and Methods). The diploid and tetraploid SK1 strains used in this study displayed a sporulation rate >80%. To obtain comparable results, experiments with lower sporulation rates were excluded from analysis.

To determine the timing of meiotic divisions in the different SK1 strains, DAPI-stained aliquots of cells that were collected at consecutive time points in sporulation and scored for bi- and tetranucleated cells as divisions. It was found that the tetraploid strain passed through the meiotic divisions with a significant delay (~2 hours) with respect to the diploid wild type. This was observed in three independent experiments (Fig. 1) and argues against an acceleratory effect of increased ploidy on meiosis I progression in yeast.

Centromere cluster resolution is delayed in tetraploid meiosis

To determine whether changes in nuclear architecture are affected by the prolonged prophase of the tetraploid yeast strain, we next measured the dissolution of the centromere cluster upon entry into meiosis. In the premeiotic (vegetative) yeast nucleus centromeres are tightly clustered at the spindle pole body (SPB; the fungal microtubule-organizing centre), whereas induction of meiosis leads to centromere dispersion throughout the nucleus (Hayashi et al., 1999; Jin et al., 1998; Jin et al., 2000). In tetraploid meiosis, we noticed that the frequency of nuclei with one centromere cluster (premeiotic nuclear architecture) dropped only gradually, which contrasted

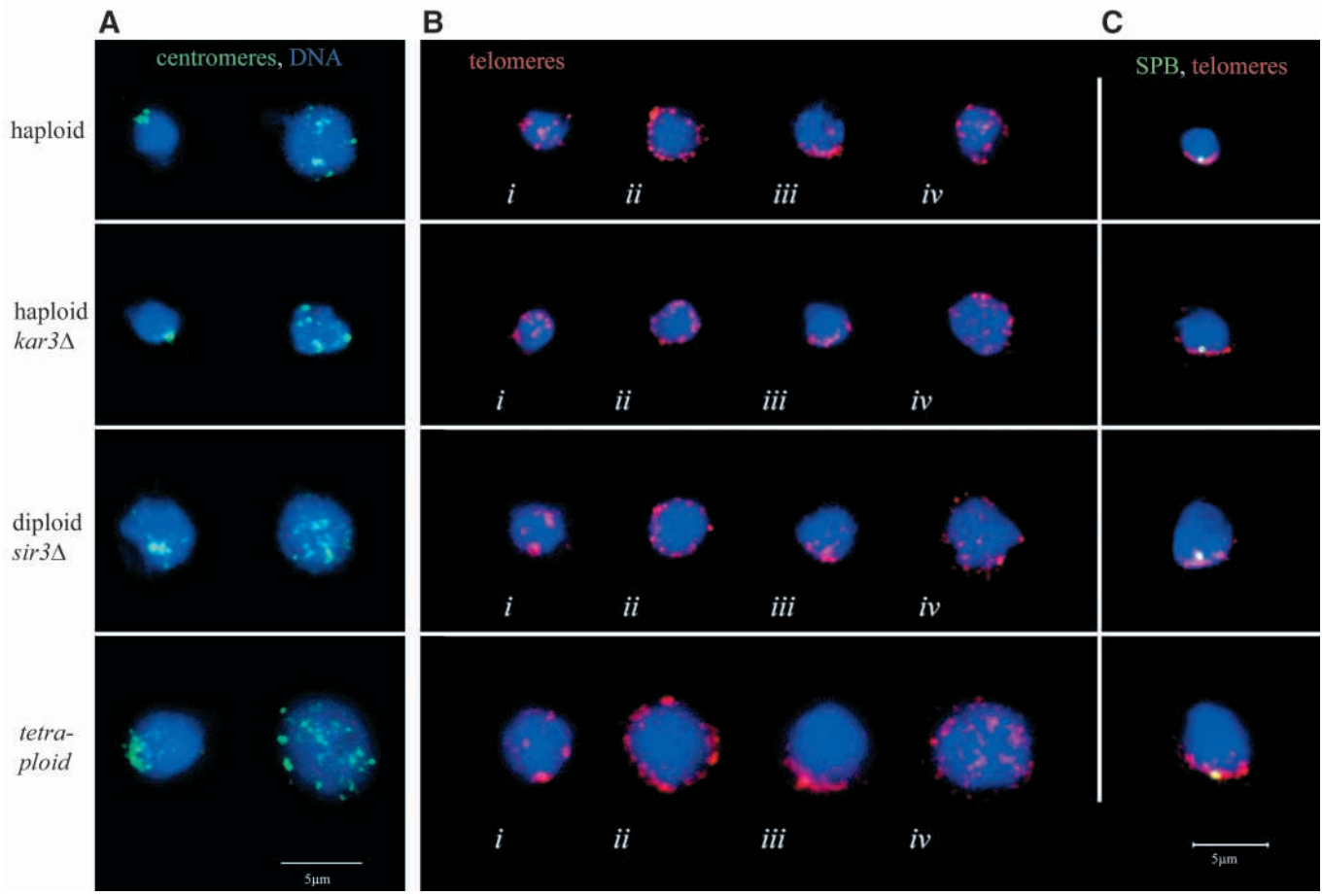

Fig. 2. (A) Representative centromere (green) and (B) XY′ telomere FISH signal patterns (red) in mildly spread nuclei (DAPI, blue) obtained from synchronized meiotic cultures of meiosis-proficient haploid *sir3Δ spo13Δ* control strain (haploid), a corresponding haploid *kar3Δ sir3Δ spo13Δ* strain (haploid *kar3Δ*), a diploid *sir3Δ* strain (diploid *sir3Δ*) and a tetraploid strain (tetraploid). All strains were of identical SK1 background. The FISH patterns observed are ordered according to their deduced sequential occurrence and match those observed in diploid wild-type SK1 meiosis (Trelles-Sticken et al., 2000). (A, left) Premeiotic nuclei (0 minutes, fixed at transfer to SPM) with a single centromere signal cluster (green). (A, right) Meiocyte nuclei (taken at 300 minutes) exhibit dispersed centromere signals. Notice the increase in cen-cluster size from haploid to tetraploid. (Bi) Premeiotic nuclei (0 minutes) of all strains display a few telomere FISH signal clusters (red). (Bii) Early meiocytes exhibiting a meiosis-specific rim-like telomere distribution (Hayashi et al., 1999; Trelles-Sticken et al., 2000). (Biii) Bouquet nuclei of all strains, which exhibit telomere FISH signals clustered at a limited region of the nuclear periphery. (Biv) Meiocyte nuclei showing dispersed telomere FISH signals, which is typical for pachytene (Trelles-Sticken et al., 1999). (C) Colocalization of clustered telomeres (red) and the SPB (green). In the diploid *sir3Δ* and tetraploid SK1 strain, telomeres and SPB were visualized by XY′ telomere FISH in combination with Tub4 IF, whereas telomeres in the haploid control and haploid *kar3Δ* strain were stained by Ndj1-HA IF. Notice the rise in nuclear diameter and signal numbers with increase in ploidy. Bar, 5 μm.

with rapid dissolution of centromere clustering in diploid wild-type SK1 meiosis (Fig. 3). The former aligns with the delayed meiotic divisions in tetraploid meiosis (Fig. 1).

Bouquet formation is delayed in tetraploid meiosis

A chromosomal hallmark of early prophase I is the temporal clustering of meiotic telomeres at the SPB during the leptotene-zygotene transition (Trelles-Sticken et al., 1999). To address the question whether a bouquet forms in tetraploid yeast meiosis, we performed combinatorial telomere (telo)-FISH with the pan XY′ telomere probe and SPB immunostaining (IF) on mild nuclear spreads obtained at 270 minutes after initiation of sporulation (when bouquets were abundant). Like previously seen in diploid yeast strains (Trelles-Sticken et al., 1999), telomeres were found to aggregate at the tetraploid SPB (Fig. 2C). Altogether, these data disclose the occurrence of a bouquet in tetraploid meiosis of budding yeast. Time-course experiments showed that meiotic telomere clustering occurred with a delay in tetraploid meiosis (Fig. 4), with bouquet frequencies peaking at 270-300 minutes and 360 minutes. This biphasic nature suggests the presence of sub-populations in the cultures (e.g. mother and daughter cells), which, probably because of size differences (Rupes, 2002; Trelles-Sticken et al., 1999), initiate and progress through meiosis somewhat asynchronously.

Altogether, the significantly delayed timing of nuclear divisions and chromosomal events in tetraploid yeast meiosis, like centromere cen-cluster dissolution and bouquet formation (Figs 3, 4), suggests that an increase in ploidy retards prophase

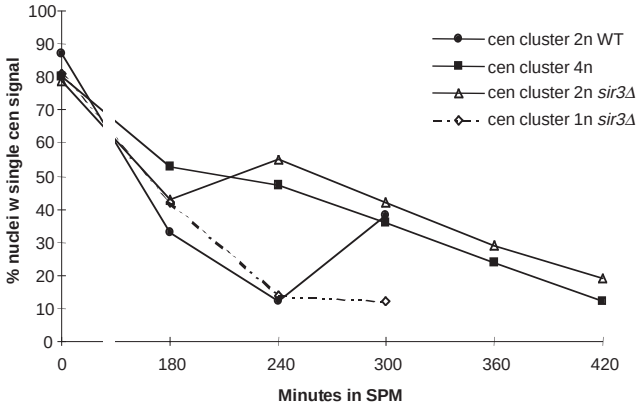

Fig. 3. Frequencies of mildly spread nuclei that display a single centromere FISH signal (cen cluster) at the indicated time points after induction of meiosis in a diploid wild-type strain (2*n* WT), a tetraploid (4*n*), a *sir3Δ* diploid (2*n sir3Δ*) and a *sir3Δ spo13Δ* haploid (1*n sir3Δ*) SK1 strain. In the diploid wild-type and the 1*n sir3Δ* strain, centromere clustering is resolved at a similar pace. In tetraploid meiosis, cen clustering resolves only gradually, as is the case for the 2*n sir3Δ* strain after 180 minutes in sporulation medium (SPM; compare Fig. 5).

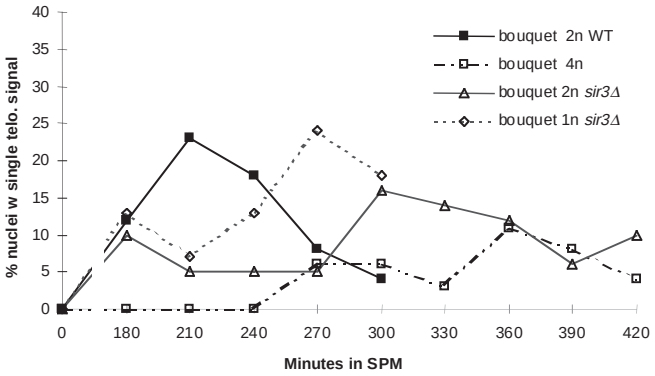

Fig. 4. Relative frequencies of nuclei with a single telomere FISH cluster (bouquet) obtained in limited nuclear spreads of the following SK1 strains. Diploid wild-type (2*n* WT; data taken from Trelles-Sticken et al., 1999), tetraploid (4*n*) and *sir3Δ* diploid (2*n sir3Δ*) and haploid *sir3Δ spo13Δ* (1*n sir3Δ*). The data shown are corrected for the initial frequencies in that the *t*=0 values (6% in diploid WT, 2% in 2*n sir3Δ*, 0% in tetraploid and 2% in haploid) were subtracted from all values of the subsequent time points. Initial increase in bouquet frequencies is similar in diploid WT, haploid and diploid *sir3Δ* meioses, whereas tetraploid meiosis displays a substantial delay in bouquet occurrence. In *sir3Δ*, haploid and diploid meiosis a further increase in bouquet frequency pauses after 180 minutes Two peaks in the mutant and tetraploid time course suggest that physiological sub-populations progress through meiosis consecutively.

I progression in yeast meiosis, which contrasts with the situation in plants.

Chromosomal events in haploid meiosis

In yeast, meiosis can also be induced in haploid and disomic strains, and genetic analysis suggests that these strains might undergo bouquet formation (Loidl et al., 1991; Rockmill and Roeder, 1998). To see whether a bouquet forms in the absence of homologous chromosomes, we investigated meiosis in a haploid *spo13Δ sir3Δ* SK1 strain that expresses both mating types and skips the reductional division (Klapholz and Esposito, 1980; Wagstaff et al., 1982).

Two independent experiments revealed that meiotic divisions in the haploid *spo13Δ sir3Δ* strain occurred with a delay compared with the diploid wild type (Fig. 1). However, centromere cluster dissolution progressed with wild-type kinetics after induction of meiosis in the haploid SK1 strain, which suggests that earliest chromosomal events of haploid prophase I are not or only mildly affected by *SPO13* or *SIR3* mutation (see below). Our findings agree with the *SPO13* mutation causing a delay in the meiosis I division (Klapholz and Esposito, 1980; McCarroll et al., 1994) through the meiosis-I spindle checkpoint (Lee et al., 2002; Shonn et al., 2001). The low frequency of divisions seen in the haploid time courses might be due to the low frequency of sporulation, which, in agreement with earlier reports (Loidl et al., 1991), never exceeded 40% and might indicate the presence of a synaptic checkpoint that arrests the cells before meiosis I (see Discussion).

Combinatorial telo-FISH and SPB immunostaining on mild nuclear spreads obtained 270 minutes after induction of sporulation revealed that telomeres aggregate at the haploid SPB (Fig. 2C), which demonstrates the occurrence of a bouquet in haploid yeast meiosis. Meiotic time courses in the haploid *spo13Δ sir3Δ* SK1 strain reproducibly revealed peak bouquet frequencies at 300 minutes (12.9±2.6% SD, three time courses). This timing matches with a bouquet peak at 300 minutes in diploid *sir3Δ* meiosis but contrasts with bouquet formation in our diploid wild-type SK1 time courses, where peak bouquet frequencies (20.5±4% SD, three time courses) formed reproducibly around 210 minutes in sporulation medium (Fig. 4). Hence, nuclei with bouquet topology accumulate late in haploid *spo13Δ sir3Δ* prophase I, which is probably due to heterosynapsis and delayed repair of double-stranded breaks (DSBs) in the haploid condition (De Massy et al., 1994; Loidl and Nairz, 1997) and to pleiotropic effects elicited by the deletion of *SIR3* (see below).

SIR3 deletion disturbs centromere cluster resolution in diploid meiosis

Because Sir3p is a structural component of telomeric heterochromatin (Hecht et al., 1996; Moretti et al., 1994), whose absence could potentially influence telomere positioning at meiosis, we determined whether a *SIR3* deletion in the SK1 strain background has an impact on meiotic chromosome behavior. Like the haploid *SIR3* mutant, diploid *sir3Δ* meiosis exhibited delayed meiotic divisions (Fig. 1). Cen FISH to meiotic time courses revealed that induction of sporulation in SK1 strain leads to a drop of cells with one centromere cluster over the first 180 minutes, which matches the course of centromere (cen) cluster resolution in haploid meiosis. Subsequently, the decrease in nuclei with one cen cluster slowed to follow the more gradual reduction seen in the tetraploid time course (Fig. 3). Repeated time courses confirmed a reproducible delay of cen-cluster resolution in the diploid *sir3Δ* time courses compared with the diploid wild type

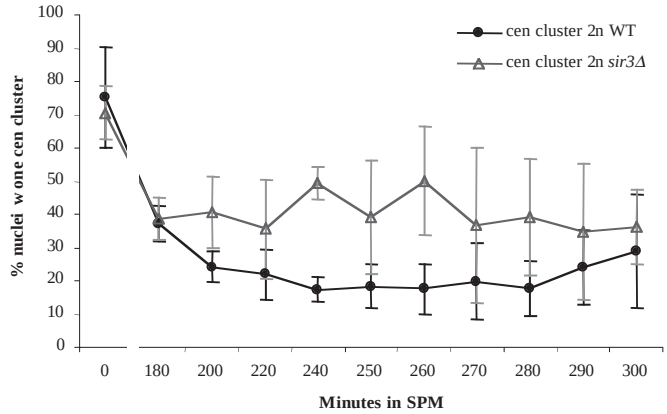

Fig. 5. Frequency of nuclei with one centromere cluster (cen cluster) in repeated time course experiments in the WT diploid (2*n* WT) and *sir3Δ* diploid (2*n sir3Δ*) SK1 strains. The values are the mean of four experiments in the wild-type and three experiments in the *sir3Δ* diploid. The standard deviation reflects considerable variability between the time courses in the mutant, whereas cen cluster resolution in the diploid WT is less variable, particularly at early time points.

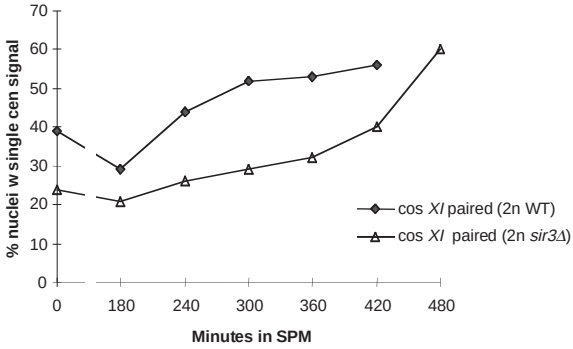

Fig. 6. FISH analysis of homologue pairing during meiotic time courses of wild-type (2*n* WT) and *sir3Δ* (2*n sir3Δ*) SK1 strains. Cosmid probe *h*, which maps near centromere XI (Trelles-Sticken et al., 2000), was hybridized to spread preparations obtained at various times after transfer to sporulation medium (Minutes in SPM). Pairing values (%) reflect the proportion of nuclei containing cosmid signals that touched each other or showed an enlarged coalesced signal. More than 100 FISH signal pairs were scored per time point and strain. The frequencies of nuclei with paired signals increased more gradually in the absence of Sir3p, reaching nearly wild-type levels with a ~3 hour delay. In the wild type, maximal signal pairing values were reached at 300 minutes and remained nearly constant up to 420 minutes The occurrence of meiotic divisions after 240 minutes and 300 minutes in 2*n* WT and 2*n sir3Δ* meiosis, respectively, might explain why pairing values never exceeded ~60%.

(Fig. 5). The differences between the two data sets were highly significant (the Wilcoxon rank sum test and the one-sided *t* test gave values of 0.000398 and 0.0001 at P=0.05, respectively). These observations suggest an over-all delayed generation of meiotic nuclear architecture in a significant proportion of *sir3Δ* diploid cells, which might in turn retard events downstream of cen clustering, such as bouquet formation and homologue pairing (see below). We consider it unlikely that failed initiation of meiosis in a significant subset of cells could be a cause of the changes observed, because sporulation was >80% in all diploid *sir3Δ* and wild-type time courses investigated. Rather, it seems that a proportion of diploid *sir3Δ* cells enters meiosis and initiates changes in nuclear architecture with a delay.

Meiotic telomeres cluster in the absence of Sir3p

Combinatorial telo-FISH and SPB IF to diploid Sir3p-deficient meiocytes obtained at 300 minutes after induction of meiosis revealed a single telomere FISH signal cluster at the diploid *sir3Δ* SPB (Fig. 2C). Frequencies of nuclei with one telo-FISH signal cluster increased similar to the wild type over the first 180 minutes after meiosis induction, then dropped and formed a second peak at 270 and 300 minutes (Fig. 4). The biphasic nature of bouquet frequency probably relates to the tardy dissolution of centromere clustering in a substantial proportion of *sir3Δ* cells (Figs 3, 5) and is consistent with a delayed occurrence of meiotic divisions in diploid *sir3Δ* meiosis (Fig. 1). In line with the haploid data (Figs 2, 4), it is evident that bouquet formation bypasses the requirement for Sir3p.

SIR3 mutation delays homologue pairing

Given the promotion of homologue pairing by bouquet formation (Rockmill and Roeder, 1998; Trelles-Sticken et al., 2000), delayed telomere clustering would predict retarded homologue pairing. Thus, we determined the pairing of a cosmid-tagged centromere-close region on the left arm of chromosome XI (Trelles-Sticken et al., 2000). In the wild type, we noticed that significant levels of premeiotic homologue pairing were reduced between 60 and 120 minutes after induction of meiosis, which probably reflects the resolution of vegetative homologue contacts during the premeiotic S phase (Weiner and Kleckner, 1994). Pairing gained meiosis-specific levels over the next 180 minutes, with the maximum values in this wild-type time course (52-56%) being reached after 300 minutes in sporulation medium (Fig. 6). In diploid *sir3Δ* meiosis, wild-type pairing values were reached only after 480 minutes in sporulation (Fig. 6). A second meiotic time course experiment revealed 62% pairing at 300 minutes in the wild type and 63% in the *sir3Δ* diploid strain at 420 minutes After induction of meiosis, which confirms a delay in homologue pairing in the mutant and suggests that the slow prophase I progression retards homologue pairing in the *sir3Δ* diploid strain. Eventually, however, wild-type levels of homologue pairing were reached in the absence of Sir3p.

Chromosomal events in Kar3p-deficient haploid meiosis

Motor proteins are expected to move meiotic telomeres, and so we investigated the role of Kar3p kinesin in bouquet formation. For this analysis, we used the bouquet-proficient haploid SK1 strain, because we repeatedly failed to obtain sporulating *kar3Δ* diploids in the SK1 background. Kar3p is a kinesin-like motor protein (Meluh and Rose, 1990) that is required for proper positioning of the metaphase spindle (for review, see Hildebrandt and Hoyt, 2000) and karyogamy (the congression stage when parental nuclei migrate and fuse with each other during syngamy) (Rose, 1996). In addition to its role in mitosis,

it has been shown that Kar3p deficiency leads to reduced meiotic recombination rates and defects in chromosome synapsis (Bascom-Slack and Dawson, 1997), which has led to the assumption that Kar3p could drive meiotic telomere clustering (Bascom-Slack and Dawson, 1997; Zickler and Kleckner, 1998). To assess the requirement of bouquet formation for Kar3p, we deleted *KAR3* in the bouquet-proficient *spo13Δ sir3Δ* haploid SK1 strain. The *kar3Δ spo13Δ sir3Δ* triple mutant and a *KAR3 spo13Δ sir3Δ* haploid control strain were induced to undergo meiosis. Although mitotic growth was not dramatically affected, repeated meiotic experiments revealed sporulation rates of 10% in the *kar3Δ* haploid, whereas the haploid control strain showed 40% sporulation. The reduced sporulation in the *kar3Δ* haploid agrees with the disruptive effect of the *KAR3* deletion on diploid meiosis (Bascom-Slack and Dawson, 1997).

KAR3-deficient meiosis shows wild-type centromere cluster resolution

To investigate the order of chromosomal events in *kar3Δ* meiosis, we first determined resolution of vegetative nuclear architecture after induction of meiosis (i.e. the drop of frequency of nuclei with one centromere cluster; see above). Time-course experiments at 30-minute intervals up to 7 hours after transfer to sporulation medium revealed that, at 0 minutes, 58% and 56% of nuclei displayed one centromere FISH cluster in the haploid control and *kar3Δ* strain, respectively. The cen cluster frequencies decreased similarly in both strains but with a slight delay between 180 and 420 minutes in *kar3Δ* sporulation (Fig. 7). Eventually, both time courses reached similar values of cen clustering in the control (17%) and *kar3Δ* (16%) at 420 minutes. The more gradual cen-cluster resolution between 180 and 420 minutes in a proportion of *kar3Δ spo13Δ sir3Δ* triple mutant cells compared with the *spo13Δ sir3Δ* double mutant meiocytes (Fig. 7) could result from altered microtubule dynamics in the absence of Kar3p (Cottingham et al., 1999). Altogether, it appears that Kar3p is dispensable for centromere cluster resolution after induction of meiosis.

To determine the frequency of cells that enter meiosis in the haploid state, we transfected a HA-tagged version of the meiosis-specific telomere protein Ndj1p (also known as Tam1p) (Chua and Roeder, 1997; Conrad et al., 1997) into the haploid *kar3Δ* and corresponding control SK1 strain. Ndj1p IF enables us to distinguish meiocytes and their telomere distribution in cell suspensions from meiotic time courses (Trelles-Sticken et al., 2000).

Expression of Ndj1p and Zip1p is altered in the absence of Kar3p

Time-course analysis revealed a rapid increase in Ndj1p-HA-positive nuclei after 150 minutes in sporulation in the haploid control, whereas the increase in *kar3Δ* meiosis was more gradual over the entire meiotic time course, with Ndj1-HA-positive *kar3Δ* meiocytes being more abundant at early time points (Fig. 8). The frequencies of meiotic cells peaked at 49% at 420 minutes in the *kar3Δ* haploid, whereas 74% of cells in the haploid control strain were Ndj1-HA positive after 420 minutes in sporulation (Fig. 8). The latter figure indicates that far more cells enter haploid meiotic prophase (74% in control, 49% in *kar3Δ*) than sporulate – 40% in the control and 10% in *kar3Δ*, respectively. To validate these results, we also monitored the expression of Zip1p, which is an synaptonemal complex (SC) protein that connects homologous chromosomes partially during zygotene and entirely during pachytene (Sym et al., 1993). As for Ndj1p, a more gradual increase in meiocytes expressing Zip1p was noted in the *kar3Δ* haploid (Fig. 8). The observed expression profiles for Ndj1p and Zip1p agree with the report that *kar3*-mutant cells display derepression of premeiotic *IME1* transcription and thus ectopic sporulation, whereas induction of meiosis does not further increase *IME1* expression in prophase I (Meluh, 1992; Keeney, 2001). The recombination defects in diploid *kar3Δ* meiosis (Bascom-Slack and Dawson, 1997) might thus relate to a more gradual entry of fewer cells into meiosis (see above). Furthermore, it is possible that a checkpoint responds to the absence of Kar3p function (Hardwick et al., 1999; Shanks et al., 2001).

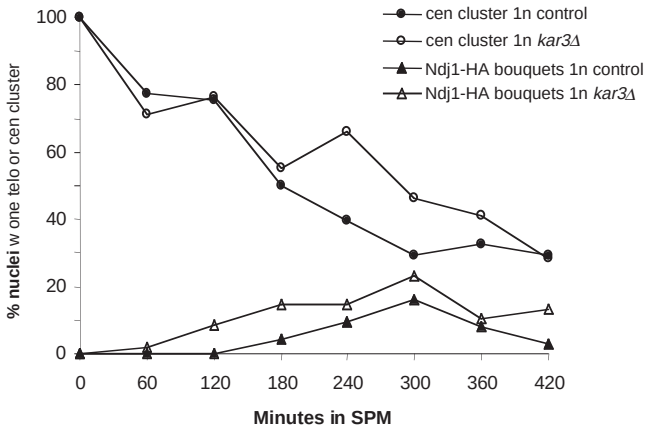

Fig. 7. Centromere and telomere analysis of Ndj1-HA-expressing haploid control and *kar3Δ* SK1 strains after transfer to sporulation medium (SPM). The frequencies (based on >100 nuclei per time point) of nuclei with one centromere FISH cluster in meiotic time courses of haploid *sir3Δ spo13Δ* control [cen cluster (1*n* control)] and haploid *kar3Δ sir3Δ spo13Δ* [cen cluster (1*n kar3Δ*)] drop at similar rates after the induction of meiosis. In the 1*n kar3Δ* time course, cen-cluster dissolution stalled between 180 and 240 minutes. All values were normalized with the values at 0 minutes (58% in WT; 56% in *kar3Δ*) set to 100%. The frequency of bouquet nuclei with one peripheral Ndj1-HA IF telomere cluster peaks at 300 minutes in the haploid control [Ndj1-HA bouquets (1n control)] and *kar3Δ* [Ndj1-HA bouquets (1n *kar3Δ*)] SK1 strain. Ndj1-HA bouquet nuclei are more abundant at all time points in *kar3Δ* meiosis. The bouquet frequencies shown are given as the percentage of haploid Ndj1-HA-expressing (meiotic) cells (74% in the WT; 48% in *kar3Δ* mutant; Fig. 8). It appears that bouquet nuclei accumulate in absence of Kar3p.

Bouquet formation in *kar3Δ* meiosis

To test whether a Kar3p-deficient strain can cluster meiotic telomeres, we performed HA-Ndj1p IF together with SPB IF on meiotic time courses of the haploid control and the corresponding *KAR3*-deleted SK1 strain. The formation of a single telomere signal cluster at the SPB disclosed the formation of a true bouquet in haploid *kar3Δ* meiosis (Fig. 2C).

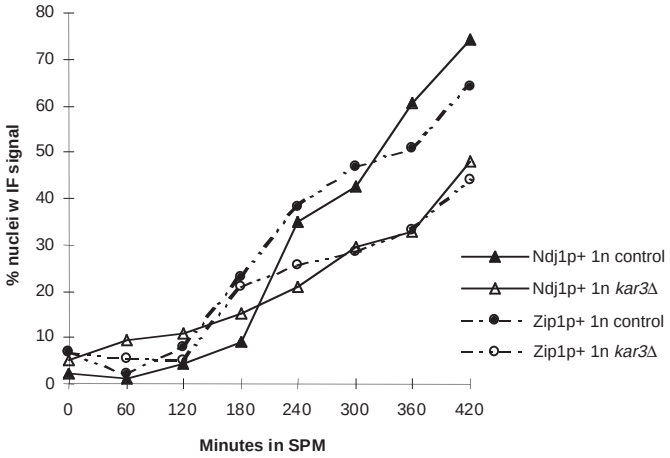

Fig. 8. The frequency of nuclei exhibiting Ndj1-HA fluorescence in meiotic time courses of haploid *sir3Δ spo11Δ* control (Ndj1-HA+ hapl. control) and *kar3Δ sir3Δ spo11Δ* (Ndj1-HA+ hapl. *kar3Δ*) SK1 strains. In the haploid control meiosis, fewer Ndj1p-HA-expressing cells (Ndj1p-HA+) are present at early time points, whereas most cells rapidly enter meiosis after 180 minutes in sporulation medium (SPM). A high frequency of *kar3Δ* cells expressing Ndj1-HA are seen before 180 minutes in SPM, whereas the increase of Ndj1-HA+ cells occurs only gradually over the entire time course. Similar observations are seen when the expression of Zip1p is monitored by IF and all cells displaying Zip1p signals of any nature were scored as Zip1p+. As in the Ndj1-HA study, induction of meiosis in the *kar3Δ* strain failed to induce a significant increase in Zip1p-expressing cells. The Ndj1-HA+ cells at 0 minutes probably result from ectopic sporulation of *kar3Δ* meiocytes.

When the proportion of bouquet cells with a single Ndj1p signal cluster was determined among NDJ1-HA-expressing haploid *kar3Δ* and control meiocytes, it was found that chromosomal bouquets formed in the two strains, with more bouquet nuclei being present in the *kar3Δ* mutant from early time points onwards. This was also seen by telo-FISH in preparations from the same time courses (not shown). Because more cells enter meiosis in the control strain than in the *kar3Δ* strain (see above), it appears that the Kar3p-deficient time courses contain more bouquet nuclei (Fig. 7). Telo-FISH time-course experiments on strains that lacked the HA-tagged version of Ndj1p (not shown) revealed a similar excess of bouquet nuclei in haploid *kar3Δ* meiosis (peak value 27.5%) compared with controls (peak value 22.5%). Altogether, it appears that telomere cluster formation in budding yeast meiosis tolerates the absence of Kar3p. Higher frequencies and earlier appearance of bouquet nuclei in *kar3Δ* haploid meiosis indicate altered bouquet duration. Thus, Kar3p might be involved in the control of tubulin-dependent telomere dynamics and release of telomere clustering.

Discussion

Increased ploidy prolongs prophase I progression in yeast meiosis

Previously, it has been observed that an increase in ploidy accelerates meiosis progression in plants (Bennett and Smith, 1972; Martinez-Perez et al., 2000). Here, we have investigated whether epigenetic effects caused by increased ploidy influence the prophase I progression in closely related SK1 strains of budding yeast. Meiosis induction in the diploid SK1 strain and the tetraploid SK1 strain derived therefrom revealed that prophase I progression, bouquet formation and meiotic divisions were delayed in tetraploid meiosis compared with diploid wild-type meiosis – which contrasts with the situation in diploid versus allopolyploid plants (Martinez-Perez et al., 2000; Moore, 2002) and indicates that accelerated prophase I progression in polyploid plants is not a universal feature that extends to other kingdoms.

Besides other possibilities, the prophase I delay in the polyploid yeast is in agreement with the assumption that a genome with increased chromosome number will require a more elaborate and time-consuming homologue search during prophase I (Dorninger et al., 1995; Pfeifer et al., 2001). How, then, according to such a scenario, is prophase I progression accelerated in polyploid plants? This is probably related to the fact that the homologue search and alignment process and the correction of non-homologous premeiotic centromere associations in polyploid plants takes place prior to the onset of meiotic prophase (Martinez-Perez et al., 2001). By contrast, the redistribution of pericentromeric heterochromatin (centromeres) in mammals and yeast occurs during the onset of first meiotic prophase (Hayashi et al., 1999; Pfeifer et al., 2001; Scherthan et al., 1996; Trelles-Sticken et al., 1999). Therefore, the elaborate architectural changes in the diploid meiocyte nucleus that occur after the initiation of meiosis probably extend the time required for the transit though prophase I, whereas the rapid prophase I progression in polyploid plants obviously benefits from the premeiotic alignment of homologous pericentromeres (Martinez-Perez et al., 2001).

In our polyploid yeast, an extended meiotic cell cycle might also be induced by altered amounts of the microtubule/kinetochore-associated proteins like Bik1p, which have been shown to be limiting in cells of higher ploidy (see below) (Lin et al., 2001), and the repression of G1 cyclin genes (Galitski et al., 1988). The latter requires the cell to be larger before it passes through START and replication (for a recent review, see Rupes, 2002). Accordingly, cells of higher ploidy will require more time before they enter premeiotic S-phase and initiate first meiotic prophase. This could explain the generally retarded entry into prophase I, signified by the delay of cen-cluster resolution and bouquet formation in the tetraploid strain. Thus, the transient increase of the frequency of cells with a single cen-cluster between 180 and 240 minutes after induction of meiosis (Fig. 3) probably relates to a delayed entry of a subpopulation of polyploid cells, possibly smaller daughter cells that will require longer to pass through the premeiotic divisions (Rupes, 2002). This feature will probably reduce synchrony in the tetraploid and other time courses, which becomes particularly evident when cultures are investigated by cytology (see below) (Trelles-Sticken et al., 1999).

Haploid meiocytes undergo wild-type telomere redistribution

We also investigated prophase I progression in haploid yeast meiosis. Our haploid SK1 strain can undergo meiosis because of the deletion of *SIR3*, which simulates heterozygosity of the

mating type locus (Rine and Herskowitz, 1987), and *SPO13* disruption, which induces the cell to skip the meiosis I division, leading to the formation of two viable spores (Klapholz and Esposito, 1980; Wagstaff et al., 1982). We consider the *spo13* mutation not to be relevant to the analysis of chromosomal events during the onset of prophase I in the haploid SK1 strain, because the *spo13*-dependent delay is mediated through the meiosis I spindle checkpoint (Lee et al., 2002; Shonn et al., 2002) and because centromere cluster resolution occurred at a similar pace in diploid wild-type and haploid meiosis (Fig. 3). Further analysis is required to tease apart the *spo13Δ*- and *sir3Δ*-induced defects in haploid prophase I.

Little, however, is known about the effects of the *SIR3* deletion on chromosomal events in meiosis. SIR (silent information regulator) proteins mediate transcriptional silencing at silent mating type loci and in the vicinity of telomeres, the latter being known as telomere position effect (TPE) (Gottschling et al., 1990; Stone and Pillus, 1998). Telomeric heterochromatin is built on the interactions of Sir2p, Sir3p and Sir4p with Rap1p and histones H3 and H4 (Hecht et al., 1996; Kyrion et al., 1993; reviewed by Grunstein et al., 1998).

Although it has been shown that *sir3* mutations lead to moderately shortened telomeres and delocalize Rap1p in diploid cells (Palladino et al., 1993), Sir3p and Sir4p have been found to be dispensable for the localization and clustering of vegetative telomeres (Gotta et al., 1996). We also observed that the haploid *sir3Δ* SK1 meiosis displayed centromere dispersion comparable to that in the diploid wild type, whereas telomeres congregated at the haploid *sir3Δ* SPB. This indicates that the programme for bouquet formation is triggered by nitrogen starvation and the expression of both mating type loci in the same cell. These observations agree with genetic data indicating that bouquets form in haploid yeast (Rockmill and Roeder, 1998). Because a chromosomal bouquet has been observed in haploid rye (Santos et al., 1994), it appears that the signal for telomere clustering in synaptic meiosis does not depend on the presence of homologous chromosomes.

During diploid wild-type SK1 sporulation, telomere clustering peaked reproducibly around 210 minutes (see Results) (Trelles-Sticken et al., 1999), whereas peak values were seen at 300 minutes after induction of haploid meiosis. Increased bouquet frequencies also persisted in prophase I of diploid *spo11Δ* and *rad50S* recombination mutants, in which DSB formation or repair, respectively, is blocked (Trelles-Sticken et al., 1999). In haploid meiosis, DSB formation is reduced and repair is delayed compared with diploid meiosis (De Massy et al., 1994; Loidl and Nairz, 1997; Loidl, 1995). Given the similar pace of centromere cluster resolution in the control and *kar3Δ* haploid compared with diploid wild-type SK1 meiosis, the prevalence of haploid bouquet cells at later time points might relate to the absence of appropriate conditions or signals from the machinery that monitors the initiation and progression of meiosis-specific events like DSB formation and/or synapsis (Roeder and Bailis, 2000; Keeney, 2001).

In the absence of homologous chromosomes, one escape route from a potential checkpoint that may monitor chromosomal events (synapsis) (Odorisio et al., 1998; Roeder and Bailis, 2000) could be heterosynapsis between non-homologous chromosomes or chromosome arms (foldback pairing) and repair of DSBs among sister chromatids. It is intriguing that foldback pairing is observed in haploid meiosis of plants and yeast (Levan, 1942; Loidl et al., 1991; Santos et al., 1994). Heterosynapsis is thought to occur late in first meiotic prophase, when homology is no longer required (Moses et al., 1984; von Wettstein et al., 1984). However, some sort of checkpoint seems to operate during haploid yeast meiosis, because only 54% of the cells that entered meiosis passed the MI division.

Sir3p is dispensable for telomere clustering in diploid meiosis

Because haploid meiosis represents an experimental system, we also investigated the impact of *SIR3* disruption on diploid meiosis. Centromere cluster dissolution was biphasic during diploid *sir3Δ* sporulation. Delayed resolution of the centromere cluster suggests that the switch from vegetative to meiotic nuclear architecture be perturbed by the *SIR3* mutation in the diploid condition. It is possible that this is a consequence of the distorted gene expression that occurs in the absence of functional Sir3p – deletion of *SIR3* has been reported to lead to transcriptional repression of, for example, the *RME1* repressor of meiosis (Wyrick et al., 1999; Mitchell and Herskowitz, 1986). *SIR3* deletion has also been shown to alter the expression of several genes required for S-M phase progression, like *BRN1* (Ouspenski et al., 2000; Lavoie et al., 2000), *BIK1* (Berlin et al., 1990; Lin et al., 2001) and *Cdc15* (Schweitzer and Philippsen, 1997; Tinker-Kulberg and Morgan, 1999). Interestingly, Bik1p, a yeast mitogen-associated protein (MAP) that is required for microtubule attachment at the kinetochore (Berlin et al., 1990; Lin et al., 2001), interacts with Ndc10p (Cbf2p), a component of the yeast kinetochore complex (Lin et al., 2001), which is necessary for vegetative centromere clustering (Jin et al., 2000). This makes it likely that kinetochore detachment (centromere cluster resolution) at meiosis could be actively controlled. Semi-quantitative RT-PCR data indicate derepression of *BRN1* and *BIK1* expression that persists into diploid *sir3Δ* meiosis (E.T-S. and H.S., unpublished). An altered pool of the yeast condensin Brn1p and the kinteochore MAP Bik1p could influence the progression of *sir3Δ* diploids through the last premeiotic division and/or distort centromere dispersion during prophase I. Interestingly, the administration of microtubule-disrupting drugs 120 minutes after induction of meiosis (when premeiotic mitoses have ceased) delayed centromere-cluster resolution (E.T-S. and H.S., unpublished). Thus, it seems likely that centromere cluster resolution during prophase I is an active process that in some way requires intact microtubules.

Probably as a consequence of the retarded resolution of centromere clustering and/or altered gene expression in the absence of Sir3p, peak frequencies of bouquet nuclei and homologue pairing were also delayed in diploid *sir3Δ* meiosis. The observation that the telomeres cluster at the haploid and diploid *sir3Δ* SPBs suggests that the telomeric heterochromatin protein Sir3p is not directly required for meiotic telomere movements.

Role of Kar3p in meiotic telomere clustering

Bouquet formation in *S. cerevisiae* has been shown to depend

on the presence of the meiosis-specific telomere protein Ndj1p (Chua and Roeder, 1997; Conrad et al., 1997; Trelles-Sticken et al., 2000). Furthermore, it has long been assumed that the repositioning of telomeres during meiotic prophase requires the action of motor proteins (Loidl, 1990; Zickler and Kleckner, 1998). In the asynaptic prophase of the fission yeast *S. pombe*, it has been found that microtubule-disrupting drugs and mutations in the myosin heavy chain motor protein disrupt nuclear motility during prophase I and reduce the rate of recombination and homologue pairing (Svoboda et al., 1995; Yamamoto et al., 1999).

In budding yeast, the kinesin-like motor protein Kar3p (Meluh and Rose, 1990) is required for the congression step of haploid nuclei, during which these approach each other to form a zygote nucleus (Rose, 1996). Based on the observation that a *KAR3* deletion can lead to reduced levels of recombination and deterioration of synapsis, it has been suggested that Kar3p could be a telomere motor (Bascom-Slack et al., 1997). Thus, we directly tested whether a *KAR3* deletion does impair meiotic telomere clustering. We had to make use of haploid meiosis because several attempts to generate sporulating diploid *kar3Δ* strains failed in the SK1 strain background. Analysis of the expression of the meiosis-specific telomere protein Ndj1p in haploid control and *kar3Δ* cultures allowed to investigate exclusively meiotic cells. It was found that meiotic telomere clustering at the *kar3Δ* SPB, which provides physical evidence that the meiotic defect mediated by *KAR3* deletion does not impair bouquet formation in the haploid and likely in other conditions too. Surprisingly, the frequency of haploid bouquet-stage nuclei was elevated in the *kar3Δ* condition, which indicates that the bouquet stage might be extended in absence of Kar3p. Thus, Kar3p might be involved in the control of meiotic telomere clustering and especially in its release, which is compatible with the microtubule-destabilizing properties of Kar3p (Cottingham et al., 1999; Saunders et al., 1997).

Given that the induction of haploid meiosis is less effective in the absence of *Kar3*, it appears that the reduced levels of recombination observed by molecular analysis in diploid *kar3Δ* meiosis (Bascom-Slack et al., 1997) might relate to the reduced levels of meiosis-specific events at any time point in meiosis compared with the control, and/or to the response of a meiotic checkpoint (Shanks et al., 2001). This reasoning is supported by the observation that the *ndj1Δ* bouquet mutant, unlike *kar3Δ* (Bascom-Slack et al., 1997), only displays minor defects in recombination (Chua and Roeder, 1997). Although meiotic telomere clustering occurs in the absence of Kar3p, it remains to be seen whether other yeast microtubule motor proteins (Hildebrandt and Hoyt, 2000) could play a more dominant role in telomere movements at meiosis.

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