Set1- and Clb5-deficiencies disclose the differential regulation of centromere and telomere dynamics in *Saccharomyces cerevisiae* meiosis

Edgar Trelles-Sticken[1,*], Sandrine Bonfils[2], Julie Sollier[2], Vincent Géli[2], Harry Scherthan[1,‡,§] and Christophe de La Roche Saint-André[2,§]

[1]MPI for Molecular Genetics, Ihnestr. 73, 14195 Berlin, Germany
[2]LISM, CNRS, 31 chemin Joseph Aiguier, 13402 Marseille, Cedex 20, France
*Present address: Schuetzenstrasse 31, 12105 Berlin, Germany
‡Present address: Bundeswehr Institute of Radiobiology, Neuherbergstr. 11, 80937 Munich, Germany
§Authors for correspondence (e-mail: laroche@ibsm.cnrs-mrs.fr; scherth@web.de)

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Summary

The entry into meiosis is characterized by a lengthy premeiotic S phase and a reorganization of the nuclear architecture. Analysis of centromere and telomere dynamics in wild-type *Saccharomyces cerevisiae* meiosis suggests that resolution of vegetative centromere and telomere clusters are independent events differently connected to premeiotic S phase. Absence of the B-type cyclin Clb5 or the Set1 histone methyltransferase leads to a delay of premeiotic S phase by separate mechanisms. In *clb5Δ* cells, centromere cluster resolution appears normal, whereas dissolution of the vegetative telomere clusters is impaired and meiosis-specific clustering of telomeres, i.e. bouquet formation, is grossly delayed. In *set1Δ* cells, centromere and telomere redistribution are both impaired and bouquet nuclei are absent, despite proper location of the meiosis-specific telomere protein Ndj1. Thus, centromere and telomere redistribution at the onset of prophase I is differentially regulated, with centromere dispersion occurring independently of premeiotic S phase. The normal kinetics of dissolution of the vegetative telomere clusters in a *set1Δ mec1-1* mutant suggests the presence of a checkpoint that limits the dispersion of telomeres in absence of Set1.

Key words: Set1, C1b5, Meiosis, Centromere, Telomere, Bouquet

Introduction

Sexually reproducing eukaryotes utilize a specialized cell division cycle, meiosis, to generate haploid spores or gametes from diploid progenitor cells to compensate for the genome doubling that occurs at fertilization. Meiosis starts with an extended premeiotic S phase, the initiation of which is controlled by the Ime2 meiosis-specific protein kinase (Foiani et al., 1996), followed by a lengthy G2-equivalent prophase in which homologous chromosomes have to find, pair and recombine with each other to ensure their proper segregation at the first meiotic division (reviewed by Petronczki et al., 2003; Roeder, 1997; von Wettstein, 1984). Checkpoint mechanisms ensure that events at the DNA and chromosomal level are completed before cells enter metaphase I, which is the case for the Mec1-dependent S-phase checkpoint that restrains meiotic M phase until DNA replication has been completed (Stuart and Wittenberg, 1998).

Fluorescent in situ hybridization (FISH) studies with pan-centromeric and pan-telomeric DNA probes, as well as live cell observations in the yeast *Saccharomyces cerevisiae*, have shown that induction of the meiotic cycle leads to a dramatic reorganization of nuclear architecture, which resembles the chromosome dynamics observed in meiosis of higher eukaryotes (reviewed by Loidl, 1990; Zickler and Kleckner, 1998; Scherthan, 2001). In mitotic yeast nuclei, all centromeres are clustered near the spindle pole body (SPB), a topology which is only reduced when cells are kept stationary for an extended period (Jin et al., 1998). Soon after induction of meiosis, centromeres lose their intimate association with the SPB and become dispersed throughout the nucleus (Hayashi et al., 1998; Jin et al., 1998). By contrast, telomeres, which are grouped into several perinuclear clusters in vegetatively growing cells (Gotta et al., 1996), disperse over the nuclear periphery and transiently congregate in the vicinity of the SPB to form the so-called bouquet at the leptotene/zygotene transition (Trelles-Sticken et al., 1999). After the bouquet stage, telomeres again become redistributed randomly over the nuclear periphery during pachytene. Formation of the bouquet depends on the presence of the meiosis-specific telomere protein Ndj1/Tam1 (Chua and Roeder, 1997; Conrad et al., 1997; Trelles-Sticken et al., 2000) and actin polymerization (Trelles-Sticken et al., 2005). It has been proposed that formation of the bouquet catalyzes the meiotic homologue pairing process (Loidl, 1990), an idea supported by cytological (Trelles-Sticken et al., 2000) and genetic (Goldman and Lichten, 2000; Rockmill and Roeder, 1998) evidence and clearly demonstrated in the asynaptic meiosis of *Schizosaccharomyces pombe* (Niwa et al., 2000).

Various analyses have shown an intimate relationship between premeiotic S phase and the initiation of recombination

(Borde et al., 2000). In this respect, the absence of the B-type cyclins Clb5 and Clb6, which are required for premeiotic S phase, leads to a defect in double-strand break (DSB) induction, recombination and synaptonemal complex (SC) formation (Smith et al., 2001; Stuart and Wittenberg, 1998). These results suggest that the meiosis-specific chromatin configuration established during premeiotic S phase might be a precondition for later chromosomal interactions.

Set1 belongs to an eight-protein complex responsible for the specific methylation of lysine 4 of histone H3 (Miller et al., 2001; Nagy et al., 2002; Roguev et al., 2001). The Set1-mediated H3 lysine 4 methylation has been linked to transcriptional elongation (Krogan et al., 2003; Ng et al., 2003). Inactivation of *SET1* affects telomere structure and DNA repair activities in mitotic yeast cells (Corda et al., 1999; Nislow et al., 1997; Santos-Rosa et al., 2004; Schramke et al., 2001) as well as sporulation of diploid cells (Nislow et al., 1997). The defect in meiotic progression combines with a delay of premeiotic S phase onset, a severe impairment of DSB formation and a limited induction of middle meiotic genes (Sollier et al., 2004).

To investigate the relationships that exists between replication and chromosomal movements during the meiotic prophase, we followed nuclear dynamics and premeiotic S phase in wild-type SK1 cells as well as in different mutants in which premeiotic replication is delayed, namely *clb5Δ*, *ime2Δ* and *set1Δ*.

Materials and Methods

Yeast strains

SET1, *CLB5* and *IME2* were disrupted in the SK1 strain (Kane and Roth, 1974) to exploit the relatively synchronous progression of meiosis in this background (Padmore et al., 1991; Trelles-Sticken et al., 1999). The *sml1 mec1-1* allele in SK1 that is used to eliminate the functions of *MEC1* was kindly provided by V. Borde (Sollier et al., 2004). A diploid strain derived from MDY431 and 433 (Conrad et al., 1997) was used for hemagglutinin (HA)-tagged Ndj1 and Rap1 immunofluorescence studies.

Cell culture and preparation

For nuclear preparation, cultures were grown in presporulation medium to a density of 2×10^7 cells/ml and then transferred to sporulation medium (2% potassium acetate) at a density of 4×10^7 cells/ml (Roth and Halvorson, 1969). Aliquots from the cultures were obtained during time course experiments at 0 minutes as well as 60, 120, 210, 270, 330 and 420 minutes after transfer to sporulation medium. For *clb5Δ*, *clb5Δ set1Δ* and *set1Δ mec1-1,* additional aliquots were taken at 480 minutes. Aliquots were immediately transferred to tubes on ice containing 1/10 volume of acid-free 37% formaldehyde (Merck). After 30 minutes, cells were removed from the fixative, washed with 1× SSC and spheroplasted with Zymolyase 100T (100 μg/ml; Seikagaku, Tokyo, Japan) in 0.8 M sorbitol, 2% potassium acetate, 10 mM dithiothreitol. Spheroplasting was terminated by adding 10 volumes of ice-cold 1 M sorbitol. Meiotic spreads were obtained and subjected to FISH as described previously (Trelles-Sticken et al., 2000).

FACS analysis

Meiotic cells were fixed in 70% ethanol. After rehydration in PBS, the samples were incubated for at least 2 hours with RNase A (1 mg/ml) at 37°C. Cells were resuspended in 50 μg/ml propidium iodide in PBS for at least 15 minutes at room temperature. After a wash in PBS, cells were resuspended in 5 μg/ml propidium iodide, sonicated briefly to remove cell clumps if required, and the DNA content was determined by FACS with a Becton Dickinson FASCalibur (BD, Franklin Lakes, NJ).

DNA probes and labeling

A composite pan-centromeric DNA probe was used to delineate all yeast centromeres (Jin et al., 1998). Two plasmids containing a conserved core fragment of the subtelomeric X and Y′ element, respectively (Louis et al., 1994), were used to probe all yeast telomeres (Gotta et al., 1996; Trelles-Sticken et al., 1999). Cosmid probes were used to determine pairing of homologous chromosome regions. The small chromosome *III* was tagged with a cosmid probe hybridizing to HML near the left telomere of chromosome *III* (cos *m*; ATCC 70884). The internal chromosome *XI* cosmid pEKG151, cos *f* (Thierry et al., 1995), mapping to 231.8-264.9 on the left arm of chromosome *XI*, was used to monitor meiotic pairing at a telomere-distant chromosomal region (Trelles-Sticken et al., 1999). Probes were labeled either with dig-11-dUTP (Roche Biochem., Mannheim, Germany) or with biotin-14-dCTP (Life Technologies, Gaithersburg, MD) using a nick translation kit according to the supplier's instructions (Life Technologies).

Fluorescence in situ hybridization (FISH)

All preparations were subjected to two color FISH as described previously (Trelles-Sticken et al., 2000). The hybridization solution contained a yeast pan-telomere probe, which delineates all telomeres (2n=32) and a pan-centromeric DNA probe delineating all centromeres in SK1 strains (Jin et al., 1998; Trelles-Sticken et al., 2000). Pairing of homologous regions was analyzed by two-color FISH to spread nuclei, using differentially labeled #*III*- and #*XI*-specific cosmid probes. Immunofluorescent detection of hybrid molecules was carried out with Avidin-FITC (Sigma) and rhodamine-conjugated sheep anti-dig Fab fragments (Roche Biochemicals) (Scherthan et al., 1992). Prior to microscopic inspection, preparations were embedded in antifade medium (Vector labs, Burlingame, CA) containing 0.5 μg/ml DAPI (4′6-diamidino-2-phenylindole) as a DNA-specific counterstain.

Immunostaining

Immunostaining with a rabbit antiserum (kind gift from S. Roeder, Yale University, CT, USA) against Zip1 transverse filament protein of the yeast synaptonemal complex (Sym et al., 1993) was performed as previously described (Trelles-Sticken et al., 2000). An anti-rabbit secondary FITC-conjugated antibody was used to identify nuclei with synapsis in progress. Ndj1-HA was stained using a monoclonal anti-HA-tag antibody (Biotec Santa Cruz) and secondary anti-mouse Cy3-conjugated antibodies (Dianova, Hamburg, Germany). Immunostaining against Rap1 was done with a rabbit antiserum (kind gift from S. Schwalader and D. Shore, University of Geneva, Switzerland) and a secondary FITC-conjugated anti-rabbit antibody (Dianova).

Microscopy

Preparations were evaluated using a Zeiss Axioskop 1 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, Rockingham, VT). Signal patterns in spread nuclei were investigated using a 100× plan-neofluoar lens (Zeiss, Jena, Germany). For each time point and probe combination, fluorescence signal patterns were analyzed in more than 100 nuclei that displayed an undisrupted, homogeneous appearance in the DAPI image. Digital images were

obtained using a cooled gray-scale CCD camera (Hamamatsu, Herrsching, Germany) controlled by the ISIS fluorescence image analysis system (MetaSystems, Altlussheim, Germany). Contrast and brightness were adjusted using Adobe Photoshop 6.0 (Adobe) to match the fluorescent image seen in the microscope.

Results

Temporal relationships of premeiotic S phase and nuclear dynamics during meiosis

In order to gain insight into the possible functional links that exist between chromosome reorganization and replication during the early steps of meiotic prophase, the dynamic centromere and telomere relocalization was followed together with premeiotic S-phase progression in a diploid wild-type SK1 strain (Fig. 1). According to FACS analysis, premeiotic S phase was initiated between 120 minutes and 210 minutes after transfer into sporulation medium (Fig. 1), with most of the cells having completed replication by 420 minutes. Wild-type cells started meiotic divisions between 270 and 330 minutes and the sporulation rate after 48 hours was 91% (not shown). Mildly spread meiotic nuclei preparations were subjected to two-color FISH with differentially labeled pan-centromeric and pan-telomeric probes (Trelles-Sticken et al., 2000) to estimate the fraction of nuclei with various centromere and telomere distribution patterns throughout the time course (Fig. 1). At 0 minutes, most of the nuclei displayed a single prominent centromere FISH signal cluster and several telomere clusters at the nuclear periphery (Fig. 1), which are typical features of vegetative nuclear architecture (Gotta et al., 1996; Jin et al., 1998). Resolution of the centromere cluster started around 120 minutes after transfer to SPM, with only a few percent of nuclei showing a cluster at 330 minutes, followed by an increasing fraction of nuclei with dispersed telomeres, indicating an effective resolution of vegetative telomere organization. Time points later than 330 minutes could not be evaluated because of a high incidence of cells that had already undergone meiotic divisions. Furthermore, the presence of a single telomere cluster (bouquet) was noted in some nucleoids, with a maximum frequency at 210 minutes (Fig. 1), consistent with the transient nature of the bouquet (Trelles-Sticken et al., 1999). Meiotic pairing was investigated by FISH with a cosmid probe corresponding to an internal region of the left arm of chromosome *XI* (Trelles-Sticken et al., 2000). The proportion of nuclei with paired FISH signals remained low until 210 minutes, followed by a sharp increase between 210 and 270 minutes (Fig. 1). Similar results where obtained with a probe specific for chromosome *III* (not shown).

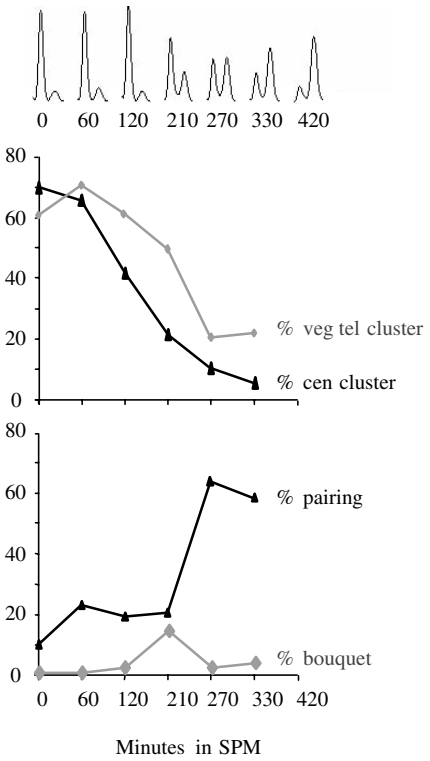


Fig. 1. Analysis of centromere and telomere dynamics during meiosis in wild-type diploid SK1 cells. Frequencies of nuclei displaying a single centromere FISH signal (% cen cluster), of nuclei with two to eight perinuclear vegetative telomere FISH signals (% veg tel cluster), of nuclei containing a single telomere FISH signal (% bouquet) and of nuclei with paired signals with a cosmid probe hybridizing to an internal region of the left arm of chromosome *XI* (% pairing), at different time points (minutes) after transfer into sporulation medium (SPM). Top: FACS profiles corresponding to the same time points.

The initiation of centromere redistribution in the absence of detectable DNA synthesis and the fact that relative kinetics of centromere and telomere dispersion are not strictly fixed (compare wild-type kinetics in Figs 1 and 2) indicate that the centromere and telomere dynamics may be subjected to different controls, possibly connected to premeiotic S phase. To gain a better understanding of this matter a genetic investigation was pursued (see below).

Centromere cluster resolution is independent of meiotic replication

To test for a possible link between premeiotic S phase and vegetative centromere and telomere cluster resolution, we followed meiotic chromosome dynamics in the context of a *CLB5* deletion strain, since the absence of the B-type cyclin Clb5 greatly delays premeiotic S phase (Stuart and Wittenberg, 1998). The wild-type meiotic progression was slightly faster than in the time course shown in Fig. 1, with most cells having completed replication after 330 minutes (Fig. 2) and the first nuclear divisions were already detectable at 210 minutes (not shown). As expected, *clb5Δ* cells displayed a meiotic arrest with the great majority of cells not having replicated their DNA and only a few percent of binucleate cells at 480 minutes (Fig. 2, and not shown). The sporulation rate at 48 hours was 93% and 6% for the wild type and *clb5Δ*, respectively.

While centromere cluster resolution in *clb5Δ* cells occurred with kinetics and magnitude similar to that of the wild type (Fig. 2), the dispersion of telomeres was severely limited, with more than half of *clb5Δ* nuclei maintaining a vegetative telomere topology throughout the time course. This differential impact of the *clb5Δ* mutation on centromere and telomere dynamics was reproducible (see Fig. 5) and indicates that centromere and telomere dispersion are not co-regulated. Centromere dispersion is independent from premeiotic S phase, while telomere dispersion requires a Clb5-dependent

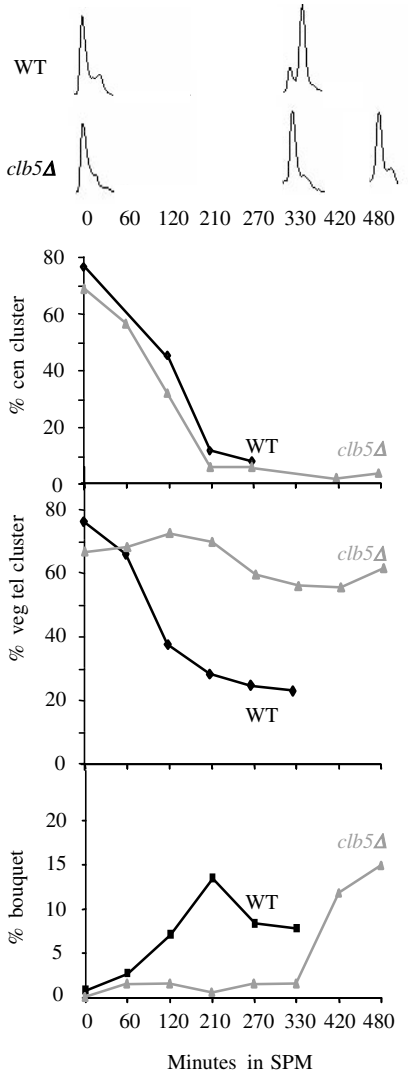


Fig. 2. Centromere and telomere dynamics during meiosis in *clb5Δ*. Frequencies of nuclei displaying a single centromere FISH signal (% cen cluster), of nuclei with two to eight perinuclear vegetative telomere FISH signals (% veg tel cluster) or with a single telomere signal (% bouquet) in wild-type and *clb5Δ* strains, at the indicated time points after transfer into sporulation medium. Top: wild-type and *clb5Δ* FACS profiles aligned to the corresponding time points.

function, possibly premeiotic S phase. However, installation of a single meiotic telomere cluster (bouquet formation) still occurred in a fraction of *clb5Δ* meiocytes, but with significant delay relative to wild type. This delay was reproducible (see Fig. 5) with the accumulation of late bouquet-like meiocytes suggesting the persistence of this stage in absence of Clb5.

Centromere and telomere dynamics are severely affected in *set1Δ* cells

To extend our analysis, two other mutants impinging on premeiotic S phase were studied. Ime2 is a meiosis-specific protein kinase that is critical for proper initiation of meiotic progression (Foiani et al., 1996). Accordingly, premeiotic S phase is delayed in *ime2Δ* cells (Fig. 3). The consequences of

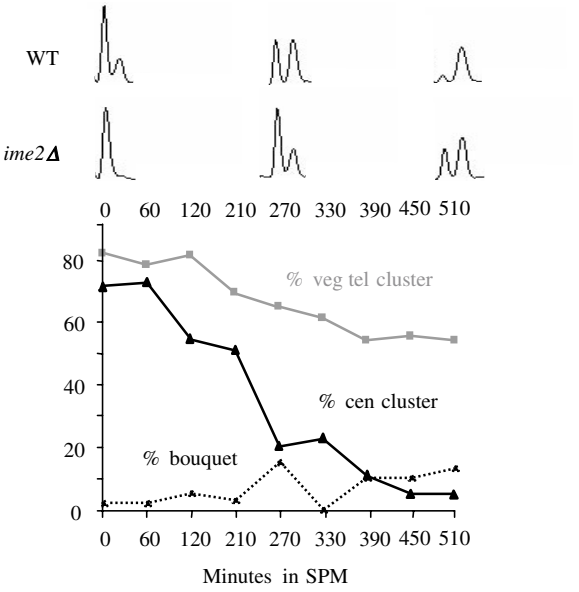


Fig. 3. Centromere and telomere dynamics during meiosis in *ime2Δ*. Frequencies of *ime2Δ* nuclei displaying a single centromere FISH signal (% cen cluster), with two to eight perinuclear vegetative telomere FISH signals (% veg tel cluster) or with a single telomere FISH signal (% bouquet), at the indicated time points after transfer into sporulation medium. Top: wild-type and *ime2Δ* FACS profiles aligned to the corresponding time points.

the *ime2Δ* mutation on chromosome behavior were similar to that of *clb5Δ* with a full dissolution of the centromere cluster, a limited dispersion of vegetative telomere clusters and persistence of bouquet nuclei at late time points (Fig. 3).

Another mutant compromised in premeiotic S phase is *set1Δ*. The absence of the Set1 histone methyltransferase leads to a 2-hour delay of premeiotic S phase (Fig. 4; Sollier et al., 2004). The sporulation rate after 24 hours was 87% for wild-type cells and 76% for *set1Δ* cells, with a larger proportion of dyads in *set1Δ* cells. The proportion of *set1Δ* nuclei displaying a single centromere cluster remained high (for length of the experiment) with only half of the cells resolving their centromere cluster by the end of the time course (Fig. 4). This limited resolution of the centromere cluster distinguishes *set1Δ* from *clb5Δ* and *ime2Δ* meiosis (Figs 2, 3). However, *set1Δ* is much more similar to *clb5Δ* in the restriction of vegetative telomere cluster dispersion. Bouquet formation was never detected in repeated *set1Δ* time courses (Fig. 4), a situation clearly different from *clb5Δ* and *ime2Δ*. Altogether, the reproducible defects elicited by Set1 deficiency, i.e. the partial centromere cluster resolution, the very limited dissolution of vegetative telomere clusters and the absence of bouquet formation, show that Set1 is required for many aspects of meiotic nuclear dynamics. Such a combination of phenotypes has not been reported before.

Epistasis relationships between the *set1Δ* and *clb5Δ* mutations

The differences in centromere and telomere dynamics between *set1Δ* and *clb5Δ* suggests that, as for premeiotic S phase (Sollier et al., 2004), Set1 and Clb5 activities are

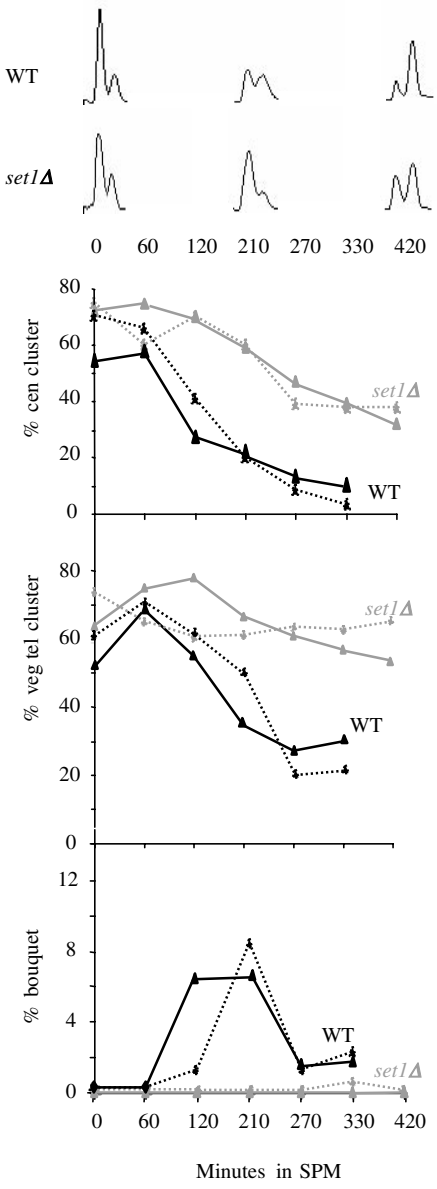


Fig. 4. Loss of Set1 limits chromosome dynamics during the meiotic prophase. Frequencies of nuclei displaying a single centromere FISH signal (% cen cluster), with two to eight perinuclear vegetative telomere FISH signals (% veg tel cluster) or with a single telomere FISH signal (% bouquet) in wild-type and *set1Δ* strains, at the indicated time points after transfer into sporulation medium. Solid and dotted lines: data from two independent time course experiments. Top: wild-type and *set1Δ* FACS profiles from the experiment shown by the solid line, aligned to the corresponding time points.

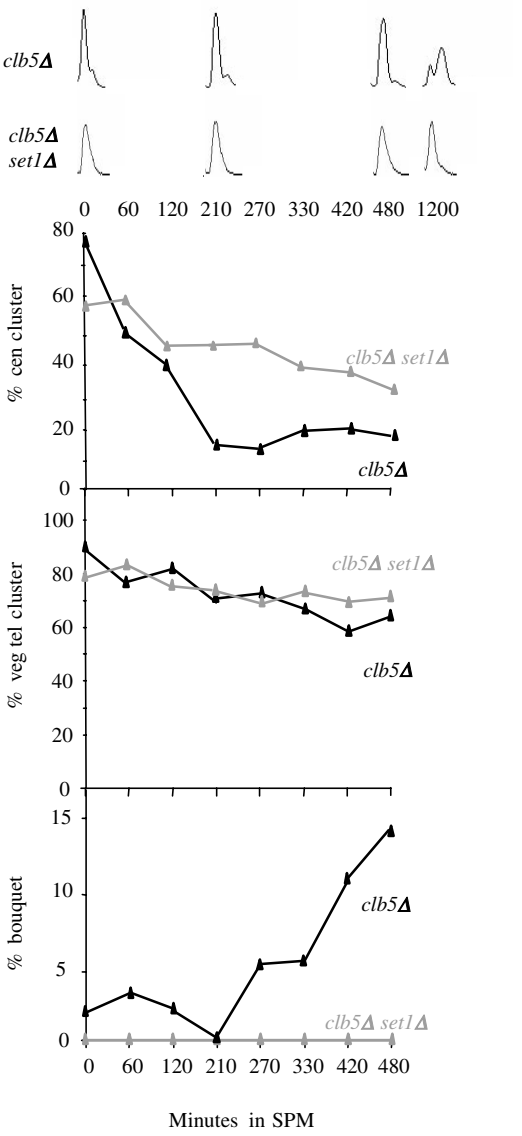


Fig. 5. Epistasis analysis of the *set1Δ* and *clb5Δ* mutations. Frequencies of nuclei displaying a single centromere FISH signal (% cen cluster), with two to eight perinuclear telomere FISH signals (% veg tel cluster) or with a single telomere FISH signal cluster (% bouquet) in *clb5Δ* and *set1Δ clb5Δ* strains, at the indicated time points after transfer into sporulation medium. Top: *clb5Δ* and *set1Δ clb5Δ* FACS profiles aligned to the corresponding time points.

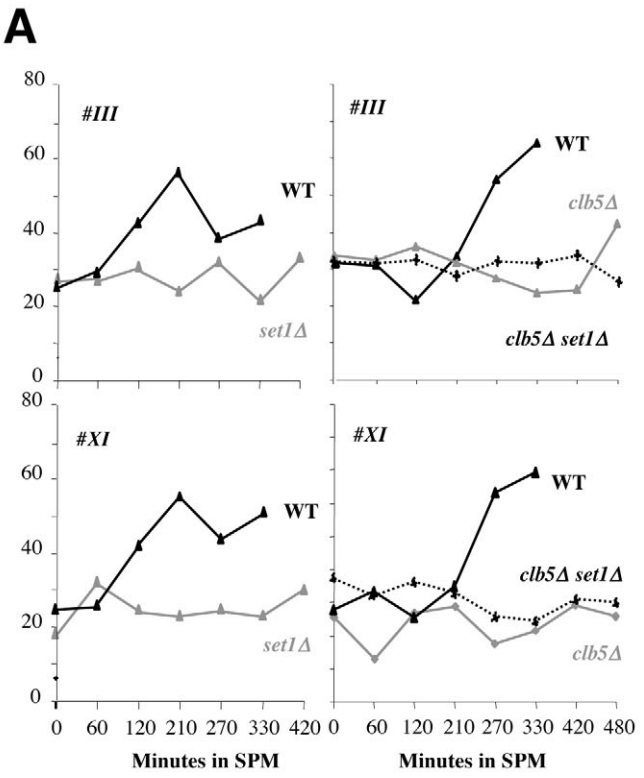
required in different aspects of nuclear reorganization during meiotic prophase. The *clb5Δ set1Δ* double mutant behaves much like *set1Δ* cells with a delayed and limited redistribution of centromeres, a severe restriction of vegetative telomere cluster dissolution and a total lack of bouquet formation (Fig. 5). Thus, the *set1Δ* defect is upstream of that of *clb5Δ* with respect to centromere dispersion and bouquet formation. However, as previously shown (Sollier et al., 2004), this epistasis relationship does not apply to premeiotic S phase, which never occurs in the case of *clb5Δ set1Δ* (see 1200 minutes on Fig. 5).

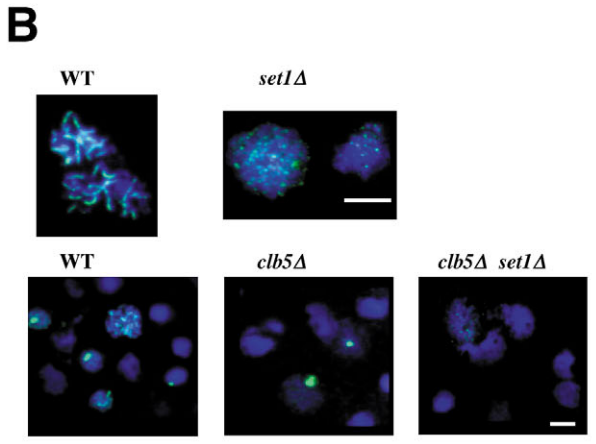
Chromosome pairing is defective in *set1Δ* meiotic cells

Since meiotic centromere and telomere dynamics were strongly affected in *set1Δ* meiocytes, we asked whether the pairing of homologues was also defective. To this end, nuclei were analyzed by FISH with two differentially labeled cosmid probes specific for chromosome *III* and *XI* (Trelles-Sticken et al., 2000). The fraction of paired FISH signals was measured on nuclei prepared from cells used for the meiotic time course

experiments presented on Fig. 4 (Fig. 6A, left diagrams). In wild-type meiocytes, the raise of the pairing values for the two probes was similar, increasing from 120 minutes to reach a maximum at 210 minutes. No significant increase of the pairing value occurred along the *set1Δ* time course. The same FISH analysis was performed with wild-type, *clb5Δ* and *clb5Δ set1Δ* nucleoids from independent time courses (Fig. 6A, right diagrams). It was found that the maximum pairing values for *clb5Δ* and *clb5Δ set1Δ* stayed well below that of the wild type.

Next, nuclei were analyzed through immunostaining of the Zip1 transverse filament protein (Sym et al., 1993). In the wild type, meiocyte nuclei were found to display Zip1 thread-like immunofluorescence patterns, indicative of a full synapsis (Fig. 6B). In *clb5Δ* meiocyte nuclei Zip1 was found accumulated in one dot, whereas *set1Δ* and *clb5Δ set1Δ* nuclei displayed numerous Zip1 speckles (occasionally with one small dot, as in the case of *clb5Δ set1Δ*). Dots correspond to aggregates of Zip1 and have previously been detected in *clb5Δ* meiocytes (Smith et al., 2001) as well as in many meiotic mutants that cannot form SCs (Sym and Roeder, 1995). The fraction of *clb5Δ* nuclei with Zip1 speckles increased over time whereas fewer Zip1-positive nuclei were detected in *set1Δ* and *clb5Δ set1Δ* (not shown). Taking in account the pairing data above, the ZIP1 staining results indicate that homologous synapsis is largely defective in the absence of Clb5 or Set1.





MEC1 inactivation alters telomere dynamics in *set1Δ* meiosis

We wondered whether the exacerbated defect in nuclear dynamics seen in *set1Δ* was the consequence of the activation of a checkpoint mechanism. As a *MEC1*-dependent DNA replication checkpoint operating during meiosis has been described (Stuart and Wittenberg, 1998), we tested the effect of the deletion of *SET1* in combination with the *mec1-1* mutation. While occurring normally in *mec1-1*, no premeiotic DNA synthesis was detected in *set1Δ mec1-1* (Fig. 7), consistent with previous data (Sollier et al., 2004). Meiotic divisions were nearly absent in *set1Δ mec1-1* meiosis, whereas the first meiotic division occurred in the majority of wild-type cells at 420 minutes (not shown). As in *set1Δ* meiosis (Fig. 4), centromere cluster resolution was very limited in *set1Δ mec1-1* meiosis (Fig. 7).

With respect to telomere dynamics it appeared that the decrease of vegetative telomere patterns in *set1Δ mec1-1* nuclei paralleled that of the wild type (Fig. 7), in contrast to *set1Δ* or *clb5Δ set1Δ* meiosis (Figs 4, 5). The frequency of bouquet-stage nuclei remained low in *set1Δ mec1-1* meiocytes (Fig. 7). The limited increase (~5%) seen at 480 minutes could reflect a grossly delayed bouquet formation. Altogether, these results show that Mec1 inactivation leads to a specific alleviation of the block of vegetative telomere cluster dissolution in *set1Δ* nuclei. This suggests that Set1 deficiency leads to activation of a Mec1-dependent checkpoint that restricts the departure from vegetative telomere organization. Nevertheless, the release of telomeres from premeiotic clusters in *set1Δ mec1-1* is not accompanied by a concomittant increase in bouquet formation, suggesting that an additional Set1-dependent activity is required to cluster meiotic telomeres.

Fig. 6. Analysis of synapsis and pairing in *set1Δ*, *clb5Δ* and *set1Δ clb5Δ* cells. (A) Frequencies of nuclei with paired cosmid signals during meiotic time courses; wild-type and *set1Δ* strains (left graphs), or the wild-type, *clb5Δ* and *set1Δ clb5Δ* strains (right graphs). Top graphs: frequencies of nuclei displaying only a single signal due to pairing of a cosmid probe corresponding to a telomeric region of the chromosome *III* left arm (cos m; 37). Bottom graphs: frequencies of nuclei displaying paired signals with a cosmid probe hybridizing an internal region of the chromosome *XI* left arm (cos f; 37). (B) Representative images of Zip1-positive nuclei at 330 minutes (top) or 270 minutes (bottom) after induction of meiosis. WT, wild-type nuclei displaying thread-like Zip1 signals (green); *set1Δ* nuclei displaying Zip1 speckles only. In *set1Δ clb5Δ* this is only the case of a few nuclei in the culture, whereas in *clb5Δ* a few nuclei display a Zip1 polycomplex (bright green oblong). Bar, 5 μm.

Telomeric proteins Rap1 and Ndj1 locate to meiotic *set1Δ* telomeres

The *set1Δ* mutation that resulted in an absence of bouquet formation (this study), was associated with an abnormal telomere structure, apparent notably through the loss of silencing (Corda et al., 1999; Nislow et al., 1997). As the meiosis-specific telomeric protein Ndj1 is required for bouquet formation (Trelles-Sticken et al., 2000), immunolocalization of Ndj1 was performed on wild-type and *set1Δ* diploid cells that expressed a HA-tagged version of Ndj1. This was done in the MDY strain background (Conrad et al., 1997), which displays wild-type nuclear divisions kinetics as well as *set1Δ*-associated defects (delay of premeiotic S phase and of prophase I progression, mild sporulation defect) similar to that observed in SK1 (not shown). The appearance of Ndj1-HA immunofluorescence foci was significantly delayed in *set1Δ* nuclei (Fig. 8A). Since Ndj1 is required for bouquet formation, this could explain the absence of bouquet formation associated with the loss of Set1. However, even the *set1Δ* Ndj1-positive nuclei, which are expected to be able to cluster their telomeres, showed no sign of bouquet formation (Fig. 8 and not shown). To assess that Ndj1 localization is not affected in *set1Δ*, we colabeled the XY′ telomere repeats using FISH in combination with Ndj1 immunostaining (Fig. 8B). The colocalization or overlap of Ndj1 foci with XY′ FISH signals in wild-type nuclei (Trelles-Sticken et al., 2000) was also observed in *set1Δ* meiocytes.

The Rap1 protein binds to telomeric sequences and is involved in meiotic telomere structure and function (Kanoh and Ishikawa, 2003). Genetic evidence suggests a role for telomeric Rap1 in *S. cerevisiae* meiosis (Alexander and Zakian, 2003), and telomeric localization of Rap1 is necessary for the telomere bouquet in *S. pombe* meiosis (Chikashige and Hiraoka, 2001). Co-staining experiments of Ndj1-HA and Rap1 (Fig. 8C) revealed that these proteins colocalize and, except for some rare Rap1-free Ndj1 spots, no discernible difference was found between wild-type and *set1Δ* nuclei. This suggests that properly localized telomere proteins require Set1 function to bring about meiotic telomere clustering.

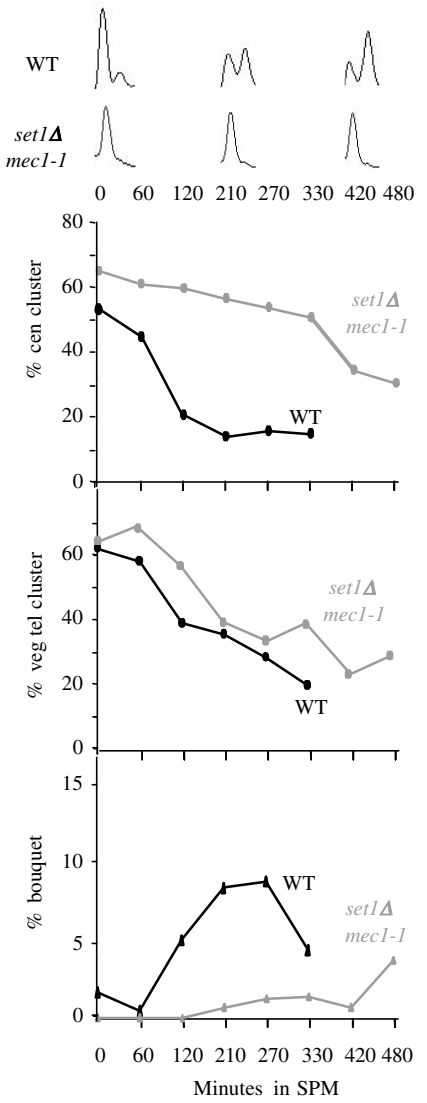


Fig. 7. Role of Mec1 in restricting chromosome reorganization in *set1Δ* cells. Frequencies of nuclei displaying a single centromere FISH signal (% cen cluster), two to eight perinuclear telomere FISH signals (% tel cluster) or a single telomere FISH signal cluster (% bouquet) in wild-type and *set1Δ mec1-1* strains, at the indicated time points after transfer into sporulation medium. Top: wild-type and *set1Δ mec1-1* FACS profiles aligned to the corresponding time points.

Discussion

Centromere dispersion is independent from premeiotic S phase

Our results show that centromere dispersion and premeiotic S phase represent two independent events of the meiotic prophase. This is first suggested when one compares the kinetics of centromere cluster resolution and completion of premeiotic S phase in wild-type time courses. Centromere dispersion was found to occur ahead of premeiotic S phase in some time courses (see Figs 1, 7). The *clb5Δ* mutation confirms the independence between the two events. In this mutant, the dissolution of the centromere cluster occurs with normal kinetics, in absence of premeiotic S phase. The precedence of centromere dispersion is also apparent in *ime2Δ* meiosis. Altogether, these data suggest the existence of independent triggers for the centromere cluster resolution and the premeiotic S phase.

Telomere dispersion is independent from centromere dispersion

The behavior of *clb5Δ* and *ime2Δ* nuclei, with normal centromere dispersion in the absence of, or limited, vegetative telomere resolution, fits with the view that the two events are independently controlled. As the two mutations impact on premeiotic S phase, the dispersion of vegetative telomeres could be temporally linked to premeiotic S phase. This goes with the fact that in *ime2Δ* the timing of premeiotic S phase and vegetative telomere dispersion is intermediate between those of wild type and *clb5Δ*. Moreover, the persistence of vegetative-like telomere clusters in live cells arrested in premeiotic S phase by hydroxyurea suggests that telomere dispersion occurs at the end of, or shortly after, premeiotic S

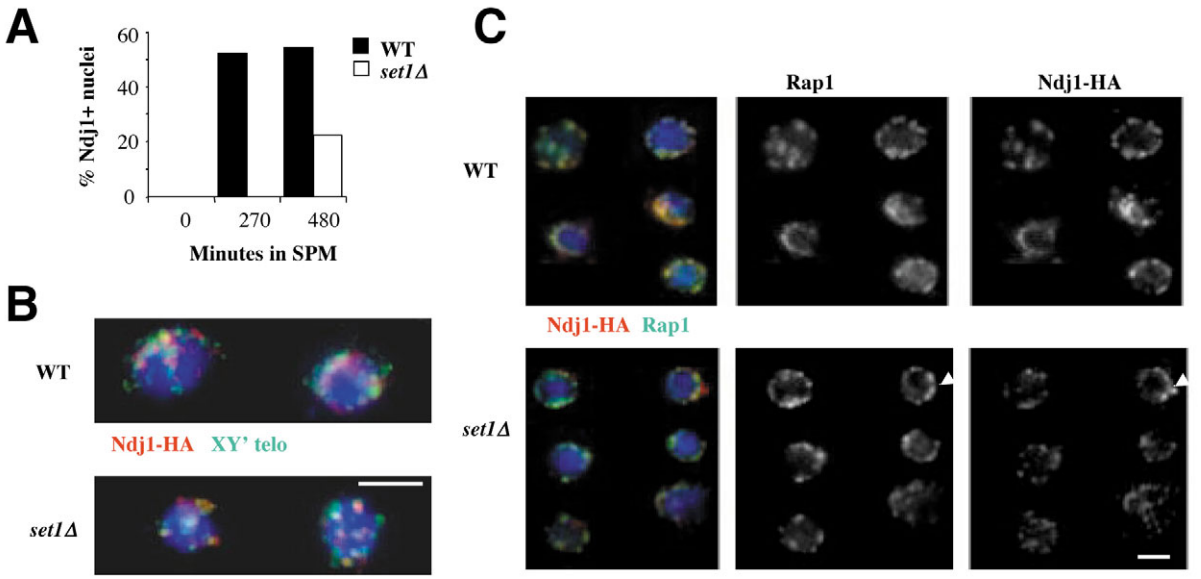


Fig. 8. Localization of Ndj1 and Rap1 is normal in *set1Δ* meiotic nuclei. (A) Frequency of nuclei from wild-type and *set1Δ* diploid (HA)-tagged Ndj1 strains displaying Ndj1-HA signal at the indicated time point in sporulation medium. (B) Colocalization of Ndj1-HA immunofluorescence signals (red) and XY′ telo-FISH signals (green) in representative wild-type and *set1Δ* nuclei. Bar, 5 μm. (C) Immunolocalization of Rap1 (middle panels) and Ndj1-HA (right panels) in mildly spread wild-type (top panels) and *set1Δ* (bottom panels) nuclei. Single immunofluorescence channels are shown in gray scale for better sensitivity. Left panels: merged color images of immunofluorescence (green: Rap1; red: Ndj1-HA). A Ndj1-HA-positive spot with no detectable Rap1 signal is indicated (white arrowhead). Bar, 5 μm.

phase (Trelles-Sticken et al., 2005). This reinforces the idea that premeiotic S phase, or some associated event, is required for telomere dispersion.

Meiotic telomere cluster formation and resolution require Clb5 function

The other important consequence of *CLB5* ablation was a delayed appearance of a single and seemingly persistent meiotic telomere cluster. Assuming that this clustering of telomeres corresponds to a genuine bouquet, its detection in unreplicated *clb5Δ* meiocytes suggests that meiotic telomere clustering can occur independently of premeiotic S phase. This clustering took place at a time when the fraction of *clb5Δ* meiocytes displaying telomere dispersion is limited. This may suggest that a bouquet can form without prior dispersion of telomeres, through the peripheral sliding of vegetative telomere clusters. However, the follow-up of telomere movements in live cells suggest that, at least in wild-type nuclei, telomeres normally disperse before they cluster in the bouquet (Trelles-Sticken et al., 2005). Since there are never 100% of cells with a vegetative telomere distribution, another possibility is that the bouquet-like topology can arise from the fraction of cells with no apparent vegetative telomere clustering.

The persistence of bouquet nuclei in *clb5Δ* could signify that the resolution of meiotic telomere clustering is defective in *clb5Δ*. A similar persistence was observed in *spo11Δ* and *rad50S* meiotic time courses (Trelles-Sticken et al., 1999) and in *spo11 Sordaria* mutants (Storlazzi et al., 2003), suggesting that its dissolution requires normal recombination processes. As *clb5Δ* is also defective in recombination initiation (Smith et al., 2001), this fits with the view that the bouquet is normally released after the progression or completion of recombination (Scherthan, 2003; Storlazzi et al., 2003). Alternatively, since the bouquet is present in unreplicated *clb5Δ* meiocytes and cohesin has been found to be required for the exit from meiotic telomere clustering (Trelles-Sticken et al., 2005), the accumulation of bouquet nuclei could be due to the lack of appropriately organized cohesin cores along unreplicated chromosomes.

Severe impairment of nuclear dynamics in absence of Set1

The limited delay of premeiotic S phase (Fig. 4) (Sollier et al., 2004) and the good sporulation rate of *set1Δ* cells, suggests that the meiotic program is less affected by the absence of Set1 than by the absence of Clb5. As compared to *clb5Δ*, nuclear dynamics are more perturbed in *set1Δ* meiosis which displays limited centromere dispersion, no clear evidence for vegetative telomere cluster dissolution and absence of bouquet formation. The differences between the two mutants have been summarized in Fig. 9. First, centromere dispersion is limited, as was observed in all strains where the *SET1* gene was deleted, whether singly (*set1Δ*) or in combination with other mutations (*set1Δ clb5Δ*, *set1Δ mec1-1*). A delayed centromere dispersion in *sir3Δ* meiosis that is also defective in vegetative telomere metabolism (Trelles-Sticken et al., 2003) has been correlated to the altered expression of genes involved in vegetative centromere clustering, and a similar mechanism may occur in the absence of Set1.

The other important difference between *set1Δ* and *clb5Δ* meiosis concerns the establishment of a bouquet, which was never detected in *set1Δ*, with an epistatic relationship of *set1Δ* over *clb5Δ*. As telomeres form a bouquet at the spindle pole body where centromeres are aggregated during vegetative

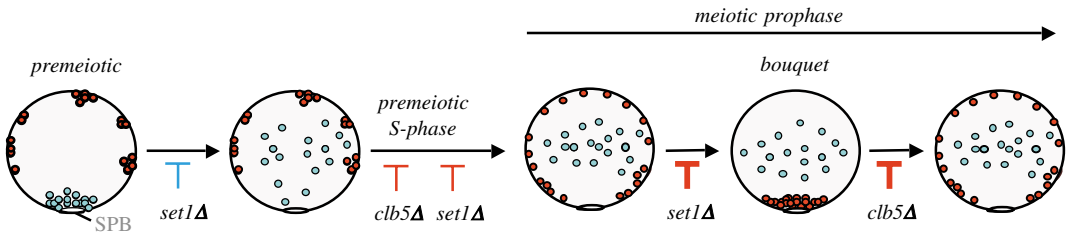


Fig. 9. Scheme showing the sequential steps of centromere and telomere redistribution that occur during transit from vegetative nuclear architecture into meiotic prophase. It is based on observations made in this and earlier work (see text). In the premeiotic nucleus, telomeres (red) form several perinuclear clusters and all centromeres (blue) locate tightly near the spindle pole body (SPB). Induction of meiosis leads to the dissolution of the centromere cluster and perinuclear telomere clusters. The dispersion of centromeres and telomeres differ relative to their dependence on premeiotic S phase. Telomeres cluster transiently near the SPB (bouquet stage) before scattering again over the periphery of the nucleus. The steps where centromere and telomere behavior are affected by the loss of Clb5 and Set1 are indicated by blue and red T-bars, respectively. The absence of Clb5 affects only the movements of telomeres with a severe impairment of vegetative telomere cluster dissolution, a delayed occurrence of meiotic telomere clustering and a persistence of meiotic telomere clustering. The lack of Set1, on the other hand, induces defects in centromere and telomere dispersion from their respective vegetative clusters and blocks meiotic telomere cluster formation. Accordingly, the thin T-bars represent a leaky effect which can be bypassed by some cells to reach the next stage of nuclear topology, whereas the bold T-bars represent a block that induces a cell to remain with a topological pattern of the previous stage, while prophase I may still be progressing. Note that the relative duration of the transitions is not drawn to scale.

growth (Jin et al., 1998; Trelles-Sticken et al., 1999), disruption of the centromere cluster could be limiting for bouquet formation. However, this is not consistent with the lack of bouquet nuclei at the later time points in *set1Δ* meiosis, where a substantial fraction of meiocytes exhibit dispersed centromeres. That centromere dispersion is not limiting is furthermore suggested by the observation that telomeres and centromeres can co-cluster in a subset of meiocytes (Trelles-Sticken et al., 1999). The meiotic clustering of telomeres occurs in *clb5Δ*, although the vegetative telomere dispersion defect is similar to that in *clb5Δ set1Δ* (Fig. 5B). Thus, the lack of bouquet in *set1Δ* does not appear to result from the limitation of telomere dispersion. Indeed, bouquet formation is largely defective in *set1Δ mec1-1*, despite the dissolution of vegetative telomere clusters.

Although one cannot exclude that meiotic telomere clustering could occur in *set1Δ* at later times than examined, this would be with a delay largely exceeding that of the premeiotic S phase and normal prophase I (Fig. 4). Moreover, the clustering of telomeres in unreplicated *clb5Δ* nuclei suggests that the delay of premeiotic S phase is not responsible for absence of bouquet formation in *set1Δ* meiosis. Similarly, the impairment of DSB formation in *set1Δ* (Sollier et al., 2004) is probably not involved, because a bouquet is formed in the *spo11Δ* mutant in the absence of meiotic double-strand breaks (Trelles-Sticken et al., 1999; Storlazzi et al., 2003). Delayed induction of meiotic middle gene expression (Sollier et al., 2004) as the cause of bouquet failure seems also unlikely, because meiotic telomere clustering usually precedes the completion of recombination, i.e. before the expression of middle genes. With respect to only a mild defect in sporulation, the *set1Δ* mutant is similar to the *ndj1Δ* bouquet mutant (Chua and Roeder, 1997; Conrad et al., 1997). However, a delayed appearance of Ndj1 at meiotic *set1Δ* telomeres seems insufficient to explain the lack of bouquet formation, since telomere clustering was absent in *set1Δ* meiocytes that efficiently express well-localized telomeric Rap1 and Ndj1 proteins. Whatever the molecular mechanism whereby Set1 controls meiotic telomere clustering, it could be related to telomeric heterochromatin, as suggested in *S. pombe* meiosis, in which telomere clustering at the SPB requires the methylation of histone H3 on lysine 9 (Tuzon et al., 2004). In *S. cerevisiae*, where there is no H3 lysine 9 methylation, silent heterochromatic DNA at telomeres requires the Sir proteins and a decreased binding of Sir3 at telomeres was observed in *set1Δ* (Santos-Rosa et al., 2004). However, this can certainly not be the cause of the bouquet failure, as Sir3 is dispensable for meiotic telomere clustering (Trelles-Sticken et al., 2003). This leaves the possibility that Set1-mediated H3-K4 methylation plays a yet unrecognized role in regulating meiotic telomere clustering in budding yeast.

Evidence for a checkpoint controlling telomere dispersion in *set1Δ*

Among the various defects elicited by the loss of Set1, only the dissolution of vegetative telomere clusters is alleviated by the *mec1-1* mutation. This provides an additional corroboration for the independent control of vegetative centromere and telomere cluster dissolution. The temporal relationship between telomere dispersion and premeiotic S phase may signify a functional coupling between the two processes via a checkpoint possibly related to the Mec1-dependent S-M checkpoint that inhibits meiotic M phase in the absence of fully replicated DNA (Stuart and Wittenberg, 1998).

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