S. pombe meiotic linear elements contain proteins related to synaptonemal complex components

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Accepted 8 March 2004
Journal of Cell Science 117, 3343-3351 Published by The Company of Biologists 2004
doi:10.1242/jcs.01203

Summary

The fission yeast *Schizosaccharomyces pombe* does not form synaptonemal complexes (SCs) in meiotic prophase nuclei. Instead, thin threads, the so-called linear elements (LEs), are observed at the corresponding stages by electron microscopy. Here, we demonstrate that *S. pombe* Rec10 is a protein related to the *Saccharomyces cerevisiae* SC protein Red1 and that it localizes to LEs. Moreover, a homologue to *S. cerevisiae* Hop1 does exist in *S. pombe* and we show by in situ immunostaining that it, and the kinase Mek1 (a homologue of which is also known to be associated with SCs), localizes to LEs. These observations indicate the evolutionary relationship of LEs with the lateral elements of SCs and suggest that these structures might exert similar functions in *S. cerevisiae* and *S. pombe.*

Key words: Chromosome pairing, Meiosis, Recombination, Yeast

Introduction

Cells of sexually reproducing eukaryotes normally contain two equal (homologous) sets of chromosomes, one contributed by the father and the other by the mother during the fusion of gametes and the formation of a zygote. Meiosis is the cell division that reduces the number of chromosomes by half. It produces gametes or their precursor cells, each of which contains a haploid set consisting of randomly assorted parental chromosomes. These chromosomes are mosaics, because the original parental homologues have exchanged corresponding pieces by crossing-over. Thus, the function of meiosis is twofold – it compensates for the doubling of the chromosome number at fertilization and it provides the progeny with newly assorted sets of alleles, which is the basis of their genetic diversity.

Crossing-over is initiated at multiple sites in recombining chromosomes by the enzymatic induction of double-strand breaks (DSBs). The resection of single strands at DSBs leads to the formation of gaps in the DNA. The missing bases are replenished by using the complementary sequence from the homologous chromosome as the template. This process is recombinogenic; that is, it can lead to the reciprocal exchange of DNA between the chromosomes involved (for review, see Keeney, 2001).

In order to allow crossing-over, homologous parental chromosomes must pair during meiotic prophase via a ladder-like proteinaceous structure, the synaptonemal complex (SC) (for reviews, see Loidl, 1990; Zickler and Kleckner, 1999). The SC consists of two parallel axes (the lateral elements), to each of which the two chromatin threads of a single replicated chromosome are attached. The lateral elements are connected and kept at a distance of ~100 nm by the so-called transversal filaments. The ultrastructure of the SC is evolutionarily well conserved from protists to humans, although its molecular composition is far more heterogeneous.

The fission yeast *Schizosaccharomyces pombe* features a meiosis that is unique in several respects. Most remarkably, it lacks an SC. Instead, so-called linear elements (LEs) appear during meiotic prophase (Olson et al., 1978; Bähler et al., 1993). LEs appear in the electron microscope (EM) as single lines of variable length, networks of interconnected lines or bundles of lines. These different morphological classes were found to prevail at different stages of meiotic prophase (Bähler et al., 1993), which suggests that their change in appearance is functionally related to chromosome pairing and/or recombination.

Because a *rec10* mutant lacks LEs, a structural or regulatory role of the Rec10 protein in LE formation has been proposed (Molnar et al., 2003). Apart from this indirect evidence, information on the molecular composition of LEs is scarce. Neither topoisomerase II nor Rec8 [which, in other organisms, constitute the cores along which lateral elements form (Klein et al., 1992; Klein et al., 1999)] delineate entire LEs (Hartsuiker et al., 1998; Parisi et al., 1999; Watanabe and Nurse, 1999), and *S. pombe* homologues of proteins present in SCs have not been described (Parisi et al., 1999; Davis and Smith, 2001). Therefore, the evolutionary relationship of LEs to SCs has remained unclear. Here, we have identified several molecular components of LEs and localized them in situ by immunocytochemistry. We demonstrate the similar molecular composition of the lateral elements of SCs and LEs, and we discuss possible functions of LEs.

Materials and Methods

Antibody production and testing

Amino acids 32-45 and 670-684 were selected from the translated

Table 1. Strain list

Name	Genotype	Source/reference
1-20	*h⁻ ade6-M210*	J. Kohli*
1-25	*h⁺ ade6-M216*	J. Kohli*
ALP3	*h⁺/h⁻ ade6-M216/ade6-M210*	1-20 × 1-25 / this paper
S1241	*h⁺ ade6-M216 leu1-32 mek1Δ::kanMX6*	Pérez-Hidalgo et al., 2003
S1242	*h⁻ ade6-M210 leu1-32 mek1Δ::kanMX6*	Pérez-Hidalgo et al., 2003
ALP22	*h⁺/h⁻ de6-M216/ade6-M210 mek1Δ::kanMX6/mek1Δ::kanMX6*	This paper
S1294	*h⁻/h⁻ pat1-114/pat1-114 ade6-M216/ade6-M210 leu1-32/leu1-32 mek1⁺::3HA-kanMX6/mek1⁺::3HA-kanMX6*	Pérez-Hidalgo et al., 2003
ED10	*h⁺/h⁻ ade6-M216/ade6-M210 ura4-D18/ura4-D18 rec8Δ::ura4⁺/rec8Δ::ura4⁺*	Eveline Doll*
BP841	*h⁺ ade6-M216 rec10-109*	This paper
BP843	*h⁻ ade6-M210 rec10-109*	This paper
ALP41	*h⁺/h⁻ ade6-M210/ade6-M216 rec10-109/rec10-109*	BP841 × BP843 / this paper
ED3	*h⁺/h⁻ rec10-155::LEU2/rec10-155::LEU2 leu1-32/leu1-32 ade6-M216/ade6-M210*	Molnar et al., 2003
ED4	*h⁺/h⁻ rec11-156::LEU2/rec11-156::LEU2 ade6-M210/ade6-M216 leu1-32/leu1-32*	Molnar et al., 2003
ED5	*h⁺/h⁻ rec12-152::LEU2/rec12-152::LEU2 leu1-32/leu1-32 ade6-M216/ade6-M210*	Molnar et al., 2003

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sequence of *rec10* (SPAC25G10.04c) as epitopes for the elicitation of antibodies in rabbits. Peptide synthesis and antibody production were performed by Eurogentec (Herstal, Belgium). Individual rabbits were immunized with a single peptide each. Antibodies were affinity purified on EAH-Sepharose columns to >85% purity and diluted in PBS containing 0.01% NaN_3 and 1% bovine serum albumin (BSA). The antibody against the C-terminus (amino acids 670-684) was used for immunostaining.

Amino acids 482-496 and 514-528 were selected from the translated sequence of SPBC1718.02 (Hop1) as epitopes for the elicitation of antibodies in guinea pigs. Peptide synthesis and antibody production were performed by Eurogentec. Two individuals were each injected with both peptides. The serum was purified by adsorption to acetone powder of sporulating *hop1*Δ cells (Harlow and Lane, 1988) (we will be publishing the details of construction of a *hop1*Δ strain in a future paper).

Strains, growth and sporulation

The strains used are listed in Table 1. Cells were maintained as prototrophic diploids on yeast extract agar (YEA) plates through the use of interallelic complementing auxotrophic markers *ade6-M210* and *ade6-M216* (Moreno et al., 1991). Sporulating cells for microscopic examination were prepared as follows (Molnar et al., 2003). Single colonies were transferred to yeast extract liquid (YEL) and cultivated overnight. (Liquid cultures were always kept shaking at 30°C.) This culture was used to inoculate *S. pombe* minimal medium (PM), and cells were grown to a density of 1×10^7-2×10^7 cells per ml. They were then pelleted by centrifugation and transferred to PM without NH_4Cl (PM-N) at a density of 1×10^7 cells per ml for sporulation. The strain carrying haemagglutinin (HA)-tagged Mek1 was *pat1-114*. Sporulation in this strain was induced according to a published procedure (Cervantes et al., 2000).

11 ml samples were taken at hourly intervals, of which 10 ml were used for the production of immunostained microscopic preparations and 1 ml to check meiotic progression by DAPI (4′,6-diamidino-2-phenylindole) staining (Molnar et al., 2003).

Microscopic preparation

Aliquots from sporulating cultures were centrifuged and the cells were resuspended in an enzyme solution for spheroplasting (Bähler et al., 1993; Molnar et al., 2003). 20 μl spheroplasted cells were put on a microscope slide and mixed with 40 μl fixative (4% paraformaldehyde, 3.4% sucrose) and 80 μl detergent (‘Lipsol’; LIP, Shipley, UK), which causes the cells and nuclei to swell and to expose the nuclear contents. The spreading procedure was stopped after ~30 seconds by the addition of 80 μl fixative. The slides were then dried in air and were kept in the refrigerator until use.

For electron microscope (EM) inspection, slides were stained with $AgNO_3$ and the material was transferred to EM grids as described previously (Bähler et al., 1993).

Immunostaining and detection

Slides were washed three times for 15 minutes each in 1× PBS containing 0.05% Triton X-100. After shaking off excess liquid, primary antibody was applied under a coverslip and the slides were put in the refrigerator overnight. Concentrations were 1:2000 for rabbit anti-Rec10 antibody, 1:50 for Guinea pig anti-*S. pombe*-Hop1 antibody and 1:200 for mouse anti-HA antibody. Primary antibodies were applied alone or in appropriate combinations. After incubation, the coverslip was removed and washing steps were performed as above. Incubation in appropriate secondary fluorescence-tagged (FITC, Cy3, Alexa) antibodies was for 4 hours at room temperature. After another round of washing as above, slides were mounted in antifade solution (Vectashield, Vector Labs, Burlingame, CA) supplemented with 1 μg ml^{-1} DAPI for the staining of DNA.

Immunofluoresence was detected with a Zeiss Axioskop epifluorescence microscope equipped with single-band-pass filters for the excitation of blue, green and red fluorescence. Pictures were taken with a cooled CCD camera (Photometrics, Tucson, AZ). Black-and-white images were assigned false colours and merged with the help of IPLab Spectrum software (Scanalytics, Fairfax, VA).

Results

Rec10 shows architectural and partial sequence similarity to S*accharomyces cerevisiae* Red1

S. pombe Rec10 was identified as a putative homologue of *Saccharomyces cerevisiae* Red1 from a sequence architecture-based database search. *S. cerevisiae* Red1 is an 827 amino acid protein with one clear sequence homologue found in the non-redundant database (nr) from NCBI, namely the 854 amino acid *Kluyveromyces lactis* Red1 (Smith and Roeder, 2000). Both proteins show similar sequence architecture: a lysine-rich region predicted to contain nuclear targeting signals is located around position 500-700 (residues 568-686 and 518-693) [determined by CAST (Promponas et al., 2000) and PROSITE (Sigrist et al., 2002)]. A highly helical region is found at the very C-terminus (the last ~90 residues) and contains a predicted coiled coil (in *S. cerevisiae*, amino acids 765-795)

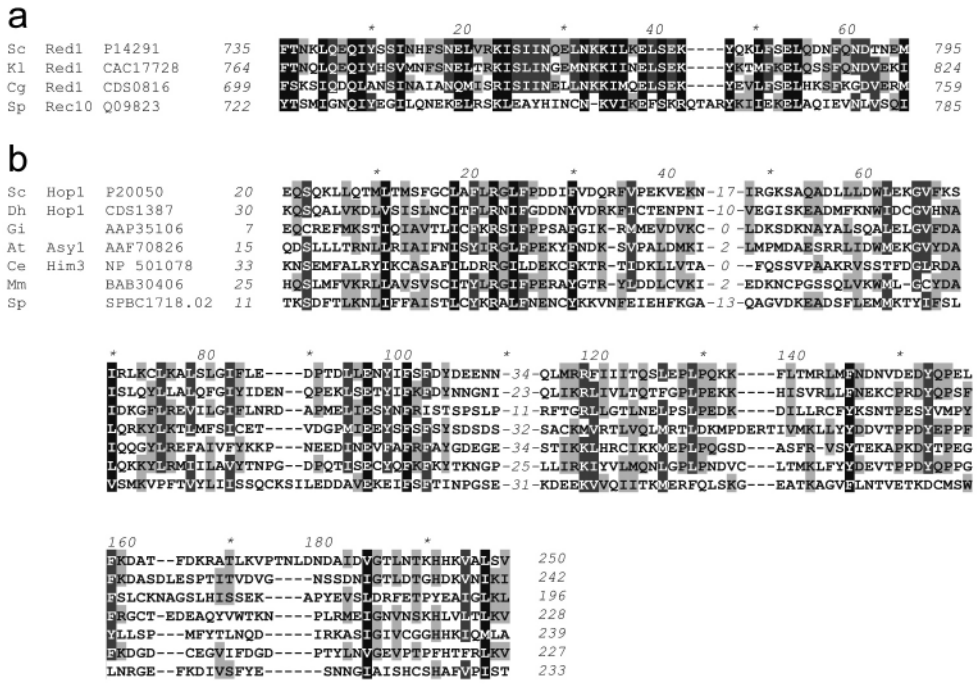

Fig. 1. (a) Multiple sequence alignment of the homologous C-termini of Red1-related proteins in *S. cerevisiae* (Sc), *Kluyveromyces lactis* (Kl), *Candida glabrata* (Cg) and *S. pombe* (Sp). Sequences are indicated with the species, followed by a database accession number [databases used are Genpept (Sc, Kl, Sp) and Génolevures (Cg) (Feldmann, 2000)]. Sequences are labelled with a four-level conservation shading using similarity groups. (b) Multiple sequence alignment of the HORMA domain (Aravind and Koonin, 1998) from Hop1-related proteins of Sc, *Debaryomyces hansenii* (Dh), *Giardia intestinalis* (Gi), *Arabidopsis thaliana* (At), *Caenorhabditis elegans* (Ce), *Mus musculus* (Mm) and Sp. Sequences are indicated with the species, followed by a database accession number [databases used are Génolevures (Dh) (Feldmann, 2000), Pompep (Sp) and Genpept (all remaining sequences)]. Sequences are labelled with a four-level conservation shading using similarity groups.

(Lupas et al., 1991). Functionally, the C-terminal 291 amino acids including the helical region are thought to mediate Red1 homo-oligomerization (Hollingsworth and Ponte, 1997; Woltering et al., 2000).

S. pombe is an ascomycete only distantly related to *S. cerevisiae*, so that the sequentially poorly conserved Red1 (*K. lactis* and *S. cerevisiae* Red1 proteins show just 26% identity over their entire length) is not found in *S. pombe* using full-length sequence similarity searches. The C-terminal 90 amino acids of the known Red1 proteins are better conserved (62%) and thus a potentially superior bait in sequence-based searches. The region from amino acid 764 to amino acid 834 in *K. lactis* (matching *S. cerevisiae* Red1 amino acids 735-805 with 66% identity) when used in WU-Blast against the *S. pombe* proteome (http://www.genedb.org/) obtains Rec10 as second best hit (E=0.017) and by far the top hit of a sequence of the expected length (791 amino acids) where the match is found at the C-terminal amino acids 722-785. [The first-listed hit in this search (SPAC7D4.14c, E=0.0052, has a similar range) is rejected as potential candidate because it is much shorter (551 residues) and the region of similarity is N-terminal (residues 41-90) in contrast to the required C-terminal location.] Shared sequence architecture was a criterion for selecting Rec10 as the potential *S. pombe* Red1 homologue (Fig. 1a). Rec10, much like Red1 proteins, contains a coiled-coil region (amino acids 760-782) and a K-rich region located in the second half of the sequence (amino acids 433-523 found with CAST) (Promponas et al., 2000), which hits a bipartite nuclear targeting sequence pattern in PROSITE (Sigrist et al., 2002).

Although a similar function has been suggested for the two animal protein families represented by vertebrate SCP3/COR1 (Heyting et al., 1987) and *Drosophila* c(2)M (DS02750.10) (Manheim and McKim, 2003), and for *S. cerevisiae* Red1 (Rockmill and Roeder, 1988), these are not conserved at the primary sequence level.

SPBC1718.02 is the likely *HOP1* orthologue

S. pombe SPBC1718.02 shows a meiotic S-phase expression peak (Mata et al., 2002). A sequence-architecture-based search similar to that applied for Rec10/Red1 identified it as the likely orthologue of budding yeast *HOP1*. Hop1, like Red1, is associated with the axial and lateral elements of the SC (Muniyappa et al., 2000). Over a length of 605 amino acids, Hop1 contains an N-terminal HORMA domain [amino acids 21-245, with a significant (E=4×10^{-27}) match in the Conserved Domain Database (CDD) (Aravind and Koonin, 1998)], and a central zinc finger motif, which is essential for its function and shows a nonsignificant hit against the PHD finger in Aravind's signalling database [amino acids 332-375, E=0.094, IMPALA – (Hollingsworth et al., 1990; Schaffer et al., 1999)]. Simple full-length WU-Blast of Hop1 against the *S. pombe* proteome (http://www.genedb.org/) obtains the 528 amino acids SPBC1718.02 as a nonsignificant hit (E=0.62) with three collinear regions of significant sequence similarity: one in the HORMA domain (amino acids 21-130), one in the PHD finger and one thereafter. Again, analogous sequence architecture is used to substantiate distant sequence similarity found between Hop1 and SPBC1718.02. SPBC1718.02, as indicated in the genedb annotation, is likely to contain an N-terminal HORMA domain (FFAS03: scop/d1goA score=–9.3) and a central PHD domain (Aravind's signalling database, E=5×10^{-5}). An N-terminal HORMA domain is typical of the Hop1 homologous group of proteins identified so far in *S. cerevisiae* (Hollingsworth et al., 1990), *Arabidopsis* (Asy1) (Caryl et al., 2000) and *Caenorhabditis elegans* (HIM-3) (Zetka et al., 1999) (Fig. 1b).

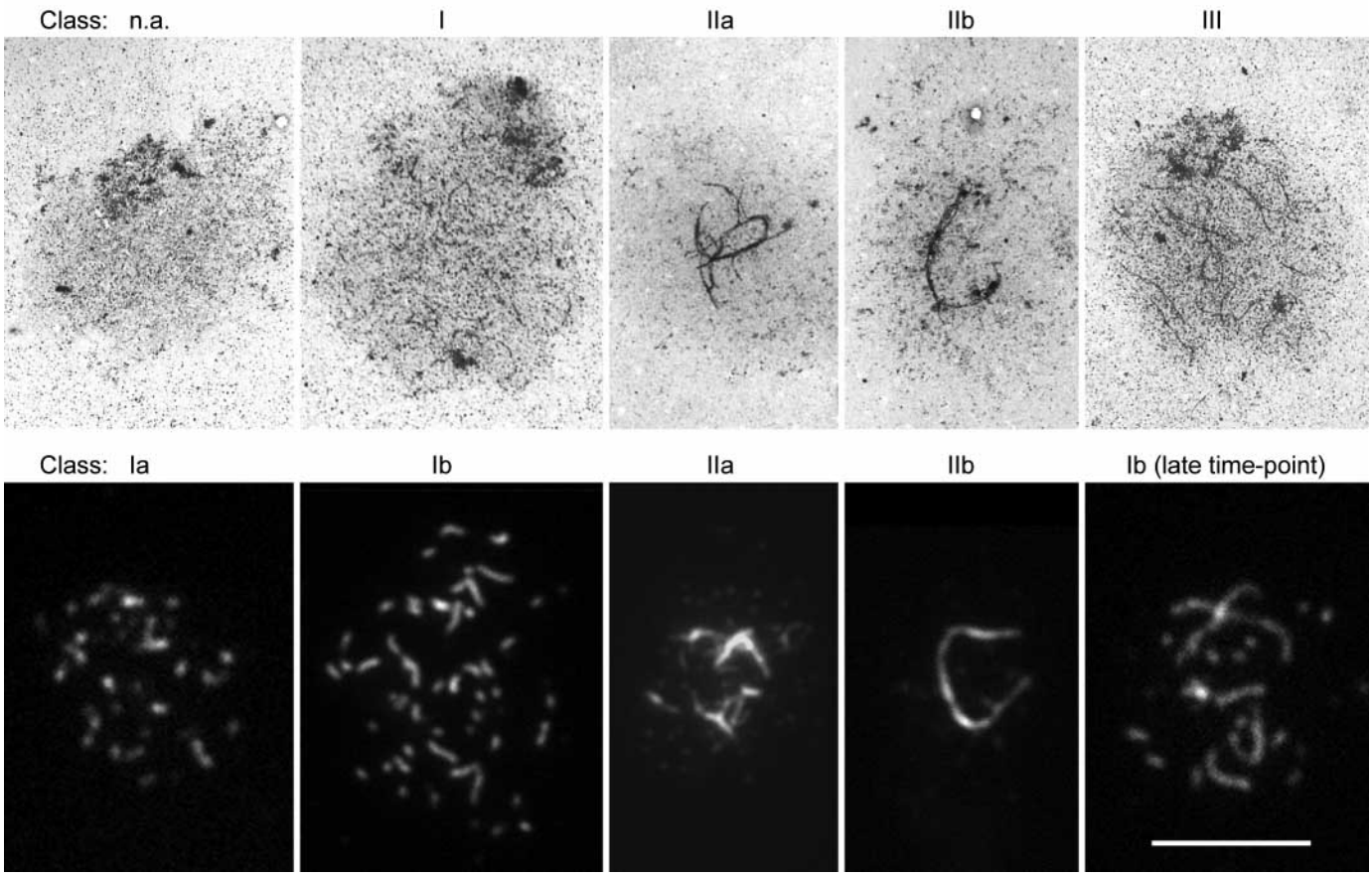

Fig. 2. LEs as observed after silver staining in the electron microscope (EM) (top) and by immunostaining of Rec10 (bottom). The classification of LEs in the EM is according to Bähler et al. (Bähler et al., 1993). Nuclei containing many (up to >50) Rec10-positive spots (designated here as class Ia) appear inconspicuous in the EM. The empty nucleus in the EM (top left, class assignment not applicable) might correspond by its shape and size to immunostained class Ia. Classes I (Ib) and IIa are identified by both immunostaining and EM as containing individual threads and meshes of threads, respectively. Class IIb appears in the EM as bundles of LEs that probably correspond to the long, thick Rec10-positive structures. Silver-stained individual LEs of heterogeneous lengths, which prevail during later stages of meiosis (7-10 hours), were designated as class III (Bähler et al., 1993). The corresponding Rec10-LEs are classified as Ib. A class-Ib nucleus from a late time point in meiosis (8 hours in sporulation medium) is shown for comparison. Scale bar, 5 μm.

Rec10 is a component of LEs and similar classes of LE morphology are observed by EM and Rec10 immunostaining

Immunostaining with Rec10 antiserum highlighted dot-shaped and linear structures in spread wild-type meiotic nuclei (Fig. 2), whereas there was no immunostaining with preimmune serum (not shown). The Rec10-positive structures correspond well with the classes of LEs that have been defined by their appearance in the EM (Bähler et al., 1993) (Fig. 2). Over a meiotic time course, there is a change in the proportion of the different Rec10 LE classes (Fig. 3). The earliest Rec10-positive structures, observed at 3 hours in sporulation medium, were dots or very short lines. Up to 58 Rec10-positive spots were present in nuclei that we denote as class Ia (63 nuclei from three different experiments were evaluated). They have no parallels in the EM. Possibly at this stage of development, the structures are not yet sufficiently elaborated to be discriminated from the high background of Ag-positive grains. Class Ia was present at ~3-10% of nuclei in all time points. Class Ib features individual LEs of various lengths. There are fewer lines than in class Ia (a maximum of 29 counted in 55 nuclei from three different experiments), which suggests that they originate by the fusion of class Ia LEs. Their frequency showed a peak at 5 hours in sporulation medium, followed by a decline and an accumulation towards 10 hours. Class IIa nuclei contain a network of connected LEs. Class IIa was the most prevalent at 5-7 hours. Class IIb appears in the EM as a dense bundle of LEs and it can be assumed that it corresponds to the thick bar seen after Rec10 immunostaining. They constitute a minor portion of nuclei at all time points. Bähler et al. (Bähler et al., 1993) had introduced a class III consisting of long single LEs appearing at late times during meiosis. Here, we did not make this distinction and assigned all single LEs to class Ib because we cannot decide whether long individual LEs observed at late times originate by the steady growth of Ia and Ib LEs or whether they are a degradation product of class II LEs. Likewise, for a class Ia nucleus at a late time, it cannot be determined whether it is the end point of a structural transformation or if it has not developed beyond the initial state. However, the fact that class Ib nuclei come in two peaks and that class IIa is most abundant in the valley between the peaks, suggests that class IIa derives from early class I and

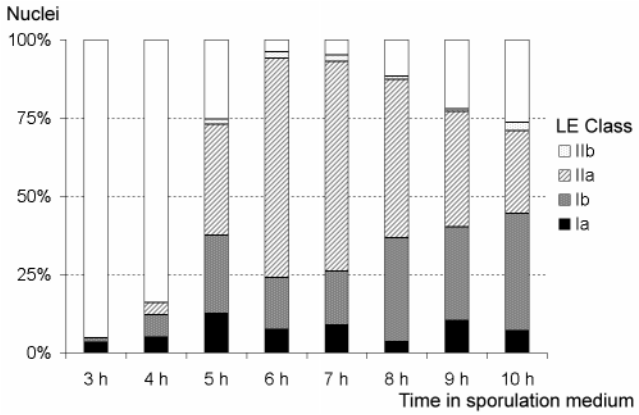

Fig. 3. Proportions of different LE classes in nuclei of the wild type at different times in sporulation medium. A typical time course is shown. For the classification of LEs, see Fig. 2. 200 nuclei were evaluated for each time point.

transforms to late class I. Neither can it be determined whether the rare class IIb represents a step in the development of all nuclei going through meiosis or if only a subset of nuclei adopt this appearance. In the former case, it must be of very short duration.

Morphology of Rec10 structures in meiotic mutants

To confirm the identity of immunostained structures and LEs as seen by EM and to determine the conditions for LE formation, we studied Rec10 localization in various mutants. In the *rec10-155* mutant, in which no LEs had been detected by EM (Molnar et al., 2003), the corresponding structures were also completely missing after immunostaining (Fig. 4b), whereas, in another mutant (*rec10-109*), up to ten Rec10-positive dots or short lines were observed (Fig. 4c) and similar rudimentary structures were also seen by EM (not shown). This is a considerably reduced LE formation compared with the wild type.

Because there is now evidence from a range of organisms that meiotic cohesins underlie the lateral elements (Klein et al., 1999; Pasierbek et al., 2001; Prieto et al., 2001), it would be interesting to know whether LE formation also depends on a cohesin scaffold. Molnar et al. (Molnar et al., 2003) reported that strains that lack meiotic cohesin components develop aberrant LEs (Molnar et al., 1995). Here, we found that the rudimentary LEs formed in a *rec8*Δ strain contain Rec10 (Fig. 4d). In spite of their superficial similarity to class IIb LEs (which are likely to develop from class I LEs), the origin of these structures must be different, because the normal precursor stages (class I) were not detected. It remains to be tested whether these aberrant LEs are at all associated with chromatin or represent aggregates of LE components that are not properly localized in the absence of chromosomal cores to which they would normally attach. Because Rec8 partners with Rec11 in arm-associated meiotic cohesin (Kitajima et al., 2003), a *rec11* mutant showed structures very similar to those in *rec8*Δ both in the EM (Molnar et al., 2003) and after Rec10 immunostaining (not shown), as expected.

In the recombination-deficient *rec12-152* mutant [*rec12* encodes the homologue of the DSB-inducing protein Spo11 (Lin and Smith, 1994)], Molnar et al. (Molnar et al., 2003) detected LEs but the frequencies of the different classes deviated from those found in the wild type, with long single threads being the most frequent phenotype. Here, we confirm that these LEs are normal in the sense of being endowed with Rec10 (Fig. 4e) and thus that LE formation is independent of the initiation of DSB formation.

We next tested the effect of the loss of Mek1 on LE formation. Mek1 was discovered in *S. cerevisiae* as a meiosis-specific protein kinase required for chromosome synapsis (Rockmill and Roeder, 1991). When the *S. pombe mek1* homologue is deleted, spore viability and recombination are reduced (Pérez-Hidalgo et al., 2003). Rec10 LEs appeared morphologically wild-type in the *mek1*Δ strain (Fig. 4f) but class IIa LEs were under-represented compared with the wild type. Whereas, in the wild type, up to ~45% of meiotic nuclei (n=619 nuclei after 7 hours in sporulation medium) were of this class, at most 3.2% of *mek1*Δ nuclei (n=400 nuclei after 6 hours in sporulation medium) contained class IIa LEs.

S. pombe Hop1 and Mek1 localize to LEs

To determine the cellular localization of Hop1, we produced antibodies against the protein for immunostaining in spread meiotic nuclei. Hop1 staining produced dotted and linear

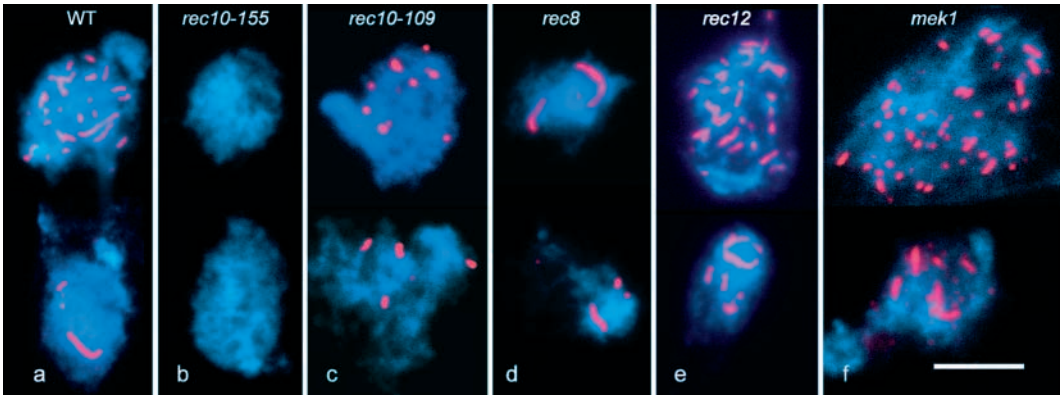

Fig. 4. Examples of Rec10-positive LEs (red) in (a) the wild type (WT) and various mutants. Two typical nuclei are shown for each strain. (b) In the *rec10-155* mutant, LEs are completely missing, whereas, in the *rec10-109* mutant (c), LEs develop into a few dots or short lines. (d) In a *rec8*Δ strain, Rec10 is organized into thick threads resembling class IIb LEs. (e) In the *rec12-152* mutant strain, all classes of LEs occur (a class IIa and a class IIb nucleus are shown). (f) In a *mek1*Δ strain, Rec10 assembles into wild-type-like LEs, whereby classes Ia and Ib are more abundant than the others. Chromatin is stained blue with DAPI. Scale bar, 5 μm.

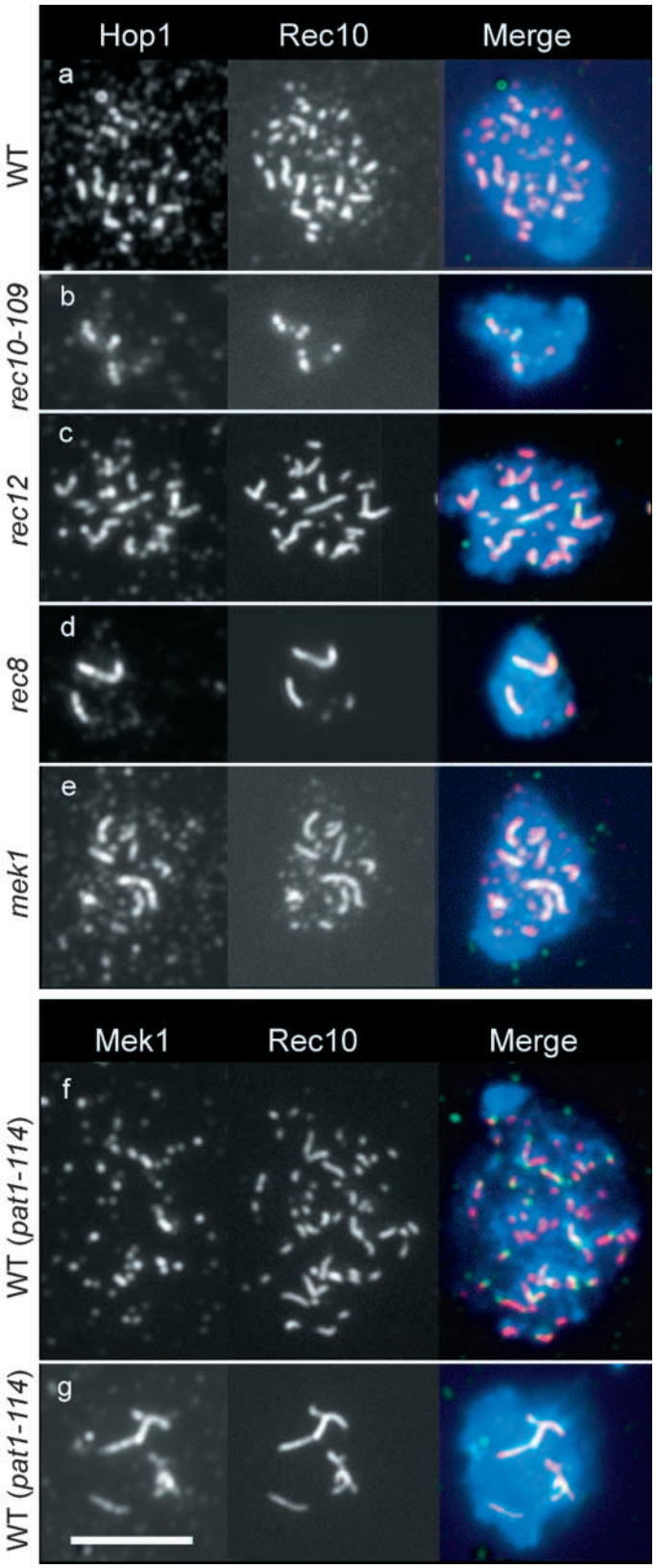

Fig. 5. Hop1 and Mek1 localization to Rec10 LEs shown by immunostaining of Rec10, Hop1 and Mek1-HA. Regions where Hop1 or Mek1 (green) colocalize with Rec10 (red) appear orange in the merge image. Chromatin is stained blue with DAPI. The images show Hop1 localization to: (a) class Ib LEs of the wild type (WT); (b) residual LEs in the *rec10-109* mutant; (c) LEs in a *rec12-152* mutant strain; (d) aberrant LEs in the *rec8*Δ mutant; and (e) LEs in a *mek1*Δ strain. Some short LEs are lacking Hop1 and Hop1 localization to longer LEs is discontinuous. Mek1 forms dots on short LEs in class I nuclei (f) but covers most parts of LE bundles in (rare) class IIb nuclei (g). Scale bar, 5 μm.

patterns similar to Rec10 LEs (Fig. 5a). Hop1 immunostaining of a *hop1* mutant (A. Lorenz et al., unpublished) was negative (not shown), which confirms that the antibody is specific. Double immunostaining revealed that, in class I nuclei, often only a subset of Rec10-positive dots is highlighted with Hop1 antibody. Of the longer class II LEs, virtually all were Hop1 positive, although Hop1 localization was somewhat discontinuous (Fig. 5a-e). The presence of class I LEs without Hop1 suggests that Hop1 is loaded onto LEs somewhat later than Rec10. In nuclei of the *rec10-155* mutant strain, which lacks LEs, no Hop1 threads were detected. However, Hop1 was present in the fragmentary LEs of the *rec10-109* mutant (Fig. 5b), supporting the notion that Hop1 associates with a pre-existing Rec10-containing scaffold.

In the *rec12-152* mutant, Hop1 delineated LEs (Fig. 5c), suggesting that wild-type LEs are independent of the formation of meiotic DSBs and, in the *rec8*Δ strain, Hop1 localized to the aberrant LEs (Fig. 5d), confirming that their molecular composition resembles that of the wild type.

Because the *S. cerevisiae* protein kinase Mek1 has been found to localize to SCs (Bailis and Roeder, 1998) and it is known that its *S. pombe* homologue is present in meiotic nuclei (Pérez-Hidalgo et al., 2003), we wanted to determine the spatial relationship of *S. pombe* Mek1 with LEs in spread nuclei. To do this, we used a strain that expresses HA-tagged Mek1 (Pérez-Hidalgo et al., 2003). In nuclei with short LEs, Mek1-HA staining highlighted many spots that localized to Rec10 LEs, whereas linear signals were rarely observed. In class II nuclei, Mek1 delineated stretches along LE bundles (Fig. 5f,g).

It had been suggested that, in *S. cerevisiae*, the interaction between Red1 and Hop1 is facilitated by Mek1-dependent phosphorylation of Red1 (De los Santos and Hollingsworth, 1999), and so we tested whether *S. pombe* Mek1 has an influence on the localization of Hop1 to LEs. In a *mek1* deletion strain, we did not observe notably reduced Hop1 immunostaining of LEs (Fig. 5e). This is in accordance with recent evidence from budding yeast that Red1 is not a Mek1 substrate (Wan et al., 2004).

Discussion

S. pombe linear elements and the axial/lateral elements of synaptonemal complexes contain similar proteins

In the budding yeast, in which the molecular composition of SCs is known best, Red1 was shown to be a basic component of SCs. However, Red1 might not be the primary building block of axial/lateral elements, because Red1 staining is discontinuous along pachytene bivalents (Smith and Roeder, 1997) and, in its absence, fragmentary axial and lateral elements are still formed (Rockmill and Roeder, 1990). It is

likely that it attaches to a pre-existing core of topoisomerase II and cohesin (Klein et al., 1992; Klein et al., 1999). The localization of Hop1, in turn, depends on Red1 (Smith and Roeder, 1997). In the absence of Hop1, extensive stretches of axial elements can be formed (Loidl et al., 1994) but both Red1 and Hop1 are required for the full development of axial elements. These are then linked by the transversal filaments consisting of Zip1 and become the lateral elements of the SC, but Hop1 dissociates from chromosomes at or before pachytene, as chromosomes synapse. The serine/threonine protein kinase Mek1 and the protein phosphatase type 1 Glc7 are also loaded onto axial elements (Bailis and Roeder, 2000; Wan et al., 2004).

Here, we show that, in *S. pombe*, immunostaining of meiotic nuclei with Rec10 antiserum highlighted structures that closely resemble those that have been visualized after silver staining in the EM (Bähler et al., 1993). Moreover, mutants with reduced or abnormal LE formation as seen by EM (Molnar et al., 2003) showed identical deficiencies of Rec10-stained structures. This is evidence that Rec10-positive structures correspond to LEs.

Our observation that *S. pombe* LEs contain Rec10 (which has some similarity to *S. cerevisiae* Red1) and homologues of *S. cerevisiae* Hop1 and Mek1 suggests that LEs are equivalents (and probably evolutionary relics) of the lateral elements of SCs. Unlike lateral elements, however, LEs do not seem to extend along the entire length of chromosomes. Moreover, it is possible that, at some time in their development, LEs become detached from the chromosomes. This is suggested by the observation that DAPI-stained chromatin occupies a much larger area than the dense LE bundle in class IIb nuclei.

The existence or nature of any underlying axial core structure to which Rec10 and Hop1 are attached is not entirely clear. Although Rec8 is part of the core structure in *S. cerevisiae* (Klein et al., 1999) to which the structural components of lateral elements are attached, Rec8 was not observed to be organized as linear structures in *S. pombe* meiotic nuclei by either green-fluorescent-protein tagging or immunostaining (Parisi et al., 1999; Watanabe and Nurse, 1999), as would be expected of a chromosomal axial scaffold. However, in the absence of Rec8, Rec10 and Hop1 form only aberrant structures resembling class IIb-like LE bundles. It is therefore possible that, in the *rec8*Δ mutant, LEs are not associated with chromosomes and resemble SC polycomplexes (Goldstein, 1987) in that they are self-organizing aggregates of unused LE components. Alternatively, the rudimentary LEs in the mutant could contain Rad21/Scc1, Rec8's mitotic counterpart, which also seems to be expressed at a low degree in meiosis (Yokobayashi et al., 2003). It therefore remains to be determined whether, in *S. pombe*, Rec8 underlies the LE structure.

Rec10 promotes homologous pairing and crossing-over

Homologous pairing was found to be reduced in the absence of Rec10 but interstitial regions of chromosomes are more severely affected than loci near the centromeres and telomeres (Molnar et al., 2003). It was proposed by Molnar et al. (Molnar et al., 2003) that the meeting of homologous centromeres and telomeres might benefit from the rigorous centromere-telomere polarization (bouquet formation) of meiotic chromosomes (Chikashige et al., 1994) and rely to a lesser extent on LEs (Scherthan et al., 1994).

In addition to pairing, Rec10 has also been implicated in recombination because genetic assays demonstrated a variable reduction in recombination for several marker pairs in the *rec10-109* mutant (De Veaux and Smith, 1994; Krawchuk et al., 1999). Here, we have found that this mutant possesses a few short LE fragments (Fig. 4b), which might account for the residual recombination detected. However, in the *rec10-155* mutant with no LEs, recombination in the single interval tested was dramatically reduced (~500 times less than the wild type) (Lin and Smith, 1995; Krawchuk et al., 1999). More recently, it was found that *rec10*Δ mutants have strongly reduced meiotic recombination in multiple intervals and lack detectable meiotic DSBs (C. Ellermeier and G. R. Smith, pers. commun.). By contrast, in the *rec8* mutant, in which only aberrant LEs (which are possibly not associated with chromosomes) are formed, recombination is less affected near chromosome ends. Therefore, it is possible that the requirement for LEs in recombination is site specific (Parisi et al., 1999).

Although LE formation is independent of DSBs (Molnar et al., 2003) and both Rec10 and Hop1 delineate wild-type LEs in a DSB-deficient *rec12* mutant (this paper), there is a similar number of incipient LEs (>50 Rec10-positive spots in class Ia nuclei) and of estimated crossovers [~45 (Munz, 1994)] per meiosis. Also, the estimated 50-150 DSB sites (Cervantes et al., 2000) per meiosis would roughly correspond to early short LEs. This invites the speculation that DSBs and the initiation of crossing-over might preferentially take place in regions where LEs start to load onto chromatin.

Possible functions for LEs

The SC has been attributed a range of functions, including strengthening the connection between homologues and mediating crossover interference (for reviews, see Loidl, 1994; Roeder, 1997; Zickler and Kleckner, 1999). Axial elements mediate the attachment of chromatin strands to transversal filaments by which the former become intimately paired. (The axial elements are referred to as lateral elements in the mature SC.) Apart from this obvious role in building the SC, axial elements have a more intricate function in conferring a loop structure on the chromatin strands and thereby providing the structural basis for the mutual exposure of homologous DNA tracts for recombination (Zickler and Kleckner, 1999; Blat et al., 2002). Moreover, in *S. cerevisiae*, the axial element components Red1, Hop1 and Mek1 direct crossing-over towards homologous non-sister chromatids (perhaps in part by actively preventing sister-chromatid recombination) (Kleckner, 1996; Roeder, 1997; Thompson and Stahl, 1999; Wan et al., 2004).

S. pombe LEs, unlike the lateral elements of canonical SCs, do not seem to be connected by transversal filaments. Therefore, the role of LEs in the stabilization of chromosome pairing is questionable. In accordance with the presynaptic structural role of axial elements, the function of LEs in *S. pombe* could be the organization of chromatin loops presenting homologous DNA regions for homology recognition and recombination and/or the prevention of recombination between sisters. For this purpose, they need not be organized into canonical tripartite SCs or extend all along the chromosomes.

If LEs have this function then one would expect them to be present at the sites of crossing-over. If, by contrast, the function of LEs was in the sensing of DSBs and the activation of a putative pachytene checkpoint (see below) then it would probably be sufficient if they were formed around only a random subset of recombination sites, because these would be representative of the status of the recombination process in the nucleus.

Recently, it was reported that DSB accumulation and other defects can cause delays in meiotic progression in fission yeast. Shimada et al. (Shimada et al., 2002) and Pérez-Hidalgo et al. (Pérez-Hidalgo et al., 2003) proposed the involvement of the fission yeast Mek1 homologue in a meiotic recombination checkpoint in *S. pombe*. Although its existence has been disputed by others (Catlett and Forsburg, 2003), the present identification of homologues of Red1 and Hop1 [which were implicated in triggering the pachytene checkpoint in budding yeast (Bailis and Roeder, 2000)], at least suggests that a putative checkpoint might use similar chromosomal components in the two yeasts.

We are grateful to J. Bähler, E. Doll, J. Kohli and P. San-Segundo for strains and plasmids. We thank the staff of the Department of Ultrastructure Research (Institute of Botany, University of Vienna) for technical support. Our special gratitude is extended to J. Kohli for critical reading of the manuscript and to G. Smith for the communication of unpublished results. This work was supported by project P16282 of the Austrian Science Fund (FWF).

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