A selfish DNA element engages a meiosis-specific motor and telomeres for germ-line propagation

Soumitra Sau,1 Michael N. Conrad,2 Chih-Ying Lee,2 David B. Kaback,3 Michael E. Dresser,2 and Makkuni Jayaram1

1Department of Molecular Biosciences, University of Texas at Austin, Austin, TX 78712
2Program in Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104
3Department of Microbiology and Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ 07101

The chromosome-like mitotic stability of the yeast 2 micron plasmid is conferred by the plasmid proteins Rep1-Rep2 and the cis-acting locus STB, likely by promoting plasmid-chromosome association and segregation by hitchhiking. Our analysis reveals that stable plasmid segregation during meiosis requires the bouquet proteins Ndj1 and Csm4. Plasmid relocalization from the nuclear interior in mitotic cells to the periphery at or proximal to telomeres rises from early meiosis to pachytene. Analogous to chromosomes, the plasmid undergoes Csm4- and Ndj1-dependent rapid prophase movements with speeds comparable to those of telomeres. Lack of Ndj1 partially disrupts plasmid–telomere association without affecting plasmid colocalization with the telomere-binding protein Rap1. The plasmid appears to engage a meiosis-specific motor that orchestrates telomere-led chromosome movements for its telomere-associated segregation during meiosis I. This hitherto uncharacterized mode of germ-line transmission by a selfish genetic element signifies a mechanistic variation within the shared theme of chromosome-coupled plasmid segregation during mitosis and meiosis.

Introduction

Meiosis is the process by which diploid nuclei undergo two distinct divisions, meiosis I and II, to form four haploid nuclei. During prophase of meiosis I, replicated homologues pair, undergo recombination, and form chiasmata, which physically link them to promote their biorientation on the metaphase spindle and subsequent anaphase separation. Meiosis II is a mitotic-like division in which sister chromatid segregation completes the production of four haploid gametes (Petronczki et al., 2003).

Meiosis in the ascomycete Saccharomyces cerevisiae displays the general features of meiosis in other eukaryotes, and culminates in the production of four haploid ascospores. Prophase I in S. cerevisiae can be divided into leptotene, zygotene, pachytene, and diplotene-like substages, as defined by the state of chromosome pairing and condensation. The sequential events that characterize these stages include clustering of perinuclear telomeres (TELS) near the spindle pole body (SPB) to shape chromosomes into a bouquet, introduction of double strand DNA breaks, assembly and maturation of synaptonemal complexes (SCs), and formation, progression, and resolution of recombination intermediates (Baker et al., 1976; Trelles-Sticken et al., 1999; Zickler and Kleckner, 1999). After dissolution of the array of cohesin complex that bridges sister chromatids from chromosome arms, but without disassembly of cohesin from centromeric regions, monopolar spindle attachment of sister chromatids and their cosegregation are ensured by the maintenance of sister kinetochore cohesion through the collaborative action of the monopolin complex, Spo13, Sgo1, and the Ipl1 kinase (Klein et al., 1999; Tóth et al., 2000; Rabitsch et al., 2003; Katis et al., 2004; Lee et al., 2004; Monje-Casas et al., 2007; Yu and Koshland, 2007). Haploidization is completed by segregation of sister centromeres (CENs) during meiosis II.

An important dynamic feature of meiosis I is the manifestation of rapid prophase movements (RPMs) of chromosomes, driven presumably by cytoskeletal actin via nuclear envelope proteins (Conrad et al., 2008; Koszul et al., 2008; Wanat et al., 2008). The anchoring of TELs to the envelope and bouquet formation, their attachment to the “nuclear envelope motor,” and the transduction of mechanical energy from the cytoplasm to the nucleus are promoted by the Mps3-Ndj1-Csm4 (MNC) complex...
The accompanying TEL-led movements result in the pairing of homologous chromosomes and formation of SCs by Zip1 polymerization along their axial elements, assisted by the synapsis initiating complex (SIC; Sym et al., 1993; Tsubouchi et al., 2008; Lee et al., 2012). Being the integral SC component that cross-links paired axial elements, Zip1 serves as a marker for synapsis, and its localization pattern is a measure of the extent of SC formation. In the absence of a functional MNC complex, chromosome dynamics are impaired, subsequent events of meiosis become delayed or disrupted, and the fidelity of chromosome segregation is compromised (Chua and Roeder, 1997; Conrad et al., 1997, 2007, 2008; Koszul et al., 2008; Wanat et al., 2008; Sonntag Brown et al., 2011).

We describe the segregation behavior of the multicopy 2 micron plasmid of S. cerevisiae during meiosis as a paradigm for the germ-line propagation of selfish extrachromosomal genomes. The 40–60 plasmid copies per haploid cell, and approximately twice as many per diploid cell, reside in the nucleus as mini-chromatin assemblies (Velmurugan et al., 2003; Jayaram et al., 2004b; Ghosh et al., 2006). The nearly chromosome-like stability of the plasmid is conferred by a partitioning system consisting of the plasmid proteins Rep1 and Rep2 and the partitioning locus STB (Jayaram et al., 2004a). The Rep–STB system ensures equal or nearly equal plasmid segregation by overcoming a diffusion barrier that causes replicated plasmid molecules to be trapped disproportionately in the mother (Murray and Szostak, 1983; Shcheprova et al., 2008; Gehlen et al., 2011; Khmelinskii et al., 2011). The plasmid also houses an amplification system, which rectifies copy number declines resulting from rare missegregation events. A termination-free mode of replication, analogous to rolling circle replication and central to amplification, is thought to be triggered by the inversion of one of the bidirectional forks as a result of a recombination event mediated by the site-specific recombinase Flp (Futcher, 1986; Volkert and Broach, 1986).

The native 2 micron plasmid or a multicopy STB reporter plasmid probed by FISH or by operator–fluorescent repressor interaction, respectively, is revealed in mitotic cells as a relatively small number of foci (Velmurugan et al., 2000; Heun et al., 2001). The segregation features of the STB reporter plasmid are consistent with each focus, which likely comprises a group of plasmid molecules, being an independent unit of segregation (Liu et al., 2013). Current evidence suggests that the plasmid segregates by physically associating with chromosomes and hitchhiking on them. Furthermore, plasmid sisters formed by the replication of a single-copy 2 micron derivative segregate as if they were tethered to sister chromatids (Ghosh et al., 2007; Liu et al., 2013).

Several chromosome segregation factors are found associated with both CENs and STB: the RSC2 chromatin remodeling complex, the Kip1 nuclear motor, the histone H3 variant Cse4 (CENP-A), and the cohesin complex (Mehta et al., 2002; Hajra et al., 2006; Ghosh et al., 2007, 2010; Cui et al., 2009; Huang et al., 2011a; Ma et al., 2013). Pairing of plasmid sisters by cohesin (Ghosh et al., 2007, 2010) would be consistent with their attachment to sister chromatids. However, the highly substoichiometric association of cohesin and Cse4 with STB (Ghosh et al., 2010; Huang et al., 2011b) raises concerns regarding their functional relevance, unless they act in a catalytic manner. Circumstantial evidence suggests that the atypical point CEN of budding yeasts and the STB locus might share an ancestor that once directed both chromosome and plasmid segregation (Malik and Henikoff, 2009; Huang et al., 2011a; Jayaram et al., 2013). The present day associations of CEN binding factors at STB may be relics of that shared evolutionary history.

The 2 micron plasmid is propagated efficiently during meiosis as well (Brewer and Fangman, 1980; Hsiao and Carbon, 1981). The presence of double the haploid plasmid content in a diploid cell suggests that, during meiosis, the plasmid undergoes a reductional event that parallels chromosome haploidization. It is not known whether there are common meiosis-specific host factors that interact with CEN and STB. When the monopolar complex is inappropriately expressed in mitotically dividing cells, it associates with CEN but not with STB (Liu et al., 2013). In light of the potential ancestral relatedness between STB and CEN, and between chromosome and 2 micron plasmid segregation pathways, it is possible that a subset of the proteins that regulate CEN function and behavior during meiosis may play analogous roles at STB.

Intrigued by how plasmid segregation is modulated during meiosis, we characterized the localization and dynamics of a fluorescence-tagged STB reporter plasmid during meiotic prophase, followed its segregation during meiosis I, and analyzed its distribution into spores at the end of meiosis II. Our findings are consistent with the potential association of the 2 micron plasmid with TELs by way of the envelope motor responsible for driving chromosome movements that presage homologue pairing. 2 micron plasmid segregation as a TEL appendage during meiosis I would signify hitchhiking on chromosomes as the underlying logic that unifies faithful plasmid propagation during both vegetative and germ-line divisions of the host cells.

Results
 Segregation of a multicopy STB reporter plasmid during meiosis

To characterize plasmid partitioning during meiosis, we used a fluorescence-tagged multicopy STB reporter plasmid (Mehta et al., 2002; Cui et al., 2009), with an autonomously replicating sequence (ARS) plasmid lacking STB as a control (see Materials and methods).

During meiosis I, the STB plasmid segregated equally (n:n) or almost equally (n:n ~ 1) to the two daughter nuclei ~76% of the time (Fig. 1, A and C). Unequal segregation (n:n’) was seen in ~23% of meiosis I divisions. Total plasmid missegregation (n:0) was quite low (~1%). In contrast, the values for the control ARS plasmid were ~29% equal and ~57% unequal segregation along with ~14% total missegregation events (Fig. 1, B and C). After meiosis II, ascus with all four plasmid-containing spores were ~68% and ~22% for the STB and ARS plasmids, respectively (4:0; Fig. S1, A and B). In the subset of tetrads with no less than four plasmid foci per tetrad, this difference was ~84% (STB plasmid) to ~48% (ARS plasmid; Fig. S1 C).
Furthermore, the type I subgroup of the 4:0 class, equal in plasmid foci number in all four spores or in pairs of spores (but not between pairs), was also larger for the \( STB \) plasmid (\(~35\% \text{ vs. } \sim16\%\); Fig. S1, D and E).

The distinct equal segregation frequencies for the \( STB \) and \( ARS \) plasmids during meiosis I, which are similar to those during mitosis (Velmurugan et al., 2000; Cui et al., 2009), suggest that meiosis I plasmid segregation is driven by the Rep–\( STB \) system. Because sister spores are not ordered in the ascus, the assessment of equal segregation during meiosis II is not straightforward. Nevertheless, the difference between the \( STB \) and \( ARS \) plasmids in the representation of type I tetrads (Fig. S1 E; \( P < 0.05 \)) suggests that the 2 micron plasmid partitioning system is active during meiosis II as well.

**Localization of \( STB \) plasmids in meiotic chromosome spreads**

To examine whether \( STB \) plasmids are associated with chromosomes, as expected from the hitchhiking model, we screened surface spread nuclei (chromosome spreads) from cells at the early (leptotene/zygotene) and late (pachytene) stages of meiosis I. The \( STB \) plasmid was found in all chromosome spreads from \([\text{cir}+]\) cells, whereas roughly half the spreads from \([\text{cir}0]\) cells (lacking the Rep proteins) contained no detectable plasmid (Fig. 2 A). As there was higher plasmid loss in the \([\text{cir}0]\) strain compared with the \([\text{cir}+]\) strain during the mitotic divisions preceding meiosis, the fraction of plasmid-containing cells in the spread assays was smaller for the \([\text{cir}0]\) strain. The data corrected for this difference (Fig. 2 A) suggest potential tethering of \( STB \) plasmids to chromosomes in a Rep1-Rep2–dependent manner. There is a caveat that the spreads may include, in addition to chromosomes, nuclear membrane fragments and nuclear matrix–associated proteins.

Next, we mapped plasmid foci in pachytene spreads (containing better resolved chromosomes) from \([\text{cir}+]\) (Fig. 2 B) and \([\text{cir}0]\) (Fig. 2 C) strains with respect to DAPI using the criteria described in the Materials and methods section. A considerable fraction of the \( STB \) plasmid foci (\(~52\%)\) was associated with chromosomes, <0.4 \( \mu \text{m} \) away, in the \([\text{cir}+]\) host (Fig. 2, B and D). Within this subpopulation, \(~73\%)\ was at chromosome tips, which suggests preferential plasmid association with \( TELs \) (Fig. 2 E). Plasmid–chromosome association required Rep1 and Rep2 proteins, as indicated by the \([\text{cir}0]\) strain (Fig. 2, C and D). The results were similar when Zip1, which marks the axis of paired homologues (Sym et al., 1993), served as the chromosome reference (unpublished data).

**Figure 1. Plasmid segregation during meiosis I.** (A and B) The segregation of fluorescence-tagged \( STB \) and \( ARS \) (lacking a partitioning system) reporter plasmids (Mehta et al., 2002; Cui et al., 2009) was scored in

\[ \text{cir}+ \text{ diploid cells by counting plasmid foci in each daughter nucleus at the end of meiosis I. Bars, 2 } \mu \text{m. } \text{[C] In the bar graph representation, the n:n and n:n−1 classes denote equal (or nearly equal) plasmid segregation; the n:n’ and n:0 classes denote missegregation and segregation failure, respectively. There is some uncertainty in these numbers as foci occasionally tend to overlap and the number of plasmid molecules in each focus is unknown. The mean number of plasmid foci per nucleus for each segregation class is given below the graphs. These data represent 80 and 90 binucleate cells analyzed for the \( STB \) and \( ARS \) plasmids, respectively. The error bars indicate ± SEM.} \]
Figure 2. Localization of the STB reporter plasmid in meiotic chromosome spreads. (A) Chromosome spreads were prepared from isogenic [cir+] or [cir0] cells (transformed by the STB reporter plasmid) at the leptotene/zygotene or the pachytene stage of meiosis I (3.5 h and 5.5 h after transfer to sporulation medium, respectively). The results were corrected for differences in the percentages of [cir+] and [cir0] cells harboring the reporter plasmid at the time of transfer to sporulation medium. The histograms represent data from ~200 spreads for t = 3.5 h and ~800 spreads for t = 5.5 h. In these and subsequent spread assays, the plasmid was detected using an antibody to GFP, which targets the GFP-LacI bound to the LacO array present on the plasmid.

(B and C) In the pachytene spreads, chromosomes were visualized by DAPI and the central axes of paired chromosomes by Zip1 (using an antibody to the native protein). Selected sections (boxed regions) of the spreads are enlarged 3x to highlight plasmid foci at chromosome tips. Bars, 2 µm.

(D) For the plasmid foci analyzed (>150 for [cir+] spreads; >100 for [cir0] spreads), a plasmid-to-chromosome separation of <0.4 µm was interpreted as colocalization of the two. (E) The chromosome-associated plasmid foci from the [cir+] spreads were distinguished into those at chromosome tips or away from them. The error bars indicate ± SEM. **, P < 0.01 (two-tailed t-test).
The chromosome spread patterns would be consistent with the association of the STB plasmid with TELs or with the nuclear periphery at or near sites for TEL anchoring. Membrane-associated TELs (Zickler and Kleckner, 1998; Scherthan, 2007) are responsible for propagating RPMs generated by envelope motor assemblies along chromosome arms (Scherthan et al., 2007; Conrad et al., 2008; Koszul et al., 2008). Plasmid foci detected outside chromosomes might indicate the dynamic nature of plasmid–chromosome association. Alternatively, they might denote plasmids associated with segments of the nuclear membrane that were detached from TELs.

Localization of STB plasmids with respect to Ctf19, Mps3, and Rap1
The suggested localization of STB plasmids at or proximal to TELs (Fig. 2, B, D, and E) was further verified against Ctf19 and Mps3 as CEN and TEL markers, respectively. Ctf19, an outer kinetochore protein, is a member of the COMA subcomplex (Hyland et al., 1999; Westermann et al., 2007). Mps3, a SUN domain nuclear envelope protein, is a component of the spindle pole body in mitotic and meiotic cells, and becomes associated with TELs during meiotic prophase (Conrad et al., 2007). Plasmid locations were further verified with respect to the TEL marker Rap1 (Klein et al., 1992).

Association of the STB plasmid with Mps3 and Rap1 (~53% and ~46%, respectively, at leptotene/zygotene; ~77% and ~71%, respectively, at pachytene) was conspicuously higher than that with Ctf19 (~14% at leptotene/zygotene and ~8% at pachytene; Fig. 3, A–H). In spreads prepared from cells immediately after transfer to sporulation medium, the fractions of plasmid foci colocalized with Ctf19 (~11%) and Rap1 (~41%) were not different from those at leptotene/zygotene (unpublished data). Thus, approximately half of the STB plasmid population resides at or near TELs during early meiosis, with a significant increase in this population at late stages of meiosis I (70–80%). CEN proximal localization of the plasmid during meiosis is quite rare.

Association of Rep1 with chromosomes
Rep1 and Rep2 associate with each other and localize to mitotic chromosome spreads in a mutually dependent manner (Velmurugan et al., 2000; Mehta et al., 2002). Consistent with their in vivo interaction with STB (Velmurugan et al., 1998), an STB reporter plasmid is recruited to the spreads with the assistance of both proteins. To test whether a similar mechanism operates in meiosis, the presence of Rep1 in meiotic chromosome spreads, as well as plasmid localization with respect to Rep1, was examined.

Rep1 formed a distribution of foci in pachytene chromosome spreads (Fig. 4 A), the majority (~75%) being larger and more intense than the rest. The STB reporter plasmid foci, fewer in number than the Rep1 foci, were almost always coincident with a subset of the latter (Fig. 4 A). Among the ~60% Rep1 foci that were localized on, or abutted, DAPI-stained chromosomes (~0.4 µm separation; Fig. 4 B and C), the vast majority (~70%) were present at chromosome tips (Fig. 4 C). This preferential localization was further ascertained with respect to Zip1 (Fig. 4 B).

The 2 micron plasmid appears to be delivered to its nuclear address during meiosis by the Rep proteins, presumably via their interaction with a TEL-associated protein or a membrane protein that associates with TELs.

RPMs of the STB reporter plasmid
The rapid TEL-led chromosome movements during meiotic prophase (Scherthan, 2006; Scherthan et al., 2007; Conrad et al., 2008; Koszul et al., 2008; Wanat et al., 2008) can be quantitatively described by their (a) mean speed, (b) maximum speed, and (c) bias (Conrad et al., 2008; Lee et al., 2012). Bias is a measure of chromosome displacement, with values of 0, 0, and >0 denoting random motion, the tendency to stay in place, and the tendency to travel forward, respectively. The speeds decrease in the order TEL > mid-chromosomal locus > CEN. Furthermore, paired TELs display higher mean and maximum speeds as well as larger bias than unpaired TELs (Conrad et al., 2008; Fig. 5). If the 2 micron plasmid is tethered to chromosomes, the plasmid movements should mimic chromosome movements, and also disclose the chromosomal locus that it is associated with. We characterized the prophase movements of the STB reporter plasmid with respect to either native TELs or an 81-bp stretch of the TEL repeat (G,C,T) units located in a CEN-based circular plasmid.

The maximum speed, mean speed, and bias distributions of the STB plasmid in the wild-type background were nearly identical to those of unpaired TELs or of the CEN-ARS-TEL plasmid (Fig. 5, A–C; and Videos 1 and 2), but differed from those of paired TELs (Fig. 5, D–F). In contrast, a CEN-ARS plasmid had clearly reduced values for all three parameters (Fig. 5, G–I), as expected for a lack of motor-driven mobility. However, a small fraction of the plasmids did match paired TELs in the distances traversed (bias values of 0.1–0.4; Fig. 5 I). The movements of a multicopy ARS plasmid were also quite different from those of the STB plasmid, the CEN-ARS-TEL plasmid, and unpaired or paired TELs (unpublished data).

The dynamics data suggest that an STB- or TEL-containing plasmid engages the meiotic RPM machinery, leading to their nearly identical patterns of movement. They are consistent with the STB plasmid gaining access to the envelope motor first and then associating with unpaired TELs, or vice versa. The resemblance of the STB plasmid to unpaired and not paired TELs may be a matter of timing. These measurements were done at a stage when most TELs were still unpaired. Indeed, colocalization of STB plasmid foci with telomeric marker proteins in pachytene stage chromosome spreads (Fig. 3, E and G, bottom) would suggest that the plasmid can associate with paired TELs. The contrasting prophase dynamics of the STB and ARS plasmids attest to the crucial role of the 2 micron plasmid partitioning system in promoting TEL-like plasmid movement.

The roles of bouquet-RPM proteins in the prophase dynamics of the STB plasmid
As alluded to earlier, the MNC complex functions in meiosis by promoting the bouquet formation–RPM pathway (Trelles-Sticken et al., 2005; Scherthan et al., 2007; Conrad et al., 2008; Koszul et al., 2008; Wanat et al., 2008; Lee et al., 2012). All three contribute toward timely pairing of homologues, normal
Figure 3. Localization of the STB reporter plasmid with respect to CEN- and TEL-specific marker proteins. Chromosome spreads were prepared at 3.5 h (leptotene/zygotene) and 5.5 h (pachytene) into meiosis. Plasmid foci were mapped with Ctf19 (A, C, and D) as the CEN marker and Mps3 (B, E, and F) or Rap1 (G and H) as the TEL marker. The antibodies for visualizing Ctf19 (CTF19-MYC), Mps3 (MPS3-HA), and Rap1 (RAP1-RFP) were anti-Myc, anti-HA, and anti-RFP, respectively. In each localization assay, ~150 plasmid foci were scored. Bars, 2 µm.
meiotic recombination, and curtailment of ectopic or nonallelic recombination and aneuploidy (Chua and Roeder, 1997; Conrad et al., 1997, 2007; Trelles-Sticken et al., 2000; Kosaka et al., 2008; Wanat et al., 2008). Whereas Mps3 and Ndj1 collaborate to anchor TELs at the nuclear envelope, Csm4 is essential for their bouquet organization, and is likely the force transducer. We used ndj1Δ and csm4Δ to test the pertinence of TEL–membrane association, bouquet formation, or force generation to prophase movements of the STB plasmid.

In the absence of Csm4, analogous to chromosomes, the STB plasmid foci were slowed down considerably and displayed a smaller bias (Fig. 6, A–C; and Videos 1 and 2). The decrease in maximum and average speeds was more pronounced at 7 h than at 4 h (Fig. 6, A and B). At 4 h, the STB plasmid average and maximum speeds exceeded those of unpaired and paired TELs but resembled those of the CEN-ARS-TEL plasmid (Fig. S2, A, B, D, and E). At this time point, the STB plasmid closely matched unpaired TELs and the CEN-ARS-TEL plasmid in bias, and only modestly differed from paired TELs (Fig. S2, C and F). At 7 h (for the plasmids) and 8 h (for the chromosomes; Fig. 6, D–F and G–I), the STB plasmid was most similar to paired TELs in average speeds and to the CEN-ARS-TEL plasmid in maximum speeds and bias. The STB plasmid maximum speeds were intermediate between those of unpaired and paired TELs. The points to note are the marked reduction in STB plasmid speeds as meiosis I progressed in the csm4Δ strain, and the manifestation in STB plasmid dynamics of mixed features of unpaired and paired TELs and of the CEN-ARS-TEL plasmid.

The lack of Ndj1, which reduces prophase movements of TELs less markedly than csm4Δ (Conrad et al., 2008), also altered STB plasmid dynamics in a similar manner (Videos 3 and 4). In a reversal of the trend in the csm4Δ strain, the decreased
Figure 5. Characterization of STB plasmid movements during prophase. In this through-focus time-lapse analysis of wild-type cells (Conrad et al., 2008), at least 35 plasmid foci (tagged by green fluorescence) were traced over a period of 2 min at 1 frame/2 s. The relative positions of four individual plasmid foci (marked 1–4) in a single nucleus at 10-s intervals are displayed at the top. Bars, 2 µm. Chromosome VIII TELs tagged by red fluorescence were also...
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traced over the 2-min period (1 frame/2 s) as an internal standard (shown in Fig. S3). The speeds and bias of the STB plasmid were plotted alongside those for a CEN-based plasmid harboring the TEL repeat (CEN-ARS-TEL; A–C), the chromosome IVR TELs in the unpaired (uTEL; A–C) and paired (pTEL; D–F) states, and for a CEN-based ARS plasmid (CEN-ARS; G–I). The values for chromosome dynamics used in these plots and those in Fig. 6 and Fig. S3 were taken from previously published results (Conrad et al., 2008).

STB plasmid speeds at 4 h due to ndj1Δ were ameliorated at 7 h, with a similar effect on the bias as well (Fig. S3, A–C). The STB plasmid was similar to paired TELs at 4 and 7 h (Fig. S2, J–L; and Fig. S3, G–I) and, except for modest differences in bias, to the CEN-ARS-TEL plasmid at 4 h (Fig. S2, G–I). At 4 h, the STB plasmid was also similar to unpaired TELs in maximum speeds and bias but differed in average speeds (Fig. S2, G–I). At 7 h, the STB plasmid maximum speeds and bias exceeded those of unpaired TELs and the CEN-ARS-TEL plasmid, whereas all three were similar in average speeds (Fig. S3, D–F). The higher

Figure 6. Dynamics of the STB reporter plasmid in the absence of Csm4. The mobility features of the STB plasmid in the wild-type (4 h after transfer of cells to sporulation medium) and csm4Δ (4 h and 7 h after transfer) strains were plotted side by side (A–C). These graphs were based on 1 frame/2 s time-lapse data. As csm4Δ delays the meiotic program, the 4 and 7 h time points correspond to comparable prophase stages of meiosis I between the wild-type and the mutant strains, respectively. Plots comparing the STB plasmid to the plasmid-borne TEL (D–F, CEN-ARS-TEL) or chromosome IVR TELs (D–F, unpaired = uTEL; G–I, paired = pTEL) were assembled from data obtained at 1 frame/s. The analyses for the plasmids and for chromosomal TELs were done at 7 h and 8 h, respectively, after initiation of meiosis. This time difference did not alter the dynamics of paired or unpaired TELs (unpublished data). Note that a difference in time resolution in plotting the same set of recorded movements, 1 frame/2 s versus 1 frame/1 s, changes the histogram shapes; e.g., the plots for the STB plasmid in the csm4Δ mutant at 7 h in A and D (see the Materials and methods).
consistent with the plasmid accessing the motor unassisted by chromosomes. However, plasmid mobility may be modulated by the association of TELs with the motor and potential plasmid–TEL tethering.

It is not clear why the features of STB plasmid dynamics in the mutants are split among those of unpaired and paired TELs and the CEN-ARS-TEL plasmid, and why these similarities or differences change with time. Possible reasons are differences between plasmid and chromosome loads on the motor, interactions of the plasmid with unpaired versus paired TELs, and changes in the relative fractions of these two TEL classes as a function of the stage of meiosis. The CEN-ARS-TEL plasmid also displays split features of unpaired and paired TELs. If this plasmid were autonomous in its mobility, it is expected to have like unpaired TELs. The complexity inherent in chromosome movements would be reflected in the movements of chromosome-associated plasmids as well. A chromosome at the leading edge of motion shows more dramatic translations than the "follower" chromosomes (Koszul et al., 2008). Leadership changes

Figure 7. Movement of individual STB plasmid foci in wild-type, csm4Δ, and ndj1Δ strains. The traces from three representative cells each illustrate the excursions of individual plasmid foci (denoted by different colors) over a 1-min period in the wild-type (A), csm4Δ (B), and ndj1Δ (C) strains [Fig. 5, Fig. 6, and Fig. S3; and Videos 2 and 4]. The plasmid foci were visualized at 4 h (wild type) and at 7 h (mutants) after transfer to sporulation medium. The nuclear periphery (broken lines) is an approximate representation deduced from a single frame of each 60-frame movie, based on background nuclear fluorescence from GFP-LacI. For comparison, the paths traversed by unpaired IVR TELs over a 1-min span are depicted in D–F (Videos 1 and 3). As the pertinent nuclei images were not available, the nuclear outlines in D–F were arbitrarily chosen from the plasmid movies representing the relevant genetic backgrounds. n = number of plasmid foci in a nucleus. Bar, 2 µm.

speed shoulder in the maximum speed plot for the STB plasmid at 7 h was absent at 4 h.

Except for some decrease in the maximum speeds in the csm4Δ mutant, the CEN-ARS plasmid was largely indifferent to the absence of Csm4 or Ndj1 (Fig. S4, A–I), and showed no significant time-dependent changes in its dynamics. Individual time traces highlight not only the TEL-like dynamics of the STB plasmid but also the similarity in their slowdown by csm4Δ or ndj1Δ (Fig. 7). This striking resemblance between them is most easily explained by the same motor and force transducer being responsible for their prophase dynamics.

The similarity of the STB and CEN-ARS-TEL plasmids to each other and to unpaired TELs in the wild-type host (at 4 h) suggest that these plasmids engage the RPM machinery similarly to, or hitchhike on, unpaired TELs during early prophase. The changes in the STB plasmid speeds in the csm4Δ and ndj1Δ strains at 4 h versus 7 h and the shoulder in the maximum plasmid speed histograms at 7 h, prominent in the ndj1Δ strain (Fig. S3, D and G) and less so in the csm4Δ strain (Fig. 6, D and G), would be consistent with the plasmid accessing the motor unassisted by chromosomes. However, plasmid mobility may be modulated by the association of TELs with the motor and potential plasmid–TEL tethering.

It is not clear why the features of STB plasmid dynamics in the mutants are split among those of unpaired and paired TELs and the CEN-ARS-TEL plasmid, and why these similarities or differences change with time. Possible reasons are differences between plasmid and chromosome loads on the motor, interactions of the plasmid with unpaired versus paired TELs, and changes in the relative fractions of these two TEL classes as a function of the stage of meiosis. The CEN-ARS-TEL plasmid also displays split features of unpaired and paired TELs. If this plasmid were autonomous in its mobility, it is expected to behave like unpaired TELs. The complexity inherent in chromosome movements would be reflected in the movements of chromosome-associated plasmids as well. A chromosome at the leading edge of motion shows more dramatic translations than the “follower” chromosomes (Koszul et al., 2008). Leadership changes
among chromosomes, and an occasional “maverick” leader displays particularly prominent displacements (Scherthan et al., 2007; Koszul et al., 2008). A leader may in certain instances lose its followers, thus becoming an isolated “orphan” chromosome. Because of these potential complicating factors, strict quantitative adherence of the STB plasmid to paired or unpaired TELs in its movements would be unlikely.

Localization of STB plasmid foci in ndj1Δ and csm4Δ mutants
The results thus far suggest that the STB plasmid interacts with sites at the nuclear periphery where TELs localize, or with TELs themselves. Anchoring of TELs to the nuclear envelope is stabilized by Ndj1 but does not require Csm4, which promotes clustering of TELs and their coupling to the force generator (Trelles-Sticken et al., 2000; Conrad et al., 2007, 2008; Kosaka et al., 2008). If association occurs primarily between membrane-localized plasmids and TELs, ndj1Δ is expected to be more disruptive of this association than csm4Δ. We have tested this prediction by measuring plasmid distances from the nuclear periphery in the mutants and by following the effects of ndj1Δ on plasmid colocalization with Rap1.

The majority of plasmid foci in pachytene nuclei from the wild-type and csm4Δ cells was associated with the outer edge of the DAPI zone, or was internal to it (Fig. 8, A and B). In contrast, in nuclei from ndj1Δ cells, several plasmid foci were separated from the DAPI edge, the majority being external to it (Fig. 8, A and B). Among the external foci, those farthest from the DAPI edge were higher in number in the ndj1Δ strain than in the wild-type and csm4Δ strains (Fig. 8 C). As the DAPI boundary was assigned conservatively, the circumference of the DAPI zone was likely contracted significantly in this analysis. To circumvent this potential complication, foci distances measured from Nup49-labeled nuclear membrane (Fig. 8 D) were converted to plasmid occupancy of nuclear zones 1–3, demarcating cross sections of equal areas (Meister et al., 2010; Materials and methods). The majority of plasmids were situated within the outermost zone (zone 1) in the wild type and in the mutant strains (Fig. 8 E).

Finally, the fraction of plasmid foci colocalizing with Rap1 in pachytene chromosome spreads was not reduced by ndj1Δ (Fig. 9, A and B). The average number of Rap1 foci in the deletion strain was ~45 compared with ~30 in the wild type (unpublished data), which is consistent with the disruption of the native organization of Rap1 or of its association with TELs (Conrad et al., 1997). The fraction of plasmid foci coincident with chromosomes (~0.4 µm) was reduced to 36% from 52% estimated for the wild type (Fig. 9 C; P < 0.05). Within this subset, the foci associated with chromosome tips were only 47%, a significant reduction from 73% when Ndj1 was functional (Fig. 9 D; P < 0.05).

The STB plasmid profiles in the mutant strains indicate that peripheral plasmid localization is unaffected by ndj1Δ or csm4Δ. The differences in plasmid location with respect to the DAPI boundary versus the nuclear envelope in the ndj1Δ strain can be explained by this mutation disrupting the preferential perinuclear chromosome organization seen in the wild-type and csm4Δ backgrounds (Trelles-Sticken et al., 2000; Conrad et al., 2008; Wanat et al., 2008). In the absence of Ndj1, TELs dissociate from the nuclear membrane, and Rap1 foci tend to be more internalized (Trelles-Sticken et al., 2000). However, the reduction in peripheral Rap1 foci in the mutant is modest (from 52% to 40%). At the same time, their total number increases from ~30 to between 45 and 60 (Conrad et al., 1997; this study), which indicates extra-telomeric Rap1. As there are far fewer plasmid foci compared with Rap1 foci, the ratio between the two in zone 1 would still favor Rap1. Thus, authentic plasmid colocalization with a subset of Rap1 foci in zone 1 is still possible. The integrity of TELs could be compromised by ndj1Δ, affecting TEL clustering and perhaps increasing the propensity for frayed chromosome ends. As a result, at least some of the plasmid foci associated with Rap1 may appear to be dislocated from the ends of DAPI-stained chromosomes. Considered in toto, these results suggest that the plasmid establishes a bipartite association with the nuclear membrane and with membrane-anchored TELs, with Rap1 perhaps being responsible for the latter directly or indirectly.

2 micron plasmid segregation in ndj1Δ and csm4Δ strains
The shared meiotic defects characterized for ndj1Δ and csm4Δ are consistent with the requirement of Ndj1 and Csm4 in promoting (a) partner interactions during the early phase of recombination; (b) formation, maturation, and resolution of recombination intermediates; and (c) disjunction of homologues during anaphase I (Chua and Roeder, 1997; Conrad et al., 1997; Trelles-Sticken et al., 2000; Kosaka et al., 2008; Wanatet al., 2008). Provided that 2 micron plasmid segregation during meiosis is physically coupled to chromosome segregation, ndj1Δ and csm4Δ are expected to lower the fidelity of meiotic plasmid segregation. We therefore scored STB plasmid segregation in the deletion strains during meiosis I and at completion of meiosis II.

The STB plasmid showed higher incidence of missegregation as well as higher segregation failure (n:0) during meiosis I in the absence of Ndj1 or Csm4 (Fig. 10 A). Similarly, there was a reduction in the fraction of asci in which all four spores contained plasmid (Fig. S5 A). An increase in chromosome missegregation in the ndj1Δ and csm4Δ mutants was also noted (Fig. S5, B and C), as had been previously described (Chua and Roeder, 1997; Conrad et al., 1997; Marston et al., 2004; Wanat et al., 2008).

Thus, disabling components of the envelope motor that promote chromosome dynamics and segregation diminishes the fidelity of meiotic segregation of the 2 micron plasmid.

Discussion
The molecular mechanisms for the nearly chromosome-like stability of the 2 micron plasmid have not been fully elucidated. Equal plasmid segregation during mitosis (Scott-Drew and Murray, 1998; Velmurugan et al., 2000) is unlikely to be mediated by direct microtubule attachment and spindle force or association with the spindle pole body, or with a nuclear membrane protein that is not impeded by a diffusion barrier.
Figure 8. STB plasmid localization at pachytene with respect to nuclear periphery in ndj1Δ and csm4Δ strains. (A and B) The nearest distances of individual plasmid foci from the edge of the DAPI staining zone were measured to separate the foci into two groups: "internal" (at the edge of the DAPI zone or within it) and "external" (outside the DAPI boundary). (C) The external group of foci was subdivided into three types, based on the extent of their separation from the boundary. (D and E) Plasmid distances were measured from the nuclear envelope (outlined by Nup49-mCherry) along the diameter of circular cross sections of the nucleus (Meister et al., 2010). They were distributed into three zones (1–3) of equal area by placing the zone 1–2 boundary at \( \sqrt{2/3 \times R} \) and the zone 2–3 boundary at \( \sqrt{1/3 \times R} \), where \( R \) = the radius of the circle. The dashed line in E marks the probability of the random
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occurrence (33.3%) of a plasmid focus within a zone. The experimental strains harbored fluorescence-tagged CEN VII (TetR-Td-Tomato-[TetO]$_{224}$) (red dot). Leptotene/zygotene and pachytene stages were distinguished by two red dots (unpaired homologues; cohesioned sisters) and one red dot (paired homologues), respectively. The data in all panels were each obtained by analyzing $\geq 100$ plasmid foci. Bars, 2 µm.

Figure 9. Localization of STB plasmid foci with respect to Rap1 in the ndj1Δ strain. (A) Chromosome spreads prepared from pachytene stage cells of the ndj1Δ strain (at 7.5 h into meiosis) were assayed for the fraction of STB plasmid foci colocalized with Rap1 foci (B) and that associated with chromosomes (C). Within the latter class, plasmid foci located at chromosome tips were demarcated (D). These data were collected by screening 22 spreads comprising 180 plasmid foci. The histograms for the wild-type strain in B–D were taken from Fig. 3 H (5.5 h), Fig. 2 D, and Fig. 2 E, respectively. The error bars indicate ± SEM. * P < 0.05 (two-tailed t test). Bars, 2 µm.

Conditional mutations that perturb chromosome segregation without affecting spindle pole body or spindle functions cause 2 micron plasmid missegregation (Velmurugan et al., 2000; Mehta et al., 2002). Unlike CEN, two copies of STB in cis do not lead to plasmid instabilities (unpublished data). The nuclear distribution of STB plasmid foci in mitotic cells does not
Collectively, they are consistent with motor-assisted and TEL-associated segregation of the 2 micron plasmid during meiosis I (Fig. 10 B).

**Association of the 2 micron plasmid with the envelope motor and TELs during meiosis I**

The localization patterns of the STB reporter plasmid and of Rep1 with respect to chromosomes, Mps3, and Rap1 are consistent with the Rep1-Rep2-assisted interaction of the 2 micron plasmid with TELs and/or the envelope motor. This interaction may be stabilized by the membrane anchoring of TELs and their association with the motor, as suggested by the increase in the fraction of Rap1- or Mps3-associated plasmid foci at pachytene.

The relocation of STB plasmid foci from the interior of the nuclei in mitotic cells (Heun et al., 2001; Mehta et al., 2005) to the nuclear periphery in meiotic cells (this study) reveals preferred plasmid localization at the periphery (Heun et al., 2001; Mehta et al., 2005). Cumulative evidence is consistent with the plasmid overcoming mother bias by hitchhiking on chromosomes (Velmurugan et al., 2000; Mehta et al., 2002; Liu et al., 2013). Tethering to chromosomes for stable maintenance in host cells is a strategy that viral episomes of the gammaherpes and papilloma families resort to as well (Wu et al., 2000; McBride et al., 2004; You et al., 2004).

If the meiotic segregation of the 2 micron plasmid is coupled to chromosome segregation, the plasmid has to adapt to the reductional division of meiosis I, when chromosome homologues, but not sister chromatids, separate from each other. Plasmid segregation during meiosis II may be mechanistically analogous to that during mitosis. The seminal findings from the present study are (a) repositioning of an STB reporter plasmid to the nuclear periphery as cells enter the meiotic program, (b) localization of plasmid foci at or close to TELs, (c) potential plasmid interaction with the nuclear envelope motor that also engages TELs, (d) similarities in motor-driven plasmid and TEL dynamics during prophase I, and (e) the requirement of the motor-associated proteins Ndj1 and Csm4 for normal meiotic segregation of the plasmid.
(Conrad et al., 2007). Difference in the host proteins that mediate plasmid–chromosome association may account for potentially distinct plasmid–chromosome interactions in mitotic versus meiotic cells.

The envelope motor that triggers rapid TEL movements also promotes 2 micron plasmid dynamics and segregation during meiosis I

The TEL-like prophase movements and equal meiosis I segregation of the STB reporter plasmid are dependent on Csm4 and Ndj1. Whereas rapid chromosome movements serve critical functions in the faithful segregation of homologues (Kosaka et al., 2008; Koszul et al., 2008; Wanat et al., 2008; Lee et al., 2012), a role for motor-driven dynamics themselves in plasmid segregation is hard to conceive. More likely, plasmid association with the motor segues into plasmid tethering to TELs and thus plasmid segregation by hitchhiking. However, coordinated plasmid and TEL movements may be conducive to their mutual association. If the plasmid-associated motor components are evenly partitioned, they would provide vehicles for equal plasmid segregation. It is not known how Csm4, Ndj1, and Mps3 are distributed at the end of meiosis I. Alternative plasmid segregation mechanisms, both membrane-associated and chromosome-associated, need not be mutually exclusive.

The mother bias of ARS plasmids has been attributed to the geometry of the nucleus, with its constricted neck and the relatively short duration of mitosis, or perhaps to a more direct barrier to plasmid diffusion, all of which would impede mother–daughter equilibration of plasmid molecules (Shcheprova et al., 2011). The diffusion barrier can be at least partially overcome by tethering multicopy ARS plasmids to certain nuclear pore proteins, to the nuclear envelope, or to TEL-associated proteins (Gehlen et al., 2011; Khmelinskii et al., 2011). The nuclear organization during meiosis I has no apparent geometric bottleneck; yet, the ARS reporter plasmid experiences high mis segregation (~57%) and significant failed segregation (~14%) events. Additional nongeometric constraints, such as the aggregation of plasmid molecules and/or plasmid interactions with multiprotein assemblies or with subnuclear structures, may interfere with passive plasmid segregation.

The model for plasmid-TEL coupling: Implications and limitations

While the model presented in Fig. 10 B is heuristic, the details will need to be refined. Because chromosome segregation is affected by ndj1Δ and csm4Δ, missegregation of the plasmid in these mutants can be explained, at least partly, by its association with chromosomes. Despite defective chromosome dynamics and meiotic progression, the mutants are at least 50% as efficient as the wild type in sporulation, with >60% spore viability (Conrad et al., 2008; Kosaka et al., 2008). There must be salvage pathways that rescue meiosis with moderate competence. In the current model, ndj1Δ reduces the efficiency of plasmid segregation by blocking access to TELs, as their membrane anchoring is destabilized. In contrast, csm4Δ does not seem to perturb TEL localization at the envelope (Conrad et al., 2008; Kosaka et al., 2008; Wanat et al., 2008). Csm4 is required for bouquet formation, which may facilitate plasmid–chromosome interactions. The csm4Δ effect on plasmid segregation may be largely indirect, and manifest through chromosome segregation. However, subtle changes in plasmid positioning due to the defective MNC complex, as well as subdued plasmid and/or chromosome mobility, may hinder plasmid–TEL docking.

Meiotic segregation of the 2 micron plasmid: Logic for chromosome association?

Assuming that the hitchhiking model applies to meiosis I, it is not clear what advantage TELs might offer over other chromosomal locales as plasmid tethering sites. Chromosome termini, being generally bereft of genes, may provide safe plasmid docking sites without disrupting normal gene expression and/or regulation. Infrequent double-strand breaks and low meiotic recombination frequencies at subtelomeric regions may lower the likelihood of plasmid dislodgement by assembly of the recombination–repair machinery and its DNA processing activities.

Equal plasmid segregation in association with TELs during meiosis I (and perhaps meiosis II as well) would demand a very specialized high-order organization of replicated molecules within a TEL-associated plasmid focus. Furthermore, this organization has to be refractory to chromosomal exchanges by recombination. If a plasmid focus were composed of four equivalent segregation units, each tethered to one of the four TELs of a homologue pair, plasmid segregation would follow the 2:2 rule during meiosis I and the 1:1 rule during meiosis II.

In a simpler model of random plasmid–TEL association, each plasmid focus would cosegregate with a pair of sister chromatids to one of the two daughter nuclei during meiosis I. For the 6–8 plasmid foci normally observed per cell, the probability of plasmid loss from a nucleus would be quite low: ~3% [(0.5)^6 × 2] and ~0.8% [(0.5)^8 × 2], for the 6 and 8 foci cases, respectively. However, the corresponding equal segregation frequencies, 3:3 and 4:4, would be only ~31% and ~27%, respectively. In principle, a decrease in plasmid copy number resulting from this type of segregation may be rectified subsequently by Flip-mediated amplification (Futcher, 1986; Volkert and Broach, 1986). However, amplification is seldom triggered during normal steady-state mitotic growth, with nearly every 2 micron plasmid molecule replicating once, and only once, during an S phase (Zakian et al., 1979). It is not known whether a replication control mechanism counteracts a higher-than-normal plasmid copy number within a nucleus. Cells containing very high plasmid copy numbers would be eliminated over time because of the selective disadvantage they suffer from plasmid overload (Holm, 1982; Chen et al., 2005, 2007; Dobson et al., 2005).

Single-copy derivatives of STB reporter plasmids have been successfully exploited to address the mitotic segregation of the 2 micron plasmid without the uncertainties introduced by multiple plasmid copies (Ghosh et al., 2007; Liu et al., 2013). Analogous reporters would be equally helpful in unveiling the segregation behavior of plasmid sisters during meiosis I.

Materials and methods

Strains and plasmids

Strains and plasmids used in this study are listed in Table S1 and Table S2. The relevant genotypes of strains as well as appropriate references are

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included. Strains carrying endogenous 2 micron plasmid are designated as [ciz+], whereas those cured of the plasmid are indicated as [ciz0]. The diploid strains for plasmid segregation, localization, and dynamics assays were constructed anew for each set of assays. The reporter plasmid was introduced into the desired "α" mating type strain by transformation, and the transformant was mated with the "α" mating type partner strain. Strains and plasmids were provided by A. Murray (Harvard University, Cambridge, MA), A. Johnson (University of Texas at Austin, TX), and E. Alani (Cornell University, Ithaca, NY) served as templates for the construction of a subset of those listed in Table S1 and Table S2.

Genomic manipulations

Genetic modifications at desired chromosomal loci were introduced by previously published procedures (Longtine et al., 1998). They were confirmed by PCR, Southern analysis, and, in the case of epitope addition, by Western blotting. terminal tags of 3′-HA and 3′-Myc were introduced at the corresponding native locus. Mutant strains used in plasmid and chromosome dynamics assays were constructed by replacing DNA sequences corresponding to amino acids 20–282 of Nd1 or 20–140 of Csm4 were deleted by KANMX6 insertion at the corresponding native locus. Mutant strains used in plasmid and chromosome dynamics assays were constructed by replacing DNA sequences corresponding to amino acids 14–252 of Nd1 or 27–156 of Csm4 by TRP1 (Berber et al., 1991). Expression cassettes for GFP-LacI were integrated at URA3 (under the control of the HIS3 promoter) and at LYS2 (under the control of the DMC1 promoter) for visualizing reporter plasmids harboring a [LacO]256 array. A derivative of plasmid pKS404 (Skoronski and Hieter, 1989) containing [TetO]256 and a CEN proximal segment of chromosome VII between coordinates 479055 and 4797542] or the left TEL segment of chromosome VII (between coordinates 4025 and 5037) was inserted at the corresponding chromosome location by homologous recombination. An expression vector for TettR-TdTomato (controlled by the URA3 promoter; Malos et al., 2008), modified by disrupting LEU2 by TRY1, was inserted at TRY1 to tag the TetO repeats by red fluorescence.

Meiotic regimen

Synchronization of meiotic cultures was performed as described previously (Dresser et al., 1997). In brief, fresh diploids obtained by mating the perti strains were similar in size and organization, and contained the [LacO] repeats by red fluorescence.

Chromosome spreads

Spheroplasts obtained as described in Dresser and Giroux (1988) were used for preparing nuclei (chromosome) spreads according to previously published methods (Voelkel-Meiman et al., 2012). In brief, 10 ml of a culture at a given stage of meiosis was resuspended in 2 ml of ZK buffer [25 mM Tris, pH 7.5, and 0.8 M KCl] and treated with 40 µl of 1 M DTT for 2 min. Cells were resuspended in 2 ml of ZK buffer and incubated with 15 µl of zymolase solution for 30 min at 30°C to obtain spheroplasts. Spheroplasts were pelleted, washed with cold MES/Sucrose solution (0.1 M MES-NaOH, pH 6.4, 0.1 mM EDTA, 0.5 mM MgCl2, and 1 M Sorbitol), resuspended in the same buffer, and kept in ice. One half or one fourth of the spheroplast solution was pelleted, and 80 µl of 1 x MES as well as 200 µl of 4% paraformaldehyde were added. 100–140 µl of the resuspended spheroplast solution was applied directly onto a clean superfrost plus slide (catalog No. 12-550-15; Thermo Fisher Scientific) and distributed over its entire surface using the edge of a coverslip. The slide was allowed to air dry at least for 20 min before washing it with 0.4% Photo-Flo (0.4% vol/vol solution of Photo-Flo 200 solution [Kodak] in sterile water). The spreads were used for visualizing chromosomes by DAPI staining or proteins by treating with specific antibodies followed by indirect immunofluorescence.

Antibodies

The following primary antibodies were used: mouse anti-GFP (1:300), goat anti-Myc (1:300), rabbit anti-HA (1:300), rabbit anti-RFP (1:300; all from Abcam), rabbit anti-Zip1 (1:100; a generous gift from S. Roeder, Yale University, New Haven, CT), and rabbit anti-Rep1 (1:200; Velmurugan et al., 2000). The secondary antibodies used were: donkey anti-mouse FITC (1:200) and goat anti-rabbit Texas red (1:200 and 1:400) from Jackson ImmunoResearch Laboratories, Inc., and donkey anti-goat Alexa Fluro 558 (1:500) from Invitrogen.

Fluorescence microscopy

Images were captured at room temperature in 0.4-µm (Fig. 1, Fig. 10, Fig. S1, and Fig. S5) and 0.2-µm (all other figures) z sections, with a pixel spacing of 0.129 µm using a microscope (BX-60; Olympus) with a 100x oil immersion objective lens (NA 1.3) and a camera (Photomicrotext Quantix; Roper Scientific). MetaMorph 7.5 software (Molecular Devices) was used for image analysis. Image stacks covering at least 4 µm of the nucleus were deconvolved using MetaMorph 2D deconvolution software using nearest neighbors algorithm.

Time-lapse video microscopy

Time-lapse video microscopy was performed as previously described by Conrad et al. (2008). The movements of reporter plasmids (Fig. 5, Fig. 6, Fig. S2, Fig. S3, and Fig. S4) were analyzed using the custom software OMRFQANT (Conrad et al., 2008). The assays with the fluorescence-tagged STB reporter plasmid (green) in the wild-type strain also included fluorescence- tagged chromosome VIII TEL (red) for reference. In the series of video frames captured at a rate of 1 frame/s, alternate frames represented the plasmid and TEL, respectively. Two types of trajectories were identified for the dynamics of the STB plasmid in the wild-type strain corresponding to 1 frame/2 s. The movements of the STB plasmid in the ndf1Δ and csm4Δ strains were followed at 1 frame/s. The data for paired and unpaired TELs and for the CEN-ARS/TEL plasmid in the wild-type and mutant strains were also obtained at 1 frame/s. In those plots that included the STB plasmid in the wild-type strain (Fig. 5, A–I; Fig. 6, A–C; and Fig. S3, A–C), all the frames/datasets were converted to 1 frame/2 s (by skipping alternate frames). By doing so,
the time resolution was kept constant for every pairwise comparison. However, this manipulation had the effect of altering the histogram patterns for the same dataset between different panels of a given figure, for example, the mean and maximum speeds of the STB plasmid in A and B versus D and E, respectively, of Fig. 6. The difference arises because the sum of the vectorial displacements $AB$ and $BC$ recorded over two 1-s intervals will not equal AC [unless the points ABC form a straight line], the resultant displacement when the movements are recorded over a 2-s interval. Because, by triangle inequality, $AC < AB + BC$, the same set of movements will appear slightly slower in a 1 frame/2 s plot compared with 1 frame/s plot.

Paried and unpaired TELs were distinguished as one spot and two spots, respectively (Fig. S3). In the few instances when this distinction was not as clear-cut, a TEL appearing as two spots in >6 frames out of 60 frames (in 1 frame/s movies) was defined as unpaired.

Mapping reporter plasmid or Rep1 foci with respect to chromosomes using fluorescence signals in cytological assays

The following rules were applied for mapping an STB reporter plasmid with respect to chromosomes marked by DAPI (or by Zip1) in pachytene stage nuclear spreads (Fig. 2 and Fig. 9). If the plasmid was closer to the arm (lateral location) than to the tip (end location) of a chromosome, the distance between the centroid (the brightest pixel) of the plasmid focus and the brightest chromosome pixel closest to it was registered. In the few instances when the brightest chromosome pixel could not be assessed unambiguously (intensity difference of <$5\%$), the more peripheral one was chosen to represent the chromosome edge. For mapping a plasmid focus located proximal to a chromosome tip, the end of a chromosome was defined based on the intensity changes in the longitudinal array of DAPI pixels linking it. The brightest row of pixels marking nearly the entire chromosome length showed little variation in intensity (within 2–5%). Near the ends however, the pixels became progressively fainter, making it difficult to precisely determine the boundary. The last pixel of the series beyond which the intensity fell 10% or more was taken to be located at the chromosome end. The “end” group of pixels was then delimited by the constraint that the intensity of an included pixel could not drop by >5% of that of its brightest member. Further steps were the same as those for the lateral distance measurements. Namely, the separation of the centroid of a plasmid focus from the nearest pixel representing the chromosome end was determined. The mapping procedure applied to plasmid foci was also used in measuring the distances of Rep1 foci from chromosomes (Fig. 4).

Criteria for colocalization of two fluorescence-tagged nuclear entities in cytological assays

The signals from two fluorescent foci (green in one case and red in the other) were defined as colocalized if they overlapped almost perfectly to generate a yellow signal or partially overlapped to generate a green–yellow or partially overlapped to generate a green–red signal (Fig. 3, Fig. 4, and Fig. 9). As the resolution on a single pixel level corresponds to G 129 µm, and as there is occasional ambiguity in deciding the centroid pixel within a signal, complete coincidence and partial coincidence in our estimates indicate a spacing of 0.0–0.26 µm (2 pixels) and 0.26–0.39 µm (3 pixels), respectively.

Mapping a reporter plasmid focus with respect to the nuclear boundary using fluorescence signals in cytological assays

The distances of plasmid foci from the nuclear boundary (Fig. 8, A–C) in fixed cells, which are assumed to preserve the overall three-dimensional organization of nuclei, were measured as follows. First, image stacks of a nucleus stained with DAPI were generated from slices with a step size of 0.2 µm. As the multiple plasmid foci were not colocalized in their locations, the z sections that captured the highest intensity from each individual plasmid signal were identified. The shortest distance from the plasmid centroid (the brightest pixel signifying the centroid of the plasmid focus) to the brightest DAPI pixel at the edge of the same image plane (nuclear boundary) was measured.

Localization of a plasmid focus with respect to the Nup49-mCherry signal demarcating the nuclear boundary (Fig. 8, D and E) was performed as follows. Plasmid foci situated within 20% of the focal planes from each pole were excluded from the analysis, as Nup49-mCherry signals were poorly resolved near the poles [Meister et al., 2010]. Furthermore, the Nup49 fluorescence signals were often discontinuous and nonuniform in size. The local membrane contour within an image plane was traced along the midpoints between the inner and outer edges of the relevant signals (Mehta et al., 2005). Adjacent traces were connected by smooth curves in regions lacking the Nup49 signal. The line signifying the shortest distance of a plasmid focus from the membrane was extended to the opposite membrane arc to obtain the diameter of the nuclear cross section. Each focus was assigned to one of three zones of equal areas based on its distance from the edge normalized to the radius, as described previously [Meister et al., 2010]. As the cross sections of the meiotic nuclei were often locally distorted from circularity, the radius was not constant among the different plasmid foci. However, this did not affect the zonal allocation of the foci as a function of the radius.

Other miscellaneous protocols

Standard protocols for yeast and bacterial transformations, yeast DNA and plasmid DNA preparation, curing (cir+)-strains of the endogenous 2 micron plasmid to generate corresponding (cir0) strains, cultivating yeast and bacteria, and other routine procedures have been published previously [Velurugan et al., 2000; Liu et al., 2013].

Online supplemental material

Fig. S1 shows STB and ARS plasmid distribution in spores, and classification of tetrads containing plasmid foci in all four spores. Fig. S2 shows plasmid dynamics in cms4 and ndj1 strains at 4 h after transfer to sporulation medium. Fig. S3 shows TEL and STB plasmid dynamics in the wild-type and ndj1 strains. Fig. S4 shows dynamics of a CEN4ARS plasmid in the wild type, ndj1, and cms4 strains. Fig. S5 shows plasmid distribution in tetrads and chromosome segregation in the ndj1 and cms4 strains. Tables S1 and S2 show strains and plasmids used in this study. Video 1 (related to Fig. 5, A–F; and Fig. 6, D–I) shows chromosome dynamics in wild-type and cms4 cells. Video 2 (related to Fig. 5, A–I; Fig. 6, A–I; and Fig. 53, A–C) shows STB plasmid dynamics in wild-type and cms4 cells. Video 3 related to Fig. 5, D–I) shows chromosome dynamics in wild-type and ndj1 cells. Video 4 (related to Fig. 3, A–I) shows STB plasmid dynamics in wild-type and ndj1 cells. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201312002/DC1. Additional data are available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201312002.

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