The 2 micron plasmid purloins the yeast cohesin complex: a mechanism for coupling plasmid partitioning and chromosome segregation?

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The yeast 2 micron plasmid achieves high fidelity segregation by coupling its partitioning pathway to that of the chromosomes. Mutations affecting distinct steps of chromosome segregation cause the plasmid to missegregate in tandem with the chromosomes. In the absence of the plasmid stability system, consisting of the Rep1 and Rep2 proteins and the STB DNA, plasmid and chromosome segregations are uncoupled. The Rep proteins, acting in concert, recruit the yeast cohesin complex to the STB locus. The periodicity of cohesin association and dissociation is nearly identical for the plasmid and the chromosomes. The timely disassembly of cohesin is a prerequisite for plasmid segregation. Cohesin-mediated pairing and unpairing likely provides a counting mechanism for evenly partitioning plasmids either in association with or independently of the chromosomes.

Introduction
The yeast plasmid 2 micron circle is a model selfish DNA element (Broach and Volkert, 1991) with an evolutionarily optimized structural and functional design for high-copy and high-fidelity propagation. The plasmid, with a copy number of 60 per cell, neither confers any obvious selective advantage nor imposes any significant metabolic burden upon its host (Futcher and Cox, 1983). The host replication machinery replicates each plasmid molecule once and only once per cell cycle (Zakian et al., 1979). The rate of plasmid inheritance by daughter cells at cytokinesis approaches that of the yeast chromosomes (Broach and Volkert, 1991). A recombination-based amplification system (Futcher, 1986; Volkert and Broach, 1986; Reynolds et al., 1987) and an efficient segregation system are responsible for the remarkable stability and high steady-state copy number of the plasmid.

The stability system consists of two plasmid-coded proteins, Rep1p and Rep2p, and a cis-acting locus, STB (Jayaram et al., 1983; Kikuchi, 1983). The Rep proteins show both self- and cross-interactions in vivo and in vitro, and bind to the STB DNA with assistance from host factor(s) (Ahn et al., 1997; Scott-Drew and Murray, 1998; Velmurugan et al., 1998; Sengupta et al., 2001). Recent evidence suggests that the carboxy-terminal domain of Rep2p can associate with DNA in a Southwestern assay (Sengupta et al., 2001).

Within the yeast nucleus, the Rep1 and Rep2 proteins tightly associate with STB-containing plasmids into well-organized plasmid foci that form a cohesive unit in partitioning (Scott-Drew and Murray, 1998; Velmurugan et al., 2000). It is generally accepted that the protein–protein and DNA–protein interactions engendered by the Rep–STB system are central to plasmid partitioning. Point mutations in Rep1p that knock out interaction with Rep2p or with STB simultaneously block the ability of these Rep1p variants to support plasmid stability (unpublished data).

Earlier work revealed that the kinetics of segregation of a GFP-tagged 2 micron plasmid derivative and a GFP-tagged chromosome closely parallel each other (Velmurugan et al., 2000). Consistent with a potential plasmid–chromosome connection in partitioning, the Rep–STB system also interacts with host-encoded proteins that are suspected to play a role in chromosome segregation (Velmurugan et al., 1998, 2000; Sengupta et al., 2001; unpublished data; results of this study). These include the products of at least three genes: SHF1/CST6, FUN30, and BRN1. The first two are not essential genes, and their functional roles have not been analyzed (Velmurugan et al., 1998; Ouspenski et al.,...
The relevant genotypes of strains and functional attributes of plasmids are briefly outlined. Plasmids YEpLac181, YEpLac195, YEpLac204, and YCpLac33 were obtained from the Gietz laboratory (University of Manitoba, Manitoba, Canada; Gietz and Sugino, 1988). Some of the derivatives constructed from these parent vectors have been described in our previous work (Velmurugan et al., 2000). The pESC-Trap and pGAD424 vectors were purchased from Stratagene and CLONTECH Laboratories, Inc., respectively. The gene for the nondegradable form of Mcd1p was given to us by Mike Christman (Boston University School of Medicine, Boston, MA). The smc1-2 and smc3-42 mutant strains were gifts from D. Koshland (Carnegie Institution of Washington, Baltimore, Maryland).

1999, 2000). BRN1 is essential, and encodes a component of the yeast condensin complex (Lavoie et al., 2000; Ouspenski et al., 2000; Strunnikov et al., 2001). Overexpression of CST6 and FUN30 or inactivation of BRN1 by a conditional mutation results in impaired chromosome segregation.

We discovered that the ip1-2 mutation, which leads to chromosome missegregation at the nonpermissive temperature (Chan and Botstein, 1993; Biggins et al., 1999; Kim et al., 1999; Kang et al., 2001), causes a 2 micron circle-derived plasmid to missegregate in tandem with the chromosomes in a Rep1p- and Rep2p-dependent fashion (Velmurugan et al., 2000). The Ip1 kinase appears to phosphorylate multiple substrates, including histone H3 and the kinetochore component Ndc10p, and may facilitate chromosome biorientation by altering kinetochore-spindle pole connections (Biggins et al., 1999; Kim et al., 1999; Hsu et al., 2000; Tanaka et al., 2002). Perhaps the Ip1 protein is required for a shared step in the chromosome segregation and plasmid segregation pathways. Or, it may perform independent functions in the two pathways, for example, by phosphorylating separate target protein(s).

In this paper, we demonstrate that mutations in genes required for kinetochore function or the establishment of sister chromatid cohesion (CTF7, CTF13, CTF14/SDC10, and NDC80) missegregate the 2 micron plasmid and the chromosomes in a coupled manner. We provide evidence that the cohesin complex, which holds replicated sister chromatids together until they are ready for separation, is important in plasmid partitioning. We propose that the cohesin complex is recruited to the STR locus by the cooperative action of the Rep1 and Rep2 proteins. The timings of cohesin association and dissociation during the cell cycle can be essentially superimposed between the plasmid and the chromosomes. As has been observed for chromosomes, when cohesin disassembly is intentionally blocked (Uhlmann et al., 1999), the normal splitting of the 2 micron plasmid cluster into two separate clusters (that would then rapidly move apart) fails to occur.
spindle were visualized by fluorescence microscopy in live yeast cells expressing Nup49p–CFP and Tub1p–YFP simultaneously. Red and green colors (tubulin and nuclear membrane, respectively) were added artificially using the Adobe Photoshop® software. The patterns shown here are representative of ~80% of the cells shifted to the restrictive temperature.

Results
Design of experiments and organization of data
The yeast strains and plasmids used for the present study and their salient features are compiled in Table I. In some of the experiments, the endogenous 2 micron circles served as the reporter plasmids. Yeast strains harboring them are designated as [cir<sup>+</sup>]. These plasmids provide a source of the Rep1 and Rep2 proteins for trans complementation of plasmid substrates lacking the REP1 and REP2 genes. Strains lacking 2 micron circles are designated as [cir<sup>−</sup>]. When complementation was desired in the [cir<sup>−</sup>] background, Rep1p, Rep2p, or both were expressed either from their native promoters or from the inducible GAL1 promoter, GAL10 promoter, or both. For direct visualization of plasmids by fluorescence microscopy, the association of GFP–Lac repressor (LacI) expressed in yeast to multiple Lac operators (LacO<sub>50</sub>) harbored by a plasmid of interest was exploited (Straight et al., 1997; Velmurugan et al., 2000). It should be pointed out that an STB reporter plasmid in the absence of one or both of the Rep proteins is functionally equivalent to an ARS reporter plasmid (lacking STB).

A 2 micron–derived plasmid stays with chromosomes in mutants defective for chromosome segregation
To further verify the alleged coordination between chromosome segregation and plasmid partitioning, we followed the effects of several mutations that affect fidelity of chromosome transmission on the behavior of 2 micron–derived plasmids. We describe below the results from mutations in the genes CTF7, CTF13, CTF14/NDC10, and NDC80.

For each mutant strain, logarithmically growing cells were incubated at the nonpermissive temperature for 3 h, and chromosomes and reporter plasmids were monitored in large-budded cells (Fig. 1). Cells showing pronounced missegregation of chromosomes, as judged by a large inequity in DAPI staining, constituted nearly 70–80% of the population. They were divided into two subgroups: those containing the reporter plasmid in one compartment alone (a) or in both compartments (b).

There was a striking correlation between the tandem missegregation of the chromosomes and the 2 micron–derived plasmid at the nonpermissive temperature (Fig. 1 A). The presence of the plasmid in a compartment lacking chromosomes was seen in at most 21% of the cells examined. In contrast, the ARS plasmid was found in the chromosome-free compartment in ~50% of the cells for four of the mutants and 70% of the cells for the fifth mutant (ndc10<sup>−</sup>). Nuclear elongation and spindle assembly were not affected by the nonpermissive temperature (Fig. 1 B).

For the class b cells, containing the majority of chromosomes in one cell compartment but the plasmids in both compartments, the differences in the numbers of fluorescent plasmid dots between the two compartments were counted. When plasmids did segregate, the equal distribution patterns (4:4, 3:3, etc.; Velmurugan et al., 2000) far outnumbered the unequal pattern (4:3/2/1, 3:2/1, etc.; Table II).

In large-budded cells from a wild-type strain grown at 25°C or 37°C or from the mutant strains grown at 25°C, the 2 micron plasmid was almost always present in both cell compartments (unpublished data). The near equivalence in DAPI staining in these compartments indicated normal chromosome segregation. Quantitatively, the results were similar to those published previously (see Fig. 7 and Table II in Velmurugan et al., 2000).

The products of the CTF13 and CTF14/NDC10 genes are required for kinetochore assembly as well as centromere association with the yeast cohesin complex (Doheny et al., 1993; Goh and Kilmartin, 1993; Jiang and Carbon, 1993; Jiang et al., 1993; Strunnikov et al., 1995; Russell et al., 1999; Tanaka et al., 1999). The Ndc80 protein is part of a kinetochore-associated complex (Wigge et al., 1998; Janke et al., 2001; Wigge and
Kilmartin, 2001), and the Ctf7 protein is important for the establishment of sister chromatid cohesion (Skibbens et al., 1999; Toth et al., 1999). Nonfunctionality in any one of these proteins results in impaired partitioning of chromosomes. Our data argue that the chromosome and plasmid partitioning pathways either overlap with each other in at least some of their steps or the two are coordinately regulated. For the ctf14 and ndc80 mutants, missegregation of the 2 micron test plasmid in tandem with chromosomes is dependent on the Rep proteins. In a [cir] mutant background, the 2 micron plasmid loses its strong chromosome-directed bias at the nonpermissive temperature (unpublished data). Plasmid segregation in the other mutants has not yet been tested in the [cir] background.

| Plasmid foci distribution | ctf13-30 | ndc80-1 | ctf14-43 | ndc10-2 | ctf7-203 |
|---------------------------|----------|---------|----------|---------|----------|
| 4:4                       | 86       | 85      | 86       | 88      | 79       |
| 4:3/2/1                   | 14       | 15      | 14       | 12      | 21       |
| 3:3                       | 90       | 91      | 83       | 94      | 88       |
| 3:2/1                     | 10       | 9       | 17       | 6       | 12       |
| 2:2                       | 88       | 82      | 81       | 91      | 83       |
| 2:1                       | 12       | 18      | 19       | 9       | 17       |

The data are from large budded cells obtained after shifting the indicated strains to the nonpermissive temperature for 3 h. The cells scored correspond to the small fraction (~20% for each strain) that revealed plasmids in both cell compartments, even though chromosomes were largely confined to one. The values for equal segregation (n:n) and unequal segregation (n:n-1, n-2,..., 1) are expressed as %. The n:0 class would represent tandem missegregation of the plasmids with chromosomes.

**Localization of the Rep1 and Rep2 proteins in chromosome spreads**

The observed coupling between chromosome and 2 micron plasmid segregation, and the lack of it in the case of an ARS plasmid, would imply that the Rep–STB system is the likely coupling agent. To probe the potential association (be it direct or indirect) between plasmid and chromosomes, we followed the localization of the Rep proteins and a 2 micron reporter plasmid in yeast chromosome spreads by indirect immunofluorescence (Fig. 2). Only the data for Rep1p are shown; results with Rep2p were essentially identical.

In [cir] spreads, both Rep1p (green) and the plasmid DNA (red) were localized with the chromosomes in exponentially growing (Fig. 2 A) as well as G1-arrested cells (Fig. 2 B). As was observed previously (Velmurugan et al., 2000),

![Figure 2](image_url)
plasmids were confined entirely to the Rep1p zone. The Rep1p foci were smaller and sharper relative to the more spread out pattern obtained with the Mcd1p/Sccl1 protein (Fig. 2 C), a subunit of the yeast cohesin complex (Michaelis et al., 1997). Mcd1p binds along the entire length of chromosomes, although discontinuously, to establish sister chromatid cohesion (Blat and Kleckner, 1999; Tanaka et al., 1999; Laloraya et al., 2000). The observed profiles suggest either a more restricted set of chromosome association sites for the Rep proteins or, alternatively, the overlap or at least close proximity between nuclear locales occupied by the plasmid and subchromosomal regions. The low resolution of the chromosome spread assay cannot distinguish between these two possibilities. The reporter plasmid present in a log phase population (Fig. 2 D). When Rep1p or Rep2p was expressed individually in a [cir0] strain, no chromosome association was absent in the G1-arrested cells (Fig. 2 D). When Rep1p or Rep2p was expressed individually in a [cir] strain, no chromosomal association of either protein was observed (Fig. 2 E–H). Consistent with the stage-specific expression and binding of the Mcd1p protein to the chromosomes during the cell cycle, Mcd1p–chromosome association was absent in the G1-arrested cells (Fig. 2 B) and a subset of the cells from the log phase population (Fig. 2 D). When Rep1p or Rep2p was expressed individually in a [cir] strain, no chromosomal association of either protein was observed (Fig. 2 E and F). When the two proteins were simultaneously expressed in the absence of a resident STB-containing plasmid (Fig. 2 G) or the presence of one (Fig. 2 H), colocalization of Rep1p with the chromosomes was evident. The tight association of the plasmid with Rep1p and Rep2p and the requirement of both proteins for their colocalization with the chromosomes suggest that this process is functionally relevant to plasmid partitioning. The plasmid and Rep protein patterns are independent of the yeast cohesin complex, as they show no difference between exponentially growing and G1-arrested cells. Because the Rep proteins bind to the STB locus, they may act as match makers in the potential association between the 2 micron plasmid and the chromosomes.

The Mcd1p/Sccl1 protein associates specifically with the STB DNA

The yeast cohesin complex plays a central role in chromosome segregation by establishing sister chromatid pairing during the S phase and maintaining it until chromosomes are ready to be separated during anaphase (Uhlmann and Nasmyth, 1998; Skibbens et al., 1999; Toth et al., 1999; Uhlmann et al., 1999, 2000; Wang et al., 2000; Carson and Christman, 2001). Based on the nearly synchronized segregation of the 2 micron plasmid and the chromosomes observed previously (see Fig. 3 in Velmurugan et al., 2000), we entertained the possibility that the plasmid might utilize the cohesin complex to ensure its stable partitioning. A segregation mechanism based on pairing and unpairing of plasmid clusters would be expected to mimic chromosome segregation in its timing if cohesin were responsible for bridging the clusters.

We employed chromatin immunoprecipitation (ChIP) to test (1) whether a central component of the yeast cohesin complex, Mcd1p, (Guacci et al., 1997; Michaelis et al., 1997) can associate with 2 micron plasmid, and (2) whether such binding (if observed) is discriminatory with respect to specific plasmid sequences. Chromosomal DNA, sheared to an average length of 500 bp, was immunoprecipitated using Mcd1p-directed antibodies and probed for the presence of 2 micron DNA by PCR (Fig. 3, A–C). A 350-bp amplification product signifying STB was yielded by a [cir] strain (Fig. 3 A, lane 2) or a [cir] strain expressing Rep1p and Rep2p and harboring an STB-containing plasmid (Fig. 3 B, lane 2). When the same [cir] host housed an ARS plasmid, the predicted 350-bp ARS-specific DNA product was not detected (Fig. 3 C, lane 2). Negative results were obtained with the [cir] immunoprecipitate and primer pairs specific to ~300-bp regions spanning the 2 micron circle replication origin or internal to the REPI, REP2, and FLP genes (Fig. 3, D–H). The weak signal seen for the origin (Fig. 3 E), which

*Abbreviations used in this paper: 3-AT, 3-aminotriazole; ChIP, chromatin immunoprecipitation; Mcd1p-nc, noncleavable Mcd1p.
Figure 4. **Probing for STB and CEN sequences in chromatin immunoprecipitates obtained with antibodies to Rep1p, Rep2p, Mcd1p, or kinetochore proteins.** The proteins targeted for immunoprecipitation are indicated above the respective experimental panels: Rep1p in A, C, and E; Rep2p in B and F; Mcd1p–HA in D; and Ndc10p–HA and Ndc80p–HA in G and H, respectively. As in Fig. 3, the relevant features of the yeast strains are also shown.

is ~600 bp from STB, was not surprising. Because of the proximity between the origin and STB, some cross-contamination of the two is expected in immunoprecipitates from chromosome fragments of 500 bp average size. The 309-bp PCR product expected for a cohesin binding sequence on chromosome V (Tanaka et al., 1999) was obtained with immunoprecipitates from the [cir0] strain as well as the [cir1] strain containing plasmids with or without STB (Fig. 3, A–H, lane 2).

The ChIP results demonstrate that the Mcd1 protein, and by extension the yeast cohesin complex (see below), associates specifically with the STB region, and this association does not spread significantly beyond STB.

**The association between Mcd1p and the STB locus requires the Rep proteins**

To assess the functional significance of Mcd1p–STB association, we wished to examine the potential role of Rep1p and Rep2p in this event. As a prelude, we wanted to verify the previously inferred interactions of the Rep proteins with STB by ChIP, while simultaneously probing their potential binding to cohesin targets on the chromosomes.

The results displayed in Fig. 4 (A–D) were obtained from a [cir1] strain harboring an STB-containing plasmid. The three variants of this strain contained chromosomal integrations of either REPI (Fig. 4 A) or REP2 (Fig. 4 B) under the GAL1 or GAL10 promoter, respectively, or both REPI and REP2 (Fig. 4 C) under the GAL1-GAL10 bidirectional promoter. ChIP was performed with antibodies to Rep1p (Fig. 4, A and C) or with antibodies to Rep2p (Fig. 4 B). In galactose-induced cells expressing Rep1p alone, Rep2p alone, or both proteins, the immunoprecipitates revealed the presence of STB (Fig. 4, A–C, lane 2). When the expression of these proteins was turned off with dextrose, the STB DNA was not detected in the mock immunoprecipitates (Fig. 4, A–C, lane 5). The results in Fig. 4 C could be reproduced when Rep2p antibodies were used for immunoprecipitation (unpublished data). Neither Rep1p nor Rep2p antibodies were able to bring down the Mcd1p binding region on chromosome V (Fig. 4, A–C, lanes 2 and 5).

In Fig. 4 D, we arranged the outcomes from three derivatives of a [cir0] strain expressing Rep1p alone, Rep2p alone, or both Rep1p and Rep2p, respectively, from 2 micron circle-based plasmids containing the STB locus. ChIP was performed with Mcd1p-directed antibodies. Presence of the STB DNA was not detected in the immunoprecipitate from cells expressing either protein individually (Fig. 4 D, lanes 2 and 5), but was readily seen in the immunoprecipitate from cells coexpressing Rep1p and Rep2p (Fig. 4 D, lane 8). As expected, the expression of the Rep proteins had no effect on the association between Mcd1p and the cohesin binding sequence from chromosome V (Fig. 4 D, lanes 2, 5, and 8).

No association of Rep1p or Rep2p with three centromeric sequences CEN3, CEN5, and CEN16 was detected in a [cir1] strain when ChIP was performed using Rep1p (Fig. 4 E) or Rep2p antibodies (Fig. 4 F). Conversely, antibodies to the kinetochore-associated proteins Ndc10p and Ndc80p failed to bring down STB, whereas both antibodies precipitated the CEN3 sequence (Fig. 4, G and H).

The sum of the results from Fig. 4 not only establishes the specificity of STB in the association of Mcd1p with the 2 micron plasmid but also reveals the absolute requirement of both Rep1p and Rep2p for this association, even though Rep1p can bind to STB in the absence of Rep2p and vice versa (Velmurugan et al., 1998; Fig. 4, A and B). Taken together, these data suggest that the Rep proteins act cooperatively to recruit the yeast cohesin complex to the partitioning locus of the 2 micron plasmid. Furthermore, the failure of the Rep proteins to bring down CEN sequences and of the centromere binding proteins to bring down STB indicate that the association of the plasmid directly to the kinetochore is unlikely.

As a corollary, STB and chromosomal cohesin binding sites are not functionally equivalent. Obviously, the Rep proteins have no role in cohesin assembly on the chromosomes. At least the one cohesin target tested here is not
bound by the Rep1 protein, either on its own or in the presence of the Rep2 protein. There is a caveat, though. Approximately 1.3 × 10^3 cohesin binding sites exist in the yeast genome (roughly one per 9 × 10^3 bp; Laloraya et al., 2000). If Rep1 binds to these sites randomly and with roughly equal probabilities, the relative abundance of the target site probed in our assays would be 7.7 × 10^-4. This large dilution effect could potentially have interfered with its detection. However, given the sensitivity of the PCR amplification step, we consider this to be an unlikely prospect.

Requirement of the Rep proteins for Mcd1p–STB interaction is corroborated by in vivo monohybrid assays

As a further verification of the ChIP results, we scrutinized the role of the Rep proteins in Mcd1p–STB interaction by an in vivo monohybrid assay. The assay is based on the enhanced HIS3 reporter gene expression via the interaction with STB of a test protein fused to a transcriptional activation domain. Increased production of the His3 protein allows the tester strain to overcome growth inhibition by the His3p-specific inhibitor 3-aminotriazole (3-AT).

In a [cir0] host strain, Mcd1p (fused to the activation domain) failed to interact with STB, the 3-AT challenge (10–50 mM) yielding no better growth than the control strain containing the empty vector (Fig. 5 A, compare columns 1 and 2). As expected from its ability to bind STB in a [cir+] background, Rep1p conferred 3-AT resistance (Velmurugan et al., 2000; Fig. 5 A, column 3). Coexpression of the Mcd1p fusion protein with Rep1p alone or Rep2p alone also failed to induce 3-AT resistance at 25 and 50 mM concentrations of the drug (Fig. 5 A, columns 4 and 5). The presence of all three proteins simultaneously in the same cell was required for growth at 25 and 50 mM 3-AT (Fig. 5 A, column 6). In a [cir+] strain, the Mcd1p hybrid was active by itself, the Rep1 and Rep2 proteins being supplied by the native 2 micron circles (Fig. 5 A, column 8).

The agreement between the ChIP and monohybrid assays validates the inference that both Rep1p and Rep2p are mandatory for Mcd1p–STB association. The involvement of all three components of the Rep–STB system in Mcdp1 (and likely cohesin) recruitment suggests that this step is relevant to equipartitioning of the 2 micron plasmid.

Association of Smc1 and Smc3 proteins with STB: their absence disrupts Mcd1p–STB association

Does the Mcd1p–STB interaction truly reflect the recruitment of the cohesin complex by the plasmid partitioning system? We repeated the ChIP assays with antibodies to two other integral cohesin components, Smc1p and Smc3p (Fig. 5 B). As was the case with Mcd1p, both Smc1p and Smc3p associated with STB in a [cir+] strain (Fig. 5 B, lanes 2 and 8) and not in a [cir+] strain (Fig. 5 B, lanes 5 and 11), suggesting that the process requires the Rep proteins supplied by the endogenous 2 micron plasmid. The chromosomal target site was indifferent to the presence or absence of the plasmid (Fig. 5 B, lanes 2, 5, 8, and 11). In host strains carrying temperature-sensitive smc1-2 and smc3-42 alleles, Mcd1p–STB association was normal at the permissive temperature (Fig. 5 C, lanes 2 and 8) but absent at the nonpermissive temperature (Fig. 5 C, lanes 5 and 11). As expected, the chromosomal association of Mcd1p followed suit in this case (Fig. 5 C, lanes 5 and 11).

The interaction of the STB locus with individual components of cohesin and their interdependence in establishing this interaction argue for the recruitment of the whole cohesin complex to the 2 micron plasmid. Yet the plasmid differs from the chromosomes in the mode of cohesin acquisition and in the DNA locale that functions as the cohesin recipient.

Binding of Mcd1p to STB as a function of cell cycle progression

The cohesin complex physically bridges sister chromatids as each chromosome is duplicated (Uhlmann and Nasmyth, 1998), and maintains this cohesion from the S phase to the late G2/M phase (Uhlmann et al., 1999). The parallels between the replication and segregation properties of the chromosomes and the 2 micron plasmid have encouraged us to...
test whether the plasmid also behaves similarly in its cell cycle–dependent association with Mcd1p.

G1-blocked [cir−] cells were released from α factor arrest at time zero and allowed to resume the cell cycle in pheromone-free medium. Cell aliquots were sampled at start (time zero) and at 15-min intervals thereafter by ChIP, light microscopy, and FACS® analysis (Fig. 6). The combined results indicated that Mcd1p binding to chromosomal sites and to STB DNA is essentially coincident during the cell cycle. The respective associations, established after the onset of the S phase, spanned the S to G2/M window. The timing of Mcd1p binding to and dissociation from STB observed in synchronously cycling cells would be consistent with the cohesin complex playing analogous roles in chromosome and plasmid segregation. The plasmid stability system appears to feed into a temporal program preestablished by its host so as to accomplish synchrony of segregation with the chromosomes.

Mechanism of cohesin recruitment by the chromosomes and the 2 micron plasmid can be further distinguished by inappropriate expression of cohesin during the G1 phase

One important issue that is unsettled by the experiments described so far is whether the 2 micron plasmid is actively recruiting cohesin or passively binding cohesin already assembled on chromosomal sites. As a potential means for dissociating the cell cycle synchrony between chromosomes and the plasmid in cohesin association, we induced the expression of Myc-tagged Mcd1p from the GAL1 promoter in cells arrested in G1 with α factor, and performed chromosome spread assays (Fig. 7 A) and ChIP analyses (Fig. 7, B and C). In [cir+] cells, Mcd1p was detected in the chromosome spreads as a narrowly localized spot that was completely included in the Rep1 protein–localizing region (Fig. 7 A, top). An STB-containing reporter plasmid (pSV1), when present in these cells, merged with Mcd1p (Fig. 7 A, middle). In [cir+] cells containing pSV1, neither Mcd1p nor the plasmid could be detected in the chromosome spreads (Fig. 7 A, bottom). Consistent with these observations, Mcd1p antibodies failed to bring down STB or the chromosome V cohesin target in the [cir+] strain (Fig. 7 B, compare lanes 1 and 2). On the other hand, Mcd1p antibodies precipitated STB in the [cir−] background, but not the chromosome V cohesin binding site nor a centromeric sequence (CEN3) (Fig. 7 B, compare lanes 3 and 4 and 5 and 6). In addition, binding of the G1-expressed cohesin to STB was strictly dependent on Smc1p and Smc3p being functional, when assayed in a temperature-sensitive background for either protein (Fig. 7 C, compare lanes 5 and 2).

Thus, when cohesin is overexpressed at an inappropriate phase of the cell cycle (namely G1), bulk chromosomes are free of it (at the chromosome spread level). Similarly, two chromosomal cohesin binding sites (one of them being a high occupancy centromeric region) remain vacant (by the ChIP test). In sharp contrast, a 2 micron–derived plasmid binds cohesin in G1 and displays the bound form in chromosome spreads.

2 micron plasmid segregation is blocked in cells expressing noncleavable Mcd1p

A critical step in triggering metaphase to anaphase transition in the Saccharomyces yeast is the proteolytic cleavage of Mcd1p by the Esp1 endopeptidase by the N-end rule pathway (Rao et al., 2001). When cleavage is prevented, sister chromatids fail to separate from each other (Uhlmann et al., 1999). If the chromosomes and the 2 micron plasmid utilize the cohesin complex for the same end, the noncleavable ver-
sion of Mcd1p (Mcd1p-nc) should interfere with plasmid segregation, presumably by blocking the separation of replicated plasmid clusters. We have tested this hypothesis by following the dynamics of fluorescence-tagged reporter plasmids, with or without STB, in cells expressing Mcd1p-nc.

The haploid [cir+] strain harboring the test plasmids contained a copy of the native MCD1 gene and, in addition, a copy of the variant MCD1-nc placed under the control of the GAL10 promoter. Exponentially growing cells were shifted from dextrose to galactose to induce high level expression of the mutant Mcd1p, and samples were examined by time-lapse fluorescence microscopy. We also analyzed the chromosome behavior in the same host strain lacking a reporter plasmid but containing fluorescence-tagged chromosome III instead. Each set of paired rows in Fig. 8 reports the results from 8 dextrose- and 10 galactose-grown cells.

After shifting to the galactose medium (Mcd1p-nc induced), DNA duplication was normal, but cells were arrested at the large-budded stage with a 2n DNA content, as indicated by FACS® analysis (unpublished data). For chromosome III, 7 out of 10 such cells showed nonseparation of sisters; the corresponding number for nondetached STB plasmid clusters was 8 out of 10 (Fig. 8). In sharp contrast, separation of the ARS reporter plasmid occurred in all of the cells examined (10 out of 10; Fig. 8). In the case of dextrose-grown cells, chromosome III, the 2 micron plasmid, and the ARS plasmid showed separation in every one of the eight cells of each type examined. The results were similar when the assays were done in a synchronized cell population released from α factor arrest (unpublished data). In ~95% of the cells expressing Mcd1p-nc, no separation of the 2 micron plasmid clusters was observed.

The above results are consistent with replicated plasmid clusters being held in direct union by cohesin or being bridged indirectly by cohesin because of plasmid tethering to sister chromatids. In both cases, cleavage of Mcd1p would be a prerequisite for the separation of the plasmid clusters. As explained in the Discussion, we favor the former mechanism of cluster to cluster cohesion.

Discussion
The remarkable stability of the 2 micron plasmid is achieved by a surprisingly simple molecular device consisting of two plasmid-coded proteins and a relatively small partitioning locus. The chromosomes, on the other hand, rely on an elaborate segregation apparatus and multiple biochemical steps to ensure that each progeny cell receives a full complement of the genome. We suggest that the effectiveness of the plasmid stability system derives from its ability to appropriate key components of the chromosome segregation pathway. We base this assertion on the accepted functional role of cohesin in chromosome segregation, the chromosome-independent recruitment of cohesin by the plasmid, and the absolute requirement of the Rep–STB system for this recruitment. We outline two possible models for plasmid segregation within this conceptual framework for future testing.

Coupling between chromosome and 2 micron plasmid partitioning
The tandem missegregation of chromosomes and the 2 micron plasmid in mutant backgrounds that affect chromosome segregation in different ways provides a strong case for potential molecular links between the chromosome and plasmid segregation pathways. One all-encompassing model that accounts for the above results proposes that plasmids are physically tethered to chromosomes, making the two indistinguishable in segregation. The Rep proteins may bring about this bridging by binding to STB on the one hand, and
interacting with proteins that bind to chromosomes on the other. Data from chromosome spreads are consistent with, but do not provide proof for, the tethering model.

Stable transmission by chromosome attachment is a strategy used by mammalian viruses that replicate as extrachromosomal plasmids in host cell nuclei (Harris et al., 1985; Simpson et al., 1996; Lehman and Botchan, 1998; Skiapoulou and McBride, 1998; Ballestas et al., 1999; Cotter and Robertson, 1999; Ilves et al., 1999; Kanda et al., 2001). An Epstein-Barr virus–based stable partitioning system for plasmids has been reconstituted in yeast (Kapoor et al., 2001). Chromosome tethering provides these viral genomes with a safeguard against their exclusion into the cytoplasm during the breakdown of the nuclear envelope. This argu-

Figure 8. Effect of noncleavable Mcd1p on the partitioning of an STB plasmid and an ARS plasmid. Small budded cells from an exponentially growing culture, induced for GFP–Lac repressor expression were followed by time-lapse fluorescence microscopy for 90 min in dextrose medium or for 150 min after shifting to galactose medium. The representative fluorescence patterns at the initial and final time points are shown. For each experiment, the number of cells that displayed a given pattern is expressed as a fraction of the total number of cells assayed.

Figure 9. A model for the role of the cohesin complex in the segregation of the 2 micron plasmid. The clustering of the 2 micron plasmid and the compactness of the cluster are mediated by the plasmid stability system and are not dependent on cohesin. The duplication and partitioning of the plasmid cluster may occur via mechanisms depicted in A or B or variations of these. (A) Cohesin-mediated pairing of replicated plasmid clusters occurs during the S phase as does the pairing of sister chromatids. The plasmid clusters are tethered to sister chromatids, likely by a cohesin-independent mechanism. (B) Cohesin-mediated pairing and unpairing are common to the chromosomes and the plasmid clusters as in A. The plasmids are partitioned, however, without physical attachment to the chromosomes.
The Rep system recruits the yeast cohesin complex to the STB locus: cohesin disassembly late in the cell cycle is a prerequisite for plasmid partitioning

The most significant finding from the present study is that the 2 micron plasmid stability system channels the yeast cohesin complex toward its partitioning. Our results best fit the interpretation that Rep1 and Rep2 proteins act in concert to actively recruit cohesin to the STB locus. The plasmid is not just passively associating with cohesin already loaded at chromosomal locales. When Mcd1p is inappropriately expressed in G1, it can be detected on the 2 micron plasmid but not on the chromosomes by ChIP or in chromosome spreads. However, at the onset of the ensuing S phase, association of cohesin with STB is reinitiated de novo, presumably because of the recycling of the Rep proteins on the STB DNA (unpublished data).

Multiple factors contribute to the cell cycle dependence of cohesin to STB association. Because Mcd1p is absent in G1, the plasmid would be cohesin free, even though STB is binding competent at this stage. STB loses its competence during a narrow window preceding the onset of the S phase, and regains it during early S phase. As a result, during the normal cell cycle, the timing of cohesin binding to the 2 micron circle would be indistinguishable from that of cohesin binding to the chromosomes. Cohesin recruitment to the chromosome and the plasmid, although retaining a conserved theme, differs in the details, including the site as well as the mediators of recruitment. The timing and life span of cohesin–STB association and the requirement for cohesin disassembly late in the cell cycle suggest that the yeast cohesin complex plays fundamentally similar roles in the partitioning of yeast chromosomes and the 2 micron plasmid.

The tandem missegregation of the 2 micron plasmid with the chromosomes caused by defects in the establishment of sister chromatid cohesion (ctf7-203) or the association of centromeres to the cohesin complex (ctf13-30 and ctf4-421 ndc10-2) do not follow directly from our results. Preliminary results suggest that Ctf7p is not essential for plasmid–cohesin association (unpublished data). Perhaps the plasmid may utilize a checkpoint mechanism to abort its partitioning and stay with the bulk of the chromosomes when chromosome missegregation is sensed. Avoidance of a cell bereft of chromatin could be mediated by spindle attachment, by an active transport system unrelated to the spindle, or by association with a subcellular entity that is evenly partitioned at cell division.

In the two models, cohesin provides a gross counting device to partition approximately half the total number of plasmid molecules into each of the two daughter cells. However, a more sophisticated counting mechanism in which the two “sister plasmids” resulting from one duplication event are directed toward opposite cell poles cannot be ruled out.

Persistence of the 2 micron plasmid in yeast

The present study sheds further light on the molecular basis of the evolutionary success of the 2 micron plasmid as a stably propagating extrachromosomal DNA element in yeast. By harboring a replication origin that is functionally equivalent to the chromosomal origins, the plasmid enjoys duplication by the host replication machinery. By pilfering host factors using components of its stability system, the plasmid apparently gains access to the services of a sophisticated partitioning mechanism. And by preserving a recombination-mediated amplification system in readiness, the plasmid ensures that its copy number is maintained at the steady-state value.

Why an active partitioning mechanism for a high copy plasmid?

How does one reconcile the amplification mechanism and the high copy number of the 2 micron plasmid on the one hand with the active partitioning system and the apparently low effective copy number on the other? Perhaps in its early evolutionary history, the plasmid segregated by a random mechanism. The Rep–STB system may have originated more recently in response to a reduction in the effective copy number as a result of plasmid clustering. Why does yeast still maintain a high copy extrachromosomal element that apparently makes no contribution to its fitness? The built-in sophistication of the strategies for plasmid maintenance suggests that the plasmid might have, at one time, conferred a significant selective advantage on its host. And paradoxically, this very sophistication may make it hard and slow for yeast to get rid of the plasmid now.

Plausible models for plasmid segregation

We suggest two general models for cohesin-mediated plasmid segregation that need not be mutually exclusive. Because of the timing and half-life of plasmid–cohesin association, both models assume that replicated plasmid clusters are bridged by cohesin.

In model I (Fig. 9 A), cohesin facilitates pairing between the two duplicated plasmid clusters that, in turn, are tethered to a pair of sister chromatids. Because a single plasmid cluster forms the segregation unit, attachment of the duplicated clusters to chromosomes in a random fashion cannot mediate stable partitioning. The coincident dissolution of the cohesin bridge between the sister chromatids and the plasmid clusters would dispatch each cluster in opposite directions in association with the chromosomes. The plasmid–chromosome attachment could be mediated by cohesin itself, although this is unlikely. Its disassembly at the time of segregation would negate the hitchhiking scheme, unless the cohesin bridge between plasmid and chromosome is selectively resistant to disassembly, or its dissolution is delayed until after segregation. If the chromosome spreads are boldly interpreted as evidence for plasmid–chromosome tethering, the observed G1 pattern would exclude cohesin from being the tether. By the same argument, cohesin would be exempt from any role in the primary clustered organization of the plasmid.

In model II (Fig. 9 B), the two postreplication plasmid clusters are bridged by the cohesin complex but are not tethered to chromosomes. Upon disassembly of cohesin, each unpaired plasmid cluster moves to opposite cell poles without assistance from the chromosomes. This movement may be mediated by spindle attachment, by an active transport system unrelated to the spindle, or by association with a subcellular entity that is evenly partitioned at cell division.

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Materials and methods

Strains, plasmids, and growth conditions

Standard tools of yeast genetics were employed for construction of strains with desired genotypes (Adams et al., 1997). Cells were grown in YPD or in SD dropout media at 30°C, unless otherwise mentioned.

Strains expressing the hybrid proteins Nup49–CFP and Tub1–YFP were constructed as follows. A PCR-amplified CFP DNA cassette was inserted at the NUP49 locus as described by Wach et al. (1997). A TUB1–YFP fusion cassette was integrated at the URA3 locus such that the hybrid gene was expressed from the TUB1 promoter. CFP and YFP formed the carboxy-terminal portions of the fusion proteins. The plasmids pDH3 and pDH5 obtained from the Yeast Resource Center (http://depts.washington.edu/yeastrc) provided the templates for the construction of the engineered strains. Yeast Escherichia coli shuttle plasmids with desired properties were constructed by routine procedures (Sambrook and Russell, 2001).

Chromosome spreads

Chromosome spreads from mitotic cells were prepared by following the procedure of Nairz and Klein (1997) with minor modifications. 20 μl of the yeast spheroplasts were mixed gently with 40 μl of 4% paraformaldehyde/hyde-3.4% sucrose and 80 μl of 1% lipso, and spread on glass slides. Af- after overnight incubation at room temperature, the slides were washed twice with 0.4% photol-200 (Kodak) and once with 1× PBS before im- munofluorescence assay.

Immunofluorescence

Chromosome spreads were first blocked with 1 mg/ml BSA for 15 min at room temperature. Primary antibody was added and incubated in a humid chamber at room temperature for 3 h. The slides were washed with 1× PBS, and incubated with the secondary antibody conjugated to a fluores- cent dye for 1 h at 26°C. 1 μg/ml DAPI in 1× PBS was used as the DNA stain. Slides were mounted with mounting media and cover glass, and ex- amined by fluorescence microscopy.

ChIP

ChIP assays were performed as described by Saitoh et al. (1997) with some modifications as described by Kang et al. (2001).

PCR amplification

An aliquot of the immunoprecipitated DNA (usually 1/25 of each sample obtained from 20 OD260 units of cells) was used as a template for PCR de- tection of potential Mad1p binding elements. DNA amplification was per- formed using Taq DNA polymerase, and the PCR products were resolved by electrophoresis in 2% agarose gels containing ethidium bromide for vi- sualization of DNA.

Fluorescence microscopy

Plasmids were visualized by fluorescence microscopy by tagging them in GFP–Lac repressor expressed in yeast (Strait et al., 1997; Velmur- gun et al., 2000). CFP and YFP images of the nuclear membrane and the spindle, respectively, in the appropriately engineered strains were ac- cessed using a Nikon inverted microscope and excitation and emission fil- ters recommended by the manufacturer. Images were captured using a Photometrics Quantix camera from Roper Scientific, and the MetaMorph software from Universal Imaging Corp.

Other procedures

Monohybrid genetic assays and cell cycle arrest using α-factor followed by re- start were performed as described previously (Velmurgun et al., 1998, 2000).

References

Adams, A., D.E. Gottschling, C.A. Kaiser, and T. Stearns. 1997. Methods in Yeast Genetics. A Laboratory Course Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Ballestas, M.E., P.A. Chatis, and K.M. Kaye. 1999. Efficient persistence of extra- chromosomal KSHV DNA mediated by latency-associated nuclear antigen. Science. 284:641-644.

Biggins, S., F.F. Severin, N. Bhatta, I. Sassoan, A.A. Hyman, and A.W. Murray. 1999. The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. Genes Dev. 13:532-544.

Blat, Y., and N. Kleckner. 1999. Coheins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. Cell. 98:249-259.

Brosch, J.R., and F.C. Volkert. 1991. Circular DNA plasmids of yeasts. In The Molecular Biology of the Yeast Saccharomyces. Genome Dynamics, Protein Synthesis and Energetics. J.R. Brosch, J.R. Pringle, and E.W. Jones, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 297-331.

Carson, D.R., and M.F. Christman. 2001. Evidence that replication fork compo- nents catalyze establishment of cohesion between sister chromatids. Proc. Natl. Acad. Sci. USA. 98:8270-8275.

Chan, C.S., and D. Botstein. 1993. Isolation and characterization of chromosone gain and increase-in-ploidy mutants in yeast. Genetics. 135:677-691.

Cotter, M.A., II, And E.S. Robertson. 1999. The latency-associated nuclear antigen tethers the Kaposi’s sarcoma-associated herpes virus genome to host chromo- somes in body cavity-based lymphoma cells. Virology. 264:254-264.

Doheny, K.F., P.K. Sorger, A.A. Hyman, S. Tugendreich, F. Spencer, and P. Hie- ter. 1993. Identification of essential components of the S. cerevisiae kineto- chore. Cell. 73:761-774.

Futcher, A.B. 1986. Copy number amplification of the 2 micron circle plasmid of Saccharomyces cerevisiae. J. Theor. Biol. 119:197-204.

Futcher, A.B., and B.S. Cox. 1983. Maintenance of the 2 micron circle plasmid in populations of Saccharomyces cerevisiae. J. Bacteriol. 154:612-612.

Goh, P.Y., and J.V. Kilmartin. 1993. NDC10 a gene involved in chromosome seg- gregation in Saccharomyces cerevisiae. J. Cell Biol. 121:503-512.

Guacci, V., D. Koshland, and A. Strunnikov. 1997. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47-57.

Harris, A., B.D. Young, and B.E. Griffin. 1985. Random association of Epstein- Barr virus genomes with host cell metaphase chromosomes in Burkitt’s lympho- plasma-derived cell lines. J. Virol. 56:328-328.

Hsu, J.Y., Z.W. Sun, X. Li, M. Reuben, K. Tatchell, D.K. Bishop, J.M. Gruchow, C.J. Brace, J.A. Caldwell, D.F. Hunt, et al. 2000. Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Gck7/P1 phosphatase in budding yeast and nematodes. Cell. 102:279-291.

Ilves, I., S. Kivi, and M. Ustav. 1999. Long-term episomal maintenance of bovine papillomavirus type 1 plasmid is determined by attachment to host chro- mosomes, which is mediated by the viral E2 protein and its binding sites. J. Virol. 73:4404-4412.

Janke, C., J. Ortiz, J. Lechner, A. Shevchenko, M.M. Magiera, C. Schramm, and E. Schiebel. 2001. The budding yeast proteins Spc24p and Spc25p interact with Ndc80p and Nud2p at the kinetochore and are important for kineto- chore clustering and checkpoint control. EMBO J. 20:777-791.
Jayaram, M., Y.Y. Li, and J.R. Broach. 1983. The yeast plasmid 2 micron circle encodes components required for its high copy propagation. *Cell.* 34:95–104.

Jiang, W., and J. Carbon. 1993. Molecular analysis of the budding yeast centromere/kinetochore. *Cold Spring Harb. Symp. Quant. Biol.* 58:669–676.

Jiang, W., J. Lechner, and J. Carbon. 1993. Isolation and characterization of a gene (CBF2) specifying a protein component of the budding yeast kinetochore. *J. Cell Biol.* 121:513–519.

Kanda, T., M. Otter, and G.M. Wahl. 2001. Coupling of mitotic chromosome tethering and replication competence in Epstein-Barr virus-based plasmids. *Mol. Cell. Biol.* 21:3576–3588.

Kang, J., L.M. Cheeseman, G. Kallstrom, S. Velmurugan, G. Barnes, and C.S. Chan. 2001. Functional cooperation of Dam1, Ipl1, and the inner centromere protein (INCENP)-related protein Sli15 during chromosome segregation. *J. Cell Biol.* 155:763–774.

Kapoor, P., K. Shire, and L. Frappier. 2001. Reconstitution of Epstein-Barr virus-based plasmid partitioning in budding yeast. *EMBO J.* 20:222–230.

Kikuchi, Y. 1983. Yeast plasmid requires a cis-acting locus and two plasmid proteins for its stable maintenance. *Cell.* 35:487–493.

Kim, J.H., J.S. Kang, and C.S. Chan. 1999. S15 associates with the Ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 145:1381–1394.

Laloraya, S., V. Guacci, and D. Koshland. 2000. Chromosomal addresses of the cohesin component Med1p. *J. Cell Biol.* 151:1047–1056.

Lavoie, B.D., K.M. Tuffo, S. Oh, D. Koshland, and C. Holm. 2000. Mitotic chromosome condensation requires Brn1p, the yeast homologue of Barren. *Mol. Biol. Cell.* 11:1293–1304.

Lehman, C.W., and M.R. Botchan. 1998. Segregation of viral plasmids depends on tethering to chromosomes and is regulated by phosphorylation. *Proc. Natl. Acad. Sci. USA.* 95:4338–4343.

Michaels, C., R. Ciskov, and K. Nasmyth. 1997. Cohesins: chromosomes proteins that prevent premature separation of sister chromatids. *Cell.* 91:35–45.

Nairz, K., and F. Kleijn. 1997. mei 115-a yeast mutation that blocks double-strand-break processing and permits nonhomologous synopsis in meiosis. *Genes Dev.* 11:2272–2280.

Osypenski, L.I., S.J. Eldridge, and B.R. Brinkley. 1999. New yeast genes important for chromosome integrity and segregation identified by dosage effects on genome stability. *Nucleic Acids Res.* 27:3001–3008.

Osypenski, L.I., O.A. Cabello, and B.R. Brinkley. 2000. Chromosome condensation factor Brn1p is required for chromatin separation in mitosis. *Mol. Biol. Cell.* 11:1305–1313.

Rao, H., F. Uhmann, K. Nasmyth, and A. Varshavsky. 2001. Degradation of a cohesin subunit by the N-end rule pathway is essential for chromosome stability. *Nature.* 410:955–959.

Reynolds, A.E., A.W. Murray, and J.W. Szostak. 1987. Roles of the 2 micron gene products in stable maintenance of the 2 micron plasmid of *Saccharomyces cerevisiae*. *Mol. Biol. Cell.* 7:3566–3573.

Russell, I.D., A.S. Granell, and P.K. Sorger. 1999. The unstable F-box protein p58-Ctf13 forms the structural core of the CBF3 kinetochore complex. *J. Cell Biol.* 145:935–950.

Saitoh, S., K. Takahashi, and M. Yanagida. 1997. Mis6, a fission yeast inner centromere protein, acts during G1/S and forms specialized chromatin required for equal segregation. *Cell.* 90:131–143.

Sambrook, J., and D.W. Russell. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Scott-Drew, S., and J.A. Murray. 1998. Localization and interaction of the protein components of the yeast 2 micron circle plasmid partitioning system suggest a mechanism for plasmid inheritance. *J. Cell Sci.* 111:1779–1789.

Sengupta, A., K. Blomqvist, A.J. Pickert, Y. Zhang, J.S. Chew, and M.J. Dobson. 2001. Functional domains of yeast plasmid-encoded Rep proteins. *J. Bacteriol.* 183:2306–2315.

Simpson, K., A. McGuigan, and C. Huxley. 1996. Stable episonal maintenance of yeast artificial chromosomes in human cells. *Mol. Cell. Biol.* 16:5117–5126.

Skiodopoulou, M.H., and A.A. McBride. 1998. Bovine papillomavirus type 1 genomes and the E2 transactivator protein are closely associated with mitotic chromosome. *J. Virol.* 72:2079–2088.

Skibbens, R.V., L.B. Corson, D. Koshland, and P. Hieter. 1999. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes Dev.* 13:307–319.

Straight, A.F., W.F. Marshall, J.W. Sedat, and A.W. Murray. 1997. Mitosis in living budding yeast: anaphase A but no metaphase plate. *Science.* 277:574–578.

Strunnikov, A.V., J. Kingsbury, and D. Koshland. 1995. CEP3 encodes a centromere protein of *Saccharomyces cerevisiae*. *J. Cell Biol.* 128:749–760.

Strunnikov, A.V., L. Aravind, and E.V. Koonin. 2001. *Saccharomyces cerevisiae* SMT4 encodes an evolutionarily conserved protease with a role in chromosome condensation regulation. *Genetics.* 158:95–107.

Tanaka, T., M.P. Conn, K. Wirth, and K. Nasmyth. 1999. Identification of cohesin association sites at centromeres and along chromosome arms. *Cell.* 98:487–498.

Tanaka, T.U., N. Rachidi, C. Jianke, G. Pereira, M. Galova, E. Schiebel, M.J. Stark, and K. Nasmyth. 2002. Evidence that the Ipl1-S15 (aurora kinase-INCEP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell.* 108:317–329.

Toth, A., R. Ciskov, F. Uhlmann, M. Galova, A. Schleifer, and K. Nasmyth. 1999. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes Dev.* 13:320–333.

Uhlmann, F., and K. Nasmyth. 1998. Cohesion between sister chromatids must be established during DNA replication. *Curr. Biol.* 8:1095–1101.

Uhlmann, F., J. Lottspeich, and K. Nasmyth. 1999. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Sc1. *Nature.* 400:37–42.

Velmurugan, S., X.M. Yang, C.S. Chan, M. Dobson, and M. Jayaram. 2000. Partitioning of the 2 micron circle plasmid of *Saccharomyces cerevisiae*. Functional coordination with chromosome segregation and plasmid-encoded Rep protein distribution. *J. Cell Biol.* 149:553–566.

Volkert, F.C., and J.R. Broach. 1986. Site-specific recombination promotes plasmid amplification in yeast. *Cell.* 46:541–550.

Wach, A., A. Brachet, C. Alberti-Segui, C. Rehischung, and P. Philippsen. 1997. Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast.* 13:1065–1075.

Wang, Z., I.B. Castano, A. De Las Penas, C. Adams, and M.F. Christman. 2000. Pol kappa: a DNA polymerase required for sister chromatid cohesion. *Science.* 289:774–779.

Wigge, P.A., and J.V. Kilmartin. 2001. The Nde80p complex from *Saccharomyces cerevisiae* contains conserved centromere components and has a function in chromosome segregation. *J. Cell Biol.* 152:349–360.

Wigge, P.A., O.N. Jensen, S. Holmes, S. Souez, M. Mann, and J.V. Kilmartin. 1998. Analysis of the *Saccharomyces* spindle pole by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. *J. Cell Biol.* 141:967–977.

Zakian, V.A., B.J. Brewer, and W.L. Fangman. 1979. Replication of each copy of the yeast 2 micron DNA plasmid occurs during the S phase. *Cell.* 17:923–934.