

Faithful segregation of the multicopy yeast plasmid through cohesin-mediated recognition of sisters

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The 2- μ m yeast plasmid, a benign high-copy nuclear parasite, propagates itself with nearly the same fidelity as the chromosomes of its host. Equal plasmid segregation is absolutely dependent on the cohesin complex assembled at the plasmid partitioning locus *STB*. However, the mechanism of cohesin action in the context of multiple plasmid copies, resident within two separate clusters after DNA replication, is unknown. By using “single-copy” derivatives of the 2- μ m plasmid, we demonstrate that recruitment of cohesin at *STB* during S phase indeed translates into cohesion between plasmid molecules. Through binary fluorescence tagging, we reveal that segregation of replicated plasmids occurs in a sister-to-sister fashion. Thus, cohesin serves the same fundamental purpose in plasmid and chromosome segregation.

2- μ m circle | cohesin complex | plasmid cohesion | sister-to-sister segregation

The 2- μ m plasmid, a high-copy extrachromosomal selfish DNA element resident in the yeast nucleus, propagates itself stably with the assistance of a partitioning system (1). The plasmid-coded proteins Rep1p and Rep2p and the *cis*-acting locus *STB* comprise the partitioning system, which is designed to channel central components of the chromosome segregation machinery toward plasmid segregation (2). The histone H3 variant Cse4p, which has so far been thought to be unique to centromeres (3), is present also at *STB* and is essential for equal plasmid segregation (4). The maturation of Cse4p-containing *STB* chromatin into its functional state appears to be mediated through the RSC2 chromatin remodeling complex (4–6). The yeast cohesin complex, required for one-to-one segregation of sister chromatids, is assembled at *STB* in a Rep1p- and Rep2p-assisted manner during early S phase, and this association lasts until anaphase (7, 8). Like chromosome–cohesin association, plasmid–cohesin association also requires the loading factors Scc2 and Scc4 (ref. 9; S. Mehta and M.J., unpublished work). However, in contrast to chromosomes, the plasmid fails to acquire cohesin when the mitotic spindle is disassembled (10). Consistent with a potential role for the RSC2 complex in remodeling *STB* chromatin, inactivation of the complex blocks cohesin assembly on the plasmid (5, 11). The timely recruitment of cohesin at *STB* and its timely disassembly are critical events in 2- μ m-plasmid segregation (7).

An amplification system consisting of the F1p recombinase and its target sites (FRTs) augments the partitioning system in the high-copy persistence of the 2- μ m circle. Under steady state conditions, the amplification system appears to be negatively regulated by a bipartite Rep1p–Rep2p repressor complex (12). Amplification is triggered only when a rare missegregation event leads to a copy-number drop in the plasmid. The generally accepted amplification mechanism is a carefully timed F1p recombination event that converts a pair of bidirectional replication forks into unidirectional ones by DNA inversion (13). The dual rolling-circle replication generates a plasmid concatamer that may be resolved by F1p recombination or homologous recombination.

Despite a copy number of ≈ 40 –60 per cell, the 2- μ m plasmid exists as a single tight-knit cluster of foci and segregates as a cluster (14, 15). This copy-number reduction, effectively to unity, makes an active partitioning mechanism imperative for stable propagation.

Based on the role of cohesin in chromosome segregation, one might imagine that cohesin assembly pairs duplicated plasmid clusters and cohesin cleavage triggers their disjunction (7). However, there is no direct evidence for this “pairing–unpairing” mode of plasmid segregation. Furthermore, it is not clear how pairing might be effected between twin clusters, each of which contains multiple plasmid copies. The question regarding whether plasmid clusters segregate in a chromosome-tethered state, by hitchhiking on sister chromatids, or do so independently of chromosomes remains open.

The multicopy nature of the plasmid and its clustered state have so far precluded direct demonstration of plasmid cohesion and subsequent separation. Assuming that pairing by cohesin does occur, two related questions become important: (i) Are replicated plasmid molecules randomly organized into two roughly equal clusters and then paired by cohesin assembled at *STB*? In this scheme, the intercluster pairing may be limited to a subset of plasmid molecules within the individual clusters. (ii) Alternatively, does each plasmid molecule become paired with its sister molecule in a replication-coupled manner? Neither model requires cohesin for plasmid clustering *per se*, which is likely mediated through the Rep proteins in conjunction with associated host factors. Consistent with this notion, the clustered plasmid organization is not altered in G₁-arrested cells even though they lack cohesin at *STB* (7, 15).

Using single-copy 2- μ m-circle-derived reporter plasmids, we now provide concrete evidence for plasmid pairing by cohesin assembly and unpairing by cohesin disassembly. In addition, our results strongly favor a segregation mechanism in which each plasmid molecule is paired with its sister molecule. Thus, the 2- μ m circle cluster is fundamentally analogous to a yeast chromosome in its mechanism of segregation.

Results

Single-Copy Derivatives of 2- μ m Circle Reporter Plasmids. In reporter plasmids containing both *STB* and a centromere, the latter is dominant in copy-number control. By incorporating *CEN3* into *STB* plasmids (Fig. 1A), we could bring down their copy number to unity or close to unity in >80% of the cell population. A *CEN*–*STB* plasmid, when tagged by fluorescence, appeared as a single focus in the nucleus, analogous to standard *CEN* plasmids [supporting information (SI) Fig. 7A]. The “unit-copy” status was further ascertained by Southern blot analysis (SI Fig. 7B).

Two *CEN*–*STB* reporter plasmids used in this study (Fig. 1A) harbored either the LacO (256 copies) or TetO (112 copies) arrays, and could be fluorescence-tagged in cells expressing GFP–LacI or RFP–TetR, respectively. The centromeres in these plasmids could be conditionally inactivated by driving transcription through them from the inducible *GAL1* promoter (16). As a result, in galactose

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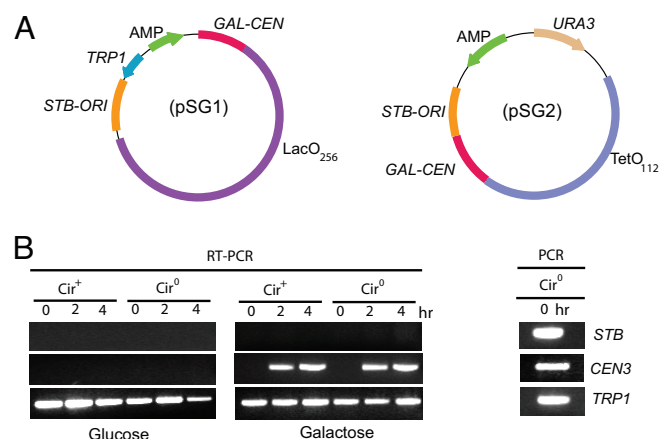


Fig. 1. *CEN-STB* containing single-copy derivatives of 2- μ m circle reporter plasmids. (A) The plasmids pSG1 and pSG2 contain, in addition to the 2- μ m circle replication origin and *STB*, the *CEN3* sequence flanked by the *GAL* promoter and the *CYC1* terminator; pSG1 harbors a [LacO]₂₅₆ array, and pSG2 harbors a [TetO]₁₁₂ array. (B) Total RNA from [cir⁺] and [cir⁰] strains harboring pSG1 was isolated after transferring raffinose-grown log-phase cells to medium containing glucose or galactose for the indicated times. RT-PCR was performed by using primers specific to the *CEN3*, *STB*, and *TRP1* sequences. A DNA sample isolated from the [cir⁰]/pSG1 strain before galactose induction was amplified with the same primers as those used in the RT-PCR assays to provide the reference bands.

medium, their segregation was under *STB* control, provided the Rep1 and Rep2 proteins were supplied in trans. We confirmed that transcription through the centromere was galactose dependent (Fig. 1B). The mitotic stability of the reporter plasmids in a [*cir*⁰]

host strain (lacking Rep1 and Rep2 proteins) was nearly 70% after 10 generations of nonselective growth in glucose and close to 5% after similar growth in galactose, verifying transcriptional inactivation of the centromere (results not shown).

The data from the experiments detailed in the sections to follow were effectively restricted to single-copy plasmids by excluding from the analyses those cells that contained more than two foci of a particular fluorescence type (either green or red). For example, in cohesion assays in metaphase cells, two fluorescent dots would indicate predominantly unpaired, although replicated, plasmids with perhaps an insignificant contribution from two unreplicated plasmid copies. Conversely, a single fluorescent dot would signal cohesion between replicated plasmid molecules, with negligible contamination due to replication failure.

Pairing of Replicated Plasmid Molecules: Does Cohesin Assembled at *STB* Mediate Plasmid Cohesion?

cohesion is assayed in G₂/M-arrested cells after microtubule depolymerization by using an antimitotic drug. The chromosomes are thus free from pulling forces exerted by the spindle. However, the test for cohesion between replicated plasmids was carried out in normally cycling cells because microtubule integrity is essential for cohesin assembly at STB (10).

Isogenic [cir⁺] and [cir⁰] wild-type strains harboring the *STB*–*CEN* reporter were released from G₁ arrest to traverse the cell cycle at 30°C (Fig. 2A) under conditions where (i) the centromere and *STB* were active, (ii) *STB* alone was active, and (iii) neither the centromere nor *STB* was active. Plasmid fluorescence, as well as DAPI staining patterns scored at progressive intervals in the assay, was grouped into four types (classes I–IV, Fig. 2B). Cells completed DNA replication by 60 min in glucose medium and 90 min in galactose medium, as judged by FACS analysis (Fig. 2C and D). At

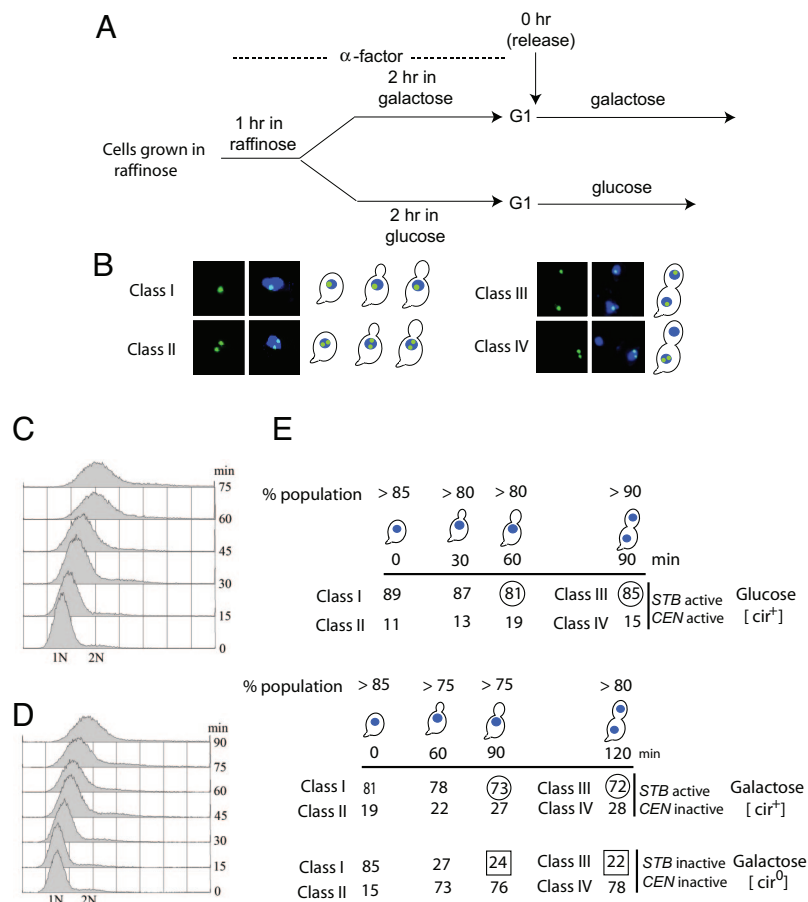


Fig. 2. Plasmid cohesion mediated through *STB* or *CEN* during metaphase in cycling cells. (A) The experimental regimen for cell cycle arrest and release is schematically outlined. (B) The four representative classes of cells scored in the assay are displayed. Classes I and II are preanaphase cells showing one and two fluorescent plasmid foci, respectively. Classes III and IV are postanaphase cells containing 1:1 and 2:0 patterns of plasmid segregation, respectively. (C and D) DNA replication was followed by FACS analysis. (E) The cell populations were assayed at the indicated times after release from G₁ arrest and binned into classes I–IV.

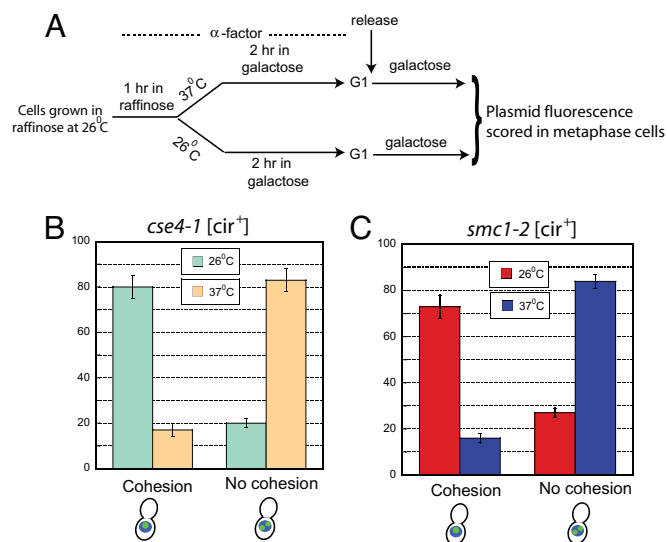


Fig. 3. Plasmid cohesion in *cse4-1* [cir⁺] and *smc1-2* [cir⁺] cells at permissive and nonpermissive temperatures. (A) The protocol for assaying plasmid cohesion is schematically indicated. (B and C) The fractions of metaphase cells displaying one fluorescent dot (cohesion) or two fluorescent dots (lack of cohesion) were estimated in the *cse4-1* and *smc1-2* strains at 26°C and 37°C.

these time points, the majority of cells (>75%) displayed an enlarged bud with a single DAPI staining zone near the bud neck within the mother. Among such cells, deemed to be in metaphase, ≈70–80% revealed plasmid pairing (class I; single fluorescence dot) when *STB* alone was active or both *STB* and *CEN3* were active (circled numbers in column 3 of Fig. 2E). This fraction dropped to 24% when both *CEN3* and *STB* were inactive (boxed number in column 3 of Fig. 2E).

At 90 min and 120 min into the cell cycle in glucose and galactose, respectively, the population comprised almost entirely (80–90%) of late anaphase or telophase cells. Among these, >80% showed equal plasmid segregation (class III) when *CEN* and *STB* were active, and >70% showed equal plasmid segregation when *STB* alone was active (circled numbers in column 4 of Fig. 2E). Lack of an active *CEN* or *STB* resulted in a precipitous fall in equal segregation to ≈20% (boxed number in column 4 of Fig. 2E).

When the Rep-*STB* system is functional, replicated plasmid molecules remain paired during metaphase and become unpaired during anaphase. The timing of these events suggests that cohesin assembly and disassembly are responsible for pairing and unpairing, respectively.

The assays carried out in [cir⁺] cells examine a single-copy fluorescent reporter plasmid resident within a cluster of native 2-μm circles that remain invisible in the analysis. By contrast, the assays in [cir⁰] cells report on a true single-copy plasmid. The observed differences in plasmid cohesion between [cir⁰] and [cir⁺] strains were unrelated to copy number. The results from the [cir⁰] strain were similar to those from a strain harboring a multicopy 2-μm-circle derivative lacking the *REP1* gene (data not shown).

We have verified that inactivating *STB* in the *GAL-CEN* and *STB*-containing reporter plasmid is functionally equivalent to deleting it. The cohesion and segregation patterns of a *GAL-CEN* plasmid (lacking *STB*) in either a [cir⁺] or a [cir⁰] strain were similar to those of the *GAL-CEN* plus *STB* reporter resident in a [cir⁰] strain (inactive *STB*). The percentage of metaphase cells showing plasmid cohesion was roughly 75% in glucose for both reporters (active *CEN*) (data not shown); this value dropped to nearly 20% in galactose (inactive *CEN*) (Fig. 2E).

Tanaka *et al.* (17) noted that duplicated copies of a fluorescence-tagged minichromosome harboring a 130-bp minimal centromere

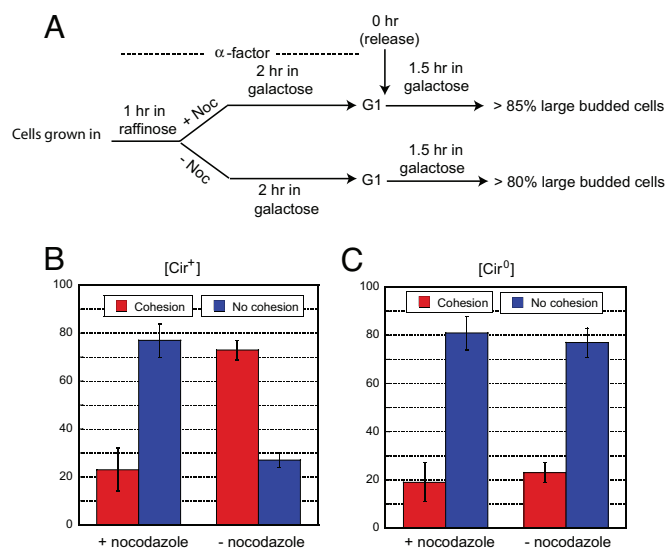


Fig. 4. Spindle integrity in the establishment of plasmid cohesion. (A) The effect of spindle disassembly on plasmid cohesion was followed according to the outlined experimental procedure. (B and C) The frequencies of one fluorescent plasmid dot (cohesion) or two fluorescent plasmid dots (lack of cohesion) were estimated in metaphase cells during the cell cycle (– nocodazole) or in cells arrested at G₂/M (+ nocodazole).

were precociously separated in more than half of the metaphase cells due to spindle force. We observed this phenotype in ≈20% of the metaphase cells when the centromere was functional. Perhaps the larger (320 bp) *CEN* fragment housed by our reporter plasmids, the simultaneous presence of *STB* and *CEN* in them, or some feature of the *GAL*-promoter-controlled *CEN* could have partially mitigated the spindle force.

Cohesin Is the Agent of Plasmid Pairing, and Plasmid Separation Follows Cohesin Disassembly. To critically verify the notion that pairing of replicated plasmid copies is cohesin mediated, we exploited previously established features of plasmid-cohesin association. Cohesin recruitment at *STB* is absolutely dependent on Rep1 and Rep2 proteins (7, 11). The *STB* chromatin harbors the histone H3 variant Cse4p (4), and inactivation of Cse4p by a temperature-sensitive mutation blocks *STB*–cohesin association. Will disrupting cohesin assembly at *STB* or directly inactivating cohesin result in lack of cohesion between replicated plasmids?

We know that withdrawal of Rep proteins (in the [cir⁰] background) results in lack of plasmid cohesion in metaphase cells (Fig. 2E). We also assayed plasmid cohesion in a [cir⁺] host strain expressing the *cse4-1* allele (18). This mutant allele, like *cse4-107* tested previously (4), fails to support cohesin assembly at *STB* at 37°C (S.H. and M.J., unpublished data). Cells released from G₁ arrest into galactose medium after centromere inactivation on the reporter plasmid (Fig. 3A) completed DNA replication by 100 min and 75 min at 26°C and 37°C, respectively (inferred by FACS analysis; data not shown). The plasmid fluorescence pattern was scored at these time points in “metaphase” cells as described for the assays shown in Fig. 2. The single-dot pattern (cohesion) was predominant over the two-dot pattern (no cohesion) at 26°C, whereas the reverse was true at 37°C (Fig. 3B). Thus, inactivation of Cse4p, which blocks *STB*–cohesin association, also prevents plasmid cohesion. Consistent with the notion that plasmid cohesion is a central step in equal plasmid segregation, at 37°C the *cse4-1* mutation caused a much larger proportion of 2:0 segregation (≈70%) relative to 1:1 segregation (SI Fig. 8). At 26°C, the principal mode of segregation was 1:1 (≈80%).

For testing the effect of disabling a cohesin component on

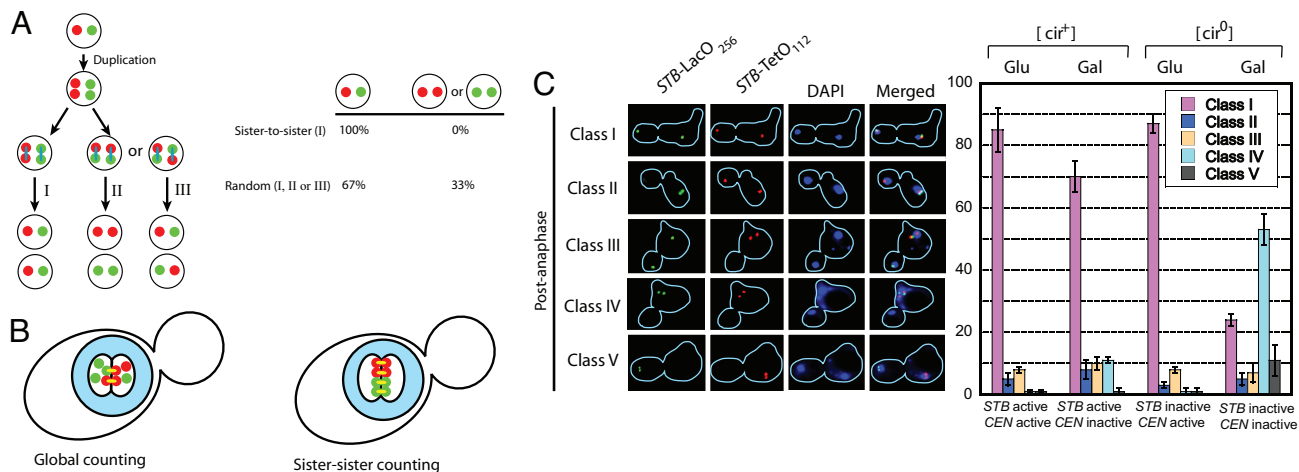


Fig. 6. A sister-to-sister binary counting mechanism for the segregation of the 2- μ m plasmid. (A) The possible modes of cohesin-mediated plasmid counting and segregation are schematically diagrammed. Replication of the single-copy reporter plasmids (one tagged by green fluorescence and the other by red fluorescence) generates two green and two red plasmid molecules. A strict sister-to-sister counting mode will pair red with red and green with green (I). A random counting mechanism may follow the pairing scheme I or mediate red-to-green pairings as indicated by II and III. Segregation according to I will not generate daughter nuclei containing two red or two green plasmids. However, equal probabilities of I, II, and III segregation modes will result in 33% frequency of such nuclei. (B) The distribution and organization of duplicated plasmid molecules according to the global counting mechanism (Left) and the sister-to-sister counting mechanism (Right) are schematically represented. The cohesin bridge (yellow) that pairs plasmid molecules (red and green) defines the boundary between the sister clusters that separate from each other upon cohesin disassembly. (C) Plasmid segregation was assayed in postanaphase cells under conditions where the plasmid-borne *CEN* and *STB* were both active, only one of the two was active, and neither one was active. The observed patterns were divided into classes I–V. Class I, with each prescient daughter cell containing one red and one green plasmid, is the only class predicted by the sister-to-sister segregation mode. Note that, even with an active centromere, class I was <100%. Class V, with would-be daughter cells containing two red or two green plasmids, is excluded by sister-to-sister segregation; however, it is a significant entity (33%) according to the random pairing mode. Note the rarity of class V in the presence of an active *CEN* or *STB*. Classes II–IV signify 3:1 and 4:0 missegregation events.

containing a single DAPI mass in the mother cell compartment. At 60 min, nearly all of the cells had traversed anaphase, with a small fraction having completed cytokinesis. As expected, cohesin was absent from *STB* at this time point. In cells displaying clearly segregated chromosomes, as indicated by DAPI staining, an *STB* plasmid (red) showed a high incidence of missegregation (2:0; cell types I, II, and V in Fig. 5D). By contrast, a coexisting *CEN* plasmid (green) segregated equally (1:1) in most of the cells (except cell types III and V in Fig. 5D).

The lack of a functional spindle at the time of replication aborts plasmid cohesion and causes high rates of plasmid missegregation. Because chromosomal cohesion is spindle-independent, once the spindle is reorganized, paired sister chromatids and *CEN* plasmids go on to segregate equally.

Segregation of Plasmid Occurs in a Sister-to-Sister Fashion. How are replicated plasmid molecules, which comprise a population of identical individuals, chosen for pairing by cohesin? Two distinct models can be envisaged (Fig. 6 *A* and *B*). In a global and approximate counting model, roughly equal pools of plasmid molecules are organized into two clusters at completion of DNA replication. The two clusters become bridged when cohesin assembled at *STB* pairs plasmid molecules across them. In this scheme, not every *STB* need participate in the bridging event (Fig. 6*B Left*). In an alternative precise counting model, each plasmid molecule is paired with its sister during the process of replication, and the two sisters then occupy separate clusters (Fig. 6*B Right*). In other words, the sisterhood between the duplicated clusters as a whole applies to every individual molecule within them. One feature common to both models is that the cohesin bridge defines the boundary between the two clusters. In their cohesed metaphase context, the clusters are referred to as “two” in a prescient sense, anticipating their binary split and segregation upon cohesin cleavage. Does 2- μ m-circle segregation conform to one of the two mechanisms diagrammed in Fig. 6 *A* or *B*?

We introduced two variants of the single-copy *STB*-reporter plasmids, pSG1 and pSG2 (see Fig. 1A), into isogenic [*cir*⁺] and

[*cir*⁰] strains expressing both GFP-LacI and RFP-TetR. Each plasmid could be distinguished by its fluorescence tag, green for pSG1 and red for pSG2. After releasing G₁-arrested cells into a synchronous cell cycle, plasmid cohesion and plasmid segregation were assayed. As expected, cohesion was observed in most metaphase cells when either *CEN* or *STB* was active or when both were active (SI Fig. 11). There was a clear lack of cohesion when neither was active. We then examined the red (R)-to-green (G) segregation profiles in postanaphase cells with clearly separated chromosomes (Fig. 6B). The 1:1 green/1:1 red (RG:RG) (class I) pattern was dominant when at least one of the two, *CEN* or *STB*, was functional. In sharp contrast, the incidence of 2:0 green/0:2 red (RR:GG) (class V) was quite low. The 3:1 and 4:0 unequal segregation patterns (RRG:G, GGR:R, and GGRR:0) (classes II–IV) were also highly infrequent. *CEN* (or the *CEN*–*STB* combination) was slightly superior to *STB* in effecting the faithful R-to-R and G-to-G segregation, ≈80–85% versus 70%. In the [*cir*⁰] host strain, with neither *CEN* nor *STB* being active, the segregation became largely unequal, with a strong tendency for both green and red to stay together in the mother cell (class IV). Of particular significance is the result that the class V (RR:GG) pattern, which should be a sizable fraction (33%) according to the random counting model (Fig. 6A), was almost nonexistent during *STB*-mediated segregation. It was as low as that encountered during *CEN*-assisted segregation.

The data from the population assays presented above were consistent with time-lapse analysis of red-green segregation carried out on individual cells [see SI Fig. 12 and time-lapse movies (SI Movies 1–3)]. The *STB*-mediated sister-to-sister segregation observed in the [cir⁺] strain was not conditioned by the reporter plasmids resident within a larger cluster of native 2- μ m circles. Nearly identical results were obtained when the two-color segregation was assayed in a [cir⁰] strain expressing Rep1 and Rep2 proteins from the bidirectional *GAL1*–*GAL10* promoter (SI Fig. 13).

In summary, the mechanisms for *CEN*-based and Rep-*STB*-

based faithful propagation of chromosome and plasmid, respectively, share the common logic of cohesin-mediated sister-to-sister segregation.

Discussion

The stable persistence of the 2- μ m plasmid in yeast derives from its ability to appropriate components of the chromosome segregation machinery and channel them for its own partitioning (1, 4, 7, 20–22). The plasmid partitioning proteins Rep1 and Rep2 mediate the incorporation of the histone H3 variant Cse4p into chromatin at the *STB* locus (4). This special nucleosome tag, regarded until recently to be unique to centromeres, is critical for assembling the plasmid partitioning complex. The maturation of *STB* chromatin into its functionally competent state, apparently mediated by the RSC2 remodeling complex (5, 6, 11), permits the recruitment of the cohesin complex as a key step in equal plasmid segregation. The mitotic spindle strongly influences the plasmid partitioning pathway by promoting the association of host factors, including cohesin, at *STB*. The mechanism by which cohesin promotes equal segregation of multiple plasmid copies arranged into two clusters has been conceptually and technically difficult to address. The work described here provides a rational solution to this problem.

The unit-copy *CEN-STB* reporter plasmids display similar temporal patterns of cohesion and separation during the cell cycle, regardless of whether they segregate using the Rep-*STB* or the *CEN* pathways. Plasmid pairing under a functional Rep-*STB* system is mediated through the cohesin complex. Inactivating cohesin directly or blocking cohesin assembly at *STB* at the appropriate time in the cell cycle abrogates plasmid pairing and leads to missegregation. Plasmid-cohesin association requires the deposition factors Scc2p and Scc4p, and the former has been detected at *STB* by ChIP. Cohesin assembly at *STB* *per se* does not lead to plasmid cohesion, as revealed by inactivation of Ctf7p or the postreplication enlistment of cohesin in cells recovering from nocodazole treatment. Thus, the 2- μ m plasmid is generally similar to chromosomes in recruitment of cohesin and establishment of cohesion (23). However, it differs conspicuously from chromosomes in using its Rep proteins and depending on spindle integrity for funneling cohesin for its selfish needs.

Despite the multicopy and clustered status of the 2- μ m circle, cohesin-mediated pairing of plasmid molecules occurs in a sister-to-sister fashion. This mechanism was suggested by the equal segregation of a fluorescently tagged single-copy reporter plasmid within a cluster of untagged native plasmids and further confirmed by the one-to-one segregation of two reporter plasmids harboring distinct fluorescence tags. The timing of plasmid cohesion during the cell cycle and the coupling between replication and cohesion are consistent with this mechanism. This pairing rule implicates a high-order organization within the plasmid cluster and suggests how that order might be engendered in the sister cluster after DNA replication. A plasmid cluster templates its sister cluster through cohesin-assisted pairing of sister molecules. Current evidence suggests that adhesion of plasmid molecules within a cluster is likely mediated by the Rep proteins, together with host factors other than cohesin (7). The function of cohesin is to bridge the two “would-be” clusters that split asunder in anaphase.

Strictly, our interpretation of sister-to-sister segregation applies to a single-plasmid focus rather than a single-plasmid molecule. Incorporating *CEN* into a reporter plasmid reduces its copy number to nearly unity (not necessarily unity). However, given the similarities established here between cohesin-mediated *CEN* and *STB* segregation mechanisms, sister-to-sister segregation of the 2- μ m circle at the single-plasmid level is a reasonable extrapolation.

The aggregation of the plasmid molecules in association with the partitioning proteins and host factors is not unlike the clustering of centromeres in association with the kinetochore complex. Both these multiDNA/multiprotein assemblies appear to remain congressed throughout the cell cycle. Yet, in the face of these organizational constraints, the plasmid molecules and centromeres replicate efficiently, acquire cohesin, and pair faithfully with their respective sisters. The size of the plasmid entity in segregation ($60 \times 6 = 360$ kbp), roughly one and a half times the size of the smallest yeast chromosome (230 kbp), further accentuates the analogy between sister plasmid clusters and sister chromatids in partitioning. What mediates the opposite movement of the sister clusters during segregation remains an open challenge. Plausible alternatives to be entertained include hitchhiking on sister chromatids, spindle-assisted segregation independent of chromosomes, and cosegregation with a nuclear entity other than chromosomes that evenly partitions between daughter cells.

Materials and Methods

Strains and Plasmids. The yeast strains and plasmids used in this study are listed in [SI Table 1](#). The strains used for ChIP harbored an integrated copy of either the *MCD1* or *SCC2* gene tagged with the HA epitope (ref. 7 and this study).

The reporter plasmids pSG1 and pSG2 were constructed as described in [SI Appendix](#).

RT-PCR. Total cellular RNA was isolated from cultures grown either in glucose or galactose at the indicated time points by using RNeasy kit (Qiagen, Valencia, CA). RT-PCR was performed by using One Step RT-PCR kit (Qiagen) with ≈ 2 μ g of RNA as template per reaction.

Antibodies. Antibodies used in this study are described in ref. 4.

Other Protocols. Synchronized cell populations were obtained by a factor-induced arrest in G₁ phase, followed by release as described in ref. 15. Nocodazole arrest and subsequent release are detailed in refs. 10 and 15. Fluorescence microscopy of reporter plasmids, indirect immunofluorescence assays for cytoskeletal structures, and ChIP followed protocols standardized in prior studies (4, 7, 15).

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