Meiosis in *Saccharomyces cerevisiae* Mutants Lacking the Centromere-Binding Protein CP1

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ABSTRACT

CP1 (encoded by the *CEP1* gene) is a centromere binding protein of *Saccharomyces cerevisiae* that binds to the conserved DNA element I (CDEI) of yeast centromeres. To investigate the function of CP1 in yeast meiosis, we analyzed the meiotic segregation of CEN plasmids, nonessential chromosome fragments (CFs) and chromosomes in *cep1* null mutants. Plasmids and CFs missegregated in 10–20% of meioses with the most frequent type of aberrant event being precocious sister segregation at the first meiotic division; paired and unpaired CFs behaved similarly. An unpaired chromosome *I* homolog ($2N + 1$) also missegregated at high frequency in the *cep1* mutant (7.6%); however, missegregation of other chromosomes was not detected by tetrad analysis. Spore viability of *cep1* tetrads was significantly reduced, and the pattern of spore death was nonrandom. The inviability could not be explained solely by chromosome missegregation and is probably a pleiotropic effect of *cep1*. Mitotic chromosome loss in *cep1* strains was also analyzed. Both simple loss (1:0 segregation) and nondisjunction (2:0 segregation) were increased, but the majority of loss events resulted from nondisjunction. We interpret the results to suggest that CP1 generally promotes chromatid-kinetochore adhesion.

YEAST (*Saccharomyces cerevisiae*) centromeres contain three highly conserved DNA sequence elements, named CDEI, CDEII and CDEIII (centromere DNA element) (FITZGERALD-HAYES, CLARKE and CARBON 1982; HIETER *et al.* 1985). Together, these elements (about 120 bp) comprise the functional centromere and when used to replace the resident centromere of a yeast chromosome are sufficient to provide full meiotic and mitotic centromere function (COTTAREL *et al.* 1989; MURPHY and FITZGERALD-HAYES 1991). CDEI is the degenerate octanucleotide sequence RTCACRTG (R = purine), CDEIII is a partially palindromic sequence 25 bp in length, and CDEII is a 78–86-bp region of highly A + T-rich (>90%) DNA which seems to provide a spacer function (FITZGERALD-HAYES 1987). Mutational analyses have revealed that CDEIII is absolutely essential for mitotic centromere function, while mutations of CDEI and CDEII impair but do not abolish function (CUMBERLEDGE and CARBON 1987; GAUDET and FITZGERALD-HAYES 1987; HEGEMANN *et al.* 1988; MCGREW, DIEHL and FITZGERALD-HAYES 1986; PANZERI *et al.* 1985). Wild-type yeast chromosomes typically are lost about once in 100,000 mitoses (HARTWELL*et al.* 1982). Deletion of CDEI results in a 10–60-fold increase in the rate of mitotic loss (CUMBERLEDGE and CARBON 1987; GAUDET and FITZGERALD-HAYES 1989; PANZERI *et al.* 1985).

Electron micrographs of mitotic yeast nuclei fail to reveal a differentiated kinetochore structure; spindle microtubules seem to attach directly to the chromatin fibers (PETERSON and RIS 1976). But nuclease sensitivity studies of native yeast chromatin show that the centromeric DNA is highly resistant to digestion and flanked on both sides by nuclease hypersensitive sites associated with highly phased nucleosome arrays, leading BLOOM and CARBON (1982) to speculate that the nuclease-resistant core represents a structurally primitive kinetochore. Two centromere-specific DNA binding factors have been identified, one which binds to CDEI and one which binds to CDEIII. The CDEIII-binding factor, CBF3, is actually a complex of three different proteins, at least one of which is phosphorylated (LECHNER and CARBON 1991). CP1 (also known as CBF1) is a relatively abundant protein which binds to CDEI (BAKER, FITZGERALD-HAYES and O'BRIEN 1989; BRAM and KORNBERG 1987; CAI and DAVIS 1989; JIANG and PHILIPPSEN 1989). Isolation of the gene encoding CP1 has revealed that the protein has a molecular weight of 39,000 and is a member of the helix-loop-helix family of DNA-binding proteins (BAKER and MASISON 1990; CAI and DAVIS 1990; MELLOR *et al.* 1990). The CP1 gene has been named *CEP1* (BAKER and MASISON 1990), *CBF1* (CAI and DAVIS 1990) and *CPF1* (MELLOR *et al.* 1990).

Disrupting *CEP1* in haploid strains of yeast has pleiotropic effects (BAKER and MASISON 1990; CAI and DAVIS 1990; MELLOR *et al.* 1990). Mitotic chromosome loss rate is increased and growth rate is decreased. The magnitude of the chromosome loss rate increase is about 10-fold, consistent with the mitotic effect of CDEI deletion but insufficient to

explain the 35% increase observed for cell doubling time. An unexpected phenotype of *cep1* null strains is that they require exogenously added methionine for growth. The basis of the methionine auxotrophy is not known, but it is likely that CP1, in addition to its function as a kinetochore protein, is also a transcription factor like other members of the helix-loop-helix family (MURRE, MCCAW and BALTIMORE 1989). Several genes encoding enzymes of the methionine biosynthetic pathway contain CDEI sites in their promoter regions (THOMAS, CHEREST and SURDIN-KERJAN 1989). The segregational defects in *cep1* strains appear to be caused directly by the lack of CP1 at the centromere. The effects of CDEI mutations on centromere function *in vivo* are quantitatively correlated with the affinity of CP1 binding *in vitro* (BAKER, FITZGERALD-HAYES and O'BRIEN 1989; CAI and DAVIS 1989), and the increases in chromosome loss rate attributable to *cis*- and *trans*-acting mutations (CDEI deletion and *cep1* gene disruption, respectively) are equivalent and nonadditive (BAKER and MASISON 1990).

CUMBERLEDGE and CARBON (1987) observed that plasmids containing CDEI-deleted centromeres displayed significantly increased rates of meiotic missegregation, more than would have been expected given the mitotic effects of the same mutations. The segregational defect observed was precocious sister segregation at the first meiotic division, suggesting that CDEI was somehow involved in maintaining sister chromatid cohesion during meiosis I (CUMBERLEDGE and CARBON 1987). [Similar findings had been obtained previously by PANZERI *et al.* (1985), but interpretation of the results was clouded by the fact that two very similar mutations gave very different meiotic segregation patterns.] The effect of deleting CDEI from the centromere of an endogenous chromosome (chromosome *III*) was also tested. When the mutation was heterozygous, segregation appeared normal, but when both homologs carried the CDEI-deleted centromere, sporulation and spore viability were poor, presumably due to missegregation of chromosome *III* (CUMBERLEDGE and CARBON 1987). A subsequent study by GAUDET and FITZGERALD-HAYES (1989) confirmed the results of CUMBERLEDGE and CARBON with respect to plasmid missegregation, but in the later study no missegregation was observed when one or both chromosomes *III* carried the CDEI-deleted centromere. To study the meiotic role of CP1 directly, we have analyzed meiosis in *cep1* null mutants. The results are quite consistent with the previous findings and indicate that CP1 is important for proper kinetochore function during meiosis.

MATERIALS AND METHODS

Plasmids: Plasmid pDK243 (KOSHLAND, KENT and HARTWELL 1985) was obtained from D. KOSHLAND, plasmid pJS2 (SHERO *et al.* 1991) from P. HIETER, plasmid pEL11 (LOUIS and HABER 1989) from E. LOUIS and plasmid pRIP1 (PARKER and JACBOSON 1990) from R. PARKER. Plasmid pDM8 was constructed by inserting (after Klenow fill-in) the 5.4-kbp *Sal*I-*Sma*I *ade3-2p* fragment from pDK243 into the *Sma*I site of pJS2, inactivating the *SUP11* gene. Plasmid pDM2 was derived from pRIP1 by inserting a Klenow-blunted 2.2-kbp *LEU2* fragment into the *Eco*RV site in *URA3*.

Yeast strains and media: The diploid yeast strains used in this study are listed in Table 1 along with the haploid parents from which they were all derived. All except the chromosome *I*-marked strains (D77-R1, D92 and D93) are congenic to strain 381G (HARTWELL 1980). Strain construction was carried out using standard methods (SHERMAN, FINK and HICKS 1986) and in all cases involved multiple backcrosses. The *cep1::URA3* alleles have been described (BAKER and MASISON 1990). Both are disruption alleles where *CEP1* sequences have been deleted and replaced with *URA3*; their *cep1* phenotypes are indistinguishable. A Ura$^-$ derivative of *cep1::URA3-10*, designated *cep1::ura3*, was obtained by selecting for 5-fluoro-orotic acid resistance. The *trp1::LEU2* allele was obtained by gene transplacement using plasmid pEL11 as described (LOUIS and HABER 1989). Chromosome *I* trisomy was introduced via strain VG31-11C of GUACCI and KABACK (1991). First, *CEP1* was disrupted to obtain strain D77-R1 (Table 1), then D77-R1 was mated with two different 381G-derived parents to obtain trisomic strains D92 and D93. D92 and D93 are thus congenic with each other, but not with the other strains used in the study.

All media were as described (BAKER and MASISON 1990) except for color indicator plates which were synthetic complete medium containing only 6 μg/ml adenine (⅓ normal concentration). Selection for *ura3* mutants was carried out with uracil dropout plates supplemented with 50 μg/ml of uracil and 1.0 mg/ml of 5-fluoro-orotic acid (PCR, Inc., Gainesville, Florida) (BOEKE, LACROUTE and FINK 1984). Sporulation medium was 1% potassium acetate supplemented with adenine, histidine, lysine, tryptophan, tyrosine, leucine, uracil and methionine at one half their normal concentrations. All strains were grown at 30° except for sporulation which was carried out at 22°. Yeast transformations were performed by the lithium acetate procedure (ITO *et al.* 1983).

Generation of chromosome fragments: Chromosome fragment CFIII(D8B.d.D30-18B) was generated by transforming strain D30-18B with *Not*I-cut pDM8 and selecting for uracil prototrophy (GERRING, CONNELLY and HIETER 1991). Several of the transformants had the expected phenotype. They formed pink colonies with rare white sectors, the result of mitotic loss of the chromosome fragment. The Ura and color phenotypes cosegregated. The expected structure of CFIII(D8B.d.D30-18B) is a long arm consisting of the left arm of chromosome *III* distal to the D8B sequence, the centromere region from chromosome *VI*, and a short arm consisting of vector sequences, *URA3* and *ade3-2p* (SHERO *et al.* 1991). Its size is about 150 kbp and for convenience we have designated it CF(*URA3*). A *LEU2* derivative of CF(*URA3*) was obtained by marker change generating CFIII(D8B.d.D30-18B.LEU2). The marker change was accomplished by one-step gene disruption using the *ura3::LEU2* disruption allele of plasmid pDM2. Strain D64-61A was transformed with *Nsi*I-*Nde*I-cut pDM2 DNA selecting for leucine prototrophy. Among the transformants were several that were phenotypically Leu$^+$ and Ura$^-$ and formed pink colonies with rare white sectors; the Leu and color phenotypes cosegregated. For convenience, CFIII(D8B.d.D30–18B.LEU2) is referred to as CF(*LEU2*).

TABLE 1

Yeast strains

Strain	Genotype[a]
381G	*MAT***a** *cry1 ade2-1 trp1 his4-580 tyr1 lys2 SUP4-3*
D1-1C	[381G] *MATα leu2 ura3 ade3 TYR1 cep1::URA3-11*
D1-11D	[381G] *MAT***a** *leu2 ura3 ade3*
D1-11D.R1	[381G] *MAT***a** *leu2 ura3 ade3 TRP1* (spontaneous *TRP1* revertant of D1-11D)
R10-2D	[381G] *MATα leu2 ura3 TYR1 cep1::URA3-10*
DJ115-12	[381G] *MAT***a** *leu2 TYR1 HIS4 cyh2 ste2-3^{u}*
R26-R1	[381G] *MAT***a** *leu2 TYR1 HIS4 cyh2 ste2-3^{u} can1* (spontaneous *can1* mutation in DJ115-12)
D32-R1	[381G] *MATα leu2 ura3 ade3 TYR1 trp1::LEU2 cep1::URA3-10*
D28-6D	[381G] *MAT***a** *leu2 ura3 TYR1 trp1 cep1::URA3-10*
D36-R3	[381G] *MAT***a** *leu2 ura3 TYR1 trp1 cep1::ura3* (spontaneous *ura3* mutation in strain D28-6D)
D30-18B	[381G] *MATα leu2 ura3 ade3 TYR1 TRP1*
D41.3	[381G] *MATα leu2 ura3 ade3 TYR1 TRP1* [CFIII(D8B.d.D30-18B)]
DJ1149	[381G] *MAT***a** *leu1 ADE2 ade3 ade5 ade6 TRP1 HIS4 cyh2*
D64.5	[381G] *MAT***a** *leu2 ura3 ade3 TYR1 TRP1 cep1::ura3 cyh2* [CFIII(D8B.d.D30-18B.*LEU2*)]
DJ1136	[381G] *MAT***a** *ADE2 ade6 his3 HIS4 leu2 TYR1 cyh2*
D24	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/tyr1 trp1/TRP1* [pDK243 (*CEN3 LEU2 ade3-2p*)]
D25	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/tyr1 trp1/TRP1 cep1::URA3-11/cep1::URA3-11* [pDK243 (*CEN3 LEU2 ade3-2p*)]
D30	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/tyr1 trp1/TRP1 cep1::URA3-11/CEP1* [pDK243 (*CEN3 LEU2 ade3-2p*)]
D38	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 trp1::LEU2/TRP1 HIS4/his4 can1/CAN1 cyh2/CYH2*
D39	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 trp1::LEU2/TRP1 cep1::ura3/CEP1 HIS4/his4 can1/CAN1 cyh2/CYH2*
D40	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 trp1::LEU2/TRP1 cep1::ura3/cep1::ura3 HIS4/his4 can1/CAN1 cyh2/CYH2*
D47	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1::LEU2 HIS4/his4 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)]
D45	[381G] *MAT***a**/*MATα leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1::LEU2 cep1::ura3/cep1::ura3 HIS4/his4 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)]
D62	[381G] *MAT***a**/*MATα LEU1/leu1 leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1::LEU2 CEP1/cep1::ura3 HIS4/his4 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)]
D63	[381G] *MAT***a**/*MATα leu1/LEU1 leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1::LEU2 HIS4/his4 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)]
D64	[381G] *MAT***a**/*MATα leu1/LEU1 leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1::LEU2 cep1::ura3/cep1::ura3 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)]
D68	[381G] *MAT***a**/*MATα LEU1/leu1 leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)/CFIII(D8B.d.D30-18B.*LEU2*)]
D69	[381G] *MAT***a**/*MATα leu1/LEU1 leu2/leu2 ura3/ura3 ade3/ade3 TYR1/TYR1 TRP1/trp1 cep1::ura3/cep1::ura3 can1/CAN1 cyh2/CYH2* [CFIII(D8B.d.D30-18B)/CFIII(D8B.d.D30-18B.*LEU2*)]
D77-R1	*MATα leu2 his3 arg4 petX cep1::URA3-10 ade1::HIS3/ade1::LEU2* (chr. I disome)
D92	*MAT***a**/*MATα leu2/leu2 his3/his3 TRP1/trp1 ADE6/ade6 ARG4/arg4 PETX/petX CEP1/cep1::URA3-10 ADE1/ade1::HIS3/ade1::LEU2*
D93	*MAT***a**/*MATα leu2/leu2 his3/his3 TRP1/trp1 ARG4/arg4 PETX/petX cep1::URA3-10/cep1::URA3-10 ADE1/ade1::HIS3/ade1::LEU2*

[a] All except the last three strains listed are congenic to 381G (HARTWELL 1980), and the genotypes given are in addition to the 381G markers.

The structures of CFIII(*URA3*) and CF(*LEU2*) were verified by pulsed field gel electrophoresis and Southern blotting (data not shown).

Meiotic analyses: Diploids containing plasmids or CFs were grown selectively, otherwise they were grown in YEPD. Cells from fresh overnight cultures were washed with 1% potassium acetate, resuspended in sporulation medium at a density of 1×10^7 cells/ml, and incubated at 22° for 4–5 days. Sporulation efficiency of both wild-type and *cep1* strains was 15–45%. Tetrads were dissected onto YEPD agar slabs and incubated for 4–5 days at which time the colony color phenotype could be scored. Cells from each colony were then picked onto YEPD master plates and after 24 hr of growth replicated onto dropout and drug-containing media to score the various phenotypes. Mating phenotype was determined by replicating the master plates onto lawns of mating type tester strains (*MAT***a** *hom3* or *MATα hom3*) and after overnight growth assaying for diploid formation by replicating to minimal plates, scoring complementation of the *hom3* marker.

The *ade2/ade3-2p* system described by KOSHLAND, KENT and HARTWELL (1985) was used to monitor the copy number of plasmids and CFs. Cells lacking plasmid or CF form white colonies. Cells containing one copy of the plasmid/CF form predominantly pink colonies with occasional white and red sectors resulting from mitotic loss/nondisjunction of the plasmid/CF. Cells with two or more copies of the plasmid/CF form predominantly red colonies with occasional pink

and white sectors. Color phenotype was most easily scored on defined media containing one third the normal amount of adenine, although scoring on standard YEPD (no added adenine) was also possible. Also, red colonies tend to grow more slowly than pink ones due to the overaccumulation of the red pigment which is somewhat toxic. In all cases where red spore colonies were identified, the presence of more than one plasmid/CF was verified genetically by crossing cells of the red colony to testers containing no plasmid/CF. The resulting diploids were sporulated, dissected, and scored for segregation of the plasmid/CF. When the original parent contains two copies of the plasmid/CF, it segregates predominantly 4:0 in the test cross.

Sister spores were identified by scoring *trp1* which is located 0.4 cM from *cen4* (MORTIMER and HAWTHORNE 1969). In experiments involving CFs, the *cen7*-linked marker *leu1* was also present. Segregation of the CF was scored first against *trp1* and then verified against *leu1*. To be counted as a CF nonsister segregation event, the CF must have displayed second division segregation with respect to both centromere-linked markers. Tetrads in which sister segregation of the CF was ambiguous were eliminated from the analysis. Since the second division segregation frequencies of *trp1* and *leu1* are 0.94% and 4.9%, respectively (MORTIMER and HAWTHORNE 1969), the expected frequency of simultaneous second division segregation of both markers is less than 0.05%. The observed frequency of tetratypes for *trp1* and *leu1* in our wild-type strains was 5.0% (ditype:tetratype, 151:8), very close to the value which would be predicted for these two markers based on their reported second division segregation frequencies. In the *cep1* mutants, the tetratype frequency for *trp1* and *leu1* is increased to 9.6% (ditype:tetratype, 189:20). This difference is not statistically significant by Chi-square test ($P > 0.05$), and it is not nearly sufficient to account for the increased nonsister segregation of CFs observed in *cep1* strains.

The procedures of LOUIS and HABER (1989) were used to screen for disomy of chromosomes *III*, *IV*, *V* and *VII*. Haploid spores disomic for one of the marked chromosomes are recognized by the following phenotypes. Chromosome *III* disomes (*MAT***a**/*MATα*) are nonmating and nonsporulating. Chromosome *IV* disomes (*TRP1/trp1::LEU2*) are both Trp^+ and Leu^+. Chromosome *V* disomes (*CAN1/can1*) form colonies which when replicated to canavanine plates are drug-sensitive but which give rise to canavanine-resistant papillae resulting from mitotic loss or conversion of the *CAN1* homolog. Likewise, chromosome *VII* disomes (*CYH2/cyh2*) give rise to papillating colonies on cycloheximide plates. The latter two phenotypes are very easy to detect in a *cep1* background where mitotic chromosome loss is increased. In all cases, the screens detect only disomes carrying heterozygous markers; therefore, some missegregation events are missed due to recombination between the marker and its centromere which produce a homozygous disome. For details on these screening procedures, see LOUIS and HABER (1989).

Chromosome *I* disomy in segregants of strains D92 and D93 was identified by scoring the *cen1*-linked markers *ADE1*, *ade1::HIS3* and *ade1::LEU2*. Haploid spores contain only one of the three markers, while disomes contain two. [For the D92 segregants, *ADE1* was scored by complementation using *ade1* testers.]A 2:2 segregation of all three markers was observed in 98% of the tetrads; a reciprocal 3:1/1:3 segregation for two of the markers was observed in the remainder. Since four CEN-linked markers were segregating (three *ADE1* alleles in addition to *trp1*), sister spores could be determined unambiguously. Recombination in the *CEN1-ADE1* interval was observed at a frequency of about 10% (21/201), and in the *CEN4-TRP1* interval at 0.5% (1/201). No significant difference was observed in recombination frequency between the wild type and *cep1* strain.

TABLE 2

Mitotic loss rates of pDK243 and CF(*URA3*)

Strain	Element	Loss rate[a]	Segregation 1:0	Segregation 2:0
D24 (*CEP1/CEP1*)	pDK243	2.0×10^{-2}	ND[b]	2.0×10^{-2}
D25 (*cep1/cep1*)	pDK243	6.5×10^{-2}	1.6×10^{-2}	4.9×10^{-2}
D47 (*CEP1/CEP1*)	CF(*URA3*)	3.0×10^{-4}	0.6×10^{-4}	2.4×10^{-4}
D45 (*cep1/cep1*)	CF(*URA3*)	7.5×10^{-3}	0.6×10^{-3}	6.9×10^{-3}

[a] Events/cell/division
[b] Not detected.

Measurement of mitotic loss rates: Mitotic plasmid loss/nondisjunction rates were measured by the half-sectored colony assay described by KOSHLAND and HIETER (1987). The results reported in Table 1 are averages of four separate experiments carried out with independent transformants. The total number of wild-type and *cep1* colonies analyzed were 1696 and 1368, respectively.

Mitotic CF loss/nondisjunction rates were determined by fluctuation analysis as previously described (HEGEMANN *et al.* 1988) except only two 150-mm indicator plates spread with approximately 1500 cells were used for each resuspended colony. The total CF loss rate was calculated from the fluctuation in the number of white colonies arising in the population. The CF nondisjunction rate (2:0 segregation) was determined from the fluctuation in the appearance of red colonies. The rate of simple loss (1:0 segregation) is the total loss rate minus the rate of nondisjunction.

RESULTS

Mitotic stability of CEN plasmids and CFs in *cep1* strains: Table 2 shows the mitotic loss rates of CEN plasmid pDK243 and CF(*URA3*) from wild-type and *cep1* diploid strains. Both elements carry *URA3* as a selectable marker and the *ade3-2p* allele to assay copy number. The plasmid is significantly less stable than the chromosome fragment, but relative to wild-type cells the *cep1* mutants displayed increased mitotic loss rates of both, 3.2- and 25-fold, respectively. The ability to monitor copy number allowed us to distinguish simple loss (1:0 segregation) from nondisjunction (2:0 segregation). In *cep1* mutants, both 1:0 and 2:0 components of mitotic loss were increased, but in all cases, even in wild-type cells, the majority of loss events resulted from 2:0 rather than 1:0 segregation.

The distribution of plasmid and CF(*URA3*) in selectively grown cell populations is shown in Table 3. The relative instability of the CEN plasmid compared to the chromosome fragment is apparent. Maintaining selective pressure minimized the fraction of cells in the population lacking plasmid, but a significant fraction (25% or so) contained two or more copies of the plasmid. The high mitotic stability of the chromosome fragment is evident. Even in the *cep1* strain, CF(*URA3*) is present at single copy in greater than 90% of the cells.

TABLE 3

Mitotic stability of pDK243 and CF(*URA3*)

Strain	Element	Percent colony type[a] Red	Pink	White
D24 (*CEP1/CEP1*)	pDK243	26.8	68.9	4.3
D25 (*cep1/cep1*)	pDK243	28.9	57.5	13.8
D47 (*CEP1/CEP1*)	CF(*URA3*)	0.5	98.5	1.0
D45 (*cep1/cep1*)	CF(*URA3*)	3.5	91.8	4.8

[a] Total colonies scored: D24, 1696; D25, 1368; D47, 28,480; D45, 8200.

Meiotic segregation of pDK243 and CFs: Normally, an unpaired plasmid or CF segregates $2^+:2^-$ at meiosis. The replicated sister plasmids or CFs remain together through meiosis I and then segregate to sister spores at meiosis II. Precocious sister segregation at meiosis I results in segregation to nonsister spores, while meiosis II nondisjunction results in $1^+:3^-$ segregation with one spore receiving two copies of the plasmid/CF. Plasmid/CF loss at either meiosis I or meiosis II also results in $1^+:3^-$ segregation, but in this case the plasmid/CF-containing spore retains only a single copy of the plasmid/CF.

Table 4 shows the meiotic segregation analysis for plasmid pDK243. Assuming that tetrads segregating plasmid 4:0 and 3:1, 2:2 and 1:3, and 0:4 (plasmid$^+$:plasmid$^-$) arose from cells containing, respectively, two or more, one, or no copies of the plasmid before entering meiosis, the relative distribution of tetrads within each class closely reflected the distribution of plasmid in the premeiotic population (Table 3). Only tetrads of the 2:2 and 1:3 classes were informative with respect to precocious sister segregation, meiosis II nondisjunction, or loss. Of 137 wild-type tetrads, no cases of 1:3 segregation were observed, and in tetrads segregating the plasmid 2:2, normal segregation of the plasmid was observed in 86 out of 87 cases. It is probable that the one case of nonsister segregation (0.9%) observed was not the result of plasmid missegregation but was due to a second division segregation of the centromere-linked *trp1* marker used to identify sister spores. [The reported second division segregation frequency of *trp1* is 0.9% (MORTIMER and HAWTHORNE 1969).] For the *cep1* diploid, there was a significant decrease in the number of tetrads segregating plasmid 2:2 and increases in the 3:1, 1:3 and 0:4 classes. The most significant difference was in the frequency of nonsister plasmid segregation. Nine of 74 tetrads (12%) segregated the plasmid to nonsisters. In addition, 6 loss events and 1 meiosis II nondisjunction were observed among the 163 meioses analyzed.

The segregation pattern of CF(*URA3*) is also shown in Table 4. The numbers of tetrads in the various classes was again reflective of the distribution of CF(*URA3*) in the premeiotic populations. The fidelity of CF(*URA3*) segregation in the wild-type strain is extremely high. One hundred percent of tetrads segregated the fragment 2:2 and only one case (0.9%) of segregation to nonsister spores was observed. This one case of nonsister CF(*URA3*) segregation was probably real, because two independent centromere-linked markers (*trp1* and *leu1*) were used to identify sister spores and the probability of both segregating to nonsisters is <0.05%. In the *cep1* mutant, aberrant meiotic events involving CF(*URA3*) occurred at about the same frequencies as observed for pDK243. Five (3.5%) and 7(5.0%) loss and meiosis II nondisjunction events were observed, respectively, among the 141 tetrads analyzed, but the most dramatic effect was the increase in the frequency of nonsister segregation (21%). This segregation pattern results from the precocious segregation of the CF sister chromatids at meiosis I.

A fundamental difference exists between the segregation of an unpaired CF and the segregation of an endogenous chromosome. The CF has no homolog, and homolog pairing is known to be important for proper segregation of chromosomes in meiosis (ENGEBRECHT and ROEDER 1990; HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1988). To determine whether the absence of a homolog contributed to the high frequency of CF(*URA3*) missegregation in *cep1/cep1* diploids, meiotic segregation was analyzed in strains containing paired CFs. The *URA3* marker on CF(*URA3*) was changed to *LEU2* by gene transplacement (see MATERIALS AND METHODS) to produce CF(*LEU2*). CF(*URA3*) and CF(*LEU2*) differed only by the insertion of the 2.2-kb *LEU2* segment on the short arm. CF(*LEU2*) displays the same mitotic stability as CF(*URA3*) (data not shown). Strains D68 and D69 each contain a single copy of both CF(*URA3*) and CF(*LEU2*). In a normal meiosis, the CFs should pair and segregate away from each other at meiosis I resulting in a tetrad with two Ura$^+$ sister spores and two Leu$^+$ sister spores. The *ade3-2p* marker will segregate $4^+:0^-$. Figure 1 illustrates the outcomes of aberrant CF segregation in meiosis. Since the fragments are nonessential, nullosomes for the fragments are viable. Fragment disomes can be detected by color phenotype (red) and, if heterozygous, by nutritional phenotype (Leu$^+$Ura$^+$). Figure 1 also depicts the outcome of reciprocal recombination occurring between the centromere and the *URA3/ura3::LEU2* loci on the CF short arm.

Table 5 shows the observed CF meiotic segregation patterns for the wild-type and *cep1* diploids. In the wild-type strain (D68), 2:2 segregation of both CFs was observed 97% of the time and only 5 of 52 tetrads contained spores disomic for one CF. By contrast, numerous cases of CF disomy were observed among the meiotic products of the *cep1/cep1* strain (D69),

TABLE 4

Meiotic segregation of unpaired chromosomal elements

Strain	Element	Distribution in tetrads[a]					Total	Segregation[b]		
		4:0	3:1	2:2	1:3	0:4		S	NS	%NS
D24 (*CEP1/CEP1*)	pDK243	35	5	87	0	10	137	86	1	1.2
D25 (*cep1/cep1*)	pDK243	37	14	75	7	30	163	65	9	12.2
D30 (*CEP1/cep1*)	pDK243	0	0	31	0	0	31	30	1	3.2
D63 (*CEP1/CEP1*)	CF(*URA3*)	0	0	110	0	0	110	109	1	0.9
D64 (*cep1/cep1*)	CF(*URA3*)	5	7	104	12	13	141	80	21	20.8
D62 (*CEP1/cep1*)	CF(*URA3*)	0	0	23	0	0	23	23	0	0.0
D92 (*CEP1/cep1*)	Chromosome *I*	0	0	109	0	0	109	109	0	0.0
D93 (*cep1/cep1*)	Chromosome *I*	0	0	92	0	0	92	85	7	7.6

[a] Tetrads with four viable spores.

[b] One D25 and three D64 tetrads were eliminated from consideration, because sister spores could not be determined unambiguously. S = sister, NS = nonsister.

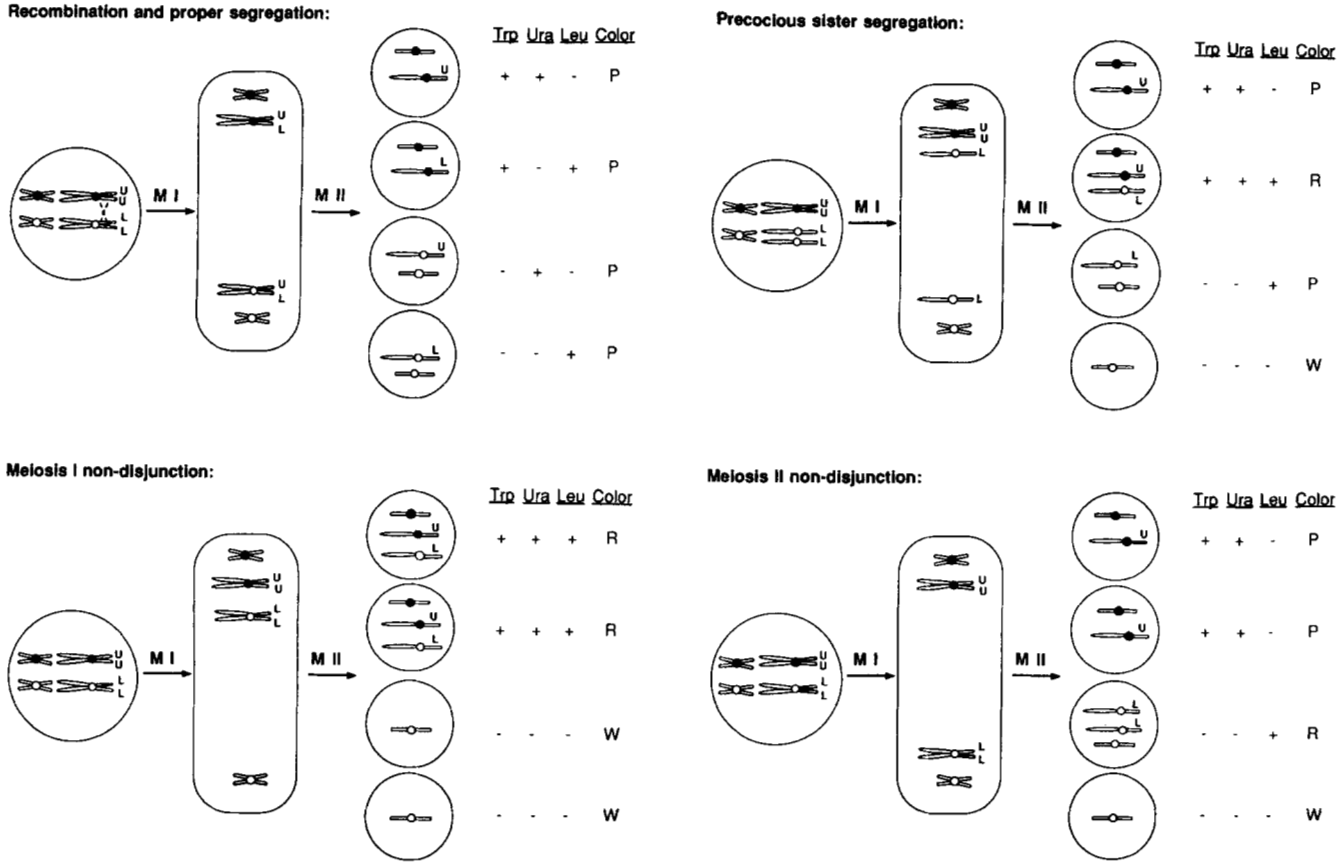

FIGURE 1.—Meiotic segregation of paired CFs. Chromosomes are not drawn to scale. The smaller chromosome represents chromosome *IV* which is used to mark sisters (*trp1/TRP1*). The larger chromosome represents a nonessential CF which carries the color marker *ade3-2p* and either *URA3* (U) or *LEU2* (L) on its short arm. Recombination occurring between the the centromere and the short arm marker results in $4^+:0^-$ segregation of the CF with both *URA3* and *LEU2* markers segregating to nonsister spores. Precocious sister segregation results in $3^+:1^-$ segregation with one CF disome (red) and one nullisome (white) occurring as nonsister spores. Meiosis II nondisjunction also results in $3^+:1^-$ CF segregation, but in this case the disome and nullisome are sisters. Meiosis I nondisjunction results in $2^+:2^-$ segregation with the production of two sister spore disomes (red) and two sister spore nullisomes (white). The Ura and Leu phenotypes indicated for the spores resulting from aberrant events assume no recombination along the CF short arm.

only some of which could be explained by cells entering meiosis with multiple CF copies. Table 6 catalogs the types of meiotic events observed for tetrads segregating a single copy of each CF homolog. For the wild-type strain, meiosis I and meiosis II nondisjunction events were observed at a frequency of around 5%. The *cep1* mutant displayed no increase in the frequency of meiosis I nondisjunction, but moderate increases in meiosis II nondisjunction (14.5% *vs.* 3.9%) and loss (1.8% *vs.* 0%) and a large increase in precocious sister segregation at meiosis I (23.6% *vs.* 0%). In addition, 10.9% of the *cep1* tetrads had under-

TABLE 5

Meiotic segregation of paired CFs

CF(*LEU2*)	CF(*URA3*) 4:0	3:1	2:2	1:3	0:4	Total
A. Strain D68 (*CEP1/CEP1*)						
4:0	0	0	0	0	0	0
3:1	0	0	0	0	0	0
2:2	0	0	49 (3)	2 (2)	0	51
1:3	0	0	0	0	0	0
0:4	0	0	1	0	0	1
Total	0	0	50	2	0	52 (5)
B. Strain D69 (*cep1/cep1*)						
4:0	0	0	1 (1)	0	1	2
3:1	0	0	0	4 (1)	1 (1)	5
2:2	0	1 (1)	38 (15)	6 (3)	5	50
1:3	1 (1)	2 (1)	5 (1)	2 (1)	1	11
0:4	0	0	4 (2)	0	0	4
Total	1	3	48	12	8	72 (28)

Data are for four viable spore tetrads. For each class, the number of tetrads containing one or more CF disome is given in parentheses (includes disomes arising through premeiotic events).

TABLE 6

Types of aberrant CF segregation

Event	No. of tetrads (%) *CEP1*	*cep1*
Proper segregation (4:0)		
Nonrecombinant	41 (80.4)	20 (36.4)
Recombinant[a]	5 (9.8)	7 (12.7)
Aberrant segregation		
Meiosis I nondisjunction	3 (5.9)	4 (7.8)
Meiosis II nondisjunction		
CF(*LEU2*)	0	4[b] (7.8)
CF(*URA3*)	2 (3.9)	4[b] (7.8)
Precocious sister segregation		
CF(*LEU2*)	0	7[b] (12.7)
CF(*URA3*)	0	6[b] (10.9)
Loss	0	1[c] (1.8)
Other	0	6 (10.9)
Total tetrads scored	51	55

Data are for tetrads containing four viable spores and segregating one copy of each CF.

[a] Inferred (see text)

[b] In four tetrads, both precocious sister segregation and meiosis II nondisjunction occurred. In the table, these events are recorded separately and are thus counted twice.

[c] CF(*URA3*).

gone multiple missegregation events, the nature of which could not be unambiguously determined. Segregation errors were observed with equal frequency for each CF, and comparing the results for strains D63 [CF(*URA3*)] and D68 [CF(*URA3*)/CF(*LEU2*)], there was no significant difference in the frequency or type of missegregation events observed. [The apparent reduction in precocious sister segregation of CF(*URA3*), 10.9% *vs.* 20.8%, is not statistically significant ($P > 0.10$).] The presence or absence of a homolog appeared to have little influence on the meiotic segregation of the CFs in *cep1* mutants.

Reciprocal recombination is often used as an indicator of functional homolog pairing at the first meiotic division (VON WETTSTEIN, RASMUSSEN and HOLM 1984). CF(*URA3*) and CF(*LEU2*) differ by only one genetic marker, making it impossible to score recombination between them; however, about 10% of tetrads from both wild-type and *cep1* diploids yielded what appeared to be the products of reciprocal recombination. As shown in Figure 1, recombination between the centromere and the markers on the short arm yields a tetrad in which both markers segregate at the second division. The identical segregation pattern would result from simultaneous precocious sister segregation of both CFs, but this event would be extremely rare in the wild-type strain where precocious sister segregation of a single CF occurs less than 1% of the time and still relatively infrequent (about 1%) in a *cep1* mutant where the precocious segregation frequency is about 10% per CF. We conclude that despite the short physical distance separating the CF centromere and the *LEU2/URA3* loci (about 2 kbp), reciprocal recombination is occurring in this interval and therefore that at least a fraction of the CFs are functionally paired at meiosis I.

Segregation of an unpaired chromosome *I*: The fact that *cep1* gene disruption affected the meiotic segregation of CF(*URA3*), unpaired or paired, suggested that CP1 was required for the accurate segregation of chromosomes in general. In order to verify this finding for a *bona fide* yeast chromosome, we analyzed the segregation of chromosome *I*. Since pairing did not to appear to significantly influence results for CF(*URA3*), chromosome *I* segregation was tested in a $2N + 1$ aneuploid. Segregation of the odd chromosome in a $2N + 1$ diploid is analogous to the seg-

regation of an unpaired CF; the extra chromosome segregates to sister spores at meiosis II. The result is a tetrad with two sister haploid spores and two sister disomic spores. Precocious segregation at meiosis I results in nonsister-spored disomes. Chromosome *I* is the smallest yeast chromosome (220 kbp), similar in size to the CFs.

Strains D92 and D93 are congenic diploids trisomic for chromosome *I* (Table 1). Each chromosome *I* homolog is marked (*ade1::LEU2/ade1::HIS3/ADE1*), so the disomic products of meiosis can be identified easily by nutritional phenotype (Leu^+His^+, Leu^+Ade^+, His^+Ade^+). The results of segregation analysis are given in Table 4. No aberrant segregation of chromosome *I* was detected for the wild-type strain; disomes were always found in sister spores. In the case of the *cep1/cep1* diploid (D93), 7 of 92 (7.6%) four-viable spore tetrads contained disomes in nonsister spores. The difference was statistically significant (chi-square test, $P < 0.05$). Qualitatively, *cep1* gene disruption had the same effect on the unpaired chromosome *I* as it did on the unpaired CF, and quantitatively, the effect was almost as great.

Spore viability: In carrying out the meiotic analyses described above, a significant difference was observed between the spore viability of wild-type and *cep1* tetrads. Table 7 shows the spore viabilities of various diploids used in this study [Strains D92 and D93 are not included, because they are not congenic with the others. However, their viabilities were not statistically different from the wild-type and *cep1* averages.] Overall, wild-type spores were 95.0% viable and *cep1* spores 73.4% viable. The difference was quite consistent day to day and between strains, indicating that the reduced viability was due to the *cep1* mutation and not to a second unrelated lesion arising by chance in the original *cep1* gene disruptant or to some nonsystematic variation in sporulation, dissection or germination conditions. The spore viability of a *cep1* homozygote was rescued to near wild-type levels (90.1%) by a CEN plasmid carrying the *CEP1* gene (data not shown), and spores obtained from *cep1* heterozygotes (D30, D62) displayed wild-type viability (Table 7), indicating that the *cep1* mutation was recessive with respect to spore viability. Microscopic examination of the germinated dissection slabs revealed that most of the spores which failed to grow did not germinate and those that did germinate (approximately 15%) stopped dividing after 2–4 generations.

The pattern of *cep1* spore inviability was not random. Table 7 shows the viability patterns we observed as well as the patterns to be expected if spore death were random. As for overall viability, the distribution of tetrads within the various viability classes (*i.e.*, 4 viable:0 inviable, 3 viable:1 inviable, etc.) was quite consistent day to day and strain to strain. Spore death in the wild-type tetrads appeared to be random; however, for all *cep1* strains the observed distribution was different from that which would have been predicted for random spore death. The differences were statistically significant in all cases (chi-square test, $P < 0.001$). (The nonrandomness actually worked to our benefit since there were many more *cep1* tetrads with 4 viable spores than would be predicted given the overall *cep1* spore viability of 74%.)

An average meiotic missegregation frequency of about 3% per chromosome pair (not greatly different from that observed for an unpaired chromosome *I*) would account for the general viability pattern observed, *i.e.*, 57% of *cep1* tetrads contain at least one dead spore. More precisely, the viability would depend on both the frequency and type of missegregation event occurring. For example, meiosis *I* nonjunction of a single chromosome would result in two dead spores per tetrad. Precocious sister segregation or meiosis II nondisjunction would produce tetrads with only one inviable spore. In all three cases, one or two disomic spores per tetrad would also be produced. If multiple missegregation events were to occur, complex segregation patterns and greater spore lethality would result, but the probability of aneuploidy in the few surviving spores would be increased.

To survey for the generation of aneuploidy, diploid strains were constructed which would allow ready detection of disomes among the dissected meiotic products. Four chromosomes were marked, chromosomes *III*, *IV*, *V* and *VII* (see MATERIALS AND METHODS). In 885 *cep1* tetrads (strains D40, D69, D64), no disomic spore colonies were observed for chromosomes *IV*, *V* or *VII*. Eight tetrads were found containing one or two chromosome *III* disomes; however, these were all obtained from CF-containing strains and are most likely due to meiosis I nondisjunction events caused by the CF (see DISCUSSION). No chromosome *III* disomes were found among the 379 D40 tetrads. If the decreased *cep1* spore viability were due solely to random chromosome missegregation, we should have detected many disomes. Consider strain D40. Of the 379 tetrads dissected, 216 tetrads having one or more inviable spores were obtained. Assuming the wild-type (D38) spore viability (96.5%), only 50 of the 379 should have contained one or more inviable spores $[(1 - (0.965)^4) \times 379]$; therefore, 166 would have contained a dead spore because of chromosome missegregation and therefore should also have contained a disome. Since we monitored 4 of the 16 chromosomes, about 40 disomes should have been obtained assuming that the missegregation event(s) involved all chromosomes at equal frequency.

DISCUSSION

Previous work has indicated that CDEI and, by inference, CP1 are required for proper centromere/

TABLE 7

Spore viability

Diploid		Tetrad classes (viable:inviable) 4:0	3:1	2:2	1:3	0:4	Total	Percent viable
D24 (*CEP1/CEP1*)	Obs.	142	17	2	0	0	161	96.7
	Exp.[a]	141	19	1	0	0		
D30 (*CEP1/cep1*)	Obs.	34	5	0	0	0	39	96.8
	Exp.	34	5	0	0	0		
D25 (*cep1/cep1*)	Obs.	123	69	57	23	7	279	74.9
	Exp.	88	118	59	13	1		
D38 (*CEP1/CEP1*)	Obs.	32	3	1	0	0	36	96.5
	Exp.	31	5	0	0	0		
D40 (*cep1/cep1*)	Obs.	163	93	80	25	18	379	73.6
	Exp.	111	160	86	20	2		
D63 (*CEP1/CEP1*)	Obs.	110	15	8	2	1	136	92.5
	Exp.	100	32	4	0	0		
D62 (*CEP1/cep1*)	Obs.	25	2	0	0	0	27	98.2
	Exp.	25	2	0	0	0		
D64 (*cep1/cep1*)	Obs.	142	73	75	42	11	343	71.4
	Exp.	89	143	86	23	2		
D68 (*CEP1/CEP1*)	Obs.	52	6	3	0	0	61	95.1
	Exp.	50	10	1	0	0		
D69 (*cep1/cep1*)	Obs.	72	38	36	14	3	163	74.9
	Exp.	51	69	35	8	1		
Totals								
CEP1/CEP1		336	41	14	2	1	394	95.0
	%	85.3	10.4	3.6	0.5	0.3		
cep1/cep1		500	273	248	104	39	1164	73.4
	%	43.0	23.5	21.3	8.9	3.4		

[a] The expected number of tetrads in each class (N_i) is obtained by expanding the polynomial $N = T\,(v + (1 - v))^4$, where T is the total number of tetrads and v is the average viability.

kinetochore function during meiosis (CUMBERLEDGE and CARBON 1987; GAUDET and FITZGERALD-HAYES 1989). In particular, CEN plasmids containing CDEI-deleted centromeres were found to exhibit high frequencies of precocious sister segregation at meiosis I. The effect was also observed for chromosome *III* containing a CDEI-deleted centromere (CUMBERLEDGE and CARBON 1987), although the latter result was not confirmed in a subsequent study (GAUDET and FITZGERALD-HAYES 1989). Our results are very consistent with these findings. Homozygous *cep1* diploids display significantly increased meiotic missegregation of a CEN plasmid, nonessential CFs and a native yeast chromosome. In all cases, the frequency of missegregation is quite high, 8–21%, and the predominant aberrant event is precocious sister segregation at meiosis I. To be segregated properly at meiosis, replicated sister chromatids must remain together throughout the first (reductional) division and then separate at anaphase of the second (equational) division. Precocious sister segregation results when sisters separate prematurely and segregate at the first division. We can envision two possible scenarios: (1) the kinetochore complex "splits" at anaphase I as it normally would at anaphase II or (2) one or both sister chromatids detach from the kinetochore complex and segregate randomly. We prefer the latter mechanism to explain the precocious sister segregation observed in *cep1/cep1* diploids, because a general defect in chromatid-kinetochore adhesion would also explain the increased mitotic and meiosis II nondisjunction observed with *cep1* mutants. In addition, since CP1 specifically binds centromere DNA, it is not hard to imagine that as part of the kinetochore complex CP1 would promote chromatid-kinetochore interaction.

The increased chromosome *I* missegregation in the $2N + 1$ *cep1* strain suggests that the *cep1* defect extends to native chromosomes as well as plasmids and recombinant CFs. Since homolog pairing does not appear to influence missegregation of the similarly sized CF(*URA3*), we infer that chromosome *I* also missegregates frequently in a normal *cep1/cep1* diploid. Some additional findings corroborate this inference. First, we have analyzed 125 tetrads from a diploid strain derived from D93 (*cep1/cep1*) but containing only two chromosome *I* homologs (*ade1::HIS3/ade1::LEU2*). Two His^+Leu^+ segregants were detected and confirmed to be chromosome *I* disomes by pulsed field gel electrophoresis. The disomes were found in separate tetrads, so the missegregation frequency was 1.1%. Second, we have used pulsed field gel electrophoresis to determine electro-

phoretic karyotypes for a number of *cep1* spores from randomly selected tetrads. While only 26 tetrads have been examined to date, one chromosome *I* disome was detected. It is premature to conclude that chromosome *I* missegregation in a 2*N cep1* strain is as high as in a 2*N* + 1 strain, but it appears to be substantial.

Both SUROSKY and TYE (1988) and SHERO *et al.* (1991) have observed increased meiosis I nondisjunction in strains containing chromosome fragments. The nondisjunction occurs when the CF pairs with an endogenous chromosome, leaving the homolog unpaired. If both chromosome homologs then segregate away from the CF, the result is two inviable sister spores lacking the chromosome and two viable disomic sister spores. This type of nondisjunction is specific for the chromosome homologous to the CF and occurs with a frequency of 4–7% depending on the CF (SHERO *et al.* 1991; SUROSKY and TYE 1988). We also observe what appears to be CF-induced meiosis I nondisjunction. In our case, the nondisjunction events involve chromosome *III* and occur at a lower frequency, about 1%. This low level of chromosome nondisjunction does not interfere in our analyses of CF segregation, because we score only tetrads containing four viable spores.

The viability of *cep1* spores is significantly reduced, and the pattern of inviability is nonrandom. While on the average, one in four spores is inviable, almost half of the *cep1* tetrads contain four viable spores, far more than would be expected. The decrease in spore viability does not appear to be caused directly by chromosome missegregation; otherwise, we should have detected many aneuploids among the viable spores from *cep1* meioses. The viability defect may be unrelated to the kinetochore function of CP1. Disrupting *CEP1* has pleiotropic effects: generation time is increased by 35% and the strains are methionine auxotrophs (BAKER and MASISON 1990). The reduced *cep1* spore viability could be related to either the nutritional or cell cycle defect.

We were surprised at being unable to detect chromosome missegregation in the genetic screen. No aneuploidy was observed for chromosomes *IV*, *V* or *VII*, and the few chromosome *III* disomes which were observed could be attributed to CF-induced meiosis I nondisjunction. Several explanations are possible for the apparent lack of aneuploids: disomic chromosomes (other than chromosomes *I* and *III*) could be extremely unstable in the *cep1* background and be lost very early during growth of the spore colony; the *cep1* defect may not affect the chromosomes we monitored; missegregation events may not be randomly distributed among all tetrads. While none of these possibilities can be ruled out, we favor a direct interpretation of the results, that for these chromosomes the level of missegregation is below detection by tetrad analysis.

Mitotically, *cep1* mutants show about a 10-fold increase in the loss rate of chromosome *III* (BAKER and MASISON 1990; CAI and DAVIS 1990) and a 25-fold increase in the loss rate of CF(*URA3*) (this study). It is difficult to quantitate the meiotic effect of *cep1*, but taking the case of the unpaired CF(*URA3*), the increase in precocious sister segregation is 20-fold (based on observing one event with the wild-type strain). Assuming a 10–25-fold increase over the wild-type meiotic chromosome nondisjunction frequency of 1×10^{-4} (measured for chromosome *V*) (SORA, LUCCHINI and MAGNI 1982), the estimated *cep1* meiotic chromosome missegregation frequency would be 1 to 3 events per chromosome per thousand meioses. If this an accurate estimate, it is not too surprising that we were unable to detect disomes in the genetic screen. It would also imply that the missegregation frequency of the typical chromosome is significantly less than that of chromosome *I*. Perhaps due to its small size chromosome *I* is differentially affected by *cep1* mutation, or more likely, the background level of chromosome *I* missegregation is higher than average. Better methods will be required to analyze endogenous chromosome segregation in the *cep1* mutants. To be applicable, the method must take into account the mitotic phenotype of *cep1* and distinguish meiotic and premeiotic events. For this reason, random spore analysis is of limited use. As mentioned above, we have begun the brute force method of electrophoretic karyotyping. Although fewer tetrads can be surveyed, most of the individual chromosomes can be monitored (in our strains, 11 chromosomes are well resolved). By continuing this analysis, we hope to be able at least to determine an upper limit for the meiotic missegregation rate of other chromosomes in *cep1* mutants.

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