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Genetics

Saccharomyces cerevisiae mutants that tolerate centromere plasmids at high copy number

(yeast centromeres/yeast transposons/G418 resistance)

GARY TSCHUMPER AND JOHN CARBON

Department of Biological Sciences, University of California, Santa Barbara, CA 93106

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ABSTRACT **Two yeast (*Saccharomyces cerevisiae*) mutants that tolerate centromere (CEN) plasmids at high copy number have been isolated. The mutations relieve the restraint normally imposed on plasmid copy number by a cloned *CEN* sequence. Our CEN plasmids specify resistance to G418 and are high copy plasmids only when the mutant host cells are grown on medium containing this antibiotic. The high copy number of the plasmids is independent of the specific cloned *CEN* sequence and recovered plasmids show no alteration in structure or function of the *CEN* DNA. The efficiency with which CEN plasmids go to high copy number is increased if the mutant cell is cotransformed by another CEN plasmid. The genomic mutation responsible for the high copy number (*COP*) is dominant and stable, and it segregates in a Mendelian manner. Homozygous *COP/COP* a/α diploids do not tolerate CEN plasmids at high copy number, suggesting that the mutation is regulated by mating type. The genomic DNA from both mutant cells contains an altered transposon (Ty) restriction fragment that cosegregates with the COP phenotype in crosses of mutant and wild-type strains. The mutations may be transposon-mediated events that identify a gene involved in centromere or mitotic spindle function.**

Plasmids containing a cloned centromere (*CEN*) from yeast (*Saccharomyces cerevisiae*) function as minichromosomes in yeast cells (1). These cloned *CEN* sequences retain both the structural and functional characteristics of chromosomal centromeres. Structurally, plasmid-borne *CEN* sequences have the same distinctive chromatin structure that occurs in the centromere region of yeast chromosomes (2). Functionally, CEN plasmids exhibit three characteristics of chromosomes in yeast cells: they are mitotically stable in the absence of selective pressure; they segregate during meiosis in a Mendelian manner; and they are found at low copy number in the host cell (1). The low copy number of CEN plasmids is not altered by the presence in cis of the yeast 2-μm plasmid amplification system that normally drives plasmids to high copy number (3). When wild-type yeast cells are forced to maintain multiple CEN plasmids bearing independently selectable markers, the cells grow slowly, and cell viability is decreased, indicating a toxic effect from the excess CEN plasmids (4). We have been using the copy control function of cloned *CEN* sequences in an attempt to identify gene products, such as components of the spindle or kinetochore, that interact with yeast centromeres. We have constructed (5) a plasmid system that permits rapid detection of high copy number plasmids by selecting host cells on antibiotic medium. These plasmids contain the phosphotransferase gene from the bacterial transposon Tn5 (6) under control of a weak promoter. The product of the phosphotransferase gene inactivates antibiotic G418 by phosphorylation (7), thereby conferring antibiotic resistance upon host cells in proportion to plasmid copy number (5). Using this plasmid system we have obtained two mutant yeast strains that tolerate CEN plasmids at high copy number. Characterization of these mutant strains suggests that the mutations result from a yeast transposon (Ty) in the genomic DNA.

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MATERIALS AND METHODS

Media and Strains. Antibiotic G418 sulfate (Geneticin) was from GIBCO at 516 μg of G418 per mg of solid material. The amounts used refer to the actual concentration of the G418, not the amount of solid material. Yeast YPD medium contains per liter of H_2O: 10 g of yeast extract, 20 g of peptone, and 20 g of glucose with 30 g of agar added for plates. YPDGen medium contains Geneticin at 500 μg of G418 per ml added after the YPD is autoclaved. Yeast minimal medium contains per liter of H_2O: 6.7 g of yeast nitrogen base without amino acids, 20 g of glucose, and each required base or amino acid at 100 mg with 30 g of agar added for plates. Yeast (*S. cerevisiae*) strains were XSB52-23C[cir°](α *trp1 gal2 leu2-3,112 cdc10 can1*) and GTSC1[cir°](**a** *leu2-3,112 trp1 his4 can1*). The *cdc10* mutation is a temperature-sensitive allele that permits growth at 25°C but not at 37°C. All yeast strains used are [cir°] (lacking the endogenous 2-μm plasmid) to prevent intermolecular recombination with CEN plasmids containing 2-μm DNA and subsequent deletion of the *CEN* DNA (3).

Plasmid Constructions and Yeast Transformation. Plasmids (Fig. 1) contain the yeast 2-μm form-B DNA opened at the *Eco*RI site within the *FLP* gene. This configuration prevents any intra- or intermolecular recombination of the plasmids across the inverted repeats of the 2-μm DNA catalyzed by the product of the *FLP* gene (8) but retains the 2-μm *REP* system that amplifies plasmid copy number under selection for plasmid markers (9). All the plasmids used are "shuttle" plasmids capable of replication and selection in both *Escherichia coli* and *S. cerevisiae*. Yeast cells were transformed using the alkali cation procedure (10) with modifications as described (5). Yeast transformants were first selected for plasmid markers on minimal medium and grown for 4 days at room temperature, then replica plated onto YPDGen to select for antibiotic resistance. This was necessary because the high salt content of yeast nitrogen base (minimal medium) renders yeast cells resistant to G418, and direct selection on YPDGen results in a low frequency of transformation (11). Homozygous **a/a** and α/α diploids were obtained by fusion of spheroplasts in polyethylene glycol (12) and by selection for complementation of markers on minimal media.

RESULTS

Selection of Cells Containing CEN Plasmids at High Copy Number. A yeast plasmid system was used for rapid detection of cells containing CEN plasmids at high copy number (5). These plasmids (Fig. 1) confer antibiotic resistance on the

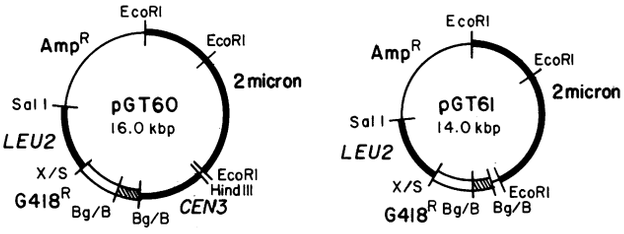

FIG. 1. Plasmids designed to indicate their copy number in yeast. Thin lines, pBR322 DNA; thick lines, yeast DNA; open segment, phosphotransferase (G418 resistance) gene; hatched segment, fragment of *Chlamydomonas reinhardtii* DNA that functions as a weak promoter for the phosphotransferase gene in yeast (5). Only those restriction sites used in construction or required to demonstrate orientation of fragments are shown. X/S, *Xho* I–*Sal* I junction; Bg/B, *Bgl* II–*Bam*HI junction. The 2-μm *FLP* gene is inactivated by the construction, but the *REP* system remains intact in both plasmids.

host cell in direct proportion to plasmid copy number. In wild-type cells, the CEN plasmid (pGT60) occurs only in low copy number, and host cells are sensitive to concentrations of G418 >150 μg/ml. A similar plasmid lacking a *CEN* sequence (pGT61) is driven to high copy number by the 2-μm DNA, and host cells grow on medium (YPDGen) containing 500 μg of G418 per ml. Although studies indicate the *FLP* gene product is required for amplification of 2-μm plasmids to high copy number under nonselective conditions (13), Flp$^-$ 2-μm plasmids that contain an intact REP system have a high copy number in selected transformants (9). The Flp$^-$,Rep$^+$ 2-μm circle derivative used here (pGT61) normally occurs at high copy number (average of >10 copies per cell) in [cir°] strains grown under leucine selection (Fig. 2), unless restrained by a *CEN* sequence (pGT60, Fig. 2). Hence, if the mechanism that maintains CEN plasmids at low copy number is disrupted, pGT60 will be driven to high copy number by the 2-μm DNA, and host cells can be isolated by selection on YPDGen agar.

In an effort to isolate yeast genes whose overexpression (due to their presence on a multicopy plasmid) would increase the copy number of CEN plasmids, yeast strain XSB52-23C containing pGT60 was supertransformed with a yeast genomic library constructed in pLC544, a TRP1,ARS1 shuttle vector (14). Leu$^+$,Trp$^+$ cotransformants were selected on minimal medium and replica plated onto YPDGen medium. From 10,000 cotransformants, ≈50 colonies that were resistant to 500 μg of G418 per ml were obtained. Plasmids from these were analyzed either by shuttling to *E. coli* followed by isolation and restriction mapping or by selective loss of the TRP1 (library) plasmid followed by testing on YPDGen. Most of the candidates contained plasmids derived from pGT60 by deletion of the centromere region, due to interaction of 2-μm sequences on pGT60 with library clones containing 2-μm DNA. (The library was constructed with sheared DNA from a yeast strain containing endogenous 2-μm DNA.) Several contained fusions of pGT60 with a library plasmid, resulting in loss or inactivation of the *CEN* sequence. At least one contained pGT60 with a yeast transposon (Ty) insertion in the promoter region of the G418 resistance gene, resulting in overexpression of the gene (5). Two candidates (colonies 11 and 21) were obtained in which the G418 resistance was diminished by the loss of the TRP1 (library) plasmid; however, some growth still occurred on YPDGen. Copy-number genomic Southern blots (3) (Fig. 2) of these two candidates show that the CEN plasmids go to high copy number when the host cells are grown in YPDGen medium, but remain at low copy number in the absence of selection for G418 resistance. The pGT60 plasmids recovered from these candidates contain *CEN* sequences that are unaltered and fully functional as shown by restriction analysis and transformation into wild-type yeast cells.

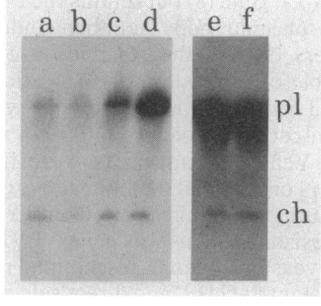

FIG. 2. Autoradiograph showing plasmid copy number in wild-type and mutant cells. Total DNA was isolated from various transformants, digested with *Sal* I, and separated by electrophoresis on a 0.8% agarose gel. A Southern blot was probed with a ^{32}P-labeled restriction fragment containing the *LEU2* gene. Transformants were grown in minimal (lanes a–d) or YPDGen medium (lanes e and f). The plasmid (pl) and chromosomal (ch) bands are indicated. Total DNA was isolated as follows: XSB52-23C(*COP-1*)/pGT60+pCNC2 (lanes a and e), XSB52-23C(*COP-2*)/pGT60+pCNC3 (lanes b and f), XSB52-23C/pGT60 (lane c), XSB52-23C/pGT61 (lane d). Both pCNC2 and pCNC3 remain at low copy number when host cells are grown in YPDGen.

G418 Resistance Mediated by CEN Plasmids Requires a Genomic Mutation. Comparison of candidates 11 and 21 revealed a consistent difference in their response to growth on YPDGen. When equal aliquots were streaked on YPDGen agar, candidate 11 always yielded more colonies resistant to G418 than did candidate 21. To quantify this difference, the cotransformants were grown in minimal medium, and equal aliquots were spread on YPD and YPDGen agar. About 1 in 10,000 cells from candidate 11 grew on YPDGen compared with ≈1 in 100,000 cells from candidate 21. Nevertheless, when colonies grown from single cells of either candidate on minimal medium were tested on YPDGen, each always gave rise to the G418 resistance phenotype with the same frequency as the original. Hence G418-resistant cells from each candidate arise consistently with a frequency that is specific for each candidate. This provides an easily observable phenotypic difference to distinguish between the two candidates. In addition, the frequency of G418-resistant cells arising from each candidate is reduced to ≈20% of the original when the cotransformant is cured of the library plasmid.

The persistence of some G418-resistant cells following selective loss of the library plasmids from cotransformants 11 and 21 suggested the high copy number of the CEN plasmids was not a simple effect of the cotransforming library plasmid on pGT60. This was confirmed by the failure of all attempts to reproduce the phenotype using wild-type cells and pGT60 alone or with the library plasmids (pCNC2 from candidate 11 and pCNC3 from candidate 21). An experiment in which XSB52-23C/pGT60 was transformed with pCNC2 or pCNC3 yielded no G418-resistant colonies when 5000 Leu$^+$,Trp$^+$ cotransformants were tested on YPDGen plates. Similar results were obtained using copies of pCNC2 and pCNC3 isolated directly from the recombinant library transformed into *E. coli* cells, indicating that no alteration of these plasmids had occurred in yeast during the initial experiment.

To determine if a genomic mutation is involved, the cotransformants 11 and 21 were cured of all plasmids, and the resulting yeast strains [XSB52-23C(*COP-1*) from candidate 11 and XSB52-23C(*COP-2*) from candidate 21] were transformed with pGT60 alone or with pGT60 and pCNC2 or pCNC3, and the resulting transformants were tested on YPDGen. All of the transformants produced the original

G418-resistant phenotype of the mutant cell (Table 1), although those containing only pGT60 did so at lower frequency than those containing pGT60 plus either pCNC2 or pCNC3. The results demonstrate that the phenotype is specific for each of the mutant host cells and independent of the specific library plasmid, although the library plasmid does contribute to the phenotype.

The Effect of the Genomic Mutation Is Enhanced by a Second CEN Plasmid. During experiments to cure the original cotransformants of the library plasmids, it was observed that both pCNC2 and pCNC3 are highly stable and are lost at a rate of only 1–3% from cotransformants streaked for single colonies onto nonselective medium. This suggested that both might contain *CEN* sequences since these are known to confer a similar degree of stability on plasmids (1). Restriction mapping of pCNC2 [3.0-kilobase-pair (kbp) yeast DNA insert] and pCNC3 (10.0-kbp yeast DNA insert) revealed a common set of restriction fragments such that the 3.0-kbp fragment was approximately in the center of the 10.0-kbp fragment. DNA sequencing on the smaller fragment showed that the common region was identical in sequence to yeast *CEN15* (15). Hence both plasmids selected from the library in our experiment contain yeast *CEN15*, although each is an independent clone of the *CEN15* region.

As noted above, the cotransforming plasmids are not essential to the mutant phenotype but provide an easily detectable enhancement of that phenotype. To determine if either *CEN15* or the surrounding DNA is crucial to this enhancement, XSB52-23C(*COP-1*)/pGT60 and XSB52-23C(*COP-2*)/pGT60 were transformed with subclones of the *CEN15* region as well as other CEN plasmids and tested on YPDGen. As shown in Table 1, any CEN plasmid in the mutant cell along with pGT60 will enhance the mutant phenotype (for example *CEN11* or another copy of *CEN3*) whereas plasmids lacking a *CEN* have no effect. The selection of two CEN15 plasmids from the library in the original cotransformation was apparently due to a large number of *CEN15* copies in the library that had been amplified several times since its construction. When *E. coli* cells transformed with the library were screened using a nick-translated CEN15 probe, ≈1 colony in 400 was positive.

Table 1. Plasmids required to produce the G418 resistance phenotype of *COP* mutants

	G418 resistance phenotype			
	XSB52-23C (*COP-1*)		XSB52-23C (*COP-2*)	
Plasmid	+ pCNC2	− pCNC2	+ pCNC3	− pCNC3
pGT60		+		+
pGT68		+		+
pGT60/pCNC2(*CEN15*)	+		+	
pGT60/pCNC3(*CEN15*)	+		+	
pGT60/pYe(*CEN3*)30	+		+	
pGT60/pYe(*CEN11*)12	+		+	
pGT60/pGT67(*CEN15*)	+		+	
pGT60/pLC544		+		+

The original cotransformants (candidates 11 and 21) were cured of both plasmids, and the resulting strains [XSB52-23C(*COP-1*) and XSB52-23C(*COP-2*), respectively] were transformed with the plasmids indicated at the left. Leu$^+$ or Leu$^+$,Trp$^+$ transformants were selected and tested on YPDGen, and the G418 resistance phenotype was compared with that of the original cotransformant (+ pCNC2 for candidate 11 and + pCNC3 for candidate 21) or with the original cotransformants that had been cured of the library plasmids (− pCNC2 for candidate 11 and − pCNC3 for candidate 21). pGT68 is pGT61 with *CEN11* inserted at the *Sal* I site. pLC544 is a *TRP1* shuttle vector that does not contain a *CEN* sequence (14). +, Phenotype obtained with each transformation. Phenotypes were determined by streaking about 5 million cells from four different transformants of each kind onto YPDGen.

Expression of the Genomic Mutation Is Dependent on Mating Type. To analyze the genomic mutation, candidates 11 and 21 were crossed with yeast strain GTSC1/pGT60+CNC2 or GTSC1/pGT60+pCNC3, and His$^+$,Cdc10$^+$ diploids were selected and tested on YPDGen. In all cases the diploids were absolutely sensitive to G418 at 500 μg/ml (Table 2), whereas tetrads obtained by sporulation of the same diploids yielded spores that grew on YPDGen in a 2+:2− Mendelian ratio (Table 3). Although these results suggested that the mutation might be a simple recessive, this was not confirmed by crosses among the spores. Vegetative cells grown from spores obtained in the above cross with genotype **a** *COP his4* were crossed with those of genotype **α** *COP cdc10*, and His$^+$,Cdc10$^+$ diploids were selected and tested on YPDGen. In all cases, the diploids were sensitive to G418 at 500 μg/ml (Table 2). Hence **a**/**α** diploids do not express the mutant phenotype, regardless of whether they are heterozygous or homozygous for the *COP* mutation, suggesting that expression of the *COP* mutation is dependent on the mating type of the cell.

To determine if the *COP* mutation is dominant or recessive and to analyze its dependence on mating type, **a**/**a** and **α**/**α** diploids were constructed by making yeast spheroplasts and fusing the cells with polyethylene glycol (12, 16). In this way, four different kinds of fusions were obtained that were homozygous for mating type (either **a** or **α**) and either homozygous or heterozygous for the *COP* mutation. Three of the fusion diploids of each kind were tested on YPDGen, and all were resistant to 500 μg of G418 per ml (Table 2). These results indicate that the *COP* mutation is dominant and that it functions in diploids that are homozygous for mating type. Hence expression of the *COP* mutation is clearly dependent on the mating type in diploid cells.

During crosses with the original cotransformants, genetic results indicated that both candidates 11 and 21 were disomic for chromosome III. In crosses of XSB52-23C(*COP HIS4*) with GTSC1(*his4*), the *HIS4* marker (located on chromosome III) did not segregate 2+:2− but rather yielded mainly tetrads with three or four His$^+$ spores (Table 3). This was further confirmed by analysis of mating type (also located on chromosome III) of the spores (Table 3), many of which were **a**/**α** steriles, consistent with the presence of a chromosome III disome in the mutant cells. This chromosome III disomy and the frequent **a**/**α** mating type of the haploid spores have no apparent effect on the *COP* mutation, since some of these haploids express the mutant phenotype. Hence, while the *COP* mutation is suppressed in **a**/**α** diploids, the COP phenotype is expressed in **a**/**α** haploids disomic for chromosome III. To determine if other chromosomes in the mutant cells were disomic, the karyotype of the mutant cells was analyzed by orthogonal-field-alternation gel electrophoresis (17) and compared with that of the wild-type cells. As shown

Table 2. Genetic crosses to analyze expression of the *COP* mutation in diploids by growth on YPDGen medium

	Haploid			
Haploid	**a** *cop*	**a** *COP*	**α** *cop*	**α** *COP*
a *COP*	+	+	−	−
α *COP*	−	−	+	+

The cross was XSB52-23C(**α** *COP-1 cdc10*)/pGT60+pCNC2 × GTSC1 (**a** *his4*)/pGT60+pCNC2. His$^+$,Cdc10$^+$ diploids from this cross were sporulated, and individual haploids were tested for growth on YPDGen, for genotype, and for mating type. Haploids were selected from among these for the crosses indicated above. Diploids heterozygous for mating type were obtained by matings, whereas diploids homozygous for mating type were obtained by fusing spheroplasts in polyethylene glycol (12). The resulting diploids were tested for growth on YPDGen. +, Growth on YPDGen; −, no growth.

Table 3. Meiotic segregation of *COP* mutations and other markers

Cross	Marker	Segregation in tetrads, no. 4+:0−	3+:1−	2+:2−	1+:3−	0+:4−
1	*COP-1*	0	2	18	5	0
	HIS4	12	10	3	0	0
	MATα	13	6	6	0	0
	CDC10	0	0	25	0	0
2	*COP-2*	0	0	36	4	0
	HIS4	18	18	4	0	0
	MATα	18	12	10	0	0
	CDC10	0	0	40	0	0

Cross 1 was XSB52-23C(α *COP-1 cdc10*)/pGT60+pCNC2 × GTSC1 (**a** *his4*)/pGT60+pCNC2. Cross 2 was XSB52-23C(α *COP-2 cdc10*)/pGT60+pCNC3 × GTSC1 (**a** *his4*)/pGT60+pCNC3. For cross 1, spore viability was 59%, and 25 complete tetrads were obtained from 96 dissected. For cross 2, spore viability was 67%, and 40 complete tetrads were obtained from 96 tetrads. *MAT***a** segregated 2+:2− in all tetrads examined. Comparison of *COP* segregation with that of the centromere-linked marker *CDC10* showed that >60% of tetrads were tetratype, indicating that *COP* is not a centromere-linked marker. *HIS4*, *MATα*, and *CDC10* are located on chromosome III.

in Fig. 3, the chromosome III disomy was confirmed, a deletion of ≈7 kbp was observed in chromosome VIII, and both chromosomal aberrations appear identical in the two mutant cells. Orthogonal-field-alternation gel electrophoresis analysis of chromosomes from all four spores of two different tetrads obtained in the crosses above showed that neither the disome nor the deletion cosegregates with the phenotype of the *COP* mutation, and hence it is unlikely that either is involved directly in expression of the COP phenotype.

Mutant Cells Have an Alteration in Their Ty-Element Pattern. The dependence of the mutant phenotype on mating type of the cell suggests that the *COP* mutation might be the result of a transposon insertion, since some Ty-mediated mutations are under mating-type control (18). To test this hypothesis, DNA was prepared from the wild-type and mutant cells, digested with various restriction enzymes, and separated on gels. Southern blots were probed with the large *Bgl* II fragment from a Ty*1* element (5) to find any variations in the pattern of Ty bands between the wild-type and mutant

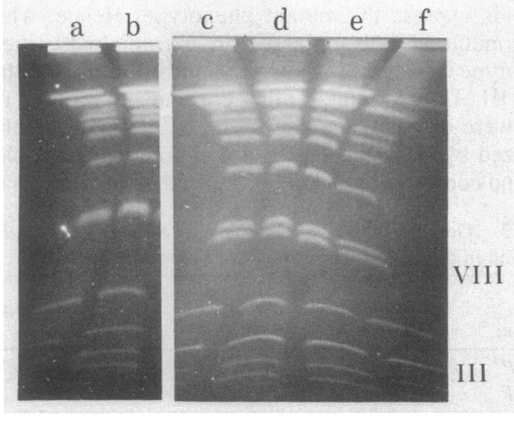

FIG. 3. Orthogonal-field-alternation gels showing chromosomal DNAs of wild-type and mutant cells. Chromosomal DNAs were separated on 1% agarose and stained with ethidium bromide. Electrophoresis was at 300 V and 130 mA for 34 hr with 30-sec switching in each direction. Other conditions and preparation of samples were as described (17). Chromosomes are as follows: XSB52-23C (lanes a and b), XSB52-23C(*COP-1*) (lanes c and d), and XSB52-23C(*COP-2*) (lanes e and f). Chromosomes III (disomic in lanes c–f) and VIII (deletion in lanes c–f) are indicated.

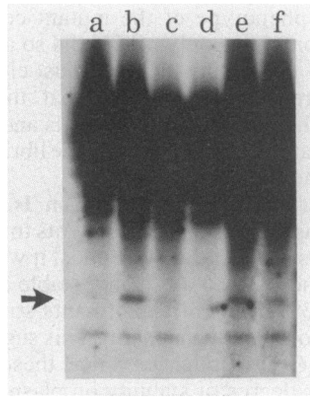

FIG. 4. Autoradiograph showing Ty fragment patterns in various yeast genomic DNAs. Total DNA was isolated from the cells indicated, digested with *Eco*RV, and separated by electrophoresis on a 0.8% agarose gel. A Southern blot was probed with a nick-translated 2.2-kbp *Bgl* II fragment from Ty*1* (19). DNAs were isolated as follows: XSB52-23C(*COP-1*) (lane a), XSB52-23C (lane b), and vegetative cells grown from tetrad spores obtained in the cross XSB52-23C(*COP-1*)/pGT60+pCNC2 × GTSC1/pGT60+pCNC2 (lanes c–f). Vegetative cells from spores (lanes c and d) grow on YPDGen, those shown in lanes e and f do not. The arrow indicates the altered Ty band.

cells. DNAs digested by *Eco*RV revealed a Ty-containing fragment that is present in wild-type cells but is altered or missing in both the mutant cells (Fig. 4). Similar analysis of DNA from haploid progeny of two tetrads indicated that the altered Ty band cosegregates with the mutant phenotype in crosses of mutant and wild-type cells. Hence the restriction fragment polymorphism detected by the Ty*1* probe should localize the *COP* mutation in the yeast genome.

DISCUSSION

Since the first isolation and characterization of yeast centromeres (1) and identification of probable attachment sites for spindle microtubules (20), various attempts have been made to isolate specific proteins that interact with *CEN* DNA (21, 22). We have been using various genetic approaches to identify genes involved in centromere function. Using a selection for strains capable of tolerating CEN plasmids at high copy number, we have isolated two strains bearing dominant mutations at a genetic locus designated *COP*. Analysis of these mutations suggests they result from a Ty transposition event(s), perhaps resulting in overexpression of a gene. In our experiment, we used a yeast strain with a temperature-sensitive *cdc10* mutation, thus necessitating growth and selection of transformants at room temperature (≈24°C). This likely contributed to the frequency of Ty transposition since there may be a 100-fold increase in Ty transposition at this lower temperature (23). Clearly, transposition did occur during our experiments as shown by the insertion of a Ty element into pGT60 in one instance during the same experiment, resulting in overexpression of the adjacent G418 resistance gene (5).

If the *COP* mutation has resulted from transposon-mediated overexpression of a gene, it is clear that some additional event or selection is also required for pGT60 to become a high copy number plasmid. As shown in Fig. 2, pGT60 is not a high copy number plasmid in the mutant cells under leucine selection and hence is not functionally equivalent to a plasmid that lacks the *CEN* sequence (pGT61, Fig. 2). It is known that Flp^-,Rep^+ 2-μm plasmids go to high copy number in selected transformants (9), unless the plasmid also contains a functional *CEN* sequence (Fig. 2). Therefore, the *CEN* sequence of pGT60 must be functional in the mutant

cells grown under leucine selection, although the presence of a high copy number plasmid in only 1 out of 10,000 cells would not be detected in Southern blots. The *leu2* mutation can be complemented by low copy number plasmids; however, growth on YPDGen requires a high copy number plasmid and, therefore, selects only cells in which pGT60 occurs in multiple copies (1 in 10,000 cells for the *COP-1* mutant and 1 in 100,000 for the *COP-2* mutant). We have noted that other CEN plasmids such as pCNC2 and pCNC3 remain at low copy number in these G418-resistant cells, indicating that the *COP* mutation does not induce high copy number in CEN plasmids, but does permit selection of those cells containing pGT60 at high copy number. This is consistent with the hypothesis that excess CEN plasmids are toxic to wild-type cells, and the copy number of CEN plasmids is low because of selection against cells containing high copy number CEN plasmids (4). In this view, the *COP* mutation would enable cells containing high copy number CEN plasmids to survive, perhaps by overexpression of a gene whose product is required in proportion to the number of centromeres in a cell. It has also been shown that multiple CEN plasmids in a cell decrease the mitotic stability of the CEN plasmids (4). This could explain the increase in frequency of G418-resistant cells observed when *COP* mutants contain a second CEN plasmid (Table 1), since aberrant segregation of pGT60 would lead to an increase in copy number, and G418 resistance would occur more frequently than in cells containing pGT60 alone.

We suspect that the *COP* mutation might be due to a Ty insertion or rearrangement because expression of the phenotype is dependent on the mating type of the cell. Overexpression of a gene due to Ty insertion in the promoter region is often suppressed in $\mathbf{a}/\alpha$ diploids but not in $\mathbf{a}/\mathbf{a}$ or α/α diploids (18). The COP phenotype is suppressed in $\mathbf{a}/\alpha$ diploids, both those heterozygous and homozygous for the mutation (Table 2). It is not suppressed, however, in diploids that are homozygous for mating type nor in the $\mathbf{a}/\alpha$ haploids disomic for chromosome III that are obtained from crosses of mutant and wild-type cells. The presence of the chromosome III disome and the chromosome VIII deletion (apparently identical in both mutant cells) resembles a situation often found in cells that have undergone transposition events, and such chromosomal aberrations are often unrelated to the resulting mutation (24). Hence, we suspect that the disome and deletion result from a general chromosomal rearrangement that may accompany a transposition event but are not essential for the persistence of the *COP* mutation. The occurrence of apparently identical chromosomal aberrations in both mutants suggests the transposition may have occurred before the transformation, since it seems unlikely that chromosomal rearrangements not required for the mutant phenotype would be reproduced exactly in two cases. It is unclear, however, why the two mutants would have different phenotypes if both resulted from the same transposition event; thus, it is possible that both are the result of independent but similar transpositions.

The strongest evidence that the *COP* mutation results from a Ty-mediated event involves comparison of Ty-containing restriction fragment patterns in genomic Southern blots of DNAs from wild-type and mutant cells. Genomic DNA from haploid yeast cells contains about 35 copies of transposons that hybridize with Ty*1* (25) and that by genomic Southern blots have many restriction fragments that overlap the ends of the transposons. From among many such digests, we located one Ty restriction fragment that was present in the DNA from wild-type cells but absent or altered in both the mutants. This is the opposite situation from what we had expected to find, since a mutation resulting from a Ty insertion would be expected to produce a new Ty band in the mutant cells rather than the removal of a band from the wild-type cell. Since Ty elements are retrotransposons and move by means of an RNA intermediate (26), the original Ty fragment locations should not be changed by a transposition event. Other explanations are possible for the apparent disappearance or movement of a Ty band on the Southern blots; for example, a Ty element may have been altered by a Ty replacement (27), yielding an altered restriction map or altered hybridization properties along with the mutant phenotype. Alternatively, insertion of a Ty may have occurred close to an existing Ty, causing the mutation and altering the restriction map, or deletion of a preexisting Ty element could have occurred, changing the expression level of a nearby gene. The fact that the missing band segregates in crosses with the mutant phenotype suggests it is a tag on the locus of the mutation. This transposon tag should permit isolation and analysis of the genome segment involved in the *COP* mutation.

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