Centromeric Regions Control Autonomous Segregation Tendencies in Single-Division Meiosis of *Saccharomyces cerevisiae*

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ABSTRACT

We have previously shown that yeast *cdc5* or *cdc14* homozygotes can be led through a single-division meiosis in which some of the chromosomes segregate reductionally whereas others, within the same cell, segregate equationally. Chromosomes *XI* tend to segregate reductionally, whereas chromosomes *IV* tend to segregate equationally. In this report we present experiments with *cdc5* homozygous strains, in which the centromeres of one or both chromosomes *XI* was replaced by the centromeric region from chromosome *IV*. Analysis of the products of single-division meioses in these strains demonstrates that the choice between reductional or equational segregation is directed by sequences in the vicinity of the centromeres. Although the choice is made separately for each individual chromosome, the analysis also reveals the existence of a system responsible for coordinated segregation of the two chromosomes of a given pair.

IN a companion study (SHARON and SIMCHEN 1990), we have shown that when diploid strains homozygous for either of the temperature sensitive mutations *cdc5* or *cdc14* are sporulated under shift-down conditions (from 34° to 23° following arrest in meiosis), they form relatively high frequency of dyads which contain two diploid spores. Genetic analysis of such spores showed that each of these dyads resulted from a single division in which centromeres had gone through a mixed segregation. Some centromeres had segregated reductionally, whereas others, within the same cell, had segregated equationally. Such meioses were also observed when cells were incubated at a semirestrictive temperature (32°) during the whole meiotic process (G. SHARON, unpublished results). Segregation analysis of four marked chromosome pairs, in a sample of more than 400 meioses, demonstrated that each of the pairs has a different, specific segregation tendency: chromosomes *IV* tend to segregate equationally, whereas chromosomes *XI* tend to segregate reductionally. Chromosomes *I* and *VII* exhibit intermediate tendencies.

In an attempt to identify the region on the chromosome responsible for the chromosome-specific segregation tendency, we have analyzed the segregation of chromosomes *XI* in *cdc5* homozygotes (which show "mixed meiotic segregation"), following the replacement of the original centromere of this chromosome by the centromere of chromosome *IV*. We find that a region at, or near, the centromere is responsible for the segregation tendency of the chromosome. Both chromosomes of a given pair segregate coordinately, unless a "tendency conflict" is generated by heterozygosity for the replacement. In such a case, the coordination may break down, leading to aberrant segregation and the generation of aneuploid trisomic and presumably monosomic spores. Thus each chromosome of a given pair is autonomous in making the choice between a reductional and an equational division. It is also suggested that some backup mechanism exists which ensures coordinated segregation within each chromosome pair.

MATERIALS AND METHODS

Yeast strains: A list of the strains used in this work, their genotypes and information about their construction is given in Table 1. Strains 11 and 22 were derived from crosses between laboratory strains of various origins. All the diploid strains are matings among derivatives of strains 11 and 22 and are therefore isogenic.

Media: 5-FOA medium has been described (BOEKE, LACROUTE and FINK 1984). The rest of the media were the same as in our companion study (SHARON and SIMCHEN 1990).

Construction of *CEN4* → *CEN11* substitution vectors: Figure 1 illustrates the construction of vectors used to replace *CEN11* by *CEN4* (designated *CEN4* → *CEN11*). The *Eco*RI-*Eco*RI fragment which includes *CEN11* and its flanking sequences was removed from the plasmid pYe(MET14)27 (FITZGERALD-HAYES, CLARKE and CARBON 1982) and cloned into pBLa (a pBR322 plasmid with a 2.2-kb DNA insert encoding the gene *LEU2* inserted into the *Sal*I site. pBLa was constructed in our laboratory, data not shown). The new plasmid, pGSH-2, had only two *Sal*I restriction sites (1.6 kb apart) bracketing *CEN11*, allowing its removal while leaving substantial flanking sequences. The *CEN11* fragment was then replaced by a 4.1-kb *Sal*I-*Xho*I fragment from the plasmid YCp50 (KUO and CAMPBELL

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TABLE 1

Strains

Strain	Derivatives
	Genotypes of parental haploids
11	*MAT***a**, *cdc5-1*, *ura3*, *can1-11*, *ade1*, *trp1*, *leu1*, *met14*
22	*MATα*, *cdc5-1*, *cdc7*, *ura3*, *lys2*
	Haploid derivatives
11R	Isogenic to strain 11, with *CEN4* replacing *CEN11* in the original orientation (with respect to CDE I, II, and III). Replacement was carried out using the substitution fragment R (see MATERIALS AND METHODS)
11W	Isogenic to strain 11, with *CEN4* replacing *CEN11* in the inverted orientation (with respect to CDE I, II and III). Replacement was carried out using the substitution fragment W (see MATERIALS AND METHODS)
22R	Isogenic to strain 22, with *CEN4* replacing *CEN11* in the original orientation (with respect to CDE I, II and III). Replacement was carried out using the substitution fragment R. The substitution deleted the 5′ region of *MET14*, converting it to *met14*.
22Ru	*ura3* derivative of strain 22R. Selected on 5-FOA medium
	Diploids
1122	Mating of 11 × 22
1122-lU	Constructed by integrating *URA3* as well as pBR322 sequences close to *CEN7* in strain 1122 (SHARON AND SIMCHEN 1990)
1122-R/O	Mating of 11R × 22
1122-R/Ru	Mating of 11R × 22Ru
1122-W/O	Mating of 11W × 22
1122-W/Ru	Mating of 11W × 22Ru

1983), containing a 1.5-kb segment of yeast DNA which includes *CEN4* and a 1.2-kb segment bearing *URA3*. The YCp50 fragment was ligated to the flanking regions of *CEN11* in two orientations. The orientation of *CEN4* (MANN and DAVIS 1986) in plasmid pGSH-3 was identical to that of *CEN11* which had been removed (with respect to the internal order of CDEI-CDEII-CDEIII). In plasmid pGSH′-3, *CEN4* was in the opposite orientation. The linear substitution fragments (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; ROTHSTEIN 1983; CLARKE and CARBON 1983) **R** and **W** (**R** standing for right orientation and **W** for wrong orientation) were formed by cleaving the plasmids pGSH-3 and pGSH-′3, respectively, by the two restriction enzymes *Eco*RI and *Bam*HI.

Substitution of centromeres in strains 11 and 22: Transformation of yeast strains was carried out according to ITO *et al.* (1983). Substitution of the centromeres was confirmed in strains 11R, 11W, and 22Ru, by Southern blotting (SOUTHERN 1975) and by genetic segregation. The orientation of the integrated *CEN4* in chromosome *XI* was also confirmed by Southern blotting (data not shown).

Shift-down experiments: Cells were grown vegetatively by shaking at 23° (the permissive temperature) in PSP2 (presporulation medium). When a titer of 10^7 cells/ml was reached, cells were transferred to SPM (sporulation medium). The cells were incubated with shaking for half an hour at 23° in order to allow the completion of mitotic cycles, and were then shifted to 34° (the restrictive temperature). At various times, subcultures were shifted down to the permissive temperature (23°) and cells were allowed to continue sporulation under permissive conditions (in most cases, the shift-down was performed 36 hr after transfer of cells to SPM). Dyads (two-spored asci) were harvested 60 hr after transfer to SPM.

Random spore analysis: See our companion study (SHARON and SIMCHEN 1990).

Detection of a/α colonies among meiotic products: Following ascus digestion and sonication, the spores were plated on canavanine plates enabling only meiotic *can1-11′/can1-11′* products to form colonies (the parent diploid was *can1-11′/CAN11*). After 5 days of incubation at 23°, colonies were replica-tested for mating ability (by mating with lawns of *MAT***a** or *MATα*) and for sporulation ability (by replication onto SPO solid sporulation medium plates). Those colonies which did not mate and had sporulation ability were assumed to have originated from **a**/α diploid spores (the background level of *can1-11/can1-1* mitotic recombinants is less then 10^{-3} and is therefore negligible).

Screening for trisomy of chromosome *XI*: Random-spore progeny of clones derived from diploid spores were examined. In such an analysis, only half of the trisomic cases can be identified. A diploid colony trisomic for a chromosome which is heterozygous for a given marker may have one dominant and two recessive alleles, or one recessive and two dominant alleles. The expected ratio between dominant and recessive phenotypes among the haploid progeny of these trisomics, is 1:1 (as among progeny of a normal diploid heterozygous colony) and 5:1, respectively (Figure 2). We screened for trisomy of chromosome *XI* in mixed meioses by checking Met^+:Met^- ratio, Ura^+:Ura^- ratio or both (depending on the strain), among random meiotic products of each diploid spore. Diploid colonies whose meiotic products showed a marked deviation from the expected ratio of 1:1 were checked further by dissection of tetrads.

Correcting the bias of tendencies due to trisomy and monosomy: Each aberrant segregation is expected to give rise to one trisomic and one monosomic diploid spores (diploids monosomic for chromosome *XI* are known to be viable, see KAWASAKI 1979). However, the screening method we used could detect spores trisomic, but not monosomic for chromosome *XI*. Monosomic diploids would seem to be homozygous for a marker on the aneuploid chromosome. In order to take into account the effect of these abnormal genotypes on the observed segregation tendencies, we subtracted the trisomic cases from the heterozygous spores, as well as an equal number of putative monosomic cases from the homozygous spores (with respect to markers on chromosome *XI*).

RESULTS

Genetic analysis of random a/α diplold spores: Previous experiments have shown that under conditions of late temperature shift-down of *cdc5* homozygotes, single-division meioses with "mixed meiotic segregation" are observed (SHARON and SIMCHEN 1990). Different chromosomes exhibit different, chromosome-specific segregation tendencies in these meioses. In order to make a quick evaluation of segregation tendencies of chromosomes in a large number of dyads, we used an alternative approach to the dissection of individual asci. Samples were obtained by direct screening for **a**/α progeny among colonies de-

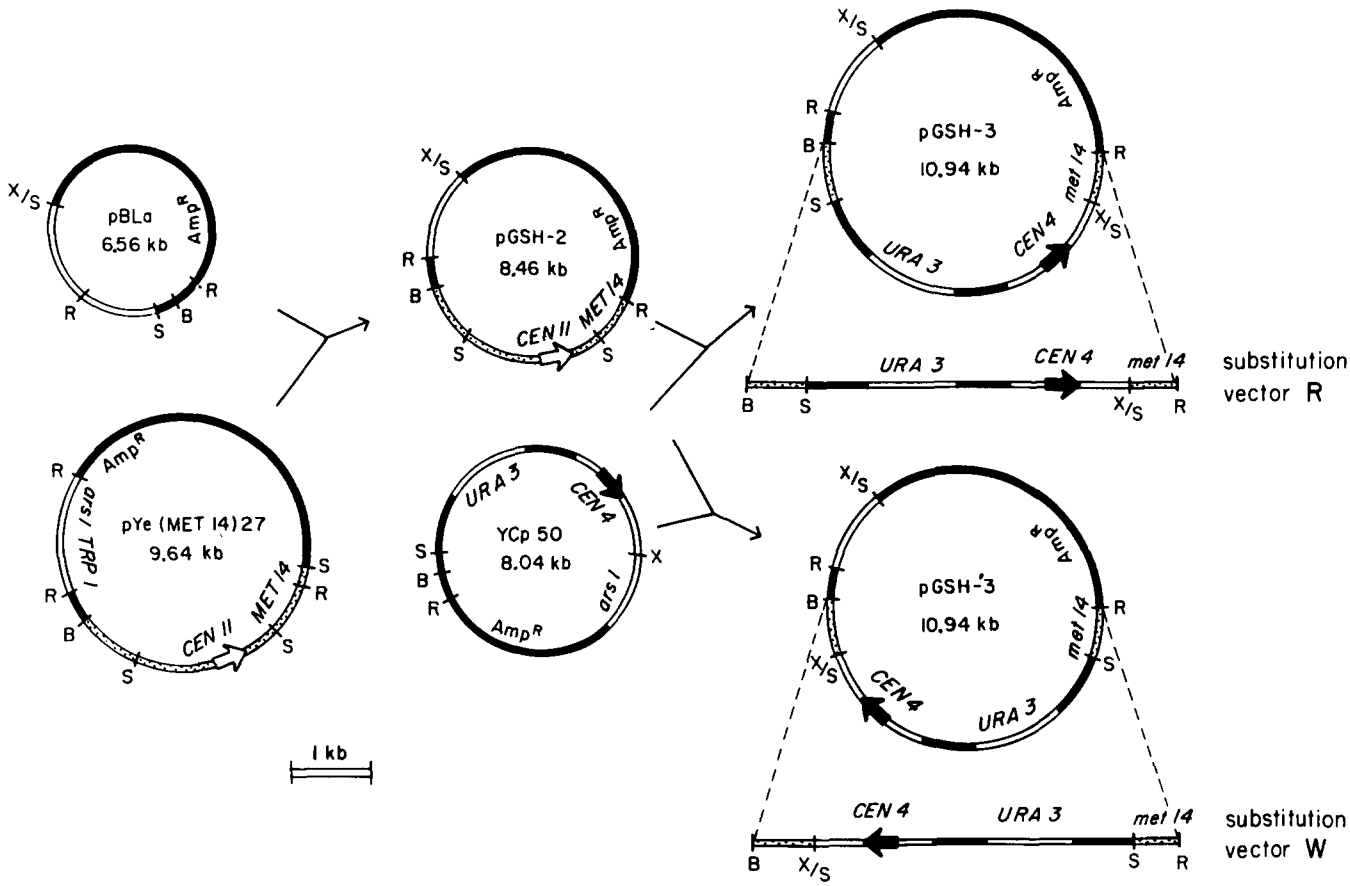

FIGURE 1.—Construction of substitution vectors **R** and **W**. *CEN11* in pGSH-2 was replaced by a fragment containing *URA3* and *CEN4* from the plasmid YCp50 (obtained from M. ROSE). Orientation of *CEN4* in pGSH-3 is the same as *CEN11* with respect to CDEI, II and III. Orientation in pGSH-3'r is inverted. Transformation was carried out using *URA3* as a selective marker. The only restriction sites shown are: *Bam*HI (B), *Hin*dIII (H), *Sal*I (S), *Eco*RI (R), *Xho*I (X). All plasmids are drawn to scale.

veloped from individual spores that were germinated on canavanine medium. After sporulation of these **a**/α colonies, random-spore analysis was carried out for each in order to determine its genotype and individual chromosome segregation pattern. A chromosome pair which was homoallelic for its centromere-linked marker had gone through a reductional segregation, whereas a chromosome pair heteroallelic for the marker had undergone an equational segregation (SHARON and SIMCHEN 1990).

We first set up two control experiments in order to test the effect of YIp5 DNA sequences introduced into a centromere linked location and in order to examine the effects of the time of the shift on the segregation tendency.

A summary of chromosome segregations in 49 random **a**/α diploid spores of strain 1122 is given in experiment 1 of Figure 3a. The bars represent the segregation tendencies of chromosomes *I*, *IV*, *VII* and *XI* (segregation tendency of a given chromosome is defined here as the percent of equational segregations among the diploid spores analyzed). Also shown are four independent experiments performed with strain 1122-lU (carrying a YIp5 insertion, see MATERIALS AND METHODS). In these experiments (2–5 in Figure 3), cells were shifted down from restrictive to permissive temperature at various times after transfer to sporulation medium. Differences in the segregation tendencies between chromosomes in each experiment were statistically highly significant (contingency chi-square tests not shown). Although the segregation frequencies of the chromosome pairs were somewhat different in each experiment, the relative tendencies of the pairs were similar in all of them. The pair of chromosomes *IV* had the highest tendency to segregate equationally and the pair of chromosomes *XI* had the lowest tendency. These relative tendencies were similar to the ones of strain 1122-lU obtained by dyad analysis (SHARON and SIMCHEN 1990). The probability of obtaining by chance such a consistent result from the five experiments shown in Figure 3a is extremely low. If each chromosome pair can have either high, medium, or low tendency, the probability of chromosomes *IV* having the highest tendency in experiments 2–5, following experiment 1, is $(1/3)^4$. Likewise, the probability for chromosomes *XI* having the lowest tendency in the same experiments is $(1/2)^3$. Multiplication of these two probabilities gives 1/1296,

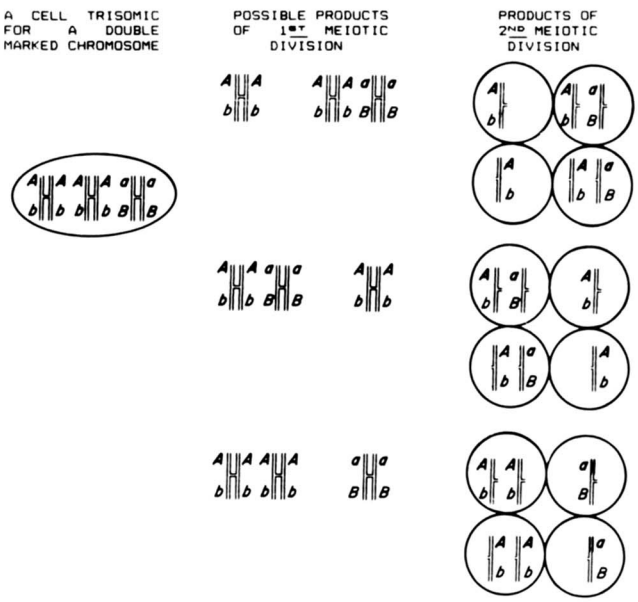

FIGURE 2.—Possible segregations of trisomic chromosome during the meiotic divisions. *A* and *a* are dominant and recessive alleles of one gene, and *B* and *b* of another. When the trisomy consists of one a and two A chromosomes, the expected ratio between a and A phenotypes among the meiotic progeny is 1:5. When it consists of one B and two b chromosomes, the expected ratio is 1:1. In cases where the trisomic chromosome is marked *in trans* by two heterozygous markers (as drawn above), all cases of trisomy can be detected by analysis of the meiotic products. In cases where the chromosome is marked by only one heterozygous marker, trisomy can be detected only if it consists of one recessive and two dominant alleles (one half of the cases).

and if the dyad analysis (SHARON and SIMCHEN 1990) is also taken into account, the probability is even lower.

Centromere substitutions on chromosome *XI*: Since the microtubules, which are attached to the chromosomes at the centromeres, are part of the segregation machinery, we wanted to study the effect of the centromeric regions on segregation tendencies. We therefore replaced the centromeric region of chromosome *XI* with that of chromosome *IV*, using the *CEN4* → *CEN11* substitution vectors **R** and **W** (see MATERIALS AND METHODS, and Figure 1). The vectors carry the yeast *URA3* gene along with *CEN4*, allowing the detection of transformants with substituted centromeres. Strains 11R, 22R and 22Ru carry a substituted *CEN4* on chromosome *XI* in an orientation identical to that of *CEN11* which has been removed (with respect to the internal order of CDEI-CDEII-CDEIII). In strain 11W, *CEN4* on chromosome *XI* has the opposite orientation.

***CEN4* substituting *CEN11* confers on chromosome *XI* a tendency for equational segregation:** Strains 11R and 22Ru were mated to each other to form a diploid strain designated 1122-R/Ru, which has *CEN4* sequences instead of *CEN11* in both of its chromosomes *XI*, and is heterozygous for the inserted *URA3*

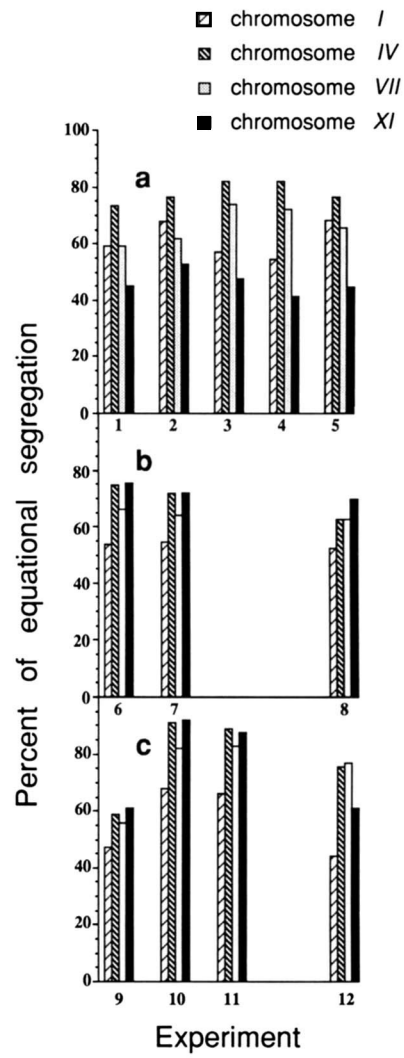

FIGURE 3.—Segregation-tendencies of four chromosomes in experiments with various strains undergoing "mixed segregation meiosis." Unless stated otherwise, the time of the temperature shift-down was 36 hr after the transfer of the cells to SPM. The chromosome segregation tendencies were determined by following the segregation of centromere-linked marker's on chromosomes *I*, *IV*, *VII* and *XI*. Each bar represents percent of equational segregations for a given centromere-linked marker among the diploid spores analyzed. (a) Experiments 1–5: Shift-down experiments which summarize the segregation of markers in 49, 38, 34, 84 and 22 random diploid spores, respectively. Experiment 1 was performed with strain 1122. Experiments 2–5 were performed with strain 1122-lU (cells were shifted-down to permissive temperature 17, 25, 34 and 39 hr after transfer to SPM medium, respectively). (b) Experiments with strains in which the centromere regions of both chromosomes *XI* were substituted. Experiments 6–7 were performed with strain 1122-R/Ru, with both inserted *CEN4* sequences in the same orientation as the original, nonsubstituted *CEN11*. Experiment 8 was carried out on strain 1122-W/Ru, with one inserted *CEN4* in the original orientation and one in the inverted orientation. Experiments 6–8 summarize the tendencies in 130, 95 and 59 random diploid spores, respectively. (c) Experiments with strains in which the centromere region on one of the *XI* chromosomes has been substituted. Experiments 9–11 were performed with strain 1122-R/O, with the inserted *CEN4* in the same orientation as *CEN11* on the homologous chromosome. Experiment 12 was carried out on strain 1122-W/O, with the inserted *CEN4* inverted in orientation relative to that of *CEN11* on the homologous

centromere-linked gene.

Chromosome tendencies in two experiments employing strain 1122-R/Ru are shown in Figure 3b. The centromere replacement resulted in a drastic change in the segregation tendency of chromosomes *XI*. In strain 1122-lU the chromosomes *XI* tended to segregate reductionally whereas in strain 1122-R/Ru they tended to segregate equationally, like chromosomes *IV*.

Substitution vectors **R** and **W** carry, besides the *CEN4* region, DNA sequences that are present in YIp5. We have shown (see above) that insertion of YIp5 sequences into a centromere-linked site of chromosome *VII* did not impair or alter the segregation-tendency of chromosomes *VII* or other chromosomes in "mixed segregation" meioses of strain 1122-lU (compare experiment 1 with experiments 2–5 in Figure 3a) It therefore seems that the differences in segregation, observed in strain 1122-R/Ru as well as in the other substituted strains, are due to the *CEN4* → *CEN11* replacement and not to the insertion of YIp5 sequences.

Coordinated segregation of the two homologous chromosomes during mixed segregation: In an analysis of 529 independent chromosome segregations of strain 1122-lU (SHARON and SIMCHEN 1990), no cases of monosomy or trisomy were detected. We checked the effect of centromere substitution on the segregation fidelity of chromosomes by screening for trisomy among the diploid spores produced in mixed meioses of strain 1122-R/Ru (see MATERIALS AND METHODS). None of the 104 spores that were checked exhibited trisomy for chromosome *XI*, nor was it found for any of the other heterozygous markers. These results were similar to those obtained for strain 1122-lU (SHARON and SIMCHEN 1990)

Heterozygosity for centromere substitution results in increased levels of meiotic nondisjunction: Chromosomes *XI* in strain 1122-lU tend to segregate reductionally whereas in strain 1122-R/Ru, the chromosomes *XI* with the substituted centromeres tend to segregate equationally, as do chromosomes *IV*. In both cases, the two chromosomes *XI* were homocentric (*i.e.*, carried the same centromeres), conferring similar segregation tendencies on both chromosomes. We wanted to test the effect of tendency conflict between the homologs. In order to do this, we constructed a heterocentric strain with one normal chromosome *XI* and one with a *CEN4* substitution by crossing strain 11R with strain 22. The resulting diploid, 1122-R/O, was led through mixed meiotic segregation. Diploid **a**/α colonies were isolated, and the segregation of the four marked chromosomes in each spore colony was determined by random-progeny analysis. Chromosome *XI* homologs were marked by two centromere-linked markers. The chromosome bearing *CEN4* was marked by *met14::URA3* and the chromosome bearing the original *CEN11* was marked by *MET14*. Thus, all cases of trisomy of chromosomes *XI* could be detected. The screening for the trisomy was carried out as described above. The results obtained were in striking contrast to the ones obtained for strains 1122-lU and 1122-R/Ru. Of 57 spores checked, 8 were trisomic for chromosome *XI* (Table 2). Furthermore, in all of the cases, the trisomy consisted of two chromosomes bearing the original *CEN11* and one bearing the substituted *CEN4*. Trisomy of other chromosomes was not detected.

TABLE 2

Coordination in segregation of the two chromosomes *XI* in the different isogenic strains

Strain	Centromeres of chromosomes *XI*[a]	No. of spores	Cases of trisomy[b]		
			No.	A	B
1122-lU	→ → *CEN11/CEN11*	89	0		
1122-R/Ru	→ → *CEN4/CEN4*	104	0		
1122-R/O	→ → *CEN4/CEN11*	57	8	8	0
1122-W/Ru	← → *CEN4/CEN4*	35	0		
1122-W/O	← → *CEN4/CEN11*	40	5	5	0

[a] →, Original orientation. ←, Inverted orientation.
[b] A, Trisomy which consists of two chromosomes with original centromeres and one with a substituted centromere. B, Trisomy which consists of two chromosomes with substituted centromeres and one with an original centromere.

Random **a**/α spores of three shift-down experiments with strain 1122-R/O were analyzed (experiments 9–11). The segregation tendencies of the marked chromosomes in each experiment are summarized in Figure 3c. The tendencies are given after correction for the bias caused by the aberrant segregation cases of chromosomes *XI* (see MATERIALS AND METHODS). The illustration shows that the tendencies were similar to those obtained for strain 1122-R/Ru. In each of the experiments the tendency of chromosomes *XI* was very similar to that of chromosomes *IV*.

Effects of inversion of substituted centromeres on segregation patterns: We wanted to see what would be the effects of a change in the orientation of one centromere relative to the other, on segregation-tendency and coordinated segregation. In order to do so, the *CEN11-URA3* fragment from plasmid pGSH'-3

chromosome. Experiments 9–12 summarize the tendencies in 34, 66, 53 and 90 random diploid spores, respectively. In these experiments, a number of chromosome *XI* trisomy cases were detected, affecting the segregation tendencies of that chromosome. The tendencies of chromosome *XI* are therefore given after correction (see MATERIALS AND METHODS).

was used for the substitution of *CEN11* of strain 11 by an inverted *CEN4* (*CEN4* being in the opposite orientation to that of the original centromere). Strain 11W was crossed to strain 22 and to strain 22Ru. The resulting diploid strains, 1122-W/O and 1122-W/Ru, had the centromeres of the two chromosomes *XI* in opposite orientations. In strain 1122-W/O, *CEN4* on one chromosome was in opposite orientation to *CEN11* on the homologous chromosome. In strain 1122-W/Ru, *CEN4* on one of the chromosomes *XI* was inverted in orientation with respect to that of *CEN4* on the other. Coordination of segregation of chromosomes *XI* is summarized in Table 2, and the relative chromosome segregation tendencies in the two strains, are shown in Figure 3, b and c (experiments 8 and 12). No chromosome *XI* trisomies were detected among the mixed segregation products of strain 1122-W/Ru. In strain 1122-W/O, on the other hand, 5 out of 40 spores checked were identified as trisomic for chromosome *XI*. In all five cases, the trisomy consisted of two chromosomes carrying the original *CEN11*, and one chromosome with *CEN4*. These results are similar to those obtained for strains 1122-R/Ru and 1122-R/O, respectively (see Table 2).

Figure 3b shows that the segregation tendencies of the marked chromosomes of strain 1122-W/Ru are similar to those of strain 1122-R/Ru. The segregation tendencies of chromosomes *XI* in 1122-W/O are somewhat different from those of strain 1122-R/O (Figure 3c) but the difference is not statistically significant. Analysis of additional samples is required in order to determine the existence of such small effects of centromere orientation on segregation tendency.

DISCUSSION

Proper meiotic segregation appears to depend on several unique features of chromosome behavior in meiosis, namely pairing of homologs and recombination prior to the first meiotic division (the reductional division), and the delay in separation of sister centromeres until the second meiotic division (the equational division). Cells of *S. cerevisiae*, homozygous for the temperature-sensitive mutations *cdc5* or *cdc14*, may undergo a single-division meiosis in which some chromosomes segregate reductionally, whereas others, within the same cells, segregate equationally (SHARON and SIMCHEN 1990). At the same time, however, both members of each bivalent appear to segregate coordinately and accurately and no cases of nondisjunction have been detected. Furthermore, different chromosomes have different segregation tendencies and these tendencies are chromosome-specific.

We suspected that the centromeres, or elements close to the centromeres, might be responsible for the differences in chromosomal segregation tendencies, because these are the microtubules attachment sites (*i.e.*, the segregation machinary). In order to test this possibility, we substituted *CEN11* by *CEN4*, using two *CEN4* → *CEN11* substitution vectors (Figure 1), in one or both of the chromosomes *XI* of our test strains (1122 and its derivatives). The centromere substitutions were done in both native and inverted orientations. The analysis showed that in nonsubstituted strains, chromosome *XI* has the lowest equational segregation frequency, chromosomes *I* and *VII* have intermediate frequencies, and chromosome *IV* has the highest frequency (Figure 3a, see also SHARON and SIMCHEN 1990). The substitution of *CEN11* by *CEN4* in both homologs of chromosome *XI* resulted in a significant change in the tendency of that chromosome to segregate equationally, compared with the other chromosomes (Figure 3). The frequency at which the chromosomes *XI* segregated equationally in the substituted strain (experiments 6 and 7) was as high as that of chromosomes *IV*. Likewise, the substitution of *CEN11* by *CEN4* in only one of the two homologs of chromosome *XI* also resulted in a rise in the equational segregation tendency of chromosomes *XI* to the level of chromosomes *IV* (Figure 3, experiments 9–11).

In strain 1122-R/Ru, where both chromosomes *XI* carried *CEN4*, the segregation of chromosomes *XI* was coordinated, as no cases of nondisjunction were observed (Table 2), and in this respect it is similar to the original strain 1122-lU. "Mixed-segregation" meioses of 1122-R/O, an isogenic strain in which only one of the *CEN11*s was substituted, differed sharply from the former strains in that respect. We identified abnormal chromosome *XI* segregation in about 1/7 of the meioses checked. All of the trisomic spores carried one substituted and two original chromosomes (see Table 2).

The inverted orientation of the implanted *CEN4* had no apparent effect on the segregation tendencies or on the accuracy of segregation. Strain 1122-W/Ru, with two substituted centromeres, one inverted in orientation with respect to the other, was similar to strain 1122-R/Ru, both in chromosomal segregation tendencies and in the fidelity of segregation (Figure 3b and Table 2, respectively). Segregation tendencies of strain 1122-W/O, with only one, inverted substituted centromere, were somewhat different from those of strain 1122-R/O (Figure 3c), but at the given sample size, the difference was not statistically significant. The frequency of abnormal chromosome *XI* segregation was also similar in these two strains (Table 2).

In the two heterocentric strains (1122-R/O and 1122-W/O), only one of two possible types of chromosome *XI* trisomy was observed (Table 2). In all cases of trisomy, the chromatids of the chromosome with the original centromere did not separate from

each other, whereas the chromatids of the one with the substituted centromere did. These results correlate with the tendency of *CEN11* to segregate reductionally and the tendency of *CEN4* to segregate equationally. The fact that trisomics were not produced by the homocentric substitution strains argues against the possibility that trisomy resulted from the substitution into chromosomes *XI per se*. The trisomic cases suggest that each of the chromosomes in a given pair is autonomous in making the choice between reductional or equational segregation. However, if there was a complete chromosomal autonomy to choose between reductional and equational segregation, higher frequencies of trisomy were expected. Considering the segregation tendencies of chromosomes *IV* and *XI* (which segregate equationally in 82% and 48% of the cases, respectively, if experiment 4 in Figure 3a is used as a reference), 50% of both homocentric (2[0.48 × 0.52]) and heterocentric ([0.48 × 0.18]+ [0.52 × 0.82]) chromosome pairs were expected to segregate abnormally. Nevertheless, no aberrant segregations of chromosomes *XI* were observed in the homocentric strains and relatively low frequencies were found in the heterocentric ones. We therefore believe that each of the chromosomes in a given pair has a limited degree of autonomy in making the choice between reductional and equational segregation. This autonomy is emphasized in cases of intrabivalent segregation conflict caused by the presence of heterocentric regions on the chromosomes *XI*. However, the low levels of trisomy, compared to what one would expect based on complete autonomy, indicate the existence of a mechanism which ensures coordinated segregation of both chromosomes of a given pair.

We postulated that putative intercentromeric interactions, required for coordinated segregation, might be affected by inverting the orientation of one centromeric region with respect to its homolog. However, no increase in the frequency of aberrant segregation was observed in strains with homologous or heterologous centromeres in opposite orientations (Table 2).

Substitution of either one or both of the *CEN11*s resulted in a noticeable change in the segregation tendency of chromosomes *XI*. The alteration in the chromosomal segregation tendency upon *CEN4* → *CEN11* substitution shows that the centromeric regions in chromosomes *XI* and *IV* contain elements responsible for making the choice between the segregation patterns. Substitution of one of the centromeres was sufficient to make the change, showing dominance of the *CEN4* effect over that of *CEN11*. Additional substitutions are needed in order to determine whether this effect is specific to the interaction between these two unique sequences or whether it reflects a general dominance of the equational segregation pattern over the reductional one.

It seems that the choice between reductional or equational segregation is made at three levels: The level of the individual chromosome, as observed in the cases of chromosome *XI* trisomy; the level of the bivalent, as demonstrated by the different segregation behavior of different chromosome pairs in single-division meiosis (mixed chromosome segregation) and the level of the whole cell as seen in normal meiosis, which consists of reductional segregation of all chromosomes in the first division, followed by equational segregation in the second. The cooperative action of regulation at all three levels ensures the accurate segregation of centromeres during meiosis.

We have shown that the centromeres, or adjacent sequences are responsible for the choice between the two segregation types during the single division meiosis in *cdc5/cdc5* strains. Additional chromosome manipulations are required in order to determine these sequences more precisely. We believe that these sequences, once identified, will prove to be elements that control the choice between reductional or equational segregation not only in "mixed meiotic segregation" but in regular meiosis as well. Studies of meiotic segregation in strains with mutated centromeres (PANZERI *et al.* 1985; MCGREW, DIEHL and FITZGERALD-HAYES 1986; CUMBERLEDGE and CARBON 1987; GAUDETO AND FITZGERALD-HAYES 1987, 1989) indicate that the subcentromeric element CDEIII is important for mitotic segregation, whereas CDEI and CDEII are important for the first meiotic segregation. These latter elements are possible candidates for the chromosomal structures which make the segregation choice in "mixed" meiosis.

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