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Acquisition and Processing of a Conditional Dicentric Chromosome in *Saccharomyces cerevisiae*

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The introduction of a conditional centromere into chromosome III of *Saccharomyces cerevisiae* provided an opportunity to evaluate phenotypic and karyotypic consequences in cells harboring dicentric chromosomes upon entry into mitosis. A mitotic pause ensued, and monocentric derivatives of chromosome III were generated at a high frequency.

The transmission of chromosomes in *Saccharomyces cerevisiae* can be manipulated effectively with a conditional centromere. The conditional centromere consists of a centromere that is regulated by the inducible *GAL1* promoter in yeast cells (6). Utilization of growth conditions that favor transcriptional induction (galactose as the carbon source) or repression (glucose as the carbon source) from the *GAL1* promoter results in centromere function that is switched off or on, respectively. In this paper, we describe the construction of a conditional dicentric chromosome. Observations made in a number of different organisms suggest diverse behaviors for dicentric chromosomes. These range from the extreme instability of chromosomes that undergo a breakage-fusion-bridge cycle through subsequent mitotic cell divisions (9, 10) to the stable transmission of a chromosome with eight centromeres (13). With an increased understanding of the molecular structure of the centromere, this diversity of behavior may be explained.

Acquisition of a dicentric chromosome results in mitotic lag. A conditional centromere was introduced into the left arm of chromosome III at the *HIS4* locus, approximately 45 kilobases (kb) from *CEN3* (Fig. 1). Logarithmically growing cultures of strains containing this construction (J178-1D#7 *MAT***a** *ade1 met14 ura3 leu2 his3 HIS4*::pBR322; *URA3*; *GALCEN3*) were switched from galactose-containing to glucose-containing medium. Cells appeared to be synchronous for mitosis after approximately 1.5 to 2 generations in the glucose-containing medium. The parent strain, J178-1D (*MAT***a** *ade1 met14 ura3 leu2 his3*), did not exhibit a similar mitotic pause. A majority of the cells had buds that were equivalent in size to those of the mother cells. Staining with the fluorescent dye Hoechst-33342 revealed that cells in the nuclear division stage were approximately three times more numerous in the experimental cultures than in control cultures raised in the galactose-containing medium.

Monocentric derivatives. Figure 2 shows the electrophoretic karyotype of chromosomes after the carbon source switch. After two generations in the glucose-containing medium, a new chromosome band smaller than chromosome III was evident (Fig. 2, lane 1). This chromosome appeared when the mitotic lag was first observed. The intensity of this chromosome increased over time, while that of its wild-type counterpart decreased. It was apparent after eight generations (Fig. 2, lane 4) that cells harboring this new chromosome constituted a major portion of the population. This 325-kb chromosome derivative contained DNA homologous to the *GAL1-GAL10* probe, while the 370-kb chromosome III band in all lanes in Fig. 2 (WT to 6), as well as the 325-kb chromosome in lanes 1 to 6, contained sequences homologous to *CEN3* (data not shown). The major rearrangement involving the dicentric chromosome is therefore a derivative of chromosome III that has retained the conditional centromere and is approximately 45 kb smaller than the wild-type chromosome III.

Primary deletion derivative is a consequence of recombination between the *CEN3* repeats. Restriction enzyme and Southern hybridization analyses were used to examine the physical structure of the conditional and wild-type *CEN3* in the rearranged chromosomes. The conditional centromere (*GALCEN3*) was contained within a 0.865-kb *Bam*HI restriction fragment, and wild-type *CEN3* resided on an 8.2-kb *Bam*HI fragment. A reciprocal recombination event mediated by the *CEN3* homology within these restriction fragments would generate both a 1.15- and a 7.9-kb *Bam*HI fragment. Figure 3A shows the results of *Bam*HI restriction analysis carried out on DNA samples isolated as described for Fig. 2. The zero time point of this experiment demonstrated the presence of both *CEN3*-containing *Bam*HI fragments. At subsequent time points a 1.1-kb *Bam*HI fragment appeared that increased in intensity. The kinetics with which this new *Bam*HI restriction fragment appeared were very similar to those for the appearance of the derivative chromosome (Fig. 2). The size of this new fragment is consistent with a crossover event between the *CEN3* repeats that serves to join the left arm of chromosome III at the *HIS4* locus to the right arm of chromosome III at the position of the wild-type centromere (Fig. 1B). In addition, the deletion derivative lacks any DNA homologous to ^{32}P-radiolabeled *LEU2* sequences that map 21 kb to the right of *HIS4* and 25 kb to the left of *CEN3* (5, 11).

The reciprocal product of an exchange between each *CEN3* is a 45-kb circle consisting of the intervening chromosomal DNA stabilized by wild-type *CEN3*. This would result in the generation of an additional 7.9-kb *Bam*HI fragment. This fragment is not readily resolved from the wild-type centromere-containing fragment (8.2 kb). An alternative restriction digest (*Hin*dIII) that would generate two distinct *CEN3*-containing fragments was performed. This restriction analysis did not reveal any evidence of a reciprocal product (data not shown). The results of direct examination for the 45-kb product by electrophoretic karyotyping were also negative. These findings indicate the lack of essential genes in the interval between *HIS4* and *CEN3* in this strain as well. This may not be the case in general, however, as chromo-

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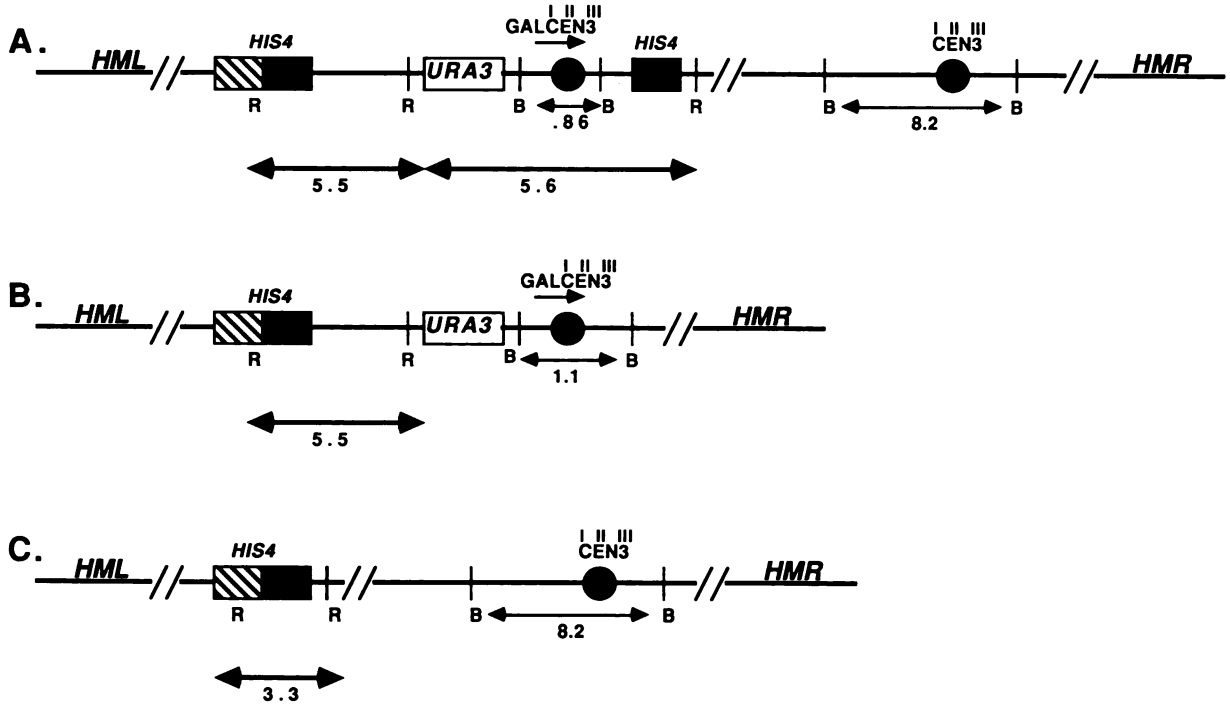

FIG. 1. Conditional dicentric chromosome III and monocentric derivatives. (A) Dicentric chromosome III resulting from the integration of pR285-GALCEN3. pR285-GALCEN3 was constructed from the substitution vector pR285 (7), which consists of a 1.56-kb *Sal*I fragment containing the 5′ end of the *HIS4* gene (▬) as well as the *URA3* gene (▭) as a selectable marker in pBR322. *GALCEN3* (●) was inserted into pR285 as a 0.865-kb *Bam*HI fragment to generate pR285-GALCEN3. *Xho*I restriction within the *HIS4* sequence served to target the conditional centromere and *URA3* into the *HIS4* locus (▨) on the left arm of chromosome III. The conditional centromere is approximately 45 kb from wild-type *CEN3* (7, 11). The arrow above *GALCEN3* denotes the direction of transcription from the *GAL1* promoter. The elements of centromere homology (centromere DNA elements I, II, and III) are indicated and are directly repeated with respect to the wild-type *CEN3* sequence in this construction. The horizontal line represents chromosome III; breaks in the line indicate that the drawing is not to scale. *HML* and *HMR* indicate the relative positions of the yeast mating-type loci on the left and right arms, respectively, of chromosome III. Numbers and arrows below the chromosome indicate sizes in kilobase pairs of the *Eco*RI and *Bam*HI restriction fragments after integration of pR285-GALCEN3. Restriction sites are *Eco*RI (R) and *Bam*HI (B). (B) Monocentric 45-kb deletion derivative resulting from recombination between the 340 base pairs of *CEN3* sequence homology. This event results in the deletion of information between *GALCEN3* and *CEN3*. The *Bam*HI fragment containing *GALCEN3* increases in size from 0.865 to 1.15 kb. (C) Monocentric derivative resulting from recombination between the 1.56-kb of *HIS4* sequence flanking *GALCEN3*. The *HIS4 Eco*RI fragment decreases from 5.5 to 3.3 kb.

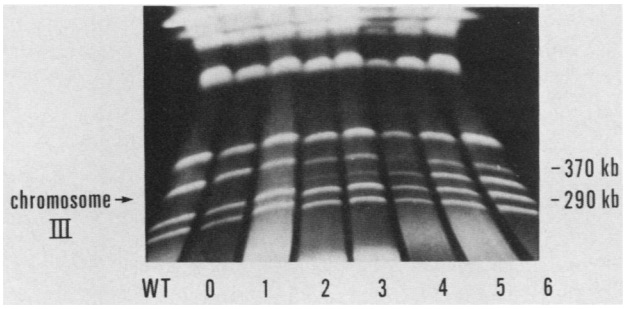

FIG. 2. Kinetics of karyotypic change. Haploid strain J178-1D#7, dicentric at chromosome III, was grown to logarithmic phase in a synthetic medium containing galactose. Cells were harvested, washed one time in sterile water, and suspended and cultured in a synthetic medium containing glucose. Samples (approximately 1.5 × 10^9 cells) were taken at intervals and prepared for orthogonal field alternating gel electrophoresis analysis (4, 12). The DNA was electrophoresed in 1% agarose at 250 V for 18 h with a 30-s pulse interval and stained in ethidium bromide. The wild-type (WT) lane represents cells of the untransformed parent strain, J178-1D. The lane for the zero time point (lane 0)represents J178-1D#7 cells grown in medium containing galactose. The remaining lanes represent chromosomes from cells harvested after the following number of hours or generations in glucose-containing medium: lane 1, 3 h or ≈2 generations; lane 2, 5.5 h or ≈2.4 generations; lane 3, 8 h or ≈2.8 generations; lane 4, 27 h or ≈7.8 generations; lane 5, 51 h or ≈14.8 generations; lane 6, 73 h or ≈21.4 generations. Generation time was determined by counting cells with a hemacytometer. Chromosome III (370 kb) is the third band from the bottom in lane WT, and chromosome VI (290 kb) is the second band from the bottom (4).

some III in the parent strain, J178-1D, is larger than chromosome III in other strains (unpublished results).

Recombination between *HIS4* repeats removes conditional centromere from chromosome III. A minor class of chromosome III rearrangements detected by these experiments is a pop-out of the conditional centromere from chromosome III. DNA was prepared from selected time points as described above and restricted with *Eco*RI. DNA fragments were electrophoretically separated, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled *HIS4* DNA fragment. The appearance of a 3.3-kb *Eco*RI fragment (Fig. 3B) with time suggests that a specific rearrangement occurred. The exci-

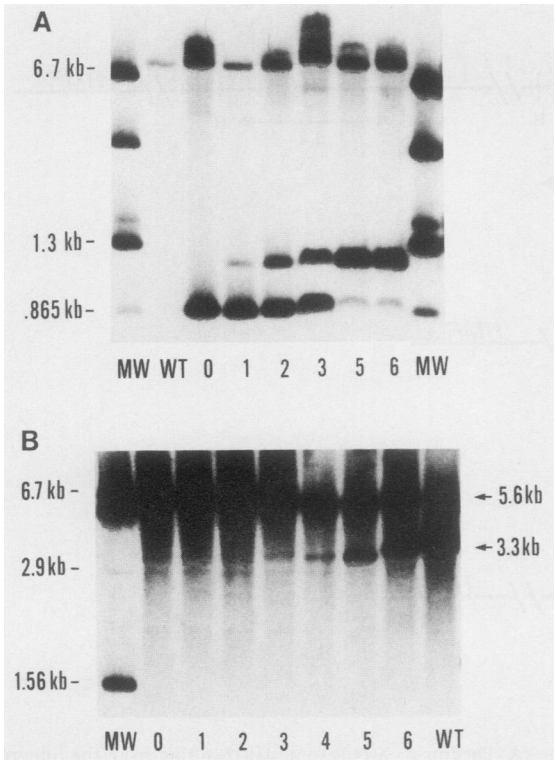

FIG. 3. Restriction analysis of *CEN3* and *HIS4* in the monocentric derivatives. Genomic DNA was prepared from J178-1D#7 cells harvested in the time course experiment described in the legend to Fig. 2. DNA was restricted with *Bam*HI (A) or *Eco*RI (B) and electrophoresed on a 1.1% agarose gel for 3 h at 150 V. The gels were transferred to nitrocellulose and hybridized with a ^{32}P-labeled DNA fragment containing the *CEN3* sequence (2) (A) or a ^{32}P-labeled fragment containing *HIS4* DNA (7) (B). The two outside lanes (MW) in panel A are molecular weight markers; the 0.865-, 1.3-, and 6.7-kb markers are indicated. The time points for lanes WT, 0, 1, 2, 3, 5, and 6 are the same as those described in the legend to Fig. 2. The zero time point demonstrates both the 8.2-kb *Bam*HI fragment that contains wild-type *CEN3* and the 0.865-kb *Bam*HI fragment that contains the conditional centromere. For panel B, the 5.5-kb *Eco*RI fragment contains unrearranged *HIS4* DNA. The cells were harvested after the following number of hours or generations in glucose-containing medium: lane 1, 3 h or ≈1.25 generations; lane 2, 5.5 h or ≈2.4 generations; lane 3, 8 h or ≈3 generations; lane 4, 23 h or ≈6.5 generations; lane 5, 29 h or ≈8.5 generations; lane 6, 47 h or ≈15 generations. WT, DNA prepared from the untransformed parent strain, J178-1D.

sion of intervening DNA between the *HIS4* repeats resulted in the loss of *GALCEN3* sequences, *URA3*, and vector DNA, generating the 3.3-kb *Eco*RI fragment (Fig. 1C). Evidence for this event was not distinct until 6.5 generations, or 23 h (Fig. 3B, lane 4). This event occurred less frequently than that for the 45-kb deletion derivative. The kinetics of appearance of the 3.3-kb *Eco*RI restriction fragment could be attributed to the presence of these recombinants in the population prior to the switch. Once the strain is switched to glucose, monocentric recombinants would not be encumbered by mitotic lag and would have a selective growth advantage over cells harboring a dicentric chromosome III.

Attachment of a functional dicentric chromosome to opposite poles is likely to result in a double-strand break. The predominant rearrangement found in dicentric strains is the product of recombination between the *CEN3* repeats (Fig. 2 and 3). Detection of the 325-kb linear product without the reciprocal product suggests that dicentric chromosome breakage followed by exonucleolytic action is the event initiating *CEN3* recombination. The efficiency with which the predominant chromosome rearrangement occurs in this strain is striking, considering the short region of DNA sequence homology shared by the two *CEN3* repeats. In *GALCEN3* there are 340 base pairs of DNA sequence derived from the wild-type *CEN3*. Approximately 250 base pairs of this sequence, in both the conditional and wild-type centromeres, are within the nuclease-protected structure associated with centromere chromatin (2, 3). This structure is a tightly associated complex of DNA and proteins (1). It is interesting that the presence of this protein-DNA complex did not have an inhibitory effect on the mitotic recombination that we observed. Recently, Liebman et al. (8) demonstrated the existence of mitotic gene conversion events extending through the centromere as well. The combined roles of centromere DNA in both chromosome attachment and genetic recombination seems to indicate a functional diversity not previously attributed to this DNA sequence.

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