

Identification and Characterization of the Centromere from Chromosome XIV in *Saccharomyces cerevisiae*

MAUREEN NEITZ AND JOHN CARBON*

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

Received 8 April 1985/Accepted 1 August 1985

A functional centromere located on a small DNA restriction fragment from *Saccharomyces cerevisiae* was identified as *CEN14* by integrating centromere-adjacent DNA plus the *URA3* gene by homologous recombination into the yeast genome and then by localizing the *URA3* gene to chromosome XIV by standard tetrad analysis. DNA sequence analysis revealed that *CEN14* possesses sequences (elements I, II, and III) that are characteristic of other yeast centromeres. Mitotic and meiotic analyses indicated that the *CEN14* function resides on a 259-base-pair (bp) *RsaI-EcoRV* restriction fragment, containing sequences that extend only 27 bp to the right of the element I to III region. In conjunction with previous findings on *CEN3* and *CEN11*, these results indicate that the specific DNA sequences required in *cis* for yeast centromere function are contained within a region about 150 bp in length.

The centromere region of eucaryotic chromosomes plays a critical role in the proper segregation of chromosomes through meiosis and mitosis. Centromeric DNA in conjunction with other nuclear components, including centromere-binding proteins and microtubules, functions in the proper distribution of chromosomes through cell division. In recent years, centromeric DNA from chromosomes III, IV, V, VI, and XI of the yeast *Saccharomyces cerevisiae* have been identified and characterized (7, 9, 17, 22, 25). When plasmids carrying centromere sequences in addition to an autonomously replicating sequence (*ARS*) are followed through cell division, these plasmids are found to behave as minichromosomes; that is, they are relatively stably maintained through mitosis, and they segregate predominantly 2+ : 2- through meiosis (10, 17, 22, 25). This plasmid assay has allowed the identification of small restriction fragments that contain centromere function. A comparison of the DNA sequences of such fragments containing *CEN3*, -4, -6, and -11 reveals three structural features common to all (see Fig. 5). These features include *CEN* sequence element I, a 14-base-pair (bp) region with a similar sequence among the centromeres, followed by a region of conserved length (77 to 84 bp) and A+T content (93 to 95%), but not of conserved sequence, termed *CEN* sequence element II. Element II is flanked on the other side by *CEN* sequence element III, a 24- to 25-bp region that is very highly conserved.

Deletion and mutation analyses have been carried out to define the boundaries of the DNA sequences essential to centromere function. Results of these studies have shown that deletions removing sequences to the left of *CEN* element I do not affect centromere function (3; L. Clarke and J. Carbon, *Annu. Rev. Genet.*, in press). A subclone of *CEN3* containing elements I, II, and III, along with flanking DNA extending 4 bp to the left of element I and 162 bp to the right of element III, retains centromere function (6). However, a subclone extending from 4 bp to the left of element I to an endpoint within the element III region such that all of elements I and II and the first 20 bp of element III are included shows only partial activity (R. Ng and J. Carbon, The Twelfth International Conference on Yeast Genetics

and Molecular Biology Abstracts, 1984, p. 46). Deletions that remove all three sequence elements completely abolish centromere function (3, 22).

We report the identification and characterization of the centromere from *S. cerevisiae* chromosome XIV. Based on a plasmid assay, centromere activity is found on a 259-bp restriction fragment containing sequence elements I, II, and III along with flanking sequences that extend only 27 bp to the right of the *CEN* element III region. In conjunction with previous findings on *CEN3* and *CEN11*, these results indicate that the specific DNA sequences required in *cis* for yeast centromere function are contained within a region approximately 150 bp in length.

MATERIALS AND METHODS

Strains, media, and enzymes. *Escherichia coli* JA226 (C600 *hsdR hsdM⁺ recBC lop-11 thi leuB6 strR*) and JA300 (*thr leuB6 hsdR hsdM trpC117 strR*) were used for transformation and preparation of plasmid DNAs. *S. cerevisiae* strain J17 (α *his2 ura3 adel met14 trp1*) was used in mitotic stability assays, strain RH218 (α *CUP2 trp1 gall mal SUC2*) was from D. Stinchcomb (19), strain K393-35C (α *spo11 ura3 his2 leu1 lys1 met4 pet8*) was from R. E. Esposito (16), strain SB7883-1C (α/α *ura3-52/lura3-52 trp1/trp1 leu2,3/LEU2,3 his4/HIS4 cry1/CRY1 MET14/met14 ADE1/adel*) was used in integration experiments, and strain AB320 (*HO ade2 lys2 trp5 leu2 can1-100 ura3 or ural met4*) was from M. Olson (21). Plasmid pCH10 was isolated by Hsiao and Carbon (14) from a yeast genomic library constructed from strain AB320 DNA in the *ARS* vector YRp7 (26). Yeast matings, sporulations, and dissections were carried out as described previously (30). Media for yeast and bacterial growth have been described previously (13). Most restriction endonucleases, bacterial alkaline phosphatase, and T4 DNA ligase were from New England Biolabs, Inc., Beverly, Mass. *DraI* was from International Biotechnologies, Inc., New Haven, Conn. Buffer and reaction conditions were those specified by the vendor. Calf intestine alkaline phosphatase was from Boehringer Mannheim Biochemicals, Indianapolis, Ind., and T4 polynucleotide kinase was from International Biotechnologies. Calf intestine alkaline phosphatase and T4 polynucleotide kinase reaction conditions were as described

* Corresponding author.

by E. F. Retzel (Ph.D. thesis, University of Minnesota, Minneapolis, 1984).

DNA preparations and transformations. Purified plasmid DNA was isolated as described by Holmes and Quigley (12). Yeast and *E. coli* transformations were as described previously (28). Yeast genomic DNA was prepared as described by Clarke and Carbon (8).

Mitotic stability assay. The mitotic stability assay has been described previously (11). Samples were taken at time points between 0 and 13 generations of growth in rich media.

DNA sequence analysis. Restriction fragments generated by cleavage of pYe(RH)10 were prepared for sequencing essentially as described by Maxam and Gilbert (18) with modifications of calf intestine alkaline phosphatase and T4 polynucleotide kinase conditions. Sequencing reactions were those described by Maxam and Gilbert (18), with the exception that the G+A depurination reaction was done with 0.3 M NaCl-0.3 N HCl instead of 4% formic acid. Samples were loaded onto either 8% standard sequencing gels or 6% buffer gradient gels (2).

Sequence comparisons. Sequence comparisons were done with the SEQ computer program (4) available through Intelligenetics, Inc., Palo Alto, Calif.

RESULTS

The plasmid pCH10 was identified as a putative centromere-containing plasmid from a yeast genomic library by directly selecting for plasmids that were stably maintained in yeast through 20 generations of mitotic growth under non-selective conditions (14). It was further demonstrated that pCH10 also possesses an important property of centromere plasmids; the plasmid genetic marker segregates predominantly 2+ : 2- through meiosis, with the plasmid segregating to sister spores. Fragments of the yeast DNA insert in pCH10 were subcloned into vector pGT27 (29) and subjected to a quick screen for mitotic stabilizing activity by an assay similar to that described by Hsiao and Carbon (14). The results of the analysis (data not shown) indicate that the centromere activity is contained within a 3.1-kilobase-pair (kbp) *Sall*-*Eco*RI restriction fragment [pYe(SR)10, (Fig. 1)].

Characterization of the *Sall*-*Eco*RI fragment. To confirm the yeast origin of the *Sall*-*Eco*RI DNA, the plasmid pBR-SR (Fig. 2C) was used to probe yeast genomic DNA. Only one genomic band in a given digest hybridized, and the *Sall*-*Eco*RI genomic band (Fig. 2C, lane 4) was identical in size to the 3.1-kbp *Sall*-*Eco*RI band in the plasmid control (Fig. 2C, lane 3). These results indicate that this centromere-containing DNA is a unique single copy sequence in the *S. cerevisiae* genome and does not cross-hybridize to other centromeres by standard Southern blot hybridizations (24), as has been shown for DNA containing *CEN3*, *-4*, *-6*, and *-11* (11, 17, 22, 25).

To demonstrate that the *Sall*-*Eco*RI cloned DNA is representative of the arrangement of this DNA in the yeast genome, blots were probed with plasmids pBR-RH and pBR-SH in addition to pBR-SR (Fig. 1 and 2). If this DNA had undergone rearrangements in cloning, then for each digest more than one band should hybridize to one or more of the probes. If this cloned DNA were identical to genomic DNA, then only one band in each genomic digest should hybridize to each probe, and the same size band should hybridize to all three probes for a given digest. The results of the Southern gel (Fig. 2) indicate that the *Sall*-*Eco*RI cloned DNA is unrearranged relative to this fragment in the yeast genome.

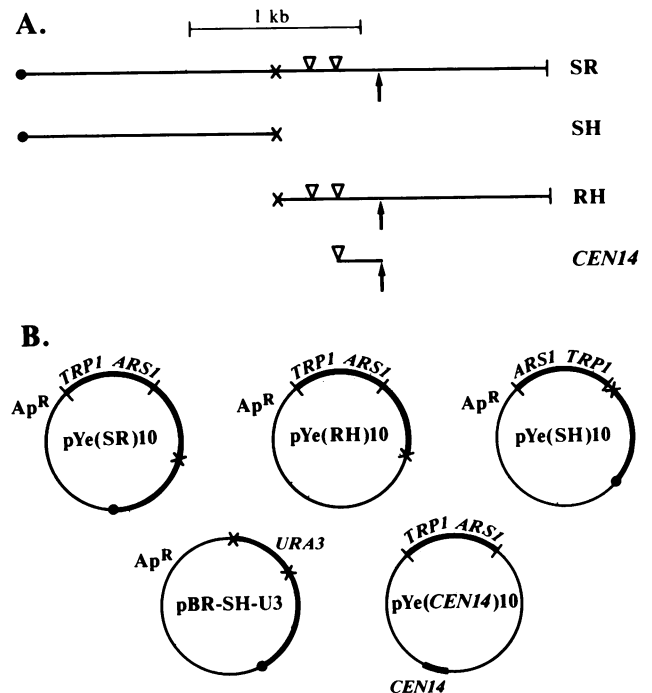


FIG. 1. (A) Restriction map and subcloning strategy of the 3.1-kbp *Sall*-*Eco*RI restriction fragment containing *CEN14*. *Eco*RI (+), *Hind*III (X), *Sall* (●), *Rsa*I (∇), *Eco*RV (↑). Not all of the *Rsa*I and *Eco*RV sites are indicated in the figure. (B) Plasmids constructed by subcloning the 3.1-kbp *Sall*-*Eco*RI DNA fragment. Plasmid pBR-SR contains the entire *Sall*-*Eco*RI restriction fragment inserted in place of the small *Sall*-*Eco*RI fragment of pBR322. Plasmid pBR-SH contains the 1.5-kbp *Sall*-*Hind*III fragment inserted in place of the small *Sall*-*Hind*III fragment of pBR322. Plasmid pBR-RH contains the 1.6-kbp *Eco*RI-*Hind*III fragment inserted in place of the small *Eco*RI-*Hind*III fragment of pBR322. Plasmids pYe(SR)10, pYe(SH)10, and pYe(RH)10 were constructed by inserting a 1.454-kbp *Eco*RI restriction fragment containing the yeast *TRP1* gene and *ARS1* (28), into the unique *Eco*RI site of pBR-SR, pBR-SH, and pBR-RH, respectively. Plasmid pYe(*CEN14*)10 was generated by blunt-end ligation of the 259-bp *Rsa*I-*Eco*RV fragment into the *Pvu*II site of vector YRp7. The orientation of the *Rsa*I-*Eco*RV fragment is not known. pBR-SH-U3 was constructed by inserting the 1.1-kbp *Hind*III fragment containing the yeast *URA3* gene (1) into the unique *Hind*III site of pBR-SH. Thin lines are pBR322 DNA, thick lines are yeast DNA. The restriction site symbols are the same as in part A, and the plasmids are not drawn to scale.

Mitotic and meiotic analysis. The *Sall*-*Eco*RI DNA fragment was subcloned as a 1.5-kbp *Sall*-*Hind*III fragment into vector YRp7 (26) to form pYe(SH)10, and as a 1.6-kb *Eco*RI-*Hind*III fragment into vector YRp7' (26) to form pYe(RH)10 (Fig. 1). These subclones were subjected to a mitotic stability assay (see above) to determine the plasmid segregation rate (loss per generation) to localize the centromere to a smaller restriction fragment. pCH10, pYe(SR)10, and pYe(RH)10 were lost at a rate of 1 to 5% per generation from yeast cells growing in rich media, a loss rate that is characteristic of centromere plasmids (Fig. 3) (3, 5). However, pYe(SH)10 was lost at a rate of 20% per generation, as was the *ARS* plasmid YRp7. These results indicate that the mitotic stabilizing activity resides on the 1.6-kbp *Eco*RI-*Hind*III DNA fragment. Results of additional subcloning experiments indicate that the mitotic stabilizing

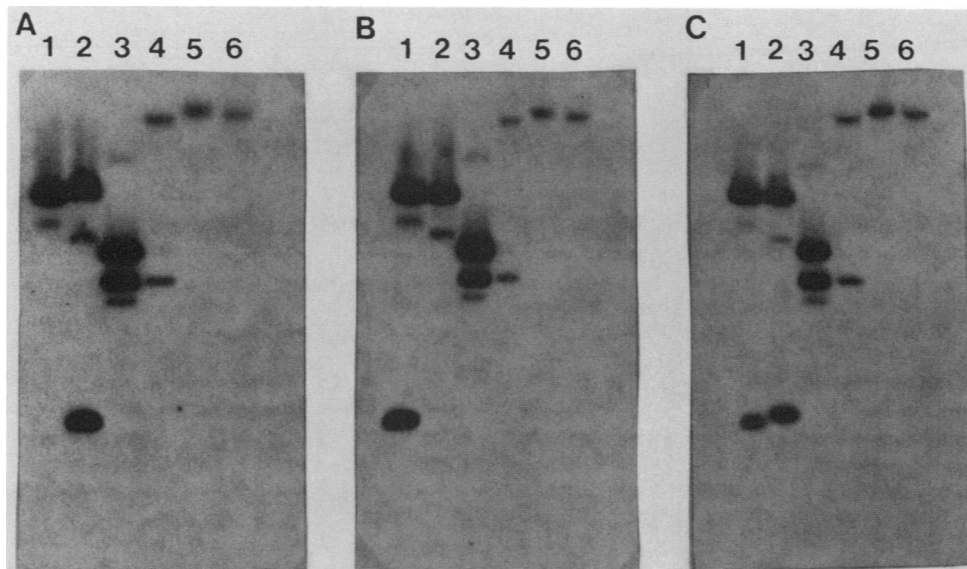


FIG. 2. Structure of the *CEN14* region in the yeast genome. Lanes for all blots are as follows: lane 1, plasmid pBR-SR digested with *Sall-HindIII*; lane 2, plasmid pBR-SR digested with *EcoRI-HindIII*; lane 3, plasmid pBR-SR digested with *Sall-EcoRI*; lane 4, yeast strain AB320 genomic DNA digested with *Sall-EcoRI*; lane 5, yeast strain AB320 genomic DNA digested with *PvuII*; lane 6, yeast strain AB320 genomic DNA digested with *BamHI*. The blots were probed as follows: (A) the probe was pBR-RH; (B) the probe was pBR-SH, (C) the probe was pBR-SR. For the *BamHI* and *PvuII* digests, only one band hybridizes to each probe, and this band is the same size for all probes. In the *Sall-EcoRI* digests of genomic DNA (lane 4), a band corresponding to the 3.1-kbp *Sall-HindIII* band in the plasmid control (lane 3) hybridized to all three probes. In this digest, a large-molecular-weight fragment also hybridizes to each probe, and this band is thought to be a partial digestion product, since it is not observed when genomic DNA is cut with other enzymes.

activity resides on a 259-bp *RsaI-EcoRV* restriction fragment. Plasmid pYe(*CEN14*)10, which contains this 259-bp fragment, was lost at a rate of only 1 to 2% per generation (Fig. 1 and 3).

Separate cultures of yeast strain J17 were transformed with each of the *ARS* plasmids shown in Fig. 1B and mated to strain RH218, and the selected diploids were subjected to standard yeast tetrad analysis (Table 1). The meiotic behavior of pCH10, pYe(SR)10, pYe(RH)10, and pYe(*CEN14*)10 was typical of centromere plasmids in that when the plasmid marker (*TRP1*) segregated 2+2-, it was almost always found in sister spores produced by the second meiotic division. Thus both mitotic and meiotic analyses support the conclusion that the 259-bp *RsaI-EcoRV* restriction fragment contains a functional centromere.

Localization of the centromere to chromosome XIV. The experimental strategy used to map the chromosomal location of the centromere found on pYe(SR)10 was to integrate a selectable genetic marker (*URA3*) adjacent to the centromere in the yeast genome by homologous recombination with the *Sall-HindIII* DNA fragment on the plasmid pBR-SH-U3 (Fig. 1) and then to localize the *URA3* gene by standard genetic methods. This was done with a homozygous *ura3-52* diploid strain (SB7883-1C), in which integration of *URA3* plasmids by homologous recombination with the endogenous *URA3* region rarely occurs (27).

Plasmid pBR-SH-U3 transforms yeast at a very low frequency, consistent with the *Sall-HindIII* fragment lacking *ARS* activity. The *Ura*⁺ transformants of strain SB7883-1C showed 100% mitotic stability of the *URA3* marker, indicating that the plasmid had integrated. Two *Ura*⁺ transformants were sporulated, the tetrads were dissected, and three *Ura*⁺ haploid spores were chosen for further mapping studies after it was confirmed by genomic Southern analysis that the plasmid had integrated by homology to the *Sall-HindIII*

DNA (data not shown). The three *URA3* haploid progeny were mated to a series of tester strains with markers on all chromosomes and subjected to genetic analysis. When mated to strain K393-35C, dissected tetrads were scored for *pet8*, a tightly centromere-linked gene on chromosome XIV (20), and *ura3*. All tetrads (59 of 59) were parental ditypes; therefore, we conclude that pYe(RH)10 contains *CEN14*.

Sequence analysis. Our sequencing strategy is summarized in Fig. 4A. The sequence of the 259-bp *RsaI-EcoRV* fragment containing *CEN14* is shown in Fig. 4B. *CEN14* possesses sequences that are characteristic of other yeast centromeres (elements I, II, and III; Fig. 4B).

A sequence homology comparison (Fig. 4B and 5) of the *CEN14* sequence with sequence element I of *CEN3*, -4, -6, and -11 reveals a sequence element I in the usual position flanking the high A+T region (element II). The sequence element II of *CEN14* is similar to that found in other yeast centromeres; it is 84 bp in length and 94% A+T. There appears to be no extensive sequence homology among the *CEN* elements II sequenced to date, although the general arrangement of blocks of A residues followed by runs of T residues as opposed to alternating A:T base pairs is similar in all centromeres.

The sequence element III region is highly conserved among yeast centromeres, and a comparison of this region in *CEN3*, -4, -6, and -11 (Fig. 5) shows that the central 11-bp core of sequence element III does not vary by more than a single A-T transversion. However, the central core of sequence element III from *CEN14* differs from *CEN3*, *CEN4*, and *CEN11* by 2 bp and differs from *CEN6* by 1 bp. An interesting point is that the fourth base pair of the central core of sequence element III is a C:G base pair in *CEN14*, whereas it is an A:T base pair in all other sequenced centromeres. This implies that either the presence of an A:T base pair at this position is unessential for proper centromere

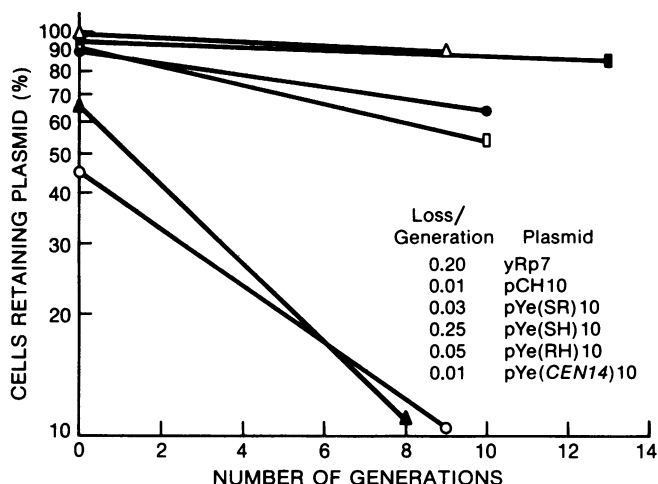


FIG. 3. Mitotic stabilities of plasmids pCH10 (Δ), pYe(SR)10 (\bullet), pYe(SH)10 (\blacktriangle), pYe(RH)10 (\square), pYe(*CEN14*)10 (\blacksquare), and YRp7 (\circ) determined in yeast strain J17. Single colonies were grown to mid-log phase in selective media (lacking tryptophan) and then diluted to an A_{660} of 0.1 to 0.2 in nonselective media. Aliquots were monitored by turbidity measurement at 660 nm. Fractions were removed at appropriate intervals, diluted and spread on nonselective agar plates. After growth at 32°C for 36 h, the colonies were replica plated to selective media. The percentage of cells retaining the plasmid was determined after incubation overnight at 32°C. Plotted is the linear regression for data from two different transformants for each plasmid. The segregation rate for each plasmid was calculated from the graph.

function or that other structural features in *CEN14* can compensate for this difference.

DISCUSSION

We identified and characterized yeast *CEN14* on a 259-bp restriction fragment containing sequence elements I, II, and III flanked by 27 bp to the right of the *CEN* element III region and 110 bp to the left of *CEN* element I.

Sequence elements I, II, and III have been shown to be essential for mitotic stabilization of *ARS* plasmids (3, 22) and for maintenance of proper chromosome segregation in mitosis and meiosis (6, 11). One approach to further our understanding of centromere function has been to study the function of smaller subclones, point mutants, and deletion mutants involving *CEN* elements I to III. A comparison of the sequence element I of *CEN3*, -4, -6, and -11, and -14 is shown in Fig. 5. The boxed sequences designate the invari-

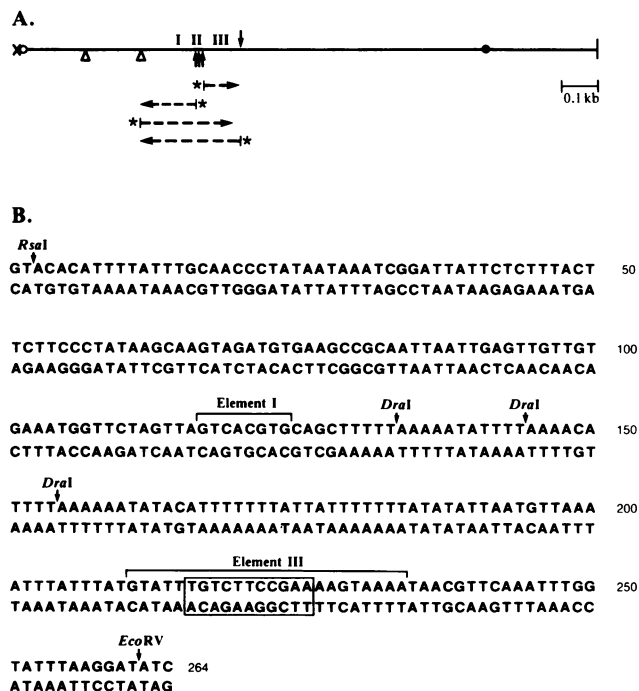


FIG. 4. (A) Strategy for nucleotide sequence analysis of the *CEN14* fragment. An asterisk indicates the position of the 5'-end label. Restriction enzyme cleavage sites are as follows: *EcoRI* (+), *EcoRV* (\downarrow), *HindIII* (X), *ClaI* (\bullet), *RsaI* (Δ), *DraI* (\uparrow), *HincII* (\circ). I, II, and III indicate the relative positions of the sequence elements I, II, and III. (B) Nucleotide sequence of the 259 bp *RsaI*-*EcoRV* restriction fragment containing *CEN14*. The *CEN* elements I and II and the element III region are indicated by brackets. The 11 bp homologous to the originally defined element III (see text [11]) is indicated by the boxed region.

ant nucleotides (PuTCACPuTG) that define the generalized sequence for element I. Subcloning analysis and genome replacement studies have shown that a 289-bp *RsaI*-*AluI* restriction fragment of *CEN3* with an endpoint 4 bp to the left of the sequence element I region (Fig. 5) retains centromere function (6). In addition, deletion analysis of *CEN11* has shown that the deletion of the first 5 bp from the left border of the sequence element I region (Fig. 5) does not affect centromere activity (10). Thus, it is clear that sequences 5' to the first invariant nucleotide of element I are not required for centromere function. However, the leftward boundary of the centromere has not been accurately deter-

TABLE 1. Meiotic segregation of centromere plasmid^a

Plasmid	Distribution in tetrads of the <i>TRP1</i> gene on plasmid (%):					Centromere linkage of the <i>TRP1</i> gene on plasmid ^b :		
	4+ : 0-	3+ : 1-	2+ : 2-	1+ : 3-	0+ : 4-	PD	NPD	T
pCH10	9 (35)	0	16 (62)	0	1 (4)	5	11	0
pYe(SR)10	31 (46)	1 (1)	28 (42)	1 (1)	6 (9)	14	13	1
pYe(RH)10	9 (33)	1 (4)	9 (33)	0	8 (30)	6	3	0
pYe(<i>CEN14</i>)10	9 (21)	2 (5)	18 (43)	1 (2)	12 (29)	8	9	1

^a In all of the crosses, the marker used to follow the plasmid (*TRP1*) was the wild type on the plasmid and the mutant in both parents. The crosses were strain J17/plasmid (α *adel his2 met14 trp1 ura3/TRP1*) with strain RH218 (*a trp1 gal2 mal CUP1 SUC*). The *CEN* plasmid genetic marker was scored versus *MET14* (20), a centromere-linked marker on yeast chromosome XI, to determine centromere linkage. Predominantly parental ditype and nonparental ditype asci were found, indicating that the genetic marker on the centromere plasmid segregates as expected for a centromere-linked gene.

^b Abbreviations: PD, parental ditype; NPD, nonparental ditype; T, tetratype.

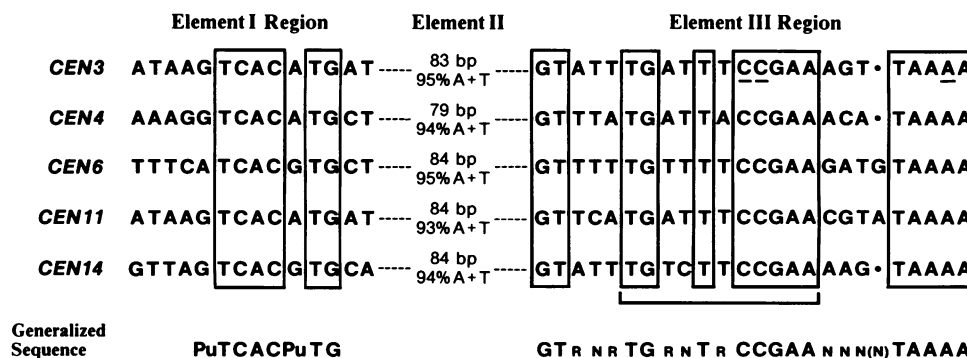


FIG. 5. Comparison of the nucleotide sequences of the *CEN* element I and element III regions and the relative spacing of these sequence elements in five yeast centromeres. The boxes indicate the highly conserved nucleotides. Generalized sequences for the element I and III regions are shown as derived from the invariant nucleotides in these regions. Pu is purine. The underlined nucleotides indicate bases that when altered by mutagenesis result in decreased centromere activity (McGrew and Fitzgerald-Hayes, Conference Abstracts, 1984, p. 47). The bracketed area indicates the 11-bp sequence homologous to the originally defined element III (see text [11]).

mined, and in fact, it is not known if any portion of element I is essential for proper centromere function.

The sequences of the A+T-rich element II regions are not conserved; however, those characterized to date are similar in length (77 to 84 bp) and in A+T content (93 to 95%). Genome replacement experiments in which the wild-type *CEN3* region is replaced by *CEN3* in which various amounts of sequence element II have been deleted have shown impaired mitotic and meiotic function (6). The length of sequence element II is critical for centromere activity. Interestingly, replacement of wild-type *CEN3* with a centromere containing a slightly longer than full-length sequence element II with a sequence arrangement that is scrambled relative to that of the wild type results in nearly normal, but clearly reduced, mitotic function. This element II construct is altered both in length and physical arrangement relative to the wild-type element II; however, it is not known whether the reduction in mitotic function is a result of one or both of these alterations.

A comparison of the sequence element III regions in *CEN3*, -4, -6, and -11, and -14 (Fig. 5) reveals several invariant nucleotides from which a generalized sequence for the *CEN* element III region can be derived. Originally, element III was defined as an 11-bp region of exact homology that occurred in *CEN3* and *CEN11* (11). Now that additional centromere sequences are available, the element III region of homology has been redefined to include homologous sequences on both sides of the original 11-bp sequence. The functional significance of several of these invariant nucleotides has been investigated with bisulfite and oligonucleotide-directed mutagenesis. Mutations altering three different invariant nucleotides (underlined in Fig. 5) affect *CEN3* function. One C:G to T:A transition (TGATT-TTCGAA) partially inactivates the centromere (for the wild-type element III sequence, see Fig. 5), whereas a similar transition in the adjacent position (TGATTTCTGAA) completely inactivates the mitotic stabilizing activity of *CEN3* (J. McGrew and M. Fitzgerald-Hayes, The Twelfth International Conference on Yeast Genetics and Molecular Biology Abstracts, 1984, p. 47), indicating that these two adjacent C:G base pairs in the *CEN3* element III region are essential for proper centromere function. We found that the *CEN14* element III region has a C:G base pair at a position occupied by an A:T pair in the other known centromeres. This naturally occurring difference indicates that the presence of

an A:T base pair at this position may not be critical for proper centromere function.

The rightward boundary of the centromere has not been exactly defined. Genomic substitution experiments indicate that a 289-bp *RsaI*-*AluI* *CEN3* restriction fragment extending from 4 bp to the left of the element I region (Fig. 5) to 162 bp to the right of the element III region contains an active centromere (6). The introduction of an A:T to C:G transversion (TAACA) in the second position from the end in the sequence element III region of *CEN3* (Fig. 5) creates a *HpaI* site and thus allows sequence elements I, II, and the first 20 bp of the element III region to be subcloned as a 121-bp *RsaI*-*HpaI* restriction fragment (Ng and Carbon, The Twelfth International Conference on Yeast Genetics and Molecular Biology Abstracts, 1984, p. 46). When this 121-bp *CEN3* region is substituted into the genome to replace the centromere on chromosome III, the resulting chromosome undergoes mitotic loss at a rate that is three orders of magnitude greater than that of the wild-type chromosome. In contrast, a larger region containing elements I and II, the element III region with this point mutation, and 162 bp of DNA flanking the element III region is only slightly reduced for mitotic stabilizing activity. Some sequences to the right of the element III region are required for full centromere activity; however, it is not known exactly how far beyond element III the essential sequences extend. Result of this study indicate that in *CEN14* the functional sequences do not extend more than 27 bp to the right of the sequence element III region.

It has been reported previously that yeast centromeres possess sequence homology to various satellite DNA sequences, most notably to the *Drosophila melanogaster* satellite DM359, a highly repetitive 359-bp sequence from the *D. melanogaster* sex chromosomes (11, 15). Figures 6A and B show a comparison of the five *CEN* element I and III regions with the homologous regions from the DM359 repeat sequence. The sequence element I region (nucleotides 108 to 130) of *CEN14* shares 50% homology with DM359 (nucleotides 201 to 222), whereas other sequence elements I share between 42 and 79% homology. *CEN14* element III and surrounding regions (nucleotides 202 to 237) share 63% homology with DM359 (nucleotides 109 to 146), whereas sequence elements III of other centromeres share between 60 and 71% homology. In Fig. 6B, the underlined sequences are regions of identity of four nucleotides or more in length

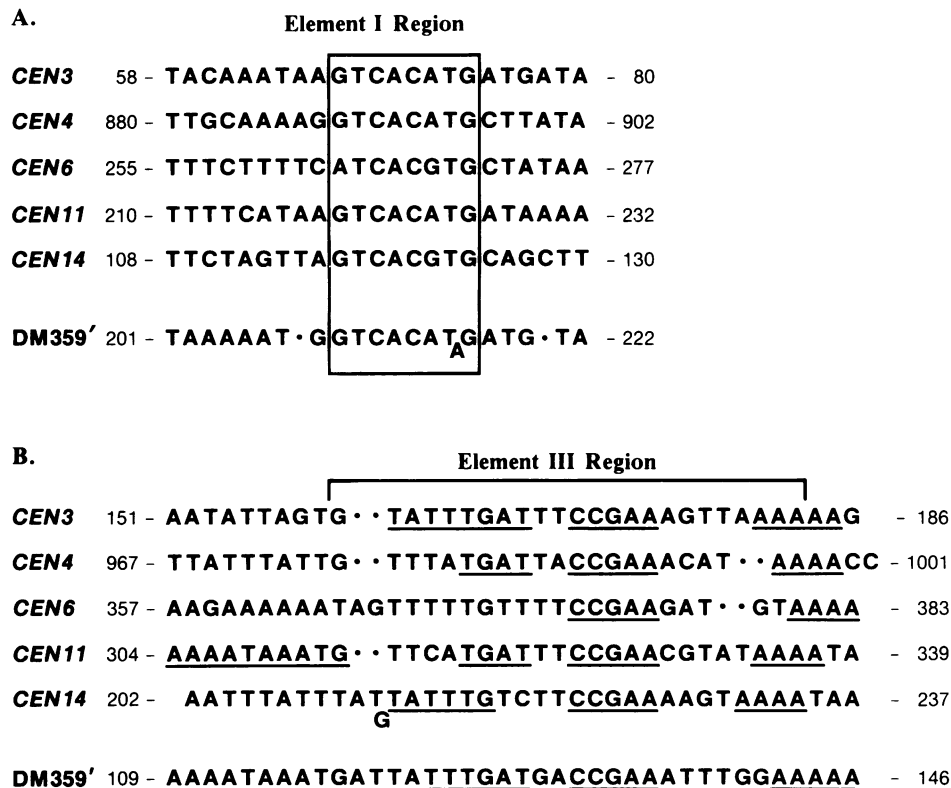


FIG. 6. (A) Comparison of sequence element I plus flanking sequences for five yeast centromeres and *D. melanogaster* satellite DM359. The highly conserved sequences are indicated by the box; the only differences are purine substitutions. The numbers refer to the beginning and end of the homologies. The numbering system for *CEN14* is that described in the legend to Fig. 4B; for *CEN3* and *CEN11* the numbering system of Fitzgerald-Hayes et al. (11) is used; for *CEN4* and *CEN6* the numbering systems are those of Mann et al. (C. Mann, M. Snyder, and R. W. Davis, The Twelfth International Conference on Yeast Genetics and Molecular Biology Abstracts, 1984, p. 52) and Panzeri and Phillippsen (22), respectively; DM359' is the bottom strand of the sequence shown previously (15). (B) Comparison of the sequence element III plus flanking regions for five yeast centromeres and DM359. Shown is the best match as determined by using the SEQ computer program (see text). Sequences longer than 4 bp occurring both in DM359 and a centromere are underlined. The numbers refer to the beginning and end of the homologies, and the numbering systems are those described in the legend to Fig. 6A.

between the DM359 sequence and any of the five yeast *CEN* element III regions. It is remarkable that nearly all of the DM359 sequence is contained in the proper order among the group of yeast *CEN* sequences. The significance of the homology between this satellite sequence and yeast centromeres is not understood. However, since DM359 occurs in the centromere region of the *D. melanogaster* sex chromosomes, it is interesting that Peacock and Miklos (23) have observed that deletions of heterochromatin in this region of the X-chromosome reduce meiotic pairing and cause abnormal segregation of the sex chromosomes.

ACKNOWLEDGMENT

This research was supported by Public Health Service grant CA-11034 from the National Cancer Institute.

LITERATURE CITED

- Bach, M. L., F. Lacroute, and D. Botstein. 1979. Evidence for transcriptional regulation of orotidine-5'-phosphate decarboxylase in yeast by hybridization of mRNA to the yeast structural gene cloned in *E. coli*. Proc. Natl. Acad. Sci. USA 76:386-390.
- Biggin, M. D., T. J. Gibson, and G. F. Hong. 1983. Buffer gradient gels and ³⁵S as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA 80:3963-3965.
- Bloom, K. S., M. Fitzgerald-Hayes, and J. Carbon. 1983. Structural analysis and sequence organization of yeast centromeres. Cold Spring Harbor Symp. Quant. Biol. 157:1175-1185.
- Brutlag, D. L., J. Clayton, P. Friedland, and L. Kedis. 1982. SEQ: a nucleotide sequence analysis and recombination system. Nucleic Acids Res. 10:279-294.
- Carbon, J. 1984. Yeast centromeres: structure and function. Cell 37:351-353.
- Carbon, J., and L. Clarke. 1984. Structure and functional analysis of a yeast centromere (*CEN3*). J. Cell Sci. Suppl. 1(Suppl.):43-58.
- Clarke, L., and J. Carbon. 1980. Isolation of a yeast centromere and construction of functional small circular chromosomes. Nature (London) 287:504-509.
- Clarke, L., and J. Carbon. 1983. Genomic substitutions of centromeres in *Saccharomyces cerevisiae*. Nature (London) 305:23-28.
- Fitzgerald-Hayes, M., J.-M. Buhler, T. G. Cooper, and J. Carbon. 1982. Isolation and subcloning analysis of functional centromere DNA (*CEN11*) from *Saccharomyces cerevisiae* chromosome XI. Mol. Cell. Biol. 2:82-87.
- Fitzgerald-Hayes, M., and J. Carbon. 1982. Identification of DNA sequences required for mitotic stability of centromere plasmids in yeast. Recent Adv. in Yeast Mol. Biol.: Recombinant DNA 2:1-12.
- Fitzgerald-Hayes, M., L. Clarke, and J. Carbon. 1982. Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. Cell 29:235-244.
- Holmes, D. S., and M. Quigley. 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. 114:

- 193–197.
13. Hsiao, C.-L., and J. Carbon. 1979. High frequency transformation of yeast by plasmids containing the cloned *ARG4* gene. Proc. Natl. Acad. Sci. USA **76**:3829–3833.
 14. Hsiao, C.-L., and J. Carbon. 1981. Direct selection procedure for the isolation of functional centromeric DNA. Proc. Natl. Acad. Sci. USA **78**:3760–3764.
 15. Hsieh, T., and D. Brutlag. 1979. Sequence and sequence variation within the 1.688g/cm³ satellite DNA of *Drosophila melanogaster*. J. Mol. Biol. **135**:465–481.
 16. Klapholz, S., and R. E. Esposito. 1982. A new mapping method employing a meiotic rec-mutant of yeast. Genetics **100**:387–412.
 17. Maine, G. T., R. T. Surosky, and B.-W. Tye. 1984. Isolation and characterization of the centromere from chromosome V (*CEN5*) of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **2**:86–91.
 18. Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
 19. Miozzari, G., P. Neiderbergen, and R. Hutter. 1978. Tryptophan biosynthesis in *Saccharomyces cerevisiae*: control of the flux through the pathway. J. Bacteriol. **134**:48–59.
 20. Mortimer, R. K., and D. Schild. 1980. The genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44**:519–571.
 21. Nasmyth, K. A., and S. I. Reed. 1980. Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. Proc. Natl. Acad. Sci. USA **77**:2119–2123.
 22. Panzeri, L., and P. Phillippsen. 1982. Centromeric DNA from chromosome VI in *Saccharomyces cerevisiae* strains. EMBO J. **1**:1605–1611.
 23. Peacock, W. J., and G. L. B. Miklos. 1973. Meiotic drive in *Drosophila*: new interpretation of segregation distorter and sex chromosome systems. Adv. Genet. **17**:361–409.
 24. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
 25. Stinchcomb, D. T., C. Mann, and R. W. Davis. 1982. Centromeric DNA from *Saccharomyces cerevisiae*. J. Mol. Biol. **158**:157–179.
 26. Stinchcomb, D. T., K. Struhl, and R. W. Davis. 1979. Isolation and characterization of a yeast chromosomal replicator. Nature (London) **282**:39–43.
 27. Stinchcomb, D. T., M. Thomas, J. Kelly, E. Selker, and R. W. Davis. 1980. Eukaryotic DNA segments capable of autonomous replication in yeast. Proc. Natl. Acad. Sci. USA **77**:4559–4563.
 28. Tschumper, G. J., and J. Carbon. 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10**:157–166.
 29. Tschumper, G. J., and J. Carbon. 1982. Delta sequences and double symmetry in a yeast chromosomal replicator region. J. Mol. Biol. **156**:293–307.
 30. Tschumper, G. J., and J. Carbon. 1983. Copy number control by a yeast centromere. Gene **23**:221–232.