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Short Communication

Isolation of centromeric DNA from *Saccharomyces bayanus*

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The centromere is a chromosomal domain that provides the attachment sites for the mitotic and meiotic spindles and functions in the segregation of chromosomes during mitosis and meiosis (Pluta et al., 1995). Until now, centromeric DNAs of three species of budding yeasts—*Saccharomyces cerevisiae* (Fitzgerald-Hayes et al., 1982; Hieter et al., 1985; Mann and Davis, 1986; Wustinger and Spevak, 1993); *Saccharomyces uvarum* (Huberman et al., 1986); and *Kluyveromyces lactis* (Heus et al., 1993)—have been cloned and sequenced. Among the yeasts, centromeric DNAs of *S. cerevisiae* have been best characterized at structural and functional levels (Olson, 1991; Pluta et al., 1995). In *S. cerevisiae*, all 16 centromeric DNAs have a short conserved element about 120 bp long. This conserved DNA element consists of three centromere DNA elements (CDEs): 8-bp CDEI [RTCACRTG (R=purine)]; CDEII (78–86-bp highly A+T rich sequence); and 25-bp CDEIII (TGTT/ATT/ATG..TTCCGAA.....AAA). The kinetochore complex is assembled by the bindings of at least Cpf1p, Cse4p, and CBF3 protein complex to these CDE sequences. *S. uvarum* and *K. lactis* also have fundamentally similar DNA elements. The evolutionary changes in centromere sequences might be restricted because of the requirements of the centromere function for propagation of cells and the association of centromeric DNA with kinetochore-protein complexes. It is of interest to examine the differences in centromere sequences and functions among various organisms. We used the budding yeast *Saccharomyces bayanus* as a model organism to study the centromere. *S. bayanus* cells can hybridize with those of *S. cerevisiae*, and the resultant hybrids can grow normally, but they are sterile (Banno and Kaneko, 1989; Naumov, 1987). The electrophoretic karyotype of *S. bayanus* is slightly different from that of *S. cerevisiae* in size and number of chromosome bands (Kaneko and Banno, 1989; Kishimoto and Goto, 1995). Southern blotting analyses of chromosomes separated by pulsed-field gel electrophoresis indicated chromosomal rearrangement in *S. bayanus* in comparison with *S. cerevisiae* (Ryu et al., 1996). DNA-DNA reassociation and Southern blotting analyses showed a low degree of genomic DNA sequence homology between *S. bayanus* and *S. cerevisiae* (Vaughan-Martini, 1989; Vaughan-Martini and Martini, 1987). Thus *S. bayanus* and *S. cerevisiae* centromeres might be functionally exchangeable during mitosis, though centromere sequences might be largely different. Therefore in this study we cloned and sequenced centromeric DNAs of *S. bayanus*.

Since centromeric DNA of *S. bayanus* is thought to be functional in *S. cerevisiae* during mitotic growth, we attempted to clone *S. bayanus* centromeric DNA by functional selection in *S. cerevisiae*, using the *SUP11* method devised by Hieter et al. (1985). The *SUP11* gene has been used to select plasmids that have low copy numbers and high stability because of centromere function. Clones with low copy numbers of the plasmid can be selected as relatively well growing transformants because of the lethality of the *SUP11* gene when present in high copy numbers. The loss of *SUP11*-containing plasmid can be estimated in the *ade2-101*^{ochre} mutant strain by the frequency of red sectors in white colonies. A gene library made by an insertion of *Hin*dIII-digested *S. bayanus* B19-3C (*HO*

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Table 1. Mitotic stability of plasmids containing centromeric DNAs in *S. bayanus* and *S. cerevisiae*.[a]

Plasmid[b]	Centromeric DNA	Stability in host	
		S. bayanus	*S. cerevisiae*
YRpH1 (A)	*SbCEN1*	77±4.1/59±6.5	70±3.3/41±6.3
YRpH1 (B)	*SbCEN6*	74±1.6/52±11	71±4.8/45±3.9
YRpH1 (C)	*SbCEN7*	88±12/63±0.8	75±5.7/49±5.9
YRpH1 (D)	*SbCEN8*	82±10/58±8.0	78±7.3/49±6.9
YRpH1 (E)	*ScCEN6*	69±9.8/46±0.7	80±14/53±7.0
YRpH1	None	63±9.4/26±4.4	32±8.0/15±4.9

[a] Stabilities are expressed as a percentage of plasmid-bearing cells; i.e., Ura$^+$ cells after precultivation in selective medium (indicated on the left of the slash) and after 10 generations in complete medium (indicated on the right of the slash). The transformants were cultivated for 10 generations at 28°C in complete (YPAD) medium with an inoculation of cells precultivated in selective medium (SGlu medium lacking uracil).

[b] Characters in parentheses of plasmid names indicate the insert DNAs.

gal4 his6 ura3; Kaneko and Banno, 1989) genomic DNA at the *Hin*dIII site of the vector pHM201 (*SUP11*, *URA3*, *ARS1*, *Amp*^{r}) was introduced into *S. cerevisiae* YP1 (*MAT***a** *ura3-52 ade2-101*^{ochre} *lys2-801*^{amber}) by transformation (Ito et al., 1983), and cells were spread on SGlu plates lacking uracil (Matsuzaki et al., 1994). Among 450 Ura$^+$ transformants forming relatively large colonies, we selected four clones shown to form a few red sectors by sectoring assay. We recovered the plasmids from these four transformants and assayed for the centromere function of each insert in *S. bayanus*. Each *Hin*dIII insert (inserts A, B, C, and D; the sizes of these inserts were 1.9, 5.4, 1.5, and 5.9 kb, respectively) was ligated into the plasmid YRpH1 (*URA3*, *ARS1*) not containing the *SUP11* gene, and the resultant plasmids were introduced into *S. bayanus* B19-3C by transformation. The mitotic stability of these plasmids in *S. bayanus* B19-3C was tested by measuring the percentage of plasmid-carrying cells, i.e., the percentage of Ura$^+$ clones after 10 generations in complete medium. All plasmids provided a higher percentage of stability (>45%, Table 1). We therefore concluded that these inserts contained centromere sequences functional in *S. bayanus*. To determine their chromosomal origins, Southern hybridization analysis was performed on each centromeric DNA against *S. bayanus* chromosomal DNA. Our results indicated that we isolated the centromeric DNA derived from chromosomes 1, 6, 7, and 8 (a, b) (Fig. 1). We could not determine whether insert D was on chromosome 8a or 8b because of chromosome comigration on contour-clamped homogeneous electric field (CHEF) electrophoresis. We designated the isolated centromeric DNAs as *SbCEN1*, *SbCEN6*, *SbCEN7*, and *SbCEN8* DNA.

The plasmid inserts were sequenced by the method of Sanger et al. (1997), followed by homology searching for centromere sequences in comparison with the *S. cerevisiae* centromere sequence. Each insert contained only one sequence, and all had typical characteristics of CDEs. The centromere function of the identified sequences was confirmed by the reduction of mitotic plasmid stability by deletion or a linker insertion within the centromere sequence in *S. bayanus* (stabilities were 22–26% after 10 generations in complete medium). The nucleotide sequences of the centromeres are summarized in Fig. 2. These sequences were not identical to any *S. cerevisiae* centromere sequences. The characteristic feature of *S. bayanus* CDEs were grossly similar to those of *S. cerevisiae* CDEs, but three differences were noted between both

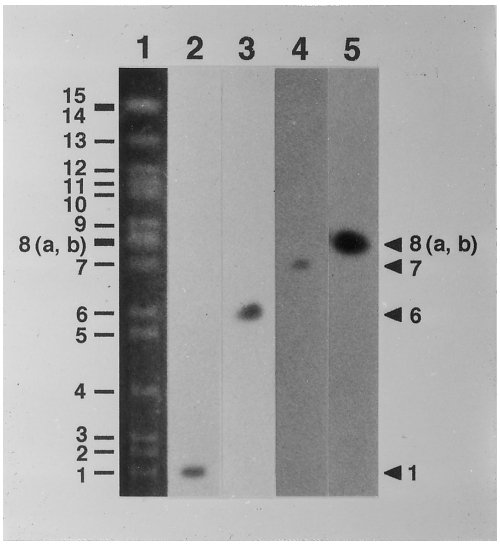

Fig. 1. Assignment of inserts to chromosomes.
The chromosomal DNA of *S. bayanus* B19-3C was separated by CHEF electrophoresis (Chu et al., 1986) and hybridized with ^{32}P-labeled insert DNAs as probes. ^{32}P-labeled probe DNAs were prepared by the random hexanucleotide priming method (Feinberg and Vogelstein, 1983). Chromosome numbers are indicated on the left. Numbering was assigned according to Ryu et al. (1996). Lane 1, ethidium bromide-stained gel; lane 2, probed with insert A; lane 3, probed with insert B; lane 4, probed with insert C; lane 5, probed with insert D.

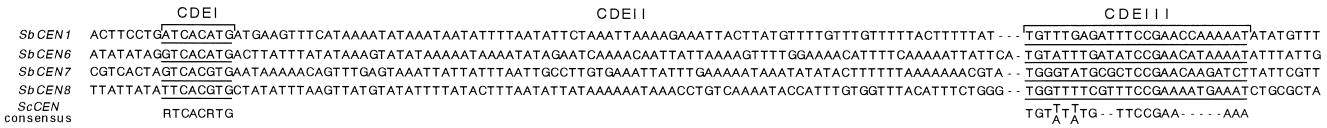

Fig. 2. DNA sequences of four centromeres of *S. bayanus*.

CDEI, CDEII, and CDEIII are indicated. Consensus sequences of *S. cerevisiae* centromeres is indicated under *S. bayanus* centromere sequences. DDBJ/EMBL/GenBank accession numbers; *SbCEN1*, AB021728; *SbCEN6*, AB021729; *SbCEN7*, AB021730; *SbCEN8*, AB021731.

CDEs. First, CDEI consensus sequence of *S. cerevisiae* is an 8-bp 5′-RTCACRTG-3′ sequence, but the nucleotide at position 1 in CDEI of *S. bayanus* is not necessarily R; the nucleotides are T and C for *SbCEN8* and another isolated *SbCEN* DNA (data not shown), respectively. Niedenthal et al. (1991) demonstrated that point mutation at position 1 (A) of *ScCEN6* resulted in little reduction in centromere activity in comparison with point mutations at positions 3 to 8. Mellor et al. (1990) showed that Cpf1p binds to CDEI sites in centromeres and in promoter sequences. The consensus sequence of the Cpf1p-binding site is 5′-TCACGTG-3′, which acts as a transcriptional activator of methionine biosynthesis gene. Therefore the nucleotide at position 1 was also not essential for centromere function in either *S. cerevisiae* or *S. bayanus*. Second, the AT-content in CDEII of *S. bayanus* is 83–88% (average 87%), which is 6% lower than that of *S. cerevisiae* (average 93%). However, the length of CDEII of *S. bayanus* (84–86 bp, average 85 bp) is similar to that of *S. cerevisiae* (average 84 bp). The GC-content of *S. bayanus* genomic DNA is 1.4% higher than that of *S. cerevisiae* (41.8% versus 40.4%). The change to GC from AT is concentrated in CDEII in comparison with the entire genome. Third, nucleotide at position 25 in CDEIII is A in *S. cerevisiae*, but it is T in *S. bayanus*. To determine the evolutionary changes in centromere sequences, it is necessary to compare sequences between centromeres from the same origin as determined by homology searching for sequences of genes close to the centromere. These studies are currently in progress in our laboratory, and the results will be reported elsewhere. Surprisingly, the *S. bayanus SbCEN6* sequence was completely identical to that of *S. uvarum CENb*, suggested that *S. bayanus* and *S. uvarum* are derived from the same origin. This was supported by the very high degree of homology in the genomic DNA between *S. bayanus* and *S. uvarum* (Vaughan-Martini and Martini, 1987).

Moreover, the functions of the *S. bayanus* centromere in mitotic and meiotic cell divisions of *S. cerevisiae* were examined by using the plasmid YRpH1 containing each cloned centromeric DNA. The plasmids all showed high mitotic stabilities (41–49% after 10 generations in complete medium, Table 1). The plasmids were introduced into an *ura3*$^-$/*ura3*$^-$ *leu2*$^-$/*LEU2*$^+$ heterozygous diploid strain by transformation. After sporulation of the resultant transformants, tetrads were dissected. The centromere-linked *LEU2* marks sister spores, the products of the second meiotic division. The segregation of plasmids was examined by monitoring the distribution of the *URA3* marker relative to the *LEU2* marker in tetrads. If meiotic segregation was faithful, the plasmids should segregate to sister spores (parental or nonparental ditypes). The plasmids segregated to sister spores at a high frequency for all inserts ($>$75% of tetrads). This result indicated that the plasmids segregated correctly at high frequency during the first and second meiotic divisions. Thus *S. bayanus* centromere was significantly functional in mitotic and meiotic cell divisions of *S. cerevisiae*. Moreover, the mitotic stability of plasmids containing *S. bayanus* centromeric DNA was higher than that of plasmid containing that of *S. cerevisiae* in *S. bayanus*, and the mitotic stability of plasmids containing *S. cerevisiae* centromeric DNA was higher than of plasmids with that of *S. bayanus* in *S. cerevisiae* (Table 1). We do not know whether these observations indicate that the centromeres are functionally more suitable in the original organism. This will be made clear by a more detailed analysis of centromere function by chromosome segregation, using chromosome fragments containing *S. bayanus* or *S. cerevisiae* centromeres.

In this study, we isolated four centromeric DNAs from *S. bayanus*. Our results indicated that *S. bayanus* centromeres are functional in *S. cerevisiae*, but characteristic features of CDEs have some differences between these organisms.

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