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Nematode Repetitive DNA with *ARS* and Segregation Function in *Saccharomyces cerevisiae*

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Several members of a repetitive DNA family in the nematode *Caenorhabditis elegans* have been shown to express *ARS* and centromeric function in *Saccharomyces cerevisiae*. The repetitive family, denoted *CeRep3*, consists of dispersed repeated elements about 1 kilobase in length, present 50 to 100 times in the nematode genome. Three elements were sequenced and found to contain DNA sequences homologous to yeast *ARS* and *CEN* consensus sequences. Nematode DNA segments containing these repeats were tested for *ARS* and *CEN* (or *SEG*) function after ligation to shuttle vectors and introduction into yeast cells. Such nematode segments conferred *ARS* function to the plasmid, as judged by an increased frequency of transformation compared with control plasmids without *ARS* function. Some, but not all, also conferred to the plasmid increased mitotic stability, increased frequency of 2+:2− segregation in meiosis, and decreased plasmid copy number. These effects are similar to those of yeast centromeric DNA. In view of these results, we suggest that the *CeRep3* repetitive family may have replication and centromeric functions in *C. elegans*.

Like the genomes of most eucaryotic organisms, the genome of the nematode *Caenorhabditis elegans* is interspersed with short, moderately repeated DNA sequences (9, 10, 31). We have characterized several of these families to gain a better understanding of their origin and role in the genome. One repetitive family that has been characterized consists of transposable elements, designated Tcl elements (11, 19). A second family has a conserved inverted repeat structure but does not appear to be an active transposon (12). We describe here a third family of short interspersed repeats containing 50 to 100 members. A striking property of this family is that at least some members contain sequences homologous to yeast *ARS* and *CEN* sequences and can express *ARS* and segregation functions in *Saccharomyces cerevisiae*. This observation raises the possibility that this repetitive family carries out similar functions in *C. elegans*.

MATERIALS AND METHODS

Nematode and yeast strains. Nematode strains were from the following sources: *C. elegans* var. Bristol (strain N2) and *Panagrellus redivivus* from D. Hirsh, *C. remanei* from E. Hedgcock, *C. elegans* var. Bergerac (strain BO) and *C. briggsae* from the *Caenorhabditis* Genetics Center, and the remaining wild-type *C. elegans* strains from the collection of R. Russell. Nematodes were cultivated on agar plates preseeded with *Escherichia coli* (4) or in liquid medium (31). *S. cerevisiae* W3031 (α *leu2 trp1 can1 ura3 ade2 his3*) was obtained from R. Rothstein. *S. cerevisiae* 332-5A (a *trp1 leu2 ura3 his2*) and 328-4A (α *trp1 ade1 met14 ura3 mal*) were provided by C. A. Michels. Yeast cultivation and genetic manipulations followed the method of Sherman et al. (26).

Nucleic acids. DNA was isolated from nematodes by the method of Emmons et al. (9) and from yeast by that of Winston et al. (34). A genomic library of DNA from *C. elegans* N2 was prepared in bacteriophage vector λ1059 by using 15- to 20-kilobase (kb) fragments generated by partial digestion with restriction endonuclease *Bam*HI, following the procedures of Karn et al. (16) and Maniatis et al. (20). Hybridization probes were labeled by nick translation (25). Southern hybridization conditions were as follows. Filters were hybridized in a solution containing 50% formamide, 5× SSC (1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7), 0.02 M sodium phosphate (pH 6.5), and 0.1% sodium dodecyl sulfate (SDS) at 37°C for 16 h. Hybridized filters were washed in 2× SSC–0.1% SDS at room temperature, followed by a wash in 0.2× SSC–0.1% SDS at 37°C. Isolation of plasmid and bacteriophage DNA was done by standard methods (20). Bacteriophage screening was carried out by the method of Benton and Davis (2). Yeast-*E coli* shuttle vectors were introduced into *S. cerevisiae* by the alkali cation method of Ito et al. (15). DNA sequences were determined by the base-specific chemical degradation protocol of Maxam and Gilbert (21).

Measurement of mitotic stability of yeast plasmids. The mitotic stability of plasmids in yeast cells was determined in strain 332-5A by the method of Clarke et al. (7). Clones to be tested were grown nonselectively in 5 ml of YEPD broth (1% yeast extract, 2% peptone, 2% dextrose) overnight at 30°C. Each culture was then plated for single colonies in YEPD agar plates (YEPD broth plus 1.5% agar), which allow nonselective growth. One hundred colonies were picked and transferred to selective medium (SD of Sherman et al. [26]) to assay for the presence of the plasmid marker gene.

Analysis of meiotic behavior of yeast plasmids. Diploid yeast strains were constructed and allowed to sporulate by the procedures of Sherman et al. (26). Strain 332-5A (α), bearing the plasmid to be tested, was mated with strain 328-4A (α). For the strain harboring plasmid YEp13, the α mating type strain used was W3031. Both parental yeast strains in the cross were mutant for a gene present on the plasmid (*leu2* or *trp1*) so that the presence of the plasmid could be scored in all four meiotic products. Strain 328-4A carries, in addition, the centromere-linked chromosomal marker *met14*. Tetrads were microdissected, and the spores were analyzed for the presence of the plasmid and chromosomal markers.

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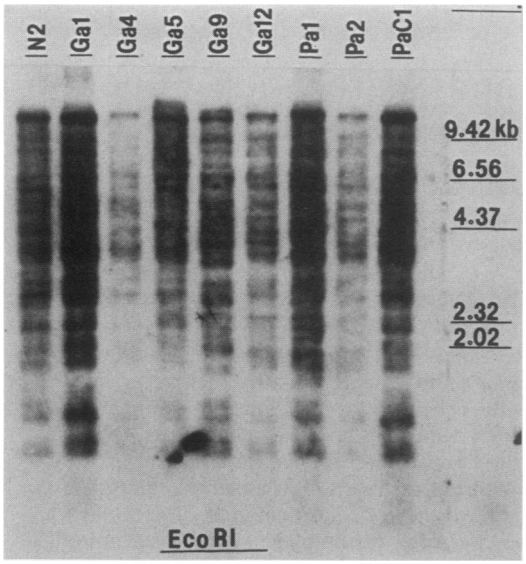


FIG. 1. Hybridization of labeled pCe17 to *Eco*RI-digested DNA from several *C. elegans* strains. The cross-homologous sequences shown define the *CeRep3* repetitive family. Southern hybridization with cloned sequences carrying other members of the family used as the probe gave patterns identical to that shown here.

Determination of plasmid copy number. Yeast strains to be tested for plasmid copy number were grown in selective medium (SD of Sherman et al. [26]) for approximately 10 generations, and a portion was plated on selective and nonselective media to determine the fraction of cells that contained any plasmid. The average number of plasmid molecules per cell in the culture was calculated after excluding from consideration cells containing no plasmid. DNA was purified from the culture, and 2 μg was fractionated on a 1% agarose gel after digestion with the restriction endonuclease *Bam*HI. DNA in the gel was transferred to nitrocellulose and hybridized to nick-translated plasmid pSZ63 (24). This plasmid consists of a 1.7-kb *Bam*HI fragment from yeast containing the *HIS3* gene, in the plasmid vector pBR322. It therefore hybridizes both to the chromosomal *HIS3* gene of the cell and to the pBR322-homologous sequences of the shuttle vector in the yeast cell. The amount of hybridization to plasmid and chromosomal sequences was measured by scanning an autoradiograph with a Quantimet 920 scanner (Cambridge Instruments). The amount of hybridization was then corrected for the amount of homology between the probe and each band and for the fraction of cells in the culture carrying the plasmid to determine the average number of copies of the plasmid per cell.

RESULTS

Properties of the *CeRep3* repetitive family. The repetitive DNA family *CeRep3* consists of a set of short repetitive elements dispersed some 50 to 100 times in the *C. elegans* genome. The family was defined originally by cross-hybridization with a 1.7-kb *Bam*HI restriction fragment cloned in plasmid pCe17. This plasmid was isolated in our earlier study of the properties of *C. elegans* genomic DNA and carries a segment of DNA from the Bristol (N2) strain (9). Southern hybridization to DNA from several *C. elegans* strains with this plasmid as probe is shown in Fig. 1. The experiment demonstrated that pCe17 carried a sequence present more than 50 times, and probably fewer than 100 times, in the *C. elegans* genome. Like other *C. elegans* repeats we have identified, with the exception of the active transposable element Tc1, this repeat defined a family of sequences that was well conserved in various wild-type *C. elegans* strains but was absent from related species of nematodes (9, 12). No cross-homology with pCe17 was found in DNA from *C. briggsae*, *C. remanei*, or *P. redivivus* under low-stringency hybridization conditions (hybridization and wash at 20 instead of 37°C; data not shown).

Thirteen members of the *CeRep3* family were isolated by screening clone banks with pCe17. The frequency of cross-hybridizing clones was consistent with wide dispersal of the members of the family in the genome. Restriction maps of four genomic segments carrying *CeRep3* repeats (from three lambda clones and pCe17) are presented in Fig. 2. The location of the repeated sequence on each clone was determined by hybridizing the clones to one another and is indicated on each map. Cross-hybridization among the clones was confined to a short segment, indicating that the *CeRep3* repeats are short and are therefore members of the interspersed class of repetitive sequences.

DNA sequence of *CeRep3* repeats. The DNA sequence in the region of cross-homology was determined for three clones carrying *CeRep3* repeats (Fig. 3). Sequence homology among the clones extended for approximately 1.4 kb. The G+C content of the repeated element (34%) was typical for *C. elegans* DNA generally (36% [31]). The boundaries of the repeated elements were not well defined, and the homologous regions differed by 25 to 30%. This degree of divergence was typical of the family as a whole, as was shown by thermal stability studies (data not shown). The sequenced elements differed from one another by both point mutations and changes affecting blocks of adjacent nucleotides. A similar spectrum of changes was found in our sequence analysis of another *C. elegans* repetitive family (12).

We analyzed the sequence of the *CeRep3* repeats for open reading frames, transcriptional signals, inverted repeats, target site duplications, and adjacent runs of poly(A), and none were found. No sequences were found that would suggest that these repeats are transposable elements or retroposons (32). No homology to RNA was seen in Northern (RNA blot) hybridization experiments or in screens of cDNA clone banks (data not shown).

However, sequences were found within the repetitive segments that have homology to both autonomously replicating (*ARS*) and centromeric (*CEN*) consensus sequences of *S. cerevisiae*. The positions of these homologies are shown in Fig. 3 and are compared with yeast sequences in Fig. 4. A single run of 12 nucleotides, present in all three repeats, was homologous to the yeast *ARS* consensus sequence, with one base inserted (5). Two runs of, respectively, 27 and 28 nucleotides in both *CeRep3.1* and *CeRep3.2*, and a run of 18 nucleotides plus a run of 27 nucleotides in *CeRep3.3*, had homology to region III of the yeast *CEN* consensus (13). A close homology to region I of the yeast *CEN* consensus (seven of eight nucleotides) was found in *CeRep3.1*, with lesser matches in *CeRep3.2* (six of eight) and *CeRep3.3* (four of eight). The region I homology was separated from the first region III homology by 360 nucleotides, 66% of which were adenine or thymine. The two sequences were ordered with respect to one another as they are in *S. cerevisiae*. Statistical analysis indicates that only the region III homologies were highly significant, that is, extremely unlikely to be found by chance in a 1,400-nucleotide sequence of the base composition of the *CeRep3* repeats. In view of these *ARS* and *CEN* homologies, we

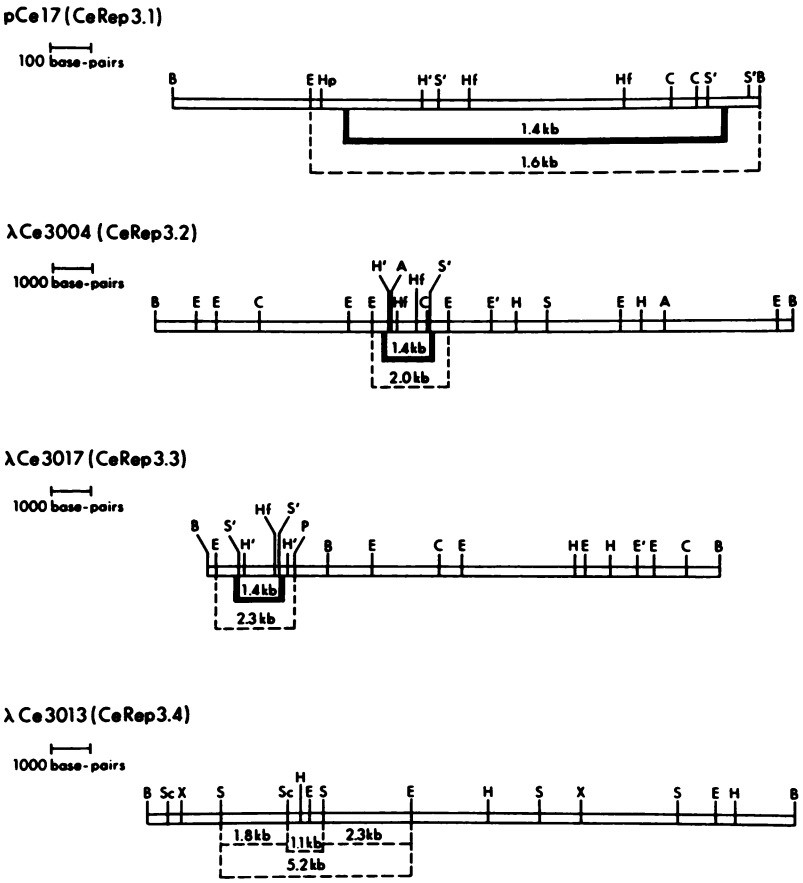


FIG. 2. Restriction maps of four genomic segments containing *CeRep3* repeats. Fragments shown to have homology to pCe17 are indicated by the dashed lines. The location of the repetitive segment as determined from the DNA sequence of three of the clones is indicated by the bold lines. Restriction sites: A, *Ava*I; B, *Bam*HI; C, *Cla*I; E, *Eco*RI; E', *Eco*RV; H, *Hin*dIII; Hf, *Hin*fI; Hp,*Hpa*I; H', *Hae*III; P, *Pst*I; S, *Sal*I; Sc, *Sac*I; S', *Sau*3A; X, *Xho*I.

tested *CeRep3* repetitive elements for *ARS* and *CEN* function in yeast cells.

DNA fragments containing *CeRep3* DNA confer a weak *ARS* phenotype. *ARS* elements are DNA sequences that increase the yeast transformation frequency of plasmids carrying them by allowing transformation to take place without chromosomal integration (see reference 33 for review). Plasmids bearing an *ARS* element can replicate autonomously in the transformed cell, possibly because the *ARS* element serves as a replication origin (27). To test for *ARS* function, *C. elegans* DNA fragments containing *CeRep3* sequences were introduced into the yeast-*E. coli* shuttle vector YIp28 (kindly provided by S. Henry). This vector consists of *E. coli* plasmid pBR322 with inserted yeast DNA containing the *LEU2* and *URA3* genes. The yeast segments do not contain an *ARS* element, and the plasmid can only transform by integration.

The *C. elegans* sequences raised the transformation frequency of YIp28 by about two orders of magnitude (Table 1). Though raised, this frequency was considerably lower than that of a plasmid containing the *ARS* of the autonomously replicating 2μm plasmid (Table 1) and in other experiments was about twofold lower than that of a plasmid containing yeast *ars1* (pLC544; data not shown). Furthermore, the plasmids containing the 2μm *ARS* sequence or yeast *ars1* gave transformants that grew well on minimal medium, producing large colonies in 2 to 4 days. In contrast, cells transformed with plasmids containing *CeRep3* sequences gave microcolonies, which appeared only after extended incubation of 4 to 7 days. The microcolony transformants were unstable and lost the Leu$^+$ phenotype under nonselective growth conditions. This instability showed that the transformants contained the plasmid sequences in unintegrated form. Overall, these properties are consistent with weak *ARS* function (17, 28).

Mitotic stabilization of yeast plasmids by *CeRep3* DNA. Yeast plasmids containing only *ARS* sequences are unstable during mitotic growth. They are inefficiently transferred to the bud cell and accumulate instead to high copy numbers in the mother cell (23). Sequences that allow segregation into

TABLE 1. *ARS* function linked to *CeRep3* repeats[a]

Plasmid	Transformation efficiency (no. of transformants/μg of plasmid DNA)
YIp28 (no *ARS* element)	0–1
YEp13 (2μm *ARS* element)	500
YIp28 + *CeRep3.1*	120
YIp28 + *CeRep3.2*	75
YIp28 + *CeRep3.3*	45
YIp28 + *CeRep3.4*	95

[a] Transformation efficiencies of plasmids carrying *CeRep3* repeats. Yeast strain 332-5A was transformed to leucine prototrophy.

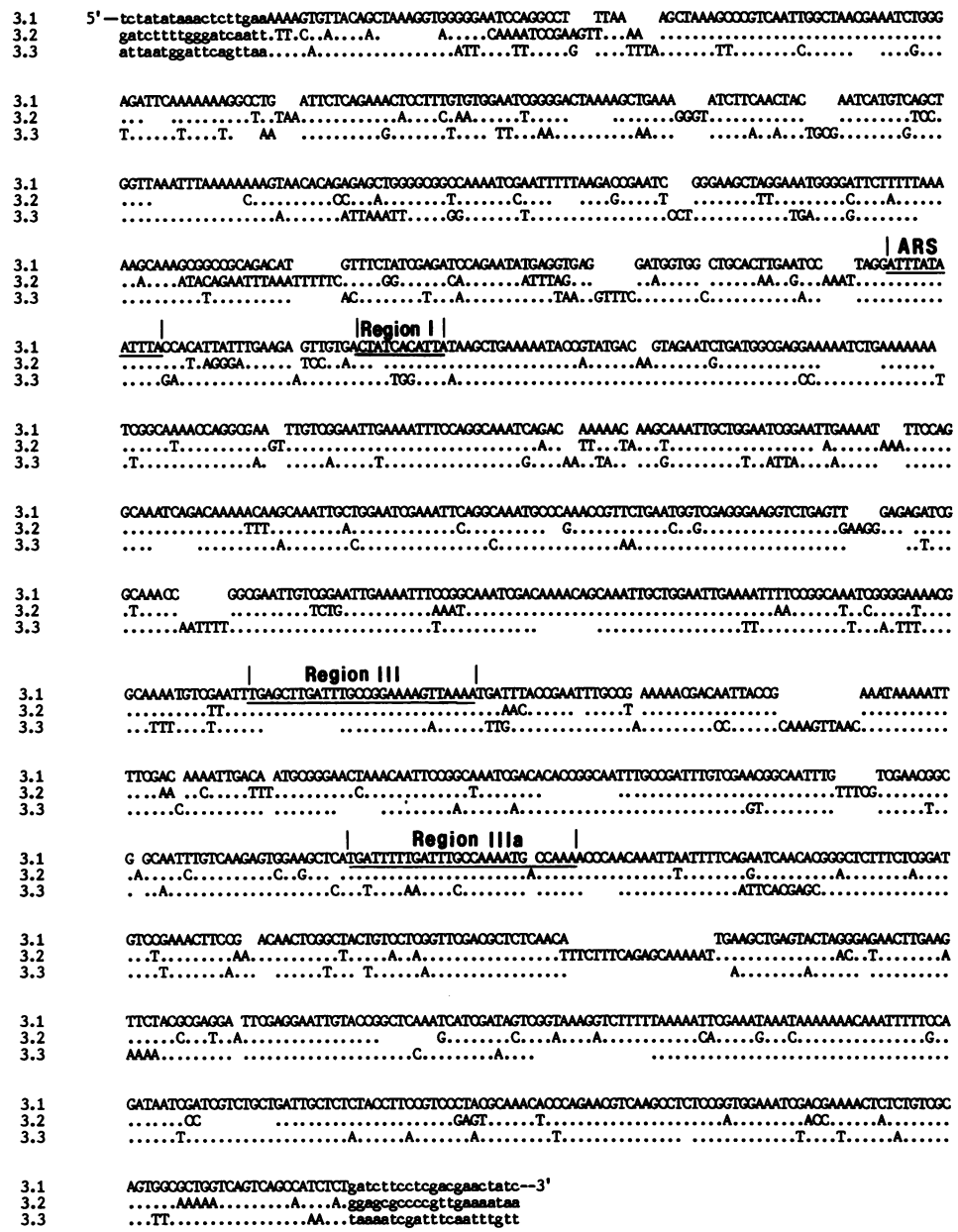


FIG. 3. DNA sequence of *CeRep3* repeats. The DNA sequence is shown in the regions of cross-homology between clones pCe17 (*CeRep3.1*), λCe3004 (*CeRep3.2*), and λCe3017 (*CeRep3.3*). Capital letters are used for significant homology among the clones. Gaps have been introduced to improve the alignment. Nucleotides homologous to yeast *ARS* and *CEN* consensus sequences are underlined.

the bud increase the mitotic stability of such plasmids and simultaneously lower their copy number. Centromeric sequences from yeast (*CEN*) have this property, as do certain sequences from other organisms, termed *SEG* sequences (29). To test *CeRep3* repeats for *SEG* function, *C. elegans* fragments were ligated into the yeast-*E. coli* shuttle vector pLC544 (6). Plasmid pLC544 contains an *ARS* sequence from yeast (*ars1*) but no *CEN* or *SEG* sequence and is unstable during mitotic growth. Some *C. elegans* restriction fragments carrying members of the *CeRep3* repetitive family stabilized pLC544 during nonselective mitotic growth, while others did not (Table 2). The stability conferred was comparable to that conferred by a yeast centromere.

Normal 2+:2− meiotic distribution can be conferred by *CeRep3*-containing DNA. To determine the effect of *CeRep3*-containing DNA on plasmid behavior during meiosis, yeast cells containing the plasmids derived from pLC544 by addition of *CeRep3*-containing DNA, used above for tests of mitotic stability, were mated to cells carrying the nuclear marker *met14*, and the resulting diploids were grown and sporulated. Tetrads were analyzed to determine the segregation pattern of the plasmid. The results of analyzing three such strains are given in Table 2. Plasmids carrying *CeRep3* sequences showed 2+:2− segregation at a significant frequency. Furthermore, 2+:2− tetrads were of the parental ditype or nonparental ditype with respect to the nuclear marker, as expected for segregation of the plasmid at the first meiotic division. Plasmids carrying *CeRep3* repeats gave higher numbers of 3:1 and 4:0 tetrads than a plasmid containing a yeast centromere. This may indicate that the

(A) ARS

Yeast consensus: ATTTATP TTTA
T T

CeRep3.1	TCCTAGGATTTATAATTTACCACATT
CeRep3.2	AATTAGGATTTATAATTTACCATAAG
CeRep3.3	TCCTAGGATTTATAATTTAGAACATT

(B) CEN

DNA	Region I	Region II	Region III
Yeast consensus	PTCACPTG	78-86 bp (>90% A+T)	TG TTTATGNTTT CCG AAANNNNAAA (T)
CEN3	GTCACATG	84 bp (93% A+T)	TG TATTTGATTT CCG AAAGTTAAAA
CEN11	GTCACATG	85 bp (94% A+T)	TG TTCATGATTT CCG AACGTATAAA
CeRep3.1	ATCACATT	360 bp (66% A+T)	TG AGCTTGATTTGCCGGAAAAGTTAAA
		(130 bp downstream)	TGATTTTTGATTTGCCA AAATGCCAAA
CeRep3.2	TTCACATT	362 bp (66% A+T)	TG AGCTTGATTTGCCGGAAAAGTTAAA
		(135 bp downstream)	TGATTTTTGATTTGCCA AAATGACCAAA
CeRep3.3	TATTGGAT	358 bp (66% A+T)	TG TGCCGGAAAAGATAAA
		(125 bp downstream)	TGTTTTTAAATTTCCCA AAATGCCAAA

FIG. 4. (A) Comparison of sequences in *CeRep3* repeats to the yeast *ARS* consensus as defined by Broach et al. (5) and by Kearsey (17). (B) Comparison of sequences in *CeRep3* repeats to the yeast centromere consensus sequence as defined by Hieter et al. (13) and to sequences of yeast *CEN3* and *CEN11*. P, Purine; Y, pyrimidine; N, any nucleotide.

CeRep3-containing plasmids were in somewhat higher copy number.

To verify that 2+:2− segregation patterns were not the result of integration of the plasmid into a chromosome, DNA of the segregants carrying the plasmid marker in 2+:2− tetrads was analyzed for the presence of the unintegrated plasmid. The presence of free plasmid DNA in these segregants was demonstrated by showing that the DNA of these cells could be used to transform *E. coli* cells to ampicillin resistance. Transformed bacterial cells, which arose at a frequency expected if the yeast strain contained free plasmid, were then shown to contain a plasmid of the original structure.

DNA containing a *CeRep3* repeat lowers the copy number of plasmids in yeast cells. A further property of *CEN* and *SEG* sequences is that they lower the copy number of *ARS*-bearing plasmids in *S. cerevisiae* (29). The copy number of various plasmids in yeast strain 332-5A was determined by simultaneously measuring the intensity of hybridization of a single probe to linearized plasmid sequences and a chromosomal restriction fragment. DNA bearing repeat *CeRep3.1* lowered the copy number of plasmid pLC544 some 30-fold, to a level comparable to that of a plasmid containing a yeast *CEN* sequence (Fig. 5). The number of plasmid molecules per cell calculated for all the strains in this experiment appeared to be low by a factor of about 5. The expected copy number for pLC544, an *ARS*-bearing plasmid, is 75 to 200, and the expected copy number for a *CEN*-bearing plasmid is 1 (29). Possible explanations for this discrepancy are lower transfer efficiency from the gel of the larger plasmid-derived fragments or hybridization efficiencies not proportional to the length of DNA homology. This systematic error does not affect the conclusion that the *CeRep3*-containing DNA segment caused a decrease in plasmid copy number comparable to that brought about by a yeast *CEN* sequence.

***ARS* and *SEG* functions reside within the *CeRep3* repeat.** To show that *SEG* and *ARS* functions were conferred by the *CeRep3* repetitive element and not by flanking *C. elegans* DNA, portions of the 1.7-kb insert in plasmid pCe17 were separately subcloned, and the resulting plasmids were tested for their transforming frequency and mitotic stability. It was found that a 0.7-kb restriction fragment, lying wholly within the *CeRep3.1* repeat, carried *ARS* and *SEG* activity, but smaller fragments lost both activities (Fig. 6).

The active 0.7-kb fragment carried all *ARS* and *CEN* consensus sequence homologies, whereas in the smaller subclones segments homologous to the *ARS* consensus sequence and to the *CEN* region I consensus sequence were separated from the segments homologous to the *CEN* region III consensus sequence. Further subcloning experiments are required to define more precisely the sequences responsible for *ARS* and *SEG* function. The data of Fig. 6 can be interpreted to imply either that both *ARS* and *SEG* activities are conferred by a short segment of DNA not containing the *ARS* and *CEN* consensus sequences (right-hand end of fragment E) or that multiple regions within the *CeRep3* repeat, possibly containing the *ARS* and *CEN* consensus sequences, are required together for these activities.

TABLE 2. *SEG function linked to CeRep3* repeats[a]

Plasmid	Plasmid marker gene	Mitotic stability		
		No. of transformants tested (total colonies)	Stabilizing DNA	Trp^+ or Leu^+ transformants remaining after nonselective growth (%)
pLC544	*trp1*	25 (2,500)	None	8
pYe(CEN3)30	*trp1*	25 (2,500)	*CEN3*	94.5
YEp13 (2μm ARS)	*leu2*	25 (2,500)	2μm	100
pLC544 + *CeRep3.1*	*trp1*	50 (5,000)	*CeRep3.1*	86.7
pLC544 + *CeRep3.2*	*trp1*	50 (5,000)	*CeRep3.2*	92.9
pLC544 + *CeRep3.3*	*trp1*	10 (1,000)	*CeRep3.3*	8.7
pLC544 + *CeRep3.4*	*trp1*	50 (5,000)	*CeRep3.4*	89.5
pLC544 + *CeRep3.5*	*trp1*	10 (1,000)	*CeRep3.5*	4.2
pLC544 + *CeRep3.6*	*trp1*	10 (1,000)	*CeRep3.6*	3.8
pLC544 + *CeRep3.7*	*trp1*	10 (1,000)	*CeRep3.7*	11.3
pLC544 + *CeRep3.8*	*trp1*	10 (1,000)	*CeRep3.8*	5.7

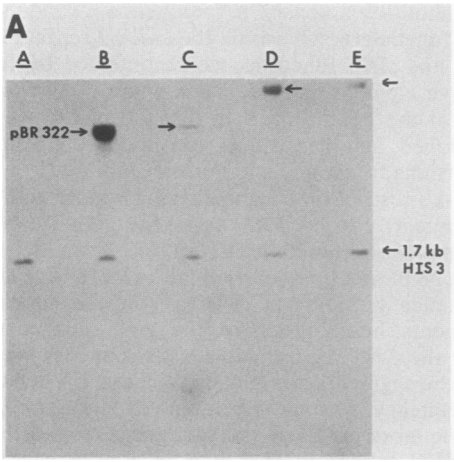
[a] Test of the effect of DNA containing *CeRep3* repeats on mitotic and meiotic behavior of plasmids. Assays for mitotic and meiotic stability are described under Materials and Methods.

DISCUSSION

Whereas many repeated DNA families in eucaryotic genomes have evident functions or origins, for example, families of expressed genes, processed pseudogenes, or transposable elements, others remain unexplained. We have characterized three nontransposon families of unknown origin and function in the *C. elegans* genome (12; K. M. Felsenstein, Ph. D. thesis, Albert Einstein College of Medicine, 1987; this work). Each is completely distinct in structure and sequence from the other two, as expected from the high complexity of this class of sequences in the *C. elegans* genome. The *C. elegans* genome contains over 100, and possibly as many as 1,000, distinct families of short, interspersed repeats, each family with fewer than 100 members (10).

The finding of sequences within *CeRep3* repeats that are homologous to yeast *ARS* and *CEN* sequences suggests a possible function for this family. *C. elegans* chromosomes are holocentric or holokinetic; that is, they attach to spindle microtubules at many points along their length rather than at a single differentiated point known as the centromere. Evidence for this dispersed attachment is provided by light and electron microscopic examination of the chromosomes (1) and by analysis of the genetic stability of chromosome fragments (1, 14). In view of this holocentric property, it might be expected that the *C. elegans* genome contains many sequences distributed along the chromosomes that can act analogously to centromeres.

Heterologous sequences with *ARS* and *SEG* function in *S. cerevisiae* have been isolated previously from *C. elegans* and other eucaryotes (28, 29). *ARS* elements are found at high frequency in many eucaryotic DNAs. Heterologous *SEG* sequences have so far been isolated only from *Chlamydomonas* spp. and *C. elegans* (3). From the frequency at which Stinchcomb et al. found *SEG* sequences in *C. elegans*, they estimated that there were 20 to 30 in the *C. elegans* genome (29). This estimate is probably not at variance with our finding of a repetitive family with *SEG* function, especially since many members of the *CeRep3* family lack *SEG* function. Several *SEG* sequences isolated by Stinchcomb et al. (29) are linked to a repetitive sequence, but the repeats



B

Lane	Cell	Plasmid	% Cells with plasmid	Measured OD (arbitrary units) plasmid/HIS3	Number of nucleotides homology with probe (kb) plasmid/HIS3	Plasmid copies per cell
A	332-5A	None	-			0
B	332-5A	Yep13	100	8691/1006	4.3/1.7	3.4
C	332-5A	pYE(CEN3)30	100	559/756	4.3/1.7	0.3
D	332-5A	pLC544	10	2069/722	4.0/1.7	12.2
E	332-5A	pLC544+CeRep3.1	90	715/908	4.0/1.7	0.4

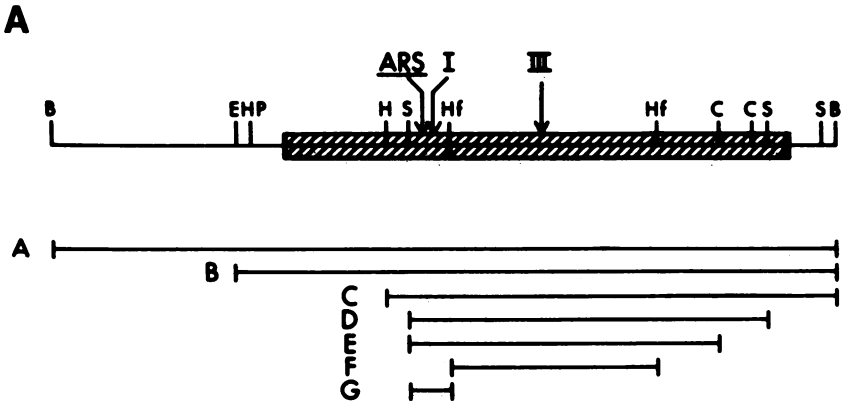
FIG. 5. Effect of *C. elegans* sequences on plasmid copy number. (A) DNA of various yeast strains was digested with *Bam*HI and fractionated on 1% agarose. After transfer to nitrocellulose, the DNA was hybridized to nick-translated plasmid pSZ63. This plasmid hybridizes to both the chromosomal *HIS3* gene on a 1.7-kb *Bam*HI fragment and pBR322-homologous sequences of the various *Bam*HI-digested plasmids (arrows). Lanes: A, DNA of strain 332-5A before transformation; B to E, DNA of strain 332-5A after transformation with the plasmids listed in panel B. (B) Calculation of copy number from intensity of hybridization, taking into consideration the measured fraction of the cells in the culture that had any plasmid and the number of nucleotides of homology between the probe and each band. OD, Optical density.

Meiotic stability							
Distribution of plasmid marker gene [no. (%) of tetrads examined]					Centromere linkage of plasmid marker		
0+:4−	1+:3−	2+:2−	3+:1−	4+:0−	Parental ditype	Nonparental ditype	Tetratype
10 (100)	0	0	0	0			
0	0	10 (91)	0	1 (9)	3	7	0
0	0	0	0	15 (100)			
0	0	6 (35)	2 (12)	9 (53)	4	2	0
0	0	3 (33)	1 (11)	5 (56)	2	1	0
0	0	5 (29)	1 (6)	11 (65)	2	3	0

they found are not homologous to *CeRep3* repeats, and the *SEG* function in their case was completely separable from the repeat, which evidently lies in flanking DNA.

Additional subcloning experiments are required to determine whether the sequences within the *CeRep3* repeats that are similar to yeast *ARS* and *CEN* consensus sequences are responsible for the effects of these repeats on yeast plasmids. As these are the only sequences within the repeats that resemble the yeast consensus sequences, it seems reasonable to suppose that they are. If they are not, then there must be other sequences besides the previously identified yeast consensus sequences that are capable of stabilizing plasmids in *S. cerevisiae*.

The sequence within *CeRep3* resembling an *ARS* element



B

Fragment	Fragment size (kb)	Regions present	ARS function	Stabilizing function
A	1.7	I,III,ARS	+	+
B	1.5	I,III,ARS	+	+
C	1.0	I,III,ARS	+	+
D	0.80	I,III,ARS	+	+
E	0.70	I,III,ARS	+	+
F	0.55	III	-	-
G	0.12	I,ARS	-	-

FIG. 6. *ARS* and *SEG* function of portions of *CeRep3.1*. The segments of the *C. elegans* insert in pCe17 shown on the map were subcloned into YIp28 and pLC544. The repetitive portion of the clone is shown as the hatched box. To test for *ARS* function, the ability of the YIp28-derived plasmids to transform yeast strain 332-5A was determined. Symbols: +, 50 to 200 transformants per microgram of plasmid; −, 0 to 1 transformant per microgram of plasmid. *SEG* function of the pLC544-derived plasmids was assayed by the mitotic stability assay described under Materials and Methods. Symbols: +, greater than 80% of the cells retained the plasmid during nonselective growth; −, less than 10% retained the plasmid. Restriction sites: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hae*III; Hf, *Hin*fI; HP, *Hpa*I; S, *Sau*3A.

was identical to the yeast sequence at every nucleotide conserved among all yeast *ARS* elements studied and at nucleotides shown to be essential by Kearsey (17). The only difference between the *C. elegans* and yeast sequences was the insertion of an additional A within the sequence. The effects of such insertions have not been studied. Several workers have shown that additional, as yet undefined flanking sequences outside the essential core sequence can have an effect on *ARS* function (17, 18, 22). Such effects could explain our finding that some subclones carrying the *ARS*-homologous sequence did not have *ARS* function (Fig. 6).

The resemblence of sequences within *CeRep3* elements to the yeast centromere consensus sequence was less close. Possibly *CeRep3* elements stabilize yeast plasmids by allowing them to utilize the 2μm plasmid partitioning system. All of the yeast strains we used contain this plasmid. This possibility appears to be unlikely in view of the meiotic segregation data. The homology within *CeRep3* repeats to the region III consensus sequence was statistically significant. In the *CeRep3.3* repeat, there was a deletion of nine nucleotides within one region III sequence, and this repeat failed to show *SEG* function (Table 2). *CeRep3* repeats showed little homology to region I and none to region II of the yeast centromeric consensus sequence. The homology to region I was not unlikely to be found on a random basis. Whether the similarities in sequence that do exist account for the effects on yeast plasmids, or whether other sequences within the *CeRep3* repeats contribute to these effects, is unknown. The effects of alterations in the yeast centromeric consensus regions on the behavior of plasmids in mitosis and meiosis is complex (see reference 8 and references therein).

Whereas sequence similarities may explain the observed properties of the *CeRep3* family of repeats in *S. cerevisiae*, they tell us nothing about the role or origin of this repetitive family in *C. elegans*. This question must be addressed in transformation studies, in which the effects of the repeats on extrachromosomal tandem DNA arrays may be tested (30).

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