Caenorhabditis elegans DNA that directs segregation in yeast cells

(centromere/nematode/Saccharomyces cerevisiae/mitosis/meiosis)

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ABSTRACT We have isolated seven DNA fragments from Caenorhabditis elegans that enhance the mitotic segregation of autonomously replicating plasmids in the yeast Saccharomyces cerevisiae. These segregators, designated SEG1-SEG7, behave like isolated yeast chromosomes: they increase the stability and simultaneously lower the copy number of circular plasmids during mitotic growth in yeast. During meiosis, plasmids containing the C. elegans segregators show higher levels of precocious or aberrant disjunction than do plasmids bearing isolated yeast centromeres. Yet one of the segregators improved the meiotic segregation of the parental plasmid. We estimate that there may be as many as 30 segregator sequences in the C. elegans genome, a value that is consistent with the polycentric nature of C. elegans chromosomes. Five of the seven segregators are linked to sequences that are repeated in the worm genome, and four of these five segregators cross-hybridize. Other members of this family of repetitive DNA do not contain segregator function. Segregator sequences may prove useful for probing the structure of centromeres of both C. elegans and S. cerevisiae chromosomes.

DNA transformation of the yeast Saccharomyces cerevisiae has been used to identify DNA sequences that direct chromosome behavior. Upon introduction into a yeast cell, hybrid molecules carrying only a yeast structural gene are not capable of autonomous replication or segregation. Addition of ARS DNA (Autonomously Replicating Sequence) to such a chimera permits its replication (1). In spite of their high copy number and their ability to replicate during each cell division (2, 3), ARS-containing hybrid molecules are not propagated efficiently during mitotic growth. Even under selective conditions, only 2–10% of the cells in a transformed culture bear the ARS hybrid molecule (1, 4). Under nonselective conditions, the hybrid plasmid is rapidly lost. Centromeric sequences, designated CEN, alleviate this instability; by allowing proper segregation, hybrids are efficiently transferred to daughter cells (5–8). The result is a significant increase in the proportion of cells that stably maintain the transformed phenotype. This stability can be used to enrich for hybrids bearing DNA sequences that aid segregation of ARS-containing molecules; we term such a sequence SEG for segregator. For instance, when hybrids bearing random fragments of yeast DNA were screened or enriched for stable segregation, the only DNA carrying segregator functions were yeast centromeres or fragments of the endogenous yeast plasmid (6, 8, 9). Herein, we describe the isolation of segregator functions from DNA of the nematode Caenorhabditis elegans. C. elegans was chosen as a source for such functions because its chromosomes are poly- or holocentric (10, 11). If the lack of a defined centromere is due to multiple sites directing chromosome segregation (and if some of these sites function in yeast), then segregator functions would be highly prevalent in worm DNA. Indeed, we isolated seven different DNA fragments from C. elegans DNA that stabilize autonomously replicating hybrid molecules.

MATERIALS AND METHODS

Strains and Materials. BNN6 (also known as HB101) was used for all Escherichia coli transformation experiments. The plasmid YRp17 consists of pBR322 sequences (12), the URA3 and TRPL genes, and the putative origin of replication ARS1 (6). YNN140 (a his3-1 trpl-289 ura3-1 ura3-2 ade2) was used in all segregator enrichment experiments and stability tests. YNN152 (a leu1 met14 ade1 ura3 trpl his2 gall, obtained from the Yeast Genetic Stock Center as X2928-3D-1C) was mated to YNN140 for meiotic analyses. Restriction endonucleases, E. coli DNA polymerase I, T4 DNA ligase, DNase, RNase, and calf alkaline phosphatase were all purchased from the usual vendors. Protocols and media for growth of E. coli, plasmids, and bacteriophage λ are described by Davis et al. (13). Yeast media were as described by Mortimer and Hawthorne (14).

DNA Preparations. Plasmid DNA was prepared by techniques described by Maniatis et al. (15). Phage DNA was prepared as described by Davis et al. (13). C. elegans var. Bristol DNA was provided by Steve Carr. Rapid yeast DNA preparations were performed as described (16). DNA fragments were purified out of agarose gels by using DEAE membranes (17) and were inserted into plasmid vectors by standard techniques (13).

Construction of Hybrid Pools. Two collections of hybrid molecules were used to enrich for segregators. For the first screen, 1 µg of YRp17 and 2.5 µg of C. elegans DNA were cut to completion with the restriction endonuclease EcoRI. The DNA samples were mixed after extraction with phenol and precipitation with ethanol and were resuspended in 50 µl of 100 mM NaCl/50 mM Tris-HCl, pH 7.4/10 mM MgSO4/1 mM ATP/10 mM dithiothreitol. The EcoRI cohesives ends were ligated with 60 units of T4 DNA ligase at 4°C for 16 hr. Then this collection of chimeric DNA molecules was used directly to transform the yeast strain YNN140 from Ura+ to Ura− by modification (16) of the original transformation procedure (18). The second pool was constructed as follows. YRp17 (0.5 µg) was cleaved with BamHI, treated with alkaline phosphatase, extracted with phenol, and mixed with 2.5 µg of BamHI-cleaved C. elegans DNA. After precipitation with ethanol, the DNA samples were ligated in 50-µl volumes as described above. The hybrid DNAs were used to transform E. coli (19) to ampicillin resistance (AmpR). The resistant clones were pooled into four separate batches and amplified for plasmid DNA purification. Plasmid DNA also was prepared from several individual ampR E. coli colonies isolated after amplification. The preparations were digested with restriction nucleases and sized by agarose gel electro-

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Abbreviation: kb, kilobase pair.

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phoresis, permitting accurate determination of the quantity of worm DNA present in the pools of hybrid plasmids. This plasmid DNA was then used to transform yeast as described.

**Enrichment for Segregators.** In the first screen (diagramed in Fig. 1), 1200 individual yeast transformants were pooled into four independent batches; 10^5 cells from each of the batch cultures were grown to saturation (3 x 10^7 cells per ml) in 5 ml of nonselective medium containing uracil. Each culture was then diluted 1:1000 into the same medium lacking uracil and again grown until saturated. A second round of growth in alternating nonselective and selective media was performed. At this time, the culture was diluted and spread on plates lacking uracil so that single Ura^+ colonies could be isolated. In subsequent screens, transformants were grown in alternating selective and nonselective media in microtiter wells. Transfers were continued until YRp17 had disappeared, while the previously isolated YRp17-SEG2 plasmid was maintained in control wells. Surviving Ura^+ cells were plated and analyzed as described in Results.

![Segregator Screen](image)

**Fig. 1.** Segregator screen. Pools of *C. elegans*/YRp17 hybrid molecules were constructed as described. The hybrid molecule collections were used to transform yeast, selecting for expression of the *URA3* gene present on YRp17. The transformants were then grown singly or in groups under nonselective conditions. Because of their failure to segregate, YRp17 molecules gradually were lost in the population. To reselect for Ura^+ transformants, the cultures then were grown under selective conditions. Growth in alternating selective and nonselective conditions thus enriches for transformants that are mitotically stable. After several cycles of growth, the surviving transformants were plated on selective media. Single Ura^+ colonies were picked, their mitotic stability was quantitated, and they were analyzed for the presence of autonomously replicating hybrid molecules (see Results).

**Mitotic Stability Tests.** Cultures grown in minimal media lacking uracil were diluted and plated onto minimal agar plates containing uracil; these were then replica-plated to selective and nonselective plates to determine the percentage Ura^+ cells. In addition, each culture was diluted 1:1000 into minimal medium with uracil and again grown until saturated. The percentage of cells that retained the Ura^+ phenotype after these 10 generations of nonselective growth was assayed by dilution and replica-plating.

**Copy Number Determinations.** Copy number measurements were performed as described (2). Briefly, Ura^+ transformants were grown in selective medium. An aliquot of each culture was diluted, plated, and replica-plated to assess how many cells carried the plasmid. Each culture was then harvested, and sheared DNA was prepared, spotted on nitrocelulose strips, and hybridized with [32P]DNA. The hybridization to each spot was quantitated by scintillation counting. We compared the hybridization of single-copy probes (fragments of the yeast *HIS3* or *ADE8* genes) to the hybridization to pBR322. We adjusted for the differential hybridization efficiencies of the probes by comparing the hybridization to known amounts of a hybrid plasmid carrying one copy of pBR322 sequences and one copy of yeast sequence. Thus, we could calculate the number of plasmid sequences per cell in the harvested populations. Dividing by the fraction of Ura^+ cells in each culture gave the copy numbers of pBR322 sequences in each transformed cell shown in Table 1.

**Meiotic Analysis.** Diploids were constructed by mating the haploid Ura^+ transformants of YNN140 with a haploid of opposite mating type (YNN152). The resulting diploid was prototrophic. URA3^+ and TRP1^+ are provided only by the extrachromosomal hybrid molecule. The strain is heterozygous for the tightly centromere-linked markers metl4 and *leu1*. Such diploids were induced to sporulate. The spores from individual tetrads were separated by microdissection techniques, germinated, and their phenotypes assessed by replica-plating (14).

**Hybridization Analysis.** Agarose gel electrophoresis (20), transfer to nitrocelulose filters (21), and nick-translations (22) were performed by modifications (13) of the original techniques. Hybridizations were routinely carried out at 32°C in 0.8 M NaCl/0.1 M Na_2HPO_4/5 mM EDTA/50% formamide/0.2% NaDodSO_4/100 μg denatured salmon sperm DNA.

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**Table 1.** Mitotic stability

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Ura^+ cells</th>
<th>Selective growth, %</th>
<th>Nonselective growth, %</th>
<th>Copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>YRp17</td>
<td></td>
<td></td>
<td></td>
<td>75-200</td>
</tr>
<tr>
<td>YRp17-SEG1</td>
<td>2-10</td>
<td>0.1-2</td>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td>YRp17-SEG2</td>
<td>30-50</td>
<td>10-20</td>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td>YRp17-SEG3</td>
<td>30-50</td>
<td>10-20</td>
<td></td>
<td>3-5</td>
</tr>
<tr>
<td>YRp17-SEG4</td>
<td>25-40</td>
<td>5-10</td>
<td></td>
<td>3-6</td>
</tr>
<tr>
<td>YRp17-SEG5</td>
<td>20-40</td>
<td>5-10</td>
<td></td>
<td>5-10</td>
</tr>
<tr>
<td>YRp17-SEG6</td>
<td>25-45</td>
<td>10-20</td>
<td></td>
<td>10-20</td>
</tr>
<tr>
<td>YRp17-SEG7</td>
<td>15-25</td>
<td>5-10</td>
<td></td>
<td>40-50</td>
</tr>
<tr>
<td>YRp17-CEN4</td>
<td>60-90</td>
<td>25-50</td>
<td></td>
<td>1-9</td>
</tr>
</tbody>
</table>

Mitotic behavior of SEG-containing hybrids. The percentage of Ura^+ cells was determined by replica-plating after growth to saturation in selective medium or after 10 generations in nonselective conditions. Here we present the range of several independent determinations. The variation was large, so subtle differences in mitotic stability would be difficult to detect. The copy number of the autonomously replicating plasmids in a population of yeast grown in selective media was measured by hybridization techniques. The average number of copies of hybrid DNA molecules per transformed cell was subsequently calculated. Again, we report the range of values obtained in several independent experiments.
RESULTS

Enrichment for Segregators. Hybrid DNA molecules bearing ARS1 replicate extrachromosomally in yeast; however, they are not transmitted efficiently to daughter cells (1). One such hybrid molecule, YRp17, bears yeast DNA sequences ARS1, TRPI, and URA3 (6). To search for segregator functions in C. elegans DNA, we inserted random pieces of worm DNA into YRp17. The collections of hybrid molecules were used to transform a ura3－yeast strain (YNN27) to Ura+. We then enriched for those transformants that retained the Ura+ phenotype under nonselective conditions (see Fig. 1). After the enrichment, individual yeast cells were cloned and tested for the stability of the transformed phenotype.

Three classes of Ura+ survivors were found. Most of the transformants were still mitotically unstable: after 10 generations of growth in nonselective medium, <5% of the cells remained Ura+. The hybrid DNA molecules were extrachromosomal (data not shown). Therefore, this first class of transformants carries unstable autonomous molecules that persisted through the enrichment procedure. A second class of transformants was quite stable (100% of the cells remained Ura+ after 10 generations). The molecular hybrids were found integrated into the yeast chromosomal DNA (data not shown). In this case, the transformants were stabilized by recombination with the host chromosomes. A third class of transformants that survived the enrichment scheme was intermediate in its stability (10–50% Ura+ cells after 10 generations) and carried autonomously replicating hybrid molecules. To test whether this increase in stability was imparted by the hybrid molecule or by the cell harboring it, we isolated the plasmids in bacteria and then retransformed a ura3－yeast strain to Ura+. Seven hybrid molecules were found that showed significantly increased mitotic stability after isolation and retransformation. These plasmids thus carry segregator function.

Mitotic Stability of Segregators. Once these plasmids were reisolated in bacteria and used to transform YNN27 to URA3, their effect on mitotic segregation was analyzed in more detail. SEG-containing hybrid molecules are significantly more stable than YRp17 during mitotic propagation in yeast. The mitotic stabilities and the copy numbers of these hybrids are shown in Table 1. Like a yeast centromere, the C. elegans segregators increased the stability and simultaneously reduced the copy number of the autonomously replicating molecules. This reduction may simply be a consequence of better segregation. Without segregator function, extrachromosomal molecules replicated before each division yet often failed to segregate to daughter cells; thus, the Ura+ cells accumulated multiple copies. With segregator function, better partitioning prevented accumulation and lowered the copy number.

Meiotic Behavior of SEG-Containing Hybrids. If a hybrid molecule with a fully functional yeast centromere is present in one copy per diploid cell, if it replicates during premeiotic S phase, and if it subsequently segregates independently of the yeast chromosomes, it should be found in two of the four meiotic products. Indeed, hybrids carrying a yeast centromere segregate 2+:2－ in over half of the tetrads (5–8); a CEN-containing hybrid molecule behaves as an independent linkage group. To analyze the behavior of SEG-containing hybrid molecules during meiosis in yeast, we constructed a/a diploids in which the YRp17/SEG chimera carried the only functional URA3 gene. These diploids were induced to undergo meiosis, and the phenotypes of the four meiotic products were analyzed upon tetrad dissection (see Table 2). Diploids carrying SEG1 and SEG2 hybrid molecules segregated 2 Ura+:2 Ura－ in 19% and 45% of the tetrads, respectively (see Table 2). Diploids carrying the other C. elegans segregators produced a high percentage of 0 Ura+:4 Ura－ tetrads. The percentage of diploids that carry a Ura+ hybrid molecule depends on the mitotic stability of the plasmid (4). Thus, the predominance of 0 Ura+:4 Ura－ tetrads may reflect the lower mitotic stability of these segregators compared to isolated yeast centromeres.

In yeast, a centromere is genetically defined as the region of a linkage group that always segregates away from its homologue in the first, reductional division of meiosis. In the 2+:2－ tetrads, the hybrid molecules are analogous to unpaired, monovalent chromosomes. If the segregators behave as yeast centromeres, both Ura+ hybrid molecules should segregate away from Ura－ (null) in the first division, and the two hybrid Ura+ chromosomes should not separate until the second equal division. First or second division disjunction can be assessed by comparing the segregation of Ura+ to two centromere-linked chromosomal markers, met14 and leu1 (see Materials and Methods and the legend to Table 3). The replicated hybrid molecules bearing a yeast centromere disjoin in the second division in 98% of the 2+:2－ tetrads; precocious separation is rare (5, 6). The chromadis of SEG2 molecules separated early in 35% of the 2+:2－ tetrads, while in 65% of the tetrads the chromatids disjoined properly in the second division. Although 35% precocious disjunction is much higher than that observed for an isolated yeast centromere, SEG2 nevertheless was behaving nonrandomly during meiosis. On the other hand, the chromatids of SEG1 hybrid molecules separated at the first division in 60% of the 2+:2－ tetrads; only 40% disjoined properly in the second division. If SEG1 were segregating randomly during meiosis, two-thirds of the 2+:2－ tetrads would show a first division segregation pattern (see the legend to Table 3).

Frequency of Segregator Sequences in C. elegans. We performed two screens that allowed us to estimate the frequency of segregator sequences in the worm genome. In the first screen, no segregators were found amongst 1300 individual yeast transformants, carrying some 2400 kilobase pairs (kb) of worm DNA. In the second screen, 12,000 transformants representing ~15,000 kb of the worm genome were screened in groups of 20. We obtained five different fragments that showed various degrees of segregator character in this second screen. On average, a segregator sequence was found every 3000 kb of C. elegans DNA. The

Table 2. Meiotic segregation of SEG-containing hybrid molecules

<table>
<thead>
<tr>
<th>Hybrid molecule</th>
<th>Ura+:Ura－ spores</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>YRp17-SEG1</em></td>
<td>11 (12%)</td>
</tr>
<tr>
<td><em>YRp17-SEG2</em></td>
<td>9 (17%)</td>
</tr>
<tr>
<td><em>YRp17-SEG3</em></td>
<td>10 (16%)</td>
</tr>
<tr>
<td><em>YRp17-SEG4</em></td>
<td>2 (4%)</td>
</tr>
<tr>
<td><em>YRp17-SEG5</em></td>
<td>9 (17%)</td>
</tr>
<tr>
<td><em>YRp17-SEG6</em></td>
<td>3 (9%)</td>
</tr>
<tr>
<td><em>YRp17-SEG7</em></td>
<td>7 (18%)</td>
</tr>
<tr>
<td><em>YRp17-SEG8</em></td>
<td>4 (12%)</td>
</tr>
<tr>
<td><em>YRp17-CEN4</em></td>
<td>14%</td>
</tr>
</tbody>
</table>

Meiotic behavior of SEG-containing hybrids. Diploids bearing the YRp17/SEG hybrid molecules were sporulated, the asci were dissected, and the Ura+ phenotype of each germinated spore was determined. The number of asci in each of the five possible classes of Ura+ segregation patterns is presented.

*The data for the segregation of hybrids containing the yeast centromere CEN4 have been published (6).
Table 3. Disjunction in 2⁺:2⁻ tetrads

<table>
<thead>
<tr>
<th>Hybrid molecule</th>
<th>First division</th>
<th>Second division</th>
</tr>
</thead>
<tbody>
<tr>
<td>YRp17-SEG1</td>
<td>9 (60%)</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>YRp17-SEG2</td>
<td>7 (35%)</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>YRp17-CEN4</td>
<td>2 (2%)</td>
<td>84 (98%)</td>
</tr>
</tbody>
</table>

Disjunction of SEG-containing hybrids in 2⁺:2⁻ tetrads. Sister spores (the daughters of the first meiotic division products) were identified by the segregation patterns of heterozygous centromere-linked markers *met14* and *leu1*. If the Ura⁺ spores were not sisters (a tetratype segregation pattern relative to *met14* and *leu1*), then the two copies of the hybrid molecules must have separated precociously in the first division. If the two Ura⁺ spores were sisters (parental ditype or nonparental ditype segregation), then the two hybrid molecules behaved like a yeast chromosome and disjoined in the second meiotic division. If two copies of a hybrid molecule were to segregate randomly during meiosis, the following products would be expected: one of four 1⁺:3⁻ tetrads (both hybrid molecules segregated to a single pole in the first and second divisions), one of four parental or nonparental ditype tetrads (the molecules segregate to a single pole in the first division and then to different poles in the second), and one of two tetratype tetrads (the molecules segregate to different poles in the first division). Since the 1⁺:3⁻ tetrads would not be considered in this analysis, we expect two of three first division disjunctions and one of three second division disjunctions amongst the 2⁺:2⁻ tetrads for a randomly segregating hybrid. SEG1 showed essentially random segregation during meiosis. However, the segregation of SEG2 significantly deviated from random behavior: the probability of observing only 35% precocious disjunction in a randomly segregating sample of this size is <1%.

*As in Table 2, the data for hybrids containing yeast CEN4 are derived from ref. 6.

haploid worm genome contains 80,000 kb (23); there may be as many as 20–30 segregator sequences in *C. elegans*.

**Genomic Structure of Segregators.** Each of the isolated segregators was labeled with 3²P by nick-translation (22) and was permitted to hybridize to a spectrum of *C. elegans* DNA fragments (21). Two of the seven segregators annealed to sequences found only once in the *C. elegans* genome (SEG3 and SEG7, data not shown). Five of the seven segregators hybridized to *C. elegans* sequences that are repeated in the genome (SEG1-2, 4-6, and 8; see Fig. 2A). Resemblances can be seen between the patterns of fragments that hybridize to different segregators (for example, compare hybridization by SEG5 and SEG6, Fig. 2A). To determine if common sequences are associated with these segregator functions, we hybridized the segregators to one another in pairwise combinations. The results are summarized in Fig. 2B. SEG4, SEG5, and SEG6 cross-hybridized; the homology between SEG5 and SEG6 was the most striking. In addition, SEG2 and SEG6 shared some sequence homology. As expected, SEG3, one of the single-copy segregators, failed to hybridize strongly to any of the repeated segregators.

The data above indicate that SEG2, SEG4, SEG5, and SEG6 are linked to a single class of repeated sequences. We isolated other members of this class by hybridization techniques to ask if they, too, would encode a segregator function. A SEG2-containing hybrid plasmid was used to isolate λ bacteriophage containing homologous worm DNA by plaque-filter hybridization (24). The DNA from one such λ chimera was mapped with restriction endonucleases, and the site of hybridization with SEG2 was localized. Two fragments that contain the repeated sequence as well as adjacent nonrepetitive DNA were inserted into YRp17. The resulting hybrid plasmids were used to transform a ura3⁻ yeast strain to Ura⁺. The Ura⁺ phenotype was unstable; neither fragment conferred mitotic stability to YRp17. A similar result was obtained with two members of the set of genome fragments that hybridize to SEG6.

### DISCUSSION

We isolated DNA fragments (segregators) from *C. elegans* that functionally resemble isolated yeast centromeres. Both segregators and centromeres enhance the mitotic stability and reduce the copy number of autonomously replicating molecules in yeast cells. However, none of the *C. elegans* sequences segregate with the surety of a yeast centromere. Their aberrant segregation is seen during both mitosis (see Table 1) and meiosis (see Table 2). The data are consistent
with the segregators representing a graded series of centromere activity: SEG2 is most similar to an isolated yeast centromere, while SEG7 barely increases the mitotic stability of the parent plasmid, YRp17.

Mutants have been isolated that destabilize isolated yeast centromeres (Phil Hieter, personal communication). The behavior of SEG-containing hybrids in such strains may address any mechanistic similarity to yeast centromeres. If these nematode DNA fragments and yeast centromeres do function by a common mechanism, then the DNA sequence responsible for segregator function may prove of interest. Yeast centromeres share defined DNA sequences (25). DNA sequences required for the modicum of stability provided by segregators may be shared with yeast centromeres; sequences present in centromeres but absent in segregators may be required for accurate centromere behavior.

On the other hand, the C. elegans sequences described herein may affect segregation through a mechanism distinct from that of centromeres. ARS-containing circular molecules appear to be so unstable mitotically because they demonstrate a strong segregation bias. In about one-third of the divisions of a transformed cell, only one of the two cells will contain any plasmid sequences. In 95% of these divisions, the plasmid sequences remain in the mother cell; segregation to the daughter is rare (4). Any sequence that would disrupt this segregation bias would allow random (and thus more equal) partitioning of circular plasmids. Such a sequence would increase mitotic stability of circular plasmids and simultaneously decrease their copy number. Murray and Szostak (4) suggested that segregation bias of circular plasmids could be due to topological constraints (catenanes) in replicated circles. If so, segregator sequences may function by permitting rapid resolution by a topoisomerase or by a recombinational mechanism. Alternatively, segregators may actively promote DNA segregation via association with a structure other than the mitotic and meiotic spindles.

Five of the seven C. elegans segregators that we isolated are linked to sequences that are repeated in the worm genome. Emmons et al. (26) observed a similar frequency of moderately repetitive sequences in restriction fragments selected at random from the worm genome. Yet four of these repeated segregators share homologous DNA sequences, suggesting a correlation between segregator function and this particular class of repetitive DNA. However, the repeated DNA is neither necessary nor sufficient for segregator function: some segregators do not carry the repeat, and some repeats do not function as segregators. Further delineation of the segregator function and the repeated DNA may uncover any cause for their association.

The identification of segregators relies on the function of the heterologous DNA in yeast cells. We can only suggest that these segregator sequences permit proper partitioning of chromosomes in C. elegans; they might be sequences of unrelated function in the worm that behave like centromeres in yeast cells. Microinjection techniques that allow the introduction of foreign DNA into the C. elegans germ line have been recently developed (unpublished data). Whether these worm segregators affect the stability and segregation of injected DNA can now be assessed. If such segregation activity can be demonstrated, the sequence and genomic organization of C. elegans segregators will elucidate the requirements for segregation of polycentric chromosomes.

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