Maintenance of the 2μm Circle Plasmid of Saccharomyces cerevisiae by Sexual Transmission: An Example of a Selfish DNA

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Manuscript received August 14, 1987
Revised copy accepted November 28, 1987

ABSTRACT

Many eukaryotic mobile elements have been identified, but few have any obvious function. This has led to the proposal that many such elements may be parasitic DNA. We have used the 2μm circle plasmid of Saccharomyces cerevisiae as a model system to investigate the maintenance of a cryptic genetic element. We find that under certain conditions this plasmid can spread through experimental populations despite demonstrable selection against it. This spread is dependent upon outbreeding, suggesting that cell to cell transmission of the plasmid during the yeast sexual cycle can counterbalance selection, and maintain the plasmid in populations. This result provides experimental support for the idea that some mobile elements may be parasitic DNA.

MANY prokaryotic plasmids and transposons encode functions such as antibiotic resistance which are advantageous for their host cells. Such functions provide an adequate explanation for the evolution and maintenance of these genetic elements. In contrast, most eukaryotic mobile elements have no obvious function, and this has led to the proposal that many such elements may be parasitic DNA (DOOLITTLE and SAPENZA 1980; ORGEL and CRICK 1980). It is difficult to prove this proposal, because the lack of an identified selective advantage does not mean that one does not exist.

To investigate the forces that maintain eukaryotic mobile elements in populations, we have used the yeast 2μm circle plasmid as a model system. The plasmid consists of a 6-kbp circular DNA molecule found at a copy number of about 60 per cell in most strains of Saccharomyces cerevisiae (GERBAUD and GUERINEAU 1980; KIELLAND-BRANDT et al. 1980). No function has been found for the plasmid and when isogenic [cir+] and [cir-] strains (i.e., containing or lacking the 2μm circle, respectively) were compared in growth rate competition experiments, it was found that [cir+] cells were at a selective disadvantage of roughly 1% (FUTCHER and COX 1983; MEAD, GARDNER and OLIVER 1986). Thus, it is not clear how the plasmid is maintained.

One explanation of 2μm circle maintenance is that the plasmid provides a subtle selective advantage of a kind not yet identified. Many workers have searched for such an advantage, but without success. A second possibility is that the plasmid is parasitic DNA. STEWART and LEVIN (1977) and HICKEY (1982) have defined conditions in which contagious genetic elements such as viruses, plasmids and transposons can be maintained in populations by transmission despite selection against them. The 2μm circle is not infective in ordinary mitotic cultures (FUTCHER and COX 1983), but it is contagious during mating (LIVINGSTON 1977). Mating occurs when two haploid cells of opposite mating types (i.e., MATa and MATα) come in contact. Cell and nuclear fusion follow. The resulting diploid can grow mitotically, but in addition is able to undertake meiosis if starved for nitrogen. Meiosis produces a cluster of four haploid spores (a tetrad) encased in an ascus shell (an ascus). Since mating type is determined by a single Mendelian locus, two of the spores are MATα and two are MATa. The spores germinate to give clones of haploid cells that may divide mitotically, or that may mate to form new diploids. When a [cir+] and a [cir-] cell mate, they form a [cir+] diploid, and when this diploid goes through meiosis, all four resulting spores are [cir+] (LIVINGSTON 1977). Thus, in a population where there is a low frequency of [cir+] cells, each round of mating and sporulation could approximately double the frequency of [cir+] cells, leading ultimately to a predominately [cir+] population. This type of transmission could be a sufficient explanation of 2μm circle maintenance in populations. Furthermore, this type of contagion during the sexual cycle is potentially available to all mobile eukaryotic genetic elements (HICKEY 1982).

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TABLE 1

<table>
<thead>
<tr>
<th>Yeast strains</th>
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<tr>
<td>Strain</td>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>RH218 [cir']</td>
</tr>
<tr>
<td>MC16 [cir+']</td>
</tr>
<tr>
<td>305-2b [cir+']</td>
</tr>
<tr>
<td>B. Diploid strains</td>
</tr>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>ER1 [cir+']</td>
</tr>
<tr>
<td>ER2 [cir+']</td>
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</tbody>
</table>

[cir'] denotes the presence, and [cir'] the absence of the 2μm circle.

MATERIALS AND METHODS

Strains: The strains used to measure 2μm circle transmission are shown in Table 1. MC16 and 305-2b were cured of their 2μm circles by transforming with plasmid pDB219 and picking [cir'] [pDB219'] clones as previously described (FUTCHER and COX 1983). This generated two new strains that were perfectly isogenic with their [cir'] parents. Two pairs of isogenic [cir'] and [cir'] diploid strains were then constructed as indicated in Figure 1. Plasmid copy number measurements were done using strains LL20 (MATa leu2 his3 can1 [cir']) and strain aa2 (MATa leu2 leu2 his4/His4 ura3/ura3 trp1/trp1 can11+/ [cir']). Strain aa2 was created by treating strain SB9882-4 (MATα/α) (CLARKE and CARBON 1983) with UV light to induce mitotic crossing over on chromosome III. Complementation tests were done to identify a segregant homozygous for MATα. This MATα/α diploid was then cured of its 2μm circles as described above.

Media: YEPD was 1% yeast extract, 2% peptone, and 2% glucose. YEPG was 1% yeast extract, 2% peptone, 2% glycerol.

DNA manipulations and hybridization procedures: DNA was extracted from yeast by the method of FUTCHER and CARBON (1986), and measured fluorimetrically (MORGAN et al. 1979). Since the 2μm circle has no known phenotype, the frequency of [cir'] colonies was assayed by yeast colony hybridization (SHERMAN, FINK and HICKS 1985). Briefly, colonies derived from single cells were transferred to nitrocellulose filters along with appropriate controls, and then lysed in situ. The filters were incubated with 35P-labeled plasmid YpR141 (from J. D. BEGGS), which contains a cloned 2μm circle. The probe hybridized only to colonies of [cir'] cells. Southern blot analyses were made by the method of SHERMAN, FINK and HICKS (1983). The ribosomal DNA probe was obtained by gel purification from BglII-digested total yeast DNA.

Mitotic competition experiments: Isogenic [cir'] and [cir'] cells were mixed in a 1:1 ratio and grown in YEPG at 30°. Cells were kept in exponential growth by a serial dilution every 24 hr (about 14 population doublings). The proportion of [cir'] cells was assayed by colony hybridization. The experiment was also done with YEPG as the growth medium.

Competition experiments with cycles of sporulation and mating: Isogenic [cir'] and [cir'] cells were mixed in a 1:9 ratio, grown overnight in YEPG (about 6 population doublings), and then grown for 3 hr (just under 2 doublings) in YEPD. A sample was plated for colony hybridization, and the remaining cells were harvested, washed and resuspended in 20 ml of 3% potassium acetate (a favorable nitrogen-starvation medium) in a Petri dish to induce sporulation. After 1 week at room temperature, the cells and asci were harvested. Appropriate treatments with diethyl ether or diethyl ether and glusulase were carried out at this point. The cells were then spread on a pH 4.5 YEPD plate at a density of 2 × 10^7 plating units per plate to allow growth and mating. After 2 days of incubation at 30°C, the population had gone through about 6 doublings, and about 40% of the cells were diploids. The cells were scraped from the plate, and the cycle was repeated (i.e., the cells were once again grown in YEPG, then YEPD, then sporulated, etc.). Samples were removed just after the second and fourth mating steps to be plated and then assayed by colony hybridization.

RESULTS AND DISCUSSION

We wished to see whether the parasitic DNA theory was sufficient to explain 2μm circle maintenance. To do this, we first constructed two pairs of isogenic [cir'] and [cir'] yeast strains. Each of the pairs was grown in competition and the frequency of [cir'] cells was monitored by colony hybridization over a period of approximately 150 generations. The results (Figure 1A) confirm the previous observations (FUTCHER and COX 1983; MEAD, GARDNER and OLIVER 1986) of a gradual decrease in the frequency of [cir'] cells consistent with a selective disadvantage of about 1%. An essentially identical result was obtained using YEPG as the growth medium (data not shown).

Next, [cir'] and [cir'] diploid cells were mixed in a 1:9 ratio, and sporulated and germinated, and the spores were allowed to mate with each other and undergo limited mitotic growth. The cycle of sporulation and mating was repeated four times. The sporulation frequency (number of cells that formed ascii divided by total number of cells) averaged about 40% in each cycle (Table 2). In S. cerevisiae, the four products of meiosis are held together within a tough ascus. Asci were not disrupted in this experiment, and so most mating occurred between spores of the same ascus—i.e., inbreeding was frequent, and most new diploids were formed either from two spores of a [cir'] parent, or from two spores of a [cir'] parent. The frequency of [cir'] cells did not change appreciably during the experiment (Fig. 1B).

The next experiment (Fig. 1C) was identical to the previous one except that an ether treatment was included after each sporulation to eliminate vegetative cells (DAWES and HARDIE 1974). Again, the frequency of [cir'] cells did not change appreciably.

The final experiment (Fig. 1D) included, in addition to the ether treatment, the disruption of asci by glusulase digestion and sonication. Microscopic examination confirmed that virtually all asci had been
cells over four cycles of sporulation and mating. This was a fourfold increase in the frequency of treatments (Table 2).

We believe that the frequency of \([\text{cir}^+\]) cells increased in the outbred populations because the plasmid was being transmitted to new hosts with each round of mating and sporulation. The rate of increase was about half the theoretically possible rate; this was probably because neither mating nor sporulation was 100% efficient.

The spread of plasmid by sexual transmission implies plasmid copy number amplification; otherwise, copy number would eventually be diluted to zero. Indeed, 2\(\mu\)m circle amplification has been documented previously (Sigurdson, Gaarder and Livingston, 1981; Volkert and Broach, 1986). We were curious to see at what stage in the process amplification occurred. To find out, we crossed a haploid $\text{MATa his3}\ [\text{cir}']$ strain to a diploid $\text{MATa/a his4/his4} [\text{cir}']$ strain. His$^+$ triploids were selected, and DNA was extracted from the haploid, diploid and triploid strains. DNA was digested with $\text{PstI}$ and $\text{BglII}$, and hybridized to the filter. After autoradiography (Figure 2), radioactive bands were cut out of the filter, and $\text{32P}$ was assayed using a scintillation counter. The ratio of the counts in the 2\(\mu\)m circle band to the counts in the rDNA band was calculated. After the ratio for the $[\text{cir}']$ haploid parent was normalized to 1, the ratio for the triploid was 0.99, plus/minus 1% counting error, indicating that the plasmid copy numbers per haploid genome were the same. Thus, the triploid had three times as many plasmids per cell as the haploid. Other experiments (data not shown) indicated that the copy number amplification was largely complete within two or three generations from the time of mating.

These results show that the parasitic DNA model is a sufficient explanation of 2\(\mu\)m circle maintenance. Whether it is the only explanation is another question. First, there is the usual caveat that the 2\(\mu\)m circle may be advantageous under some unusual condition, though there is absolutely no evidence for this. Second, the amount of horizontal 2\(\mu\)m circle transmis-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sporulation frequency$^\text{a}$</th>
<th>Untransferred spores</th>
<th>Ether treatment</th>
<th>Glusulase and ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER1 [cir$^+$]</td>
<td>0.37</td>
<td>0.54</td>
<td>0.56</td>
<td>0.39$^\text{c}$</td>
</tr>
<tr>
<td>ER1 [cir$^-$]</td>
<td>0.49</td>
<td>0.68</td>
<td>0.64</td>
<td>0.54$^\text{c}$</td>
</tr>
<tr>
<td>ER2 [cir$^+$]</td>
<td>0.46</td>
<td>0.69</td>
<td>0.50</td>
<td>0.09</td>
</tr>
<tr>
<td>ER2 [cir$^-$]</td>
<td>0.44</td>
<td>0.84</td>
<td>0.50</td>
<td>0.09</td>
</tr>
</tbody>
</table>

$^\text{a}$ Viability was measured as the frequency of survivors per 1800 plating units.

$^\text{b}$ Ratio of cells that formed asci to total cells.

$^\text{c}$ Not significantly different, due to variations in killing between different ether treatments.
sion occurring in wild populations depends on the frequency of mating and sporulation, on spore viability (because of the ascus, high spore viability leads to inbreeding), and on the frequency of homothallism. We do not know the values of these parameters for any wild population, but given the high stability of the 2μ circle plasmid (only $7 \times 10^{-5}$ loss events per generation) (FUTCHER and COX 1983), an extremely small amount of outbreeding would be sufficient for plasmid maintenance (STEWART and LEVIN 1977). For example, the proportion of [cir]$^+$ cells in a population with 99% [cir]$^-$ and 1% [cir]$^+$ cells would tend to increase at the rate of about $2 \times 10^{-4}$ per generation (1 $\times$ $10^{-4}$ from selection for the 1% [cir]$^+$ cells, and about $1 \times 10^{-4}$ from new loss events). To keep the proportion of [cir]$^+$ cells in equilibrium at 1%, cells would have to out-breed at a rate of only $2 \times 10^{-4}$ per generation (i.e., once every 5000 generations).

This many generations would take over a year of continuous growth. This calculation assumes that some mating occurs in each generation; in a pure heterothallic clone as found in a laboratory, no mating would occur during mitotic growth. Even so, it would take over 300 generations before the proportion of [cir]$^+$ cells rose to 10% (FUTCHER and COX 1983), and since strains are not normally propagated in laboratories by serial transfer, few if any laboratory strains are this far removed from the cross that generated them.

The applicability of this hypothesis to wild yeast strains is difficult to assess. Many wild and industrial strains are polyploid, and have been thought not to sporulate or mate. However, this view has been challenged by BILINSKI, RUSSELL and STEWART (1986), who were able to induce efficient sporulation of several brewing strains by simple manipulations of sporulation conditions. Mating competent spore clones were obtained. In any case, even if there are polyploid populations that seldom mate or sporulate, they may remain [cir]$^+$ because polyploids have plasmid copy numbers higher than haploids, so that the rate of plasmid loss is negligible (MEAD, GARDNER and OLIVER 1986).

We note that the yeast life-style includes features that promote inbreeding (e.g., the ascus, homothallism). These may have evolved at least partly as a defence against parasitic DNAs.

A number of theoretical models have shown that the rate of spread of bacterial plasmids (STEWART and LEVIN 1984) or eukaryotic transposons (HICKEY 1982) depends on the frequency of conjugation of host cells. The contact between the host genomes allows the intragenomic over-replication of these elements to be translated into intergenomic spread within the population (HICKEY 1984). The importance of conjugation for plasmid genome survival is illustrated by the fact that bacterial conjugation is usually dependent on plasmid-encoded genes (WILLETTS and SKURRAY 1980). It has been suggested that the origins of eukaryotic gamete conjugation may also have involved selection for horizontal gene transfer (HICKEY 1982; ROSE 1983; Hickey and ROSE 1988).

Thus, the survival strategy of the 2μ circle is analogous to that of mobilizable nonconjugative bacterial plasmids, or, more distantly, to that of pathogenic viruses. The only difference between viruses and the elements we are considering is that viruses take extracellular routes to infect new hosts, while some other mobile elements take intracellular or intranuclear routes that depend upon cell-cell or nuclear-nuclear contact.

A species that reproduces by outbreeding is liable to accumulate many elements capable of over-replication and transmission. Such elements need not contribute to survival nor to the outbreeding process itself. The principle we have illustrated here with the 2μ circle can explain the accumulation of a large variety of self-replicating elements in eukaryotic cells, including transposons as well as plasmids.

We would like to acknowledge the help of MONICA MANTCH in preliminary experiments. This work was supported by a Medical Research Council of Canada operating grant, and by an MRC Scholarship to A.B.F.

**LITERATURE CITED**


**FIGURE 2.—Plasmid amplification in triploids. MATa' [cir]$^+$ diploids (DNA run in lane 1) were mated with MATa [cir]$^+$ haploids (DNA run in lane 3) to give triploids (lane 2). Plasmid copy number was found to be proportional to ploidy. Lane M contains a molecular weight marker.**
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Communicating editor: D. CHARLESWORTH