INHERITANCE OF THE 2μm DNA PLASMID FROM SACCHAROMYCES

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ABSTRACT

A variety of Saccharomyces strains were examined for the presence of 2μ DNA and, if present, for the pattern of fragments produced by its digestion with site-specific (restriction) endonucleases. Two strains were found that did not contain detectable levels of 2μ DNA, and two strains contained 2μ DNA molecules having only one *Eco*RI restriction endonuclease recognition site rather than the usual two.—A haploid containing 2μ DNA with one *Eco*RI restriction site was mated with a haploid containing 2μ DNA with two *Eco*RI restriction sites and the resulting diploid maintained both types during vegetative growth. Sporulation of the diploid produced four spores, and the clones from these spores contained both types.—A haploid lacking 2μ DNA was mated with a haploid containing 2μ DNA and the resulting diploid contained 2μ DNA. The four clones derived from the haploid spores after sporulation of this diploid all contained 2μ DNA. A *rho*$^-$ strain without 2μ DNA was mated to a *rho*+ strain with 2μ DNA, and heteroplasmons were selected that had received the nucleus from the strain without 2μ DNA and the mitochondria from the strain with 2μ DNA. Twelve of twenty-four such clones contained 2μ DNA.—I conclude that: (1) the different types of 2μ DNA identified in these strains do not restrict one another, (2) the different types are inherited extrachromosomally, (3) lack of 2μ DNA in two strains is not due to the absence of genes needed for maintenance and (4) the approximately 100 copies of 2μ DNA contained within a single cell are probably clustered within one or a few cytoplasmic organelles.

THE yeast *Saccharomyces cerevisiae* contains approximately 100 copies of a 2μm circular DNA molecule (SINCLAIR *et al.* 1967; CLARK-WALKER and MIKLOS 1974) called 2μ DNA. GUERINEAU, SLONIMSKI and AVNER (1974) have shown that the expression of a nuclear oligomycin resistance mutation is correlated with the presence of 2μ DNA within the cell. Furthermore, HOLLENBERG *et al.* (1976), GUERINEAU, GRANDCHAMP and SLONIMSKI (1976) and LIVINGSTON and KLEIN (1977) have shown that 2μ DNA contains an inverted repeated sequence comprising 20% of the total molecular length. These two properties of 2μ DNA are interesting because of recent studies which show that inverted repetitions which form the boundaries of drug resistance genes on bacterial plasmids are important to the process of translocating the resistance genes from plasmid DNA to other cellular or phage DNA (BERG *et al.* 1975; HEFFRON, RUBENS and FALKOW 1975; KLECKNER *et al.* 1975; KOPECKO and COHEN 1975).

Little is known about the maintenance of 2μ DNA within the yeast cell. For example, the cellular location of 2μ DNA is not known, although neither isolated nuclei (CLARK-WALKER and MIKLOS 1974) nor mitochondria (CLARK-WALKER 1972) contains 2μ DNA. Also, the physiological role of 2μ DNA and its mode of inheritance are unknown. In order to investigate the formal rules governing 2μ DNA distribution during mitosis and meiosis, I have carried out crosses between strains bearing and lacking 2μ DNA and between strains harboring distinguishable forms of 2μ DNA.

MATERIALS AND METHODS

Yeast strains: Strains used in this study are listed in Table 1.

Cell growth: Cells were grown in a synthetic medium at 30° (LIVINGSTON and KLEIN 1977). Zygotes, spores, and individual cells were grown on YEPD plates until the colony contained approximately 1×10^5 cells and then transferred to liquid medium. Cultures containing 1×10^{10} cells which represent approximately thirty doublings were harvested for analysis.

Analysis of 2μ DNA: 2μ DNA was extracted from yeast spheroplasts and purified by centrifugation in the presence of CsCl and ethidium bromide as previously described (LIVINGSTON and KLEIN 1977). Purified 2μ DNA was analyzed by cleavage with three different site-specific endonucleases (restriction nucleases) followed by electrophoresis in an agarose gel (LIVINGSTON and KLEIN 1977). The three restriction endonucleases are *Pst* (from *Providencia stuartii* 164), *Eco*RI (from *Escherichia coli* RY13), and *Hinc*II (from *Haemophilus influenzae* Rc).

In order to screen strains rapidly for the presence of 2μ DNA, cell extracts were prepared from 1×10^9 cells using the procedure of HIRT (1967) as modified by LIVINGSTON and KLEIN (1977). The nucleic acids in the extract were precipitated by the addition of two volumes cold ethanol (95%). After incubation at 0° for 30 min the precipitate was collected by centrifugation at 27,000 $\times$ *g* for 15 min. The precipitate was extracted with 1.25 ml of 50mM TrisHCl buffer pH 8.0, 50mM NaCl, 10mM EDTA and the insoluble debris removed by centrifugation. The nucleic acids in the supernatant solution were once more precipitated with 2.5 ml of cold ethanol

TABLE 1

Yeast strains

Strain	Nuclear genotype	2μ DNA Plasmid phenotype*
A364A	**a** *ade1 ade2 ura1 tyr1 his7 lys2*	[*cir*+-E1,E2]
EMS 63	α *his2*	[*cir*+-E1,E2]
HQ/5C	α	[*cir*+-E2]
SS 101	**a** *pet*$^-$	[*cir*+-E2]
Y379–5D	α	[*cir*°]
NCYC74–CB11	**a** *ade1 MAL6*	[*cir*°]
M191	α *pets*-1	[*cir*+]
499	**a** *pets*-2	[*cir*+]
JC K5–25C	**a** *kar1 his4 ade2*	[*cir*+]

* The presence of 2μ DNA in a cell is designated [*cir*+] and its absence [*cir*°]. The addition of E1 and E2 after *cir*+ denotes the presence of the *Eco*RI restriction endonuclease recognition sequences *Eco*RI-1 and *Eco*RI-2, respectively, previously defined by LIVINGSTON and KLEIN (1977).

Strain NCYC74–CB11 was provided by R. NEEDLEMAN, Albert Einstein School of Medicine, New York; strains M191 and 499 were from R. WICKNER, National Institutes of Health, Bethesda, and JC K5–25C was from J. CONDE, Cornell University, Ithaca. All other strains were from the collection of L. HARTWELL at this university.

and collected as described above. The pellet containing the 2μ DNA plus other nucleic acid species was then resuspended in 200 μl 10mM Tris HCl buffer pH 7.4, 1mM EDTA. 100 μl aliquots were then subjected to agarose gel electrophoresis. To distinguish DNA from double stranded RNA species, 100 μl samples were incubated with 1 μg RNase (Bovine Pancreas 5X recrystallized Sigma) for 30 min at 37° before electrophoresis.

Genetic techniques: Standard techniques for the genetic analysis of *S. cerevisiae* have been followed (MORTIMER and HAWTHORNE 1969).

Heteroplasmons (WRIGHT and LEDERBERG 1957) were produced by first selecting a clone of Y379-5D [*cir*°] which was resistant to cycloheximide (10 μg/ml). This strain Y379-5D *cyh*^{r} [*cir*°] was then grown in YM-1 media in the presence of ethidium bromide (10 μg/ml) for 12 hrs at 30° to eliminate mitochondrial DNA. A clone of the cycloheximide resistant petite Y379-5D *kar*+ *cyh*^{r} [*rho*$^-$ *cir*°] was then mated with the grande strain JC K5-25C *kar*$^-$ *cyh*^{s} [*rho*+ *cir*+] (CONDE and FINK 1976). The *kar*$^-$ mutation in strain JC K5-25C prevents nuclear fusion in the zygote produced in this mating, and a high proportion of zygotes bud off cells with haploid nuclei and a cytoplasm that may contain extrachromosomal elements from both input strains.

RESULTS

Differences in physical structure of 2μ DNA from various strains: The yeast strains selected for examination were capable of mating with the reference strains A364A or EMS 63, and the resulting diploids were able to sporulate. The 2μ DNA of these strains was purified and subjected to an analysis of its physical structure by cleavage with restriction nucleases. Figure 1 is a map of 2μ DNA

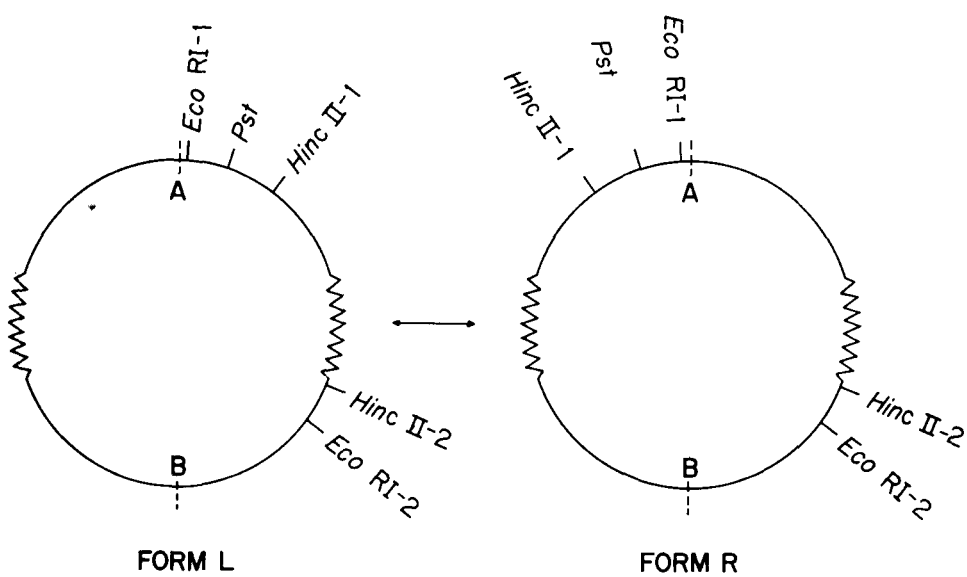

FIGURE 1.—A physical map of 2μ DNA. The sites of restriction endonuclease cleavage and the repeated sequences (jagged lines) were mapped previously as described by LIVINGSTON and KLEIN (1977). Two forms of 2μ DNA (Form L and R) are shown, which differ by the inversion of interstitial segment A, probably as a result of an intramolecular reciprocal recombinational event between the two units of the repeat. The two *EcoRI* and two *Hinc*II restriction endonuclease sites have been arbitrarily numbered (LIVINGSTON and KLEIN 1977).

from A364A showing the inverted repeated sequence and five restriction endonuclease sites. 2μ DNA from A364A contains one *Pst* recognition sequence, two *EcoRI* sites, and two *Hinc*II sites (LIVINGSTON and KLEIN 1977). All 2μ DNAs examined contain the *Pst* recognition sequence and the two *Hinc*II recognition sequences, and within the limits of experimental error these sites are located in the same positions as those in 2μ DNA from A364A.

One structural difference was found among the 2μ DNAs from the various strains, and two strains were found that lacked detectable 2μ DNA. 2μ DNA from strain SS 101 (Figure 2d) and strain HQ/5C contains only one *Eco*RI recognition site instead of the two found in the 2μ DNA from A364A (Figure

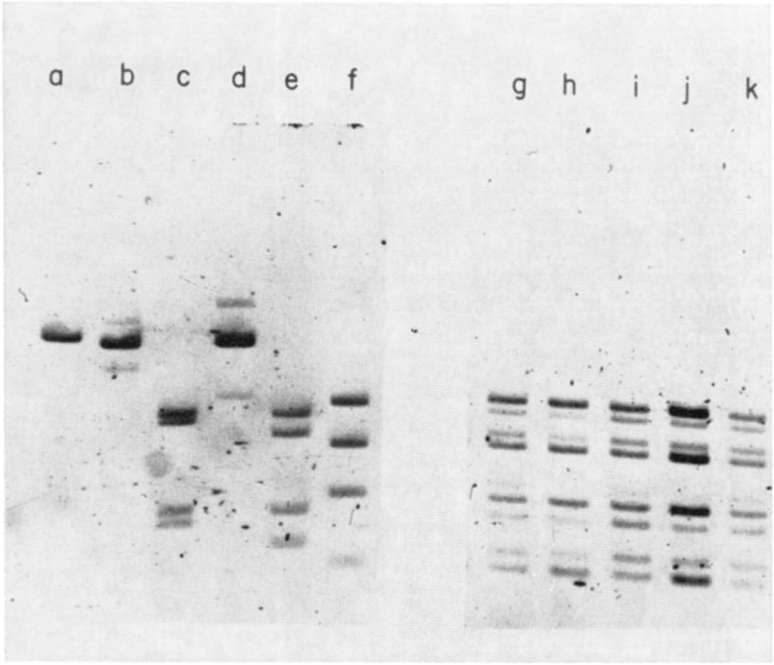

FIGURE 2.—Agarose gel electrophoresis of restriction fragments of 2μ DNA. Enzymatic digestion and electrophoresis has been previously described (LIVINGSTON and KLEIN 1977). Migration is from top to bottom. Slots a, c, and e are restriction nuclease digests of 2μ DNA purified from A364A [*cir*+-E1, E2], and b, d, and f are digests of 2μ DNA from SS 101 [*cir*+-E2]. Digests shown in slots a and b are made with the restriction nuclease *Pst*, digests in slots c and d are made with *Eco*RI, and digests in slots e and f are made with simultaneous addition of *Pst* and *Eco*RI. The single band in slots a, b, and d represent full length linear molecules of 2μ DNA and the pattern of four bands in slots c, e, and f are the result of two forms of 2μ DNA as shown in Figure 1. A fifth fragment corresponding to the segment between the *Eco*RI-1 and *Pst* sites of A364A 2μ DNA cannot be seen in slot e because it has migrated off of the gel. Minor bands probably result from cleavage of concatenates of 2μ DNA.

Slot g contains a digest of 2μ DNA from a diploid from the cross SS 101 [*cir*+-E2] $\times$ EMS 63 [*cir*+-E1, E2], and slots h–k are digests of 2μ DNA from four spore clones of a tetrad resulting from sporulation of this diploid. Digests in slots g–k are made by simultaneous cleavage with *Pst* and *Eco*RI, and the eight fragments seen are a superimposition of the four fragment patterns shown in slots e and f.

2c). The phenotype one *Eco*RI restriction site is designated [*cir*$^+$—E2] and the phenotype two *Eco*RI restriction sites is designated [*cir*$^+$—E1,E2]. Simultaneous digestion of SS 101 2μ DNA was made with *Pst* and *Eco*RI to ascertain the map location of the one remaining *Eco*RI site. Figure 2f shows that simultaneous digestion produces a pattern of four fragments much like the four fragment pattern obtained by digestion of A364A 2μ DNA with *Eco*RI alone (Figure 2c). The four fragments produced by *Eco*RI cleavage of A364A 2μ DNA are indicative of the fact that 2μ DNA exists in two forms (Figure 1), probably as a result of an intramolecular reciprocal recombination between the inverted repeated sequences. The four fragments produced by simultaneous digestion of SS 101 2μ DNA by *Pst* and *Eco*RI demonstrates that 2μ DNA from this strain also exists in two forms. The actual presence of an inverted repeat in SS 101 2μ DNA which would give rise to the two forms has been confirmed by electron microscopic examination as previously described (LIVINGSTON and KLEIN 1977). Mapping of the one remaining *Eco*RI site using *Pst* and *Hinc*II reveals that the number and order of restriction endonuclease recognition sequences in SS 101 2μ DNA is the same as that of A364A 2μ DNA except that the *Eco*RI-1 site is absent in SS 101 2μ DNA (LIVINGSTON and KLEIN 1977). Restriction cleavage also demonstrates a second difference between SS 101 2μ DNA and A364A 2μ DNA. If the only difference were the loss of the *Eco*RI-1 site, then two of the four bands produced by simultaneous digestion with *Pst* and *Eco*RI of SS 101 2μ DNA (Figure 2f) should be the same as two of the four major bands produced during a similar digest of A364A 2μ DNA (Figure 2e). One possible explanation for the absence of this correlation is that SS 101 2μ DNA differs from A364A 2μ DNA in that SS 101 2μ DNA contains a small deletion (less than 2% of the total molecular length) of a sequence within the region including the *Pst*, *Hinc*II-1, *Hinc*II-2 and *Eco*RI-2 restriction sites of the A364A 2μ plasmid. Whether the loss of one *Eco*RI site and an apparent deletion are the only differences between the 2μ DNA plasmids from these two strains will require further physical and genetic studies.

Maintenance of 2μ DNA during mitotic division: The occurrence of strains bearing 2μ DNAs which differ in the number of *Eco*RI sites permitted an analysis of the mode of inheritance of the different types of 2μ DNA. 2μ DNA from progeny obtained by crossing these strains was digested simultaneously with the restriction endonucleases *Pst* and *Eco*RI and analyzed by agarose gel electrophoresis. As shown in Figure 2e and 2f, simultaneous digestion by both restriction endonucleases produces a unique pattern of fragments which distinguishes *cir*$^+$—E2 2μ DNA with one *Eco*RI site from *cir*$^+$—E1,E2 2μ DNA with two *Eco*RI sites. Strain SS 101 [*cir*$^+$—E2] was mated with strain EMS 63 [*cir*$^+$—E1, E2] and two zygotes were removed from the mating mixture by micromanipulation. Figure 2g shows the restriction pattern of 2μ DNA obtained from one zygotic clone. The diploid clone obtained from the zygote contains both types of 2μ DNA. The second zygote from this cross as well as two independent zygotes from the cross of strains HQ/5C [*cir*$^+$—E2] and A364A [*cir*$^+$—E1,E2] also contain both types of 2μ DNA.

Since the 2μ DNA from the zygote cultures represents a collection of 2μ DNA molecules from many cells, some cells might contain one type of plasmid and other cells in the culture the second type of plasmid. To examine this possibility, cells from the diploid culture were cloned and the 2μ DNA content of the clones were examined. Six different clones from the same zygotic culture contain both types of 2μ DNA. One of the clones examined was started from a cell after more than 100 cell divisions following zygote formation.

Although both types of 2μ DNA are present in the diploid shown in Figure 2g, a predominance of 2μ DNA molecules with one *Eco*RI site is present. In many of the other diploid cultures examined a predominance of one or the other of the two types of 2μ DNA was also evident. Furthermore, cultures started by recloning individual cells from the original clone did not necessarily result in the predominance of the same molecular type as that seen in the original clone. Thus, no significant exclusion of one molecular type by another during mitotic division of the diploid was observed, and both types can reproduce in the same cell for at least 100 generations.

Maintenance of 2μ DNA during meiotic division: To investigate whether the two molecular types of 2μ DNA would segregate during meiotic division, diploid cells from the cross SS 101 [*cir*$^+$—E2] with EMS 63 [*cir*$^+$—E1,E2] were sporulated and the resulting tetrads dissected. 2μ DNA was purified from the four spore colonies from two tetrads. The restriction analysis of one tetrad is shown in Figure 2h–k. Cultures from all four spores contain both molecular types. As in diploid clones, some spore colonies show a predominance of one molecular type. Although a predominance of one type was often evident in some of the spore clones, the predominant type was not necessarily the same as that which predominated in the diploid clone from which the spore arose. Also, spores from the same tetrad did not all display predominance of the same type, nor did they appear to show any clear segregation pattern such as two spores with a predominance of one type and two spores with a predominance of the second type. Individual haploid cells contained both molecular types as evidenced by their appearance in subclones from the spore clone. Thus, the two physical types do not segregate during meiosis nor during subsequent mitotic division of the haploid cells.

Crosses with strains lacking 2μ DNA: Although most strains of Saccharomyces contain 2μ DNA, some do not. GUERINEAU, SLONIMSKI and AVNER (1974) were able to find cells without the plasmid by selecting for the loss of a particular oligomycin resistance phenotype. Other workers have been unsuccessful in curing the plasmid by growth in ethidium bromide (CLARK-WALKER 1972, GRIFFITHS, LANCASHIRE and ZANDERS 1975). Among the strains that I examined, two were found that did not contain 2μ DNA. The absence of 2μ DNA is designated [*cir*°].

Figure 3 shows an agarose gel in which extracts of cellular DNA have been subjected to electrophoresis. Extracts made from strain Y379–5D are missing the DNA species corresponding to superhelical and relaxed circular 2μ DNA. Surprisingly, this strain (as well as the other strain lacking 2μ DNA, NCYC74-CB11) also is missing the species of double stranded RNA designated as L-dsRNA

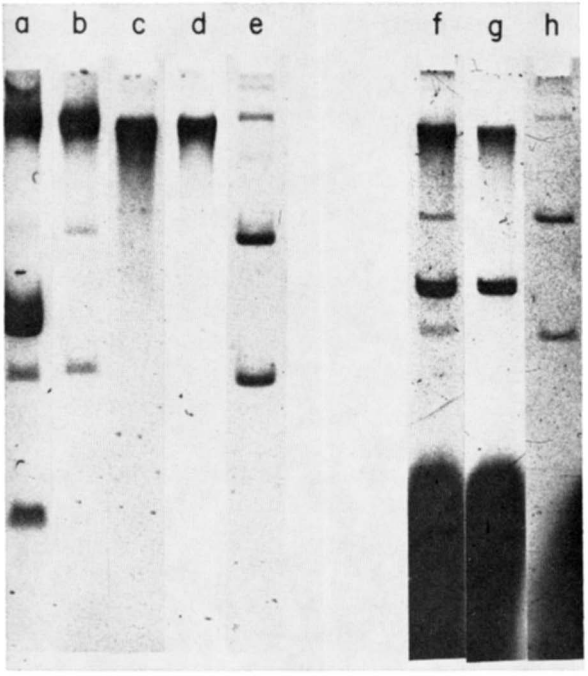

FIGURE 3.—Agarose gel electrophoresis of nucleic acid extracts from [*cir*+] and [*cir*°] strains. Nucleic acid extracts were made as described under MATERIALS AND METHODS and then subjected to electrophoresis. Slots a and b are nucleic acids from A364A and c and d from Y379–5D. The extracts in slots b and d have been treated with RNase before application to the gel. The two major bands in slot e which contains purified 2μ DNA are superhelical molecules (bottom) and circular molecules with single strand interruptions (top). These two bands can be seen in the extracts of A364A (a and b). In all extracts the stained material above the level of the relaxed circular molecules is nuclear and mitochondrial DNA fragments remaining in the extract. The heavy band in slot a between the superhelical and relaxed 2μ DNA bands is a double stranded RNA species designated L-dsRNA, and the heavy band below the superhelical 2μ DNA is *killer* associated double stranded RNA. Both RNA species are removed by RNase digestion (slot b).

Slots f and g contain extracts from two heteroplasmons of the cross JC K5–25C *kar*⁻ *cyh*^{s} [*cir*+ *rho*+] × Y379–5D *kar*+ *cyh*^{r} [*cir*° *rho*⁻]. The extracts have not been treated with RNase. Slot h contains purified 2μ DNA as a reference. The minor bands seen above the major bands in slots e and h are probably concatenates of 2μ DNA.

(BEVAN, HERING and MITCHELL 1973; VODKIN, KATTERMAN and FINK 1974; WICKNER and LEIBOWITZ 1976). To be certain that Y379–5D does not contain 2μ DNA, cells were grown in the presence of ^{14}C-uracil to label the DNA. The agarose gel containing the labeled DNA was then subjected to an analysis by autoradiography. These results demonstrate that strain Y379–5D contains less than 1% the amount of 2μ DNA that strain A364A contains. Because strain A364A contains 50 to 100 copies of 2μ DNA per cell, strain Y379–5D must not contain any free copies of 2μ DNA.

Strain Y379–5D [*cir*°] was mated with strain A364A [*cir*+]. Extracts from

a diploid produced by this mating contain 2μ DNA. Furthermore, all four spores from a single tetrad of the diploid contain 2μ DNA. The double stranded RNA species are also present in the diploid cells and all four spore colonies. These results indicate that Y379–5D [*cir*°] does not harbor either an element that prevents maintenance of 2μ DNA, or the double stranded RNA in the heterozygote, or an element which segregates during meiosis as a single chromosomal marker and prevents maintenance of the molecules in the haploid spore colonies.

To ascertain whether multiple nuclear genes were present in Y379–5D [*cir*°] which prevent the maintenance of 2μ DNA and double stranded RNAs, heteroplasmons between JC K5–25C *kar*$^-$ *cyh*^{s} [*rho*$^+$ *cir*$^+$] which contains both 2μ DNA and double stranded RNA and the strain Y379–5D *kar*$^+$ *cyh*^{r} [*rho*$^-$ *cir*°] were produced. Because JC K5–25C bears the *kar*$^-$ mutation, matings between it and *kar*$^+$ strains produce heterokaryons which bud off cells with haploid nuclei and a mixture of cytoplasm from the two parent strains. Twenty-four heteroplasmons containing nuclei from the Y379–5D [*rho*$^-$ *cir*°] parent and mitochondria from the other parent were examined and all contained the double stranded RNA species, but 12 did not appear to contain any 2μ DNA. Figure 3 shows one heteroplasmon extract which contains both 2μ DNA and double stranded RNA and another heteroplasmon extract which contains only the double stranded RNA and not 2μ DNA. Thus, the nuclear genotype of Y379–5D does not prevent 2μ DNA or double stranded RNA maintenance. These results also show that the double stranded RNA may exist independently in the cell from 2μ DNA.

Presence of 2μ DNA in strains incapable of maintaining both killer associated RNA and mitochondrial DNA: A single mutation, *pets*, (FINK and STYLES 1972) which produces in a haploid the loss of killer-associated double stranded RNA (M-dsRNA) (BEVAN, HERING and MITCHELL 1973; VODKIN, KATTERMAN and FINK 1974) and also mitochondrial DNA (WICKNER and LEIBOWITZ 1976) but not the loss of the L-dsRNA species, has been detected by assay for loss of the killer function. Examination of these strains demonstrated that they do contain 2μ DNA and the large double stranded RNA species (data not shown). Thus, the *pets* mutation, which prevents maintenance of two cytoplasmic nucleic acid species, does not eliminate 2μ DNA or another double stranded RNA species.

DISCUSSION

This study began with an investigation of physical variations in 2μ DNA from different Saccharomyces strains. Two variations were found. Some strains contained no detectable levels of 2μ DNA, while other strains contained 2μ DNA with one *Eco*RI restriction endonuclease recognition site instead of the two sites found in 2μ DNA from the reference strains. No variation in the restriction pattern made by two other restriction endonuclease was found. 2μ DNA from all strains bearing the plasmid exists as an equimolar mixture of two forms differing by an inversion. This suggests that all 2μ DNA contains an inverted repeated sequence as does 2μ DNA from A364A.

All strains of yeast tested are capable of maintaining 2μ DNA. This is true even for the two *cir°* strains. The presence of 2μ DNA in spores and some heteroplasmons from crosses of *cir°* strains with *cir+* strains show that there are no nuclear genes in the two *cir°* strains which prevent maintenance. Examination of strains bearing a single nuclear mutation, *pets*, (FINK and STYLES 1972; WICKNER and LEIBOWITZ 1976) which renders cells incapable of harboring both mitochondrial DNA and *killer* associated double stranded RNA, are capable of maintaining 2μ DNA as well as a large double stranded RNA species missing in the two *cir°* strains. Possibly some other nuclear genes necessary for mitochondrial DNA maintenance or *killer* associated RNA maintenance (WICKNER 1974; WICKNER and LEIBOWITZ 1976) will prove to be necessary for 2μ DNA maintenance.

The existence of *cir°* strains which do not contain 2μ DNA might indicate that 2μ DNA is not needed for growth under laboratory conditions. The ubiquity of its presence in diverse yeast strains, the conservation of its physical structure, and the inability to cure it by growth on ethidium bromide (CLARK-WALKER 1972; GRIFFITHS, LANCASHIRE and ZANDERS 1975) are therefore somewhat surprising. Possibly, in the strains lacking the plasmid the same genetic information is carried within the nuclear or mitochondrial genome.

The heteroplasmon formation experiments reveal that during cytoplasmic transfer, 2μ DNA is not always passed from the *cir+* strain to the *cir°* strain. Since heteroplasmons were selected by requiring the transfer of mitochondria from the *cir+*, *rho+* parent into buds bearing the nucleus from the *cir°*, *rho⁻* parent, 2μ DNA must neither be transferred with the mitochondria nor can it be sequestered exclusively in the nucleus. The failure of some heteroplasmons to receive 2μ DNA suggests that the approximately 100 copies of 2μ DNA may be clustered within a membranous organelle, as has been suggested by both CLARK-WALKER (1972) and GUERINEAU *et al.* (1971).

This study shows that two different physical types of 2μ DNA can exist simultaneously in a diploid through mitotic divisions as well as through meiotic divisions. Furthermore, haploids containing two types of 2μ DNA also maintain both types during mitotic divisions. The simplest interpretation of these results is that 2μ DNA is inherited as an extrachromosomal element and there is no preference in either haploids or diploids for either of the two types of 2μ DNA used in this study.

The lack of segregation of variant physical types of 2μ DNA during mitotic divisions is in contrast to the very rapid mitotic segregation of genes situated on the mitochondrial DNA (COEN *et al.* 1970). In the case of mitochondrial DNA, some strains apparently contain mitochondrial DNA which may suppress or exclude mitochondrial DNAs from other strains when the two strains are mated. Even in a case where the two strains contain mitochondrial DNAs which do not show this suppressive ability, their mating results in zygotes which will, upon two or three mitotic divisions, contain either of the two parental types or a recombinant type of mitochondrial DNA but will not contain a mixture of various parental and recombinant types. MICHAELIS, PETROCHILO and SLONIMSKI (1973) have shown that this rapid segregation of genetic markers on the mito-

chondrial DNA is accompanied by a change in the physical state of the DNA when strains which differ in the buoyant densities of their mitochondrial DNAs are mated. 2μ DNA does not show this rapid mitotic segregation. The fluctuations seen in the ratio of the two types of 2μ DNA either in diploid clones or progeny haploid clones may result from random sampling of the limited number of copies of 2μ DNA molecules packaged in the bud during mitotic divisions or in the spores after meiotic division.

The lack of segregation of the two types of 2μ DNA during meiosis excludes simple hypotheses in which maintenance of each of the two types is dependent on a single chromosomally inherited gene. GUERINEAU, SLONIMSKI and AVNER (1974) have advanced the hypothesis that a copy of 2μ DNA may be integrated into the nuclear genome much like a bacterial episome. If a single integrated copy served as a master copy for production of free copies of 2μ DNA, then two physically different types of 2μ DNA should exhibit chromosomal inheritance. Such a mechanism apparently operates in the case of the genes for ribosomal RNA in Xenopus, where the allelic types segregate during meiosis as single copies on one chromosome and then amplification of this chromosomally located copy takes place after zygote formation (BROWN and BLACKLER 1972). If 2μ DNA inheritance followed a similar pattern, then haploid spores from a diploid containing both types of 2μ DNA should contain only one molecular type of 2μ DNA.

The rules governing 2μ DNA inheritance are different from those describing nuclear, mitochondrial, and killer inheritance. Unlike nuclear genes, 2μ DNA inheritance does not show segregation during meiotic division. Its extrachromosomal inheritance is unlike mitochondrial inheritance because it does not exhibit rapid mitotic segregation. The heteroplasmon formation experiments, which show that 2μ DNA does not transfer with 100% efficiency, distinguishes it from both killer associated RNA and another species of double stranded RNA which do transfer with 100% efficiency (CONDE and FINK 1976). The rules found in this study for the inheritance of physical types of 2μ DNA should provide a basis for determining whether the drug resistances associated with 2μ DNA by GUERINEAU, SLONIMSKI and AVNER (1974) are in fact located on 2μ DNA. This study may also serve to determine whether other extrachromosomally inherited traits of yeast which are not associated at present with any particular nucleic acid species (COX 1965; AIGLE and LACROUTE 1975; LANCASHIRE and GRIFFITHS 1975; and SCHAMHART, TEN BERGE and VAN DE POLL 1975) may also be coded for by 2μ DNA.

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