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# Preferential inclusion of extrachromosomal genetic elements in yeast meiotic spores

(*Saccharomyces cerevisiae*/mitochondrial DNA/plasmids/double-stranded RNA/organelles)

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ABSTRACT **During meiosis and sporulation in the yeast *Saccharomyces cerevisiae*, extrachromosomal traits are efficiently transmitted to haploid spores. Although the pattern of inheritance of chromosomal traits reflects the mechanism of regular chromosomal segregation in meiosis, it is not known what processes are reflected by the efficient inheritance of extrachromosomal traits. Because extrachromosomal genetic elements in yeast are present in multiple copies, perpetuation of an extrachromosomal trait could occur by the passive envelopment of a subset of copies or by an active sequestering of all or a subset of copies within the four spores. We show that only subsets of the four extrachromosomal nucleic acids commonly found in yeast are transmitted through meiosis—55% of mitochondrial DNA copies, 82% of the 2-μm DNA plasmids, and about 70% of the L and M double-stranded RNAs. However, electron micrographs of serial sections through yeast asci indicate that the four spores enclose only 30% of the total ascus material. Thus these extrachromosomal elements are preferentially included within the spores, indicating that their inheritance is not a random process. Transmission of mitochondrial DNA can be accounted for by the observed enclosure of 52% of the mitochondrial volume within the spores. The high transmission frequencies of the double-stranded RNAs (which exist as virus-like particles in the cytoplasm) and 2-μm DNA must indicate that either these nucleic acids are actively recruited from the cytoplasm by some mechanism or they are associated in some way with the nucleus during meiosis.**

The division of chromosomes during meiosis is a regular and equal process, and chromosomally inherited traits reflect this precise segregation. However, at the same time, division of extrachromosomal cellular material is often visibly irregular and unequal. While traits determined by extrachromosomal genetic elements are efficiently transmitted, genetic variants of them often do not segregate during meiosis. Because extrachromosomal genetic elements are usually present in high copy numbers it is not possible to determine by genetic analysis alone the underlying mechanisms of their inheritance. It is therefore not known whether there is a precise segregation mechanism for all or a restricted subset of the copies, or if inheritance is a consequence of a random process relying on the large number of copies for the efficient transmission of the extrachromosomal phenotype.

The yeast *Saccharomyces cerevisiae* contains several genetic elements that exhibit the meiotic 4:0 phenotypic segregation characteristic of extrachromosomal molecules. These elements include a circular plasmid called 2-μm DNA, circular mitochondrial DNA, and two cytoplasmic linear double-stranded RNAs (dsRNAs) called L and M (1, 2). In yeast, the two meiotic divisions occur within a single intact nucleus from which four haploid nuclei bud (3, 4). As the prospore walls grow around the budding nuclei, nuclear and cytoplasmic contents are pulled into the developing spores. However, only a fraction of the cell contents is enveloped when the closing spore walls pinch the nuclei apart. Chromosomal DNA is efficiently transmitted to the spores as a result of association with the spindle apparatus. However, neither the mechanisms by which the extrachromosomal elements are put into the spores nor the efficiency of such processes is known.

We have measured quantitatively the meiotic transmission of four yeast extrachromosomal genetic elements and compared these values to the fractions of different cytoplasmic components that are enclosed in the spores. Each of the elements is transmitted at a characteristic efficiency that is higher than that expected from the fraction of cytoplasm enveloped. The results suggest that the mechanism of inheritance of extrachromosomal genetic elements is not a random process but may involve an interaction of the elements with the nucleus during meiosis.

## MATERIALS AND METHODS

**Strains and Culture Conditions.** Two diploid yeast strains, both derived from the haploid strain A364a, were used. The meiotic transmission experiments were carried out on A364A D5 $U^-$ $A^+$ (called D5), a uracil auxotroph heterozygous for several other nutritional requirements (5). Electron microscopic analysis was performed on the strain AP-1 (6).

For the meiotic transmission experiments D5 was grown to a density of $\approx 10^7$ cells per ml with 20 μCi (1 Ci = $3.7 \times 10^{10}$ becquerels) of [6-$^3$H]uracil (60 μCi/μg) per ml in 50 ml of PSP2 presporulation medium (7) containing the nonfermentable carbon source acetate. Cells were transferred to fresh PSP2 without [$^3$H]uracil for 0.5 doubling to chase labeled nucleotides into stable nucleic acids, then filtered and resuspended in 0.3% potassium acetate with 3 μg of uracil per ml at a density of $\approx 3 \times 10^6$ cells per ml. After 4 days of incubation at 30°C with vigorous shaking, 70–80% of the cell population had formed asci. The culture was collected by centrifugation, washed with sterile $H_2O$, and recentrifuged. The cell pellet was resuspended in 1.4 ml of Y-minimal medium, pH 5.8 (8) (supplemented with 3 mg of uracil per liter), 6.4 ml of Ludox HS (Du Pont) (pH 7.5), and 6.2 ml of 6% (wt/vol) dextran T 40 (Pharmacia) and centrifuged in a 15-ml tube at 12,000 rpm in a Sorvall HB-4 swinging-bucket rotor for 20 min at 4°C. The band of cells at the bottom of the tube contained ≥97% asci. The asci were washed twice in sterile $H_2O$, then incubated in 10% Glusulase (Endo Laboratories, New York) for 30 min at 37°C to remove the ascus wall. The spores were washed, sonicated, added to 1 liter of germination medium (Y-minimal medium with 0.33 μCi of [2-$^{14}$C]uracil per ml and supplemented with 25 mg of histidine, 2.5 mg of tyrosine, 25 mg of lysine, and 7.5 mg of

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Abbreviations: dsRNA, double-stranded RNA; EtdBr, ethidium bromide; VLP, virus-like particle.

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adenine per liter) to give a density of $\approx 0.3 \times 10^6$ spores per ml. The culture was grown at 30°C and harvested at a cell density of $\approx 10^7$ cells per ml, at which time 20% of the culture was diploid as a consequence of matings.

**Nucleic Acid Isolation.** Spheroplasts were formed by using Glusulase (5). Nucleic acids were isolated from cell lysates in CsCl/ethidium bromide (EtdBr) gradients as described (8). They were further purified in CsCl gradients or in 0.4% agarose gels (see *Results*). Conditions for equilibrium centrifugation and agarose gel electrophoresis have been published (8). $^3H/^{14}C$ ratios were determined for chromosomal DNA, 2-$\mu$m DNA, L and M dsRNA, and total cell RNA by slicing the gel lanes, dissolving the slices in $H_2O$, and measuring their radioactivities in Aquasol scintillation fluid (New England Nuclear). Ratios for chromosomal and mitochondrial DNAs were determined by scintillation counting of alkali-stable acid-precipitable radioactivity in CsCl gradient fractions.

**Electron Microscopy of Serial Sections.** Materials for electron microscopy were the generous gift of Loretta Goetsch and were prepared by her according to published procedures (9). A culture of AP-1 9 hr after transfer from PSP2 to 0.3% potassium acetate was pretreated with 2-mercaptoethanol, fixed in 3% (wt/vol) glutaraldehyde, and then treated with Glusulase to remove cell walls. Cells were treated with osmium tetroxide and uranyl acetate, dehydrated, and embedded in Spurr resin. Blocks were serially sectioned into 800 to 1000-Å sections. After mounting on grids, the sections were stained successively with uranyl acetate and lead citrate. The sections were examined in a Philips 300 electron microscope at a magnification of 12,500 diameters. Three asci were followed, photographed, and printed. Each section was traced onto transparent acetate sheets by outlining cellular organelles. The sheets were photocopied and all contours were cut out by hand and pooled according to the cell compartment to which each belonged. When the entire series had been cut, each "organelle" was weighed to the nearest milligram (Mettler balance PL200).

## RESULTS

**Meiotic Transmission.** The diploid strain D5 when grown in acetate presporulation medium contains approximately 70 copies of 2-$\mu$m DNA, 50 copies of mitochondrial DNA, and 1800 copies each of L and M dsRNAs per cell (10). Each nucleic acid species is stable during vegetative growth—i.e., fewer than 2% of the copies turn over each mitotic cell cycle (10). The fractions of each multiple-copy set that are transmitted from the diploid cell through meiosis and sporulation to the haploid generation were determined in double-label experiments. Strain D5 was grown in medium with [$^3$H]uracil and then transferred to sporulation medium without radiolabel for 4 days. Purified spores were germinated and grown in medium containing [$^{14}$C]uracil. Presporulation medium contained acetate as the carbon source to increase the sporulation efficiency (7). Because spores germinate poorly with acetate, glucose was used in the germination medium.

Nucleic acids were partially fractionated in a CsCl/EtdBr gradient (data not shown). To compare $^3H/^{14}C$ ratios of chromosomal DNA and mitochondrial DNA, material from the light shoulder of the chromosomal DNA band was recentrifuged in CsCl without EtdBr (Fig. 1). The dsRNAs were purified from the densest region of the CsCl/EtdBr gradient by gel electrophoresis (Fig. 2*A*). Material in the covalently closed circular DNA region of the CsCl/EtdBr gradient was subjected to electrophoresis (Fig. 2*B*) to purify 2-$\mu$m DNA away from any RNA and covalently closed mitochondrial DNA that might contaminate it. Electrophoresis of material in the chromosomal DNA region of the CsCl/EtdBr gradient (Fig. 2*C*) provided

Table 1. Quantitative transmission of yeast extrachromosomal nucleic acids

| Nucleic acid | Normalized $^3H/^{14}C$ ratios (A) Meiotic transfer experiment | (B) Meiotic mixing experiment | (C) Meiotic transmission (A/B) |
|---|---|---|---|
| Chromosomal DNA | 1.00 (±0.06)* | 1.00 (±0.05)* | 1.00 |
| Mitochondrial DNA | 1.00 | 1.79 | 0.55 |
| 2-$\mu$m DNA | 0.66 | 0.80 | 0.82 |
| L dsRNA | 0.64 | 0.90 | 0.71 |
| M dsRNA | 1.06 | 1.43 | 0.74 |
| RNA | 0.19 | 0.68 | 0.28 |

* The standard deviation of the chromosomal DNA ratio was calculated by averaging the $^3H/^{14}C$ ratios from at least 10 slices through the chromosomal DNA region of the gel and comparing the average to each individual ratio. The deviation is expressed as a fraction of the average chromosomal ratio (redefined as 1.00). The normalized ratios of the multi-copy genetic elements are assumed to have similar errors.

radiolabel data for chromosomal DNA. The $^3H/^{14}C$ ratios for each extrachromosomal nucleic acid, normalized to the chromosomal DNA ratio, are shown in Table 1, column A. In normalizing the $^3H/^{14}C$ ratios to chromosomal DNA a value of 1 indicates that the particular nucleic acid species has been replicated in the $^{14}$C-containing medium to the same extent that chromosomal DNA has been replicated; a value of <1 indicates that the nucleic acid has undergone proportionally greater replication during or after germination, or both, than chromosomal DNA. A ratio of <1 would be found for extrachromosomal genetic elements in which only a portion of the copies are transmitted through meiosis and sporulation, and the lost copies are replaced by extra replication in the postmeiotic medium.

Using $^3H/^{14}C$ ratios to measure relative nucleic acid levels compensates for the possible differential extraction of different nucleic acids. However, the $^3H/^{14}C$ ratios obtained in this experiment could reflect variables other than the efficiency of transmission. (*i*) The copy number of each nucleic acid species might not be the same in the premeiotic and postmeiotic populations. (*ii*) The postmeiotic population might not be in steady state at the time of harvest such that the copy number per ge-

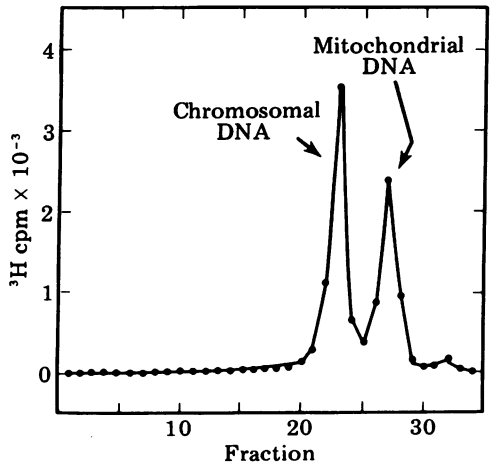


FIG. 1. CsCl gradient of mitochondrial DNA. DNA from the light shoulder of the chromosomal DNA in a CsCl/EtdBr gradient was recentrifuged in CsCl without EtdBr to separate mitochondrial DNA from contaminating chromosomal DNA. Only the $^3$H cpm profile is shown. Density decreases to the right.

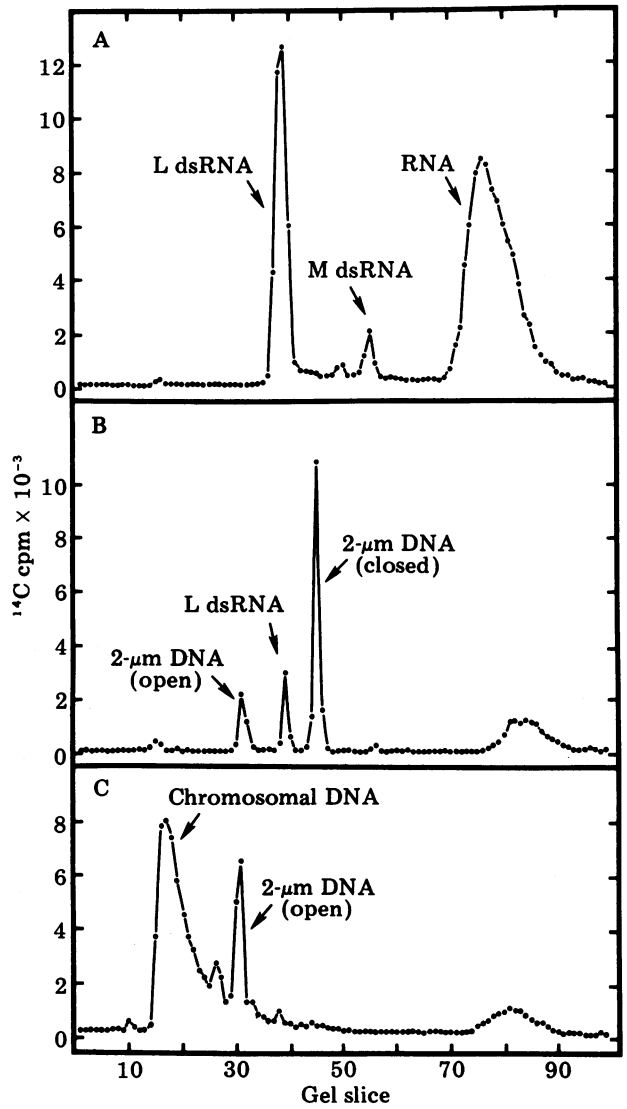


FIG. 2. Agarose gel electrophoresis of yeast nucleic acids. Nucleic acids isolated from a CsCl/EtdBr gradient were electrophoresed in 0.4% agarose gels for 16 hr at 1 V/cm. Gels were dried by suction and sliced into 1.2-mm sections, and radioactivity in each section was measured. Only the $^{14}$C cpm profiles are shown. Electrophoretic migration was from left to right. (*A*) RNA from the densest region of the gradient. (*B*) Nucleic acids from the covalently closed circular DNA region of the gradient. (*C*) Nucleic acids from the chromosomal (main band) region of the gradient. The DNA was cleaved with *Sma* I restriction endonuclease prior to electrophoresis as part of an analysis of the ribosomal RNA genes (rDNA) which will be reported elsewhere. The 2-μm DNA is not cleaved by *Sma* I.

nome of a nucleic acid species is changing. (*iii*) The precursor pools for the synthesis of the different nucleic acid species may equilibrate in the postmeiotic medium at different rates.

To control for these possible variables in the meiotic transfer experiment, a second double-label experiment was performed. One culture of D5 was grown in presporulation medium without radiolabel, then sporulated, and the spores were isolated and germinated in medium containing [$^{14}$C]uracil in a manner identical to the previous experiment. This culture was then mixed and harvested with a second culture of D5 grown in presporulation medium containing [$^{3}$H]uracil. The nucleic acids were isolated and analyzed as described above. The $^{3}$H/$^{14}$C ratios of each repeated nucleic acid set were normalized to the chromosomal DNA ratio (Table 1, column B). The normalized ratios in this experiment imitate a 100% meiotic transmission efficiency for each set. Assuming that the nucleic acids are extracted with equal efficiency from the two cell populations, the mixing experiment controls for possible variables in the meiotic transfer experiment except for the special case in which a nucleic acid species might be degraded and resynthesized from a closed precursor pool during meiosis or germination.

Comparison of the normalized ratios from the two experiments provides an estimate of the meiotic transmission efficiency. For example, there appears to be 1.8 times as much mitochondrial DNA per haploid genome in the diploid parent grown on presporulation medium, which contains the nonfermentable carbon source acetate, as there is in the germination culture grown on glucose. If all of the mitochrondrial DNA in the diploid had been transmitted to the haploid generation there would need to be preferential underreplication of mitochondrial DNA in the $^{14}$C-containing postsporulation medium until the concentration of mitochondrial DNA reached its new steady state value. Therefore, 100% transmission would have given a normalized ratio of >1 for mitochondrial DNA. Because the mitochondrial DNA ratio was 1, it follows that only a fraction of the copies were transmitted and this new copy number was maintained during the growth on glucose. The actual fraction transmitted is obtained by dividing the normalized ratio obtained in the meiotic transmission experiment (Table 1, column A) by the normalized ratio obtained in the control experiment (Table 1, column B); for mitochondrial DNA the fraction transmitted is 0.55.

The transmission efficiencies for each of the extrachromosomal elements are given in Table 1, column C. Of the molecules examined, none are transmitted with 100% efficiency. About 70% of L and M dsRNAs, 82% of 2-μm DNA, 55% of mitochondrial DNA, and 28% of the cellular RNA are transmitted to the haploid generation. If there were degradation and resynthesis of a nucleic acid species from a closed pool, these transmission frequencies would be overestimates.

**Electron Microscopy of Sporulating Yeast.** To determine the fractions of various cell organelles enveloped by spores, complete serial sections of three asci were examined and recorded by electron microscopy (see Fig. 3). The thin sections had been prepared by L. Goetsch for other purposes and were from a strain, AP-1, that has one of the same haploid parents as D5. Each ascus contained four spores bounded by a spore membrane and a spore wall of varying thickness. By following an organelle in consecutive sections, the number of organelles per ascus and their volume distribution between spores and epiplasm (the cytoplasm excluded from the spores) was determined.

The mitochondrial mass of the epiplasm was contained mostly in one or two giant highly branched mitochondria representing about 63% of the total mitochondrial volume, with an additional 5–13 smaller mitochondria containing the remainder. This organization of the mitochondrial mass is similar to that observed for diploid cells growing vegetatively on glycerol, a nonfermentable carbon source (11). In spores the mitochondrial mass was almost exclusively contained within one branched structure. Of the 12 spores examined, 8 had a single mitochondrion and the other 4 had one large mitochondrion, which represented ≥90% of the mitochondrial volume, and 1 or 2 smaller mitochondria. The presence of a single mitochondrion is characteristic of diploid cells growing exponentially on the fermentable carbon source glucose (11). This "fermentation-like" physical organization of the spore mitochondrion may account for the poor spore germination on acetate.

There was a single nucleus per spore and a single spherical membrane-bound fragment of nucleus in the epiplasm, as re-

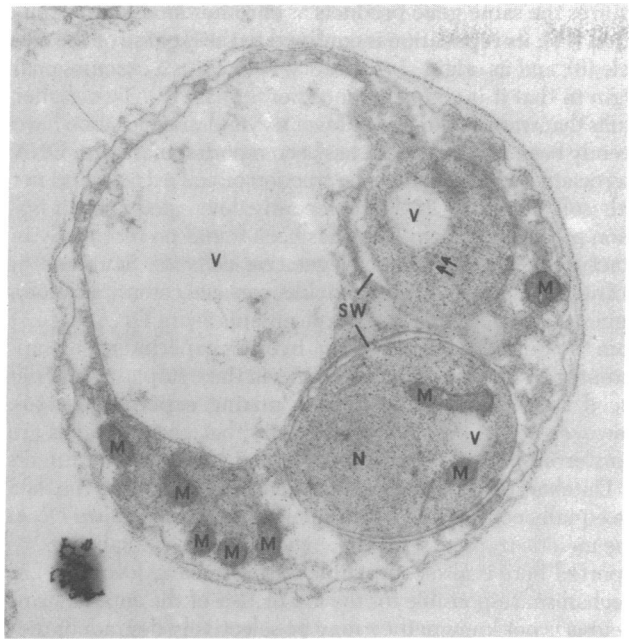


FIG. 3. Electron micrograph of a sporulating yeast cell. Thin section number 30 (out of 92 sections total) contains a nearly tangential profile of one spore and a cross section of a second spore. Visible are the spore nucleus (N), spore wall (SW), vacuoles (V), and mitochondria (M). Two virus-like particles (VLPs) (arrows) are seen in the cytoplasm of the first spore. (×13,200.)

ported previously (4). Vacuoles, defined as membrane-bound regions of the cytoplasm that were electron transparent or uniformly and lightly stained, were not counted. However, the epiplasm generally contained a few very large vacuoles and many fragments, while the spores contained a few small vacuoles. Ribosomes and VLPs were clearly present in both spore cytoplasm and epiplasm; however, no attempt was made to quantitate their distributions.

Quantitative volume data for the three asci are given in Table 2. The sizes of the four spores in ascus 1 are quite uniform,

Table 2. Distribution of ascus components by volume*

| Component | Spore 1 | Spore 2 | Spore 3 | Spore 4 | Spore total | Epiplasm |
|---|---|---|---|---|---|---|
| Ascus 1 | | | | | | |
| Cytoplasm | 3.16 | 3.30 | 3.24 | 3.17 | 12.87 | 20.50 |
| Mitochondria | 0.32 | 0.54 | 0.57 | 0.58 | 2.07 | 1.82 |
| Nucleus | 0.66 | 0.68 | 0.77 | 0.64 | 2.75 | ..14 |
| Vacuole | 0.49 | 0.44 | 0.33 | 0.35 | 1.61 | 11.33 |
| | 4.63 | 4.96 | 4.91 | 4.74 | 19.30 | 33.76 |
| Ascus 2 | | | | | | |
| Cytoplasm | 4.81 | 2.61 | 4.70 | 2.80 | 14.92 | 41.42 |
| Mitochondria | 0.61 | 0.42 | 0.74 | 0.36 | 2.13 | 1.95 |
| Nucleus | 1.11 | 0.61 | 1.12 | 0.61 | 3.45 | 0.09 |
| Vacuole | 0.99 | 0.42 | 0.80 | 0.30 | 2.51 | 21.06 |
| | 7.52 | 4.06 | 7.36 | 4.07 | 23.01 | 64.52 |
| Ascus 3 | | | | | | |
| Cytoplasm | 3.43 | 4.78 | 4.98 | 4.16 | 17.35 | 37.90 |
| Mitochondria | 0.40 | 0.60 | 0.67 | 0.44 | 2.11 | 2.09 |
| Nucleus | 1.06 | 1.09 | 1.29 | 1.17 | 4.61 | 0.09 |
| Vacuole | 0.41 | 0.50 | 0.53 | 0.40 | 1.84 | 23.25 |
| | 5.30 | 6.97 | 7.47 | 6.17 | 25.91 | 63.33 |

* The values are grams of paper cutouts and are proportional to volume. Ascus 1 was contained in 65 sections, ascus 2 in 92 sections, and ascus 3 in 94 sections.

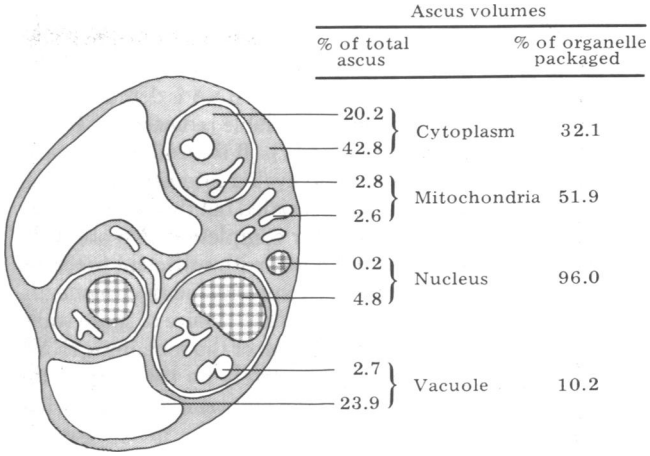


FIG. 4. Summary of ascus and spore volumes. Illustration of the volumes of nucleus, mitochondria, vacuole, and granular cytoplasm distributed between spores and epiplasm of the ascus. Percentages are averages of the three asci listed in Table 2. The average spore is 7.6% of the total ascus. Spore walls were not included in the calculations.

whereas ascus 2 contains two large spores and two small ones. Nevertheless, the percent of a spore that is granular cytoplasm (66 ± 1.8%) is constant for the 12 spores examined. Compared to the total ascus, spores contain the same percentage of granular cytoplasm but are enriched in mitochondria (2-fold) and nuclear material (4-fold) and are deficient in vacuoles (3-fold). Fig. 4 summarizes the average distributions diagramatically, showing that the organelles are distributed among the spores and epiplasm in different proportions. The four spores contain 32% of the granular cytoplasm, 52% of the mitochondria, 96% of the nucleus, and 10% of the vacuoles. Because the four spores occupy 30% of the internal ascus space (spore walls excluded from the calculations), the data indicate a random inclusion of granular cytoplasm in the spore but a nonrandom envelopment of nucleus, mitochondria, and vacuoles.

## DISCUSSION

From the behavior of the nucleus and the segregation of chromosomal genes during yeast meiosis it can be concluded that single-copy chromosomal DNA is conserved and fully transmitted to the four haploid products of meiosis. However, with multiple-copy extrachromosomal genetic elements there is no *a priori* requirement for all copies to be inherited. Indeed, the maintenance of homogeneity among the copies of a multiple copy set might be attained by the inheritance of a limited number of copies to reduce the heterogeneity brought about by random mutation. Three general mechanisms might be responsible for inheritance of a subset of copies. The genetic element could be preferentially included in spores, preferentially excluded (by the degradation of copies before spore formation, for example), or passively included as a consequence of random enveloping of regions of the parental diploid cell.

The results of the meiotic transmission experiments reported here reveal that only subsets of the copies of the extrachromosomal genetic elements are inherited. However, all are transmitted at frequencies higher than the stable nongenetic nucleic acids (rRNAs), and the fraction inherited differs for the different types of genetic elements. Assuming that there is a doubling in the number of each extrachromosomal element during meiosis, a spore receives an average of 14 copies of mitochondrial DNA, 29 copies of 2-$\mu$m DNA, and 600 copies each of L and M dsRNA. The fraction of granular cytoplasm packaged (32%) correlates well with the fraction of ribosomal RNA transmitted (29%), and, therefore, suggests that the packaging

of ribosomes is a random and passive event. However, the process may not be this simple, because there is massive degradation of RNA during meiosis and sporulation. It has been reported (6) that 50–70% of the cellular RNA is degraded and released into the culture medium as soluble radioactivity by the time sporulation is completed (24 hr). (It is possible that some of the degradation products are recycled into new RNA molecules; experiments have not been carried out that would detect reutilization.) The time courses for completion of spore walls and RNA degradation are not known with sufficient precision to permit the ordering of these two events relative to one another. If rRNA degradation precedes the closure of spore walls, then there must be a selective inclusion of the remaining rRNA in spores. This possibility seems unlikely on the basis of the abundance of ribosomes seen in the epiplasm at a time when spore walls have completely surrounded the spore contents. Therefore, we favor the interpretation that the transmission of rRNAs is accomplished by a random enclosure of cytoplasm within spores followed by the degradation of epiplasmic RNAs.

**Mitochondrial DNA.** The fraction of total mitochondria enclosed in spores (52%) correlates well with the 55% of mitochondrial DNA that is transmitted. It therefore seems likely that the inclusion of mitochondrial DNA is a passive event; the nucleic acid is included in spores because a certain fraction of the mitochondrial mass is enclosed. However, the mitochondria themselves are selectively included in the four spores, which occupy only 30% of the ascus. At a stage of sporulation before the prospore walls begin to form around the four nuclear buds the mitochondria fuse into one large highly branched mitochondrion surrounding the parental nucleus (B. Byers and L. Goetsch, personal communication). This association with the nucleus is most likely responsible for the higher than random expectation for the inclusion of mitochondria in spores.

**L and M dsRNAs.** The cytoplasmic dsRNAs have transmission values (70%) that are higher than can be accounted for by the random packaging of cytoplasm. They are, therefore, preferentially included in the spores. dsRNA particles (VLPs) could be seen in the granular cytoplasm of some thin sections (see Fig. 3) but not within nuclei or mitochondria. However, their distribution between spores and epiplasm could not be quantitated because of the difficulty in finding and positively identifying each VLP. The VLPs did not seem to have a preferred cytoplasmic location, although several cases of clustering of VLPs in the cytoplasm of a spore were noted. Even though VLPs are not seen associated with the meiotic nucleus it is possible that free dsRNA might be. It has been reported that only ≈25% of the L dsRNA in yeast is recovered when VLPs are isolated (12). The 25% recovery may reflect either poor yield or the existence of two populations of dsRNA, encapsulated and unencapsulated (12). It is tempting to speculate that if there are free dsRNA molecules they are located in the nucleus. Such a localization might provide an explanation for the preferential inclusion of dsRNAs in the spores.

**2-μm DNA.** Like dsRNA, 2-μm DNA has a high transmission frequency (82%) and a reported cytoplasmic location (13, 14). However, evidence that favors a nuclear location for 2-μm DNA has accumulated: it is condensed with histones in a manner identical to nuclear chromatin (15, 16); its replication requires the same gene products as chromosomal DNA replication (17); its replication is confined to the S phase of the cell cycle (8); and its origin of replication behaves as a chromosomal origin in that it is activated once per S phase (8). Two earlier results that were considered to favor a cytoplasmic location have recently been reexamined. It has been reported that 2-μm DNA is associated with a membrane fraction of cell extracts and not with isolated nuclei (13); with recently developed nuclear isolation procedures 2-μm DNA has been found preferentially in intact nuclei isolated from vegetative cells (C. Saunders, J. Proffitt, K. Zsebo, and K. Van Holde, personal communication), suggesting that in the earlier experiments 2-μm DNA was lost from the nuclei during isolation. In other experiments the apparent transmission of 2-μm DNA via the cytoplasm was deduced from *in vivo* cytoplasmic mixing experiments (14). However, it has been shown recently that chromosomes are transferred between the haploid nuclei in these experiments (S. Dutcher, personal communication; ref. 18), so that the data are equally compatible with a nuclear location of 2-μm DNA. The meiotic transmission efficiency of 2-μm DNA (about 82%) reported here is also compatible with a nuclear location. The mechanism responsible for the loss of 18% of the copies during meiosis is not known: they may be selectively degraded; they may simply leak out of the nucleus during meiosis; or they may be entrapped in the small piece of nucleus (4%) that is left in the epiplasm.

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