chain elongation (*11*), additional support was obtained for the interpretation that DRB shifts the sedimentation profile of hnRNA because it inhibits chain initiation. Cells were treated with a high dose of actinomycin D (10 μg/ml) for 1 minute and then exposed to [^{3}H]uridine for 45 seconds. Although the total amount of ^{3}H incorporated into RNA was decreased by 70 percent, the sedimentation profile of the remaining labeled RNA was similar to that of untreated cells (Fig. 2, A and C). In contrast, treatment with DRB (75 μM) for 3 minutes resulted in a shift (as observed earlier) in the sedimentation profile toward the longer molecules (Fig. 2B).

Because a fraction of hnRNA appears resistant to DRB inhibition [see (*2*) and Table 1] it appears possible that DRB may distinguish between two classes of hnRNA initiated by different RNA polymerases. Other experiments show that the class of hnRNA which is DRB-sensitive contains at least a substantial portion of the molecules which become messenger RNA (mRNA) because no or very little labeled polyadenylate [poly(A)]-containing mRNA reaches the cytoplasm in DRB-treated cells (*8*). Hence, in addition to its value as the only known inhibitor of hnRNA chain initiation in living mammalian cells, it appears possible that DRB may become an important tool in the analysis of the role of RNA polymerases in transcription, and in providing understanding of the regulation of cellular biosynthetic processes (*6*).

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10. For comparison, the rate of RNA chain elongation has been estimated to be at most 100 nucleotides per second for poliovirus RNA [J. E. Darnell, M. Girard, D. Baltimore, D. F. Summers, J. Maizel, in *The Molecular Biology of Viruses*, J. Colter, Ed. (Academic Press, New York, 1967)] and for the 45*S* precursor of ribosomal RNA [H. Greenberg and S. Penman, *J. Mol. Biol.* **21**, 527 (1966)] in HeLa cells. Bacterial RNA polymerases have been estimated to incorporate a maximum of 50 nucleotides per second [H. Bremer and D. Yuan, *J. Mol. Biol.* **38**, 163 (1968)] while the RNA polymerase in *Chironomus tentans* incorporates only about 30 nucleotides per second (*3*).
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Nuclear and Mitochondrial DNA Replication During Zygote Formation and Maturation in Yeast

Abstract. *Nuclear and mitochondrial DNA replication were monitored during the development of synchronous yeast zygotes. Purified first zygotic buds were also analyzed. Nuclear DNA replicated discontinuously but coincidently with bud initiation, while mitochondrial DNA replicated throughout the zygotic formation and maturation period. First zygotic buds contained the diploid level of both nuclear and mitochondrial DNA.*

Mating is initiated when haploid yeast cells of opposite mating type (*a* and α) are mixed under appropriate conditions. Soon after, cells of opposite mating type pair and, within an hour after cell mixing, clumps of *a* and α cells are evident. One to 3 hours later, after agglutination, *a* and α cell pairs fuse to produce zygotes. After cell fusion, parental cytoplasms mix and parental nuclei fuse. Approximately 0.5 hour after zygote formation, the first zygotic bud is initiated. Genetic analyses of this zygotic bud showed that it contained a diploid nucleus derived from both nuclear parental inputs. In contrast to the stable nuclear genetic composition of zygotic buds, their cytoplasmic genetic constitution can be

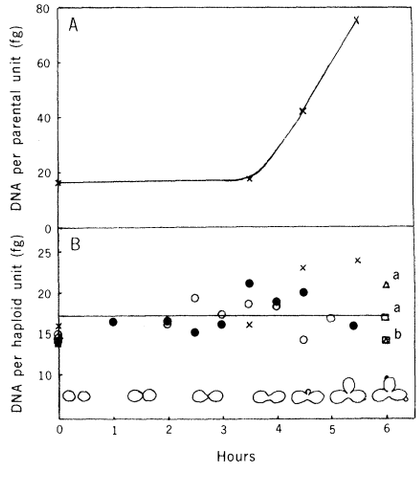

Fig. 1. DNA content during synchronous mating. Haploid single spore isolates *a* 6A *trp-4* and α 6B *trp-4* of diploid *a* 5032A × α 5032B were cultured, mated, and fractionated (*12*). The DNA content of all the samples except the diploid zygotic buds was determined chemically with the DABA method (*4*). Total DNA isolated from 1×10^8 to 2×10^8 diploid zygotic buds was estimated in the analytical ultracentrifuge by measuring the areas under the densitometer tracings relative to a known quantity of marker DNA. All samples taken during the initial 4 hours of mating were removed directly from the mating mixture. After 4 hours, the zygotes were purified (90 to 99 percent) from the nonmating cells. A sample of pure zygotes was taken, and the remainder were inoculated into fresh mating medium for further maturation. Other samples were taken during first zygotic bud formation and after the first zygotic bud was completed. The remaining mature zygotes were fractionated into a first diploid zygotic bud fraction (*b*-released diploid buds represented 10 to 15 percent of the actual formed buds) and a residual zygote fraction (*a*-mature zygotes, 10 to 15 percent of which were without their first zygotic buds) for DNA measurement. The predominant cell morphology during the experiment is diagrammed for reference. [Since cells are not maintained in medium during zygote separation, the time or abscissa positions beyond zygote isolation (4 hours) were determined by comparing the fraction morphology with the age of an unfractionated synchronously mating population.] (A) The kinetics of DNA increase during mating in a typical experiment. Values are calculated per haploid parent. Unbudded cells were counted as one parental unit, while budded cells, unbudded zygotes, and singly and multiply budded zygotes were counted as two parental units. (B) The mean of data points obtained from five synchronous mating experiments (●, ○, ×, △, □). The DNA content per haploid equivalent was calculated by counting unbudded cells as one, budded cells and unbudded zygotes as two, singly budded zygotes as four, doubly budded zygotes as six, triply budded zygotes as eight, unbudded diploid zygotic buds as two, and budded diploid zygotic buds as four. This line through the mean of the data points is not statistically different from the calculated linear regression line.

extremely heterogeneous (*1, 2*). Thus, when parental cells differing in mitochondrial markers are mated, the mitochondrial genome of the first zygotic bud can be exclusively parental, exclusively recombinant, or a mixture of both types.

These genetic data pose major questions concerning the fate of nuclear and mitochondrial DNA (mtDNA) molecules during zygote formation and maturation. Among the central questions are: What are the parental nuclear and mtDNA contributions to the zygote? and How are these DNA species replicated during zygote formation and then partitioned between the first diploid zygotic bud and the residual zygote?

To clarify the general patterns for DNA transmission during mating, we monitored the replication of nuclear and mtDNA in synchronously mating populations of aerobically sufficient (*grande*) *Saccharomyces cerevisiae* strains. DNA replication was followed during the initial mating stages, in purified zygote preparations isolated from the synchronously mating cell populations, and subsequently in purified diploid zygotic buds isolated from matured purified zygote preparations.

Chemical measurement of total DNA content per parental cell throughout mating and zygote maturation (Fig. 1A) revealed that the DNA content remained at about 16 fg per parental cell during cell pairing and initial zygote formation. The amount of DNA began to increase when first zygotic bud initiation occurred and continued to increase throughout zygote maturation. When each zygotic unit carried at least a single mature bud, it contained a total of 150 fg of DNA per zygote or 75 fg of DNA per parental cell. The calculated DNA per haploid cell equivalent averaged 17 fg throughout mating and zygote maturation (Fig. 1B), suggesting that zygotic DNA duplication occurred coincident with bud initiation. Purified first zygotic buds averaged 14 fg of DNA per haploid cell equivalent (Fig. 1B,b), only slightly less than the average value, which is within the standard error range for these calculations. These data support the prediction that first zygotic buds contain a diploid DNA content.

While estimates of total DNA per cell during mating indicate, for the greater part, replication of the nuclear DNA, both nuclear DNA and mtDNA replication can be studied when ^{15}N-labeled parental cells are mated in ^{14}N medium. Microdensitometer tracings of absorbance photographs of DNA samples isolated during a ^{15}N to ^{14}N mating shift experiment are shown in Fig. 2. Both the main nuclear DNA (ρ = 1.715 g/ml) and the mtDNA (ρ = 1.699 g/ml) were completely labeled with heavy isotope (^{15}N : ^{15}N) prior to and therefore at the initiation of mating. During the early mating reaction, although before cell fusion, the mtDNA peak exhibited a shift toward the hybrid (^{15}N : ^{14}N) density. Purified zygotes (isolated immediately after their formation) from the synchronously mating cell population contained only 40 percent hybrid density nuclear DNA (1.707 g/ml) while the mtDNA peak had a density equivalent to totally hybrid mtDNA (1.691 g/ml). After zygote maturation, nuclear DNA replication was complete (95 percent ^{15}N : ^{14}N and 5 percent ^{14}N : ^{14}N), while the mtDNA density attained a value equal to 1.689 g/ml. Table 1 shows that up to zygote formation (4.5 hours), mtDNA incorporated enough ^{14}N to account for a complete duplication and that during zygote maturation (5.5 hours) mtDNA continued to incorporate ^{14}N. We conclude that both parental mtDNA's replicated because the entire mtDNA peak shifted unimodally from the uniformly heavy to the hybrid position during zygote formation. Our conclusion that the ^{14}N incorporation data reflect the complete duplication of mtDNA during the time interval required for zygote formation, and its continued replication in the developing zygote, is supported by the percent mtDNA values given in Table 1. Also included in Table 1 are the percent mtDNA estimates for diploid zygotic buds. As nuclear DNA replication occurs in the zygote (Table 1), the mtDNA decreases to 15 percent. Two diploid zygotic bud populations obtained from zygotes bearing 99 percent centrally located zygotic buds averaged 16.5 percent mtDNA. The variability between the zygotic bud populations probably reflects the slight age differential between the populations. However, it is clear that, upon release, the diploid zygotic bud contains at least a diploid quantity of mtDNA.

Our data demonstrating that mtDNA synthesis continued throughout the early mating reaction, zygote formation, and maturation whereas nuclear DNA synthesis was delayed until after initial zygote formation conform with other related experiments and with experiments based on use of *a* and α peptides (*3*). Al-

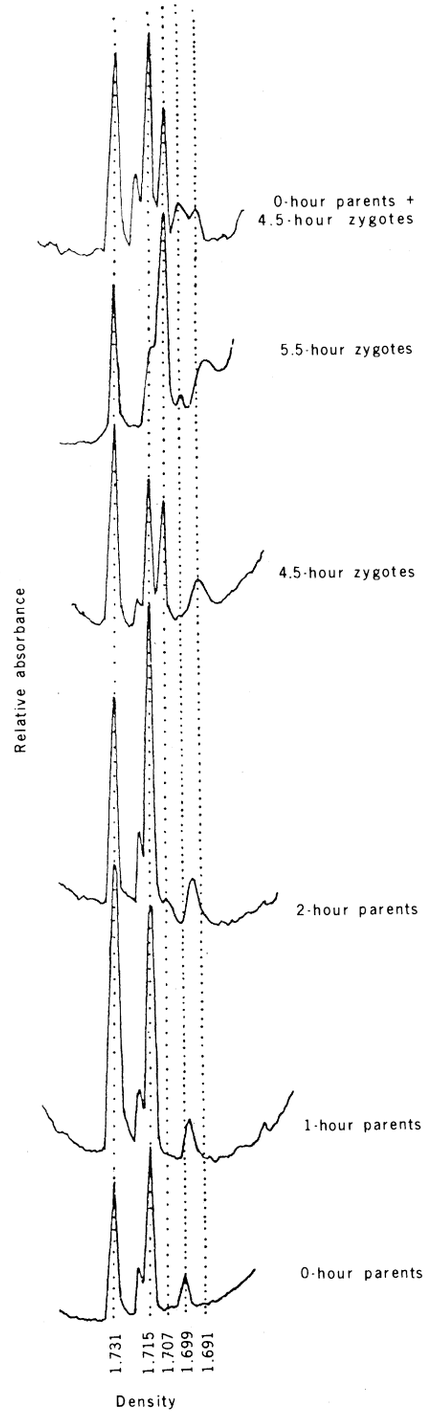

Fig. 2. Densitometer tracings of DNA from mating *Saccharomyces cerevisiae*. Cell cultures of *a* and α cells grown for at least 12 generations in medium containing 0.005 percent $(^{15}NH_4)_2SO_4$ (99 atom percent; International Chemical and Nuclear Corporation) and 0.05 percent glucose were mated synchronously in a similar medium containing 0.4 percent $(^{14}NH_4)_2SO_4$ (*4, 12*). DNA isolated from mating samples taken 1, 2, and 3 hours after mating initiation and from two zygote samples (one purified 4.5 hours after mating initiation; the other, the purified zygotes matured 1 hour) were centrifuged to equilibrium in CsCl (*4*). The density of heavy labeled nuclear DNA (^{15}N : ^{15}N) is 1.715 g/ml, and heavy labeled mtDNA is 1.699 g/ml. The hybrid nuclear DNA density (^{15}N : ^{14}N) is 1.707 g/ml, whereas the hybrid mtDNA density is 1.691 g/ml. *Micrococcus lysodeikticus* DNA (1.731 g/ml) was used as marker.

though bulk DNA synthesis proceeds exponentially after zygote formation (Fig. 1A) and nuclear fusion, a relatively constant quantity of DNA per zygote and diploid zygotic bud is maintained (Fig. 1B). The dissociation of mtDNA synthesis from nuclear DNA synthesis during mating is similar to the mtDNA synthetic pattern observed during vegetative mitosis (*4, 5*) and meiosis (*6*) and after treatment with mating-type peptides (*7*).

The unimodal density shift pattern observed for ^{15}N-labeled mtDNA replicating in ^{14}N medium was observed previously in mitotically dividing cells (*4, 8*). The hypothesized recombination mechanism (or mechanisms) producing this unimodal banding pattern may also occur during the initial mating reaction; however, recombination at the molecular level in early mating would not be genetically detectable since cell fusion has not occurred. Genetic recombination between parental mitochondrial markers is common, and recombinant-type mtDNA molecules have been isolated from descendants of zygotes (*9*). It has been postulated that most, if not all, recombination resulting in genetically detectable exchanges between parental mtDNA's occurs in the zygote (*10*). Our initial experiments designed to detect molecular recombination in the zygote are consistent with this view.

On the basis of the DNA content per parental unit shown in Fig. 1A and the mtDNA percentages per mating sample in Table 1, we calculated the average number of mtDNA molecules in cells and zygotes during various mating stages (assuming that the molecular weight of mtDNA is 50×10^6). Thus our unbudded parental cells, which contained about 28 mtDNA molecules at the initiation of mating, contained approximately 56 molecules of mtDNA at the time of cell fusion. The mtDNA content per young zygote, therefore, averaged 112 molecules each or a value representing 23 percent of the total zygote DNA (Table 1). As the zygote matures and nuclear DNA replicates, the mtDNA percentage decreases to 15 percent, and the zygote then contains about 168 molecules of mtDNA. Since zygotic synchrony is not maintained after first zygotic bud production, it is difficult to ascertain whether the mtDNA level returns to the diploid amount per nucleus. Therefore, we estimate that zygotes contain between 235 and 307 mtDNA molecules after first zygotic bud maturation (about the stage when second bud initiation is occurring). The first diploid zygotic bud itself contains an average of 44 to 67 mtDNA molecules immediately after release from the zygote.

Table 1. Replication of nuclear and mitochondrial DNA during mating. The extent of nuclear and mtDNA replication during mating is shown. The mtDNA replication was obtained from the ^{14}N content of mtDNA (Fig. 2), which was calculated from the buoyant density, assuming 1.699 g/ml as 100 percent ^{15}N and 1.684 g/ml as 100 percent ^{14}N DNA. The theoretical ^{14}N incorporation values based on specific percentages of mtDNA replication are included for comparison. Nuclear DNA replication was measured from the percentage of hybrid nuclear DNA per sample (Fig. 2). The percentage mtDNA per mating sample was measured as described (*4*). The theoretical percentage values were based on the specific replication values shown for mtDNA. For the first four theoretical values no nuclear DNA replication was assumed; while in mature zygotes with a mature first zygotic bud one round of nuclear DNA replication was assumed. The theoretical value for the first zygotic bud is based on the assumption that the bud contains the diploid DNA content derived from both *a* and α parents; Obs., observed; Theor., theoretical.

Mating samples	Replication (%)		^{14}N in mtDNA (%)		mtDNA (%)	
	Nuclear DNA	mtDNA	Obs.	Theor.	Obs.	Theor.
0 hour	0	0	0	0	13	13
1 hour	0	25	13	20	15	17
2 hour	0	50	31	33	19	18
4.5-hour zygotes	25	100	53	50	23	23
5.5-hour matured zygotes	105	150	67	67	15	18
1st diploid zygotic buds					13 to 20	13

The number of mtDNA molecules per zygotic bud, estimated from the frequency of mitochondrially homozygotic buds, is given as about three (*1*), a value significantly smaller than the estimated 44 to 67 mtDNA molecules indicated by our measurements. It is unlikely that three molecules could replicate rapidly enough during the interval required for first zygotic bud isolation to yield the 44 to 67 molecules per bud observed in our study. However, Dujon *et al.* do state that the assumption of purely random distribution of mtDNA molecules between the zygote and the bud may represent an oversimplification.

In addition, parental mtDNA inputs into the zygote may vary and affect the observed mitochondrial genetic output (*11*). Our parental mtDNA inputs into the zygote were equivalent. The average numbers of mtDNA molecules per zygotic bud derived from crosses of unequal parental inputs are unknown. Also, little is known about parameters such as cytoplasmic mixing, mitochondrial dissolution or fusion, mtDNA mixing, and mtDNA packaging, any of which could affect the distribution of mtDNA molecules within the zygote as well as between the zygote and its first bud.

The contrast between the precise control over the replication and constancy of nuclear DNA content in the zygote with the continued mtDNA replication and its variable amount during zygotic formation and maturation are striking. Clearly, the mechanisms controlling nuclear and mtDNA replication and transmission within developing zygotes are different. Projected studies on the transmission of nuclear and mtDNA during the yeast sexual cycle, which are also aimed at correlating the biochemical and structural parameters of maturing zygotes with the inheritance patterns of nuclear and mitochondrial genes will doubtless better define the contrasting and interactive features of these two genetic systems contained within a single eukaryotic cell.

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