THE REPLICATION OF MITOCHONDRIAL DNA DURING THE CELL CYCLE IN SACCHAROMYCES LACTIS*

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Data accumulated in recent years that yeast mitochondria contain DNA (mDNA) with a base composition different from that of nuclear DNA (nDNA)[1] support genetic and biochemical evidence[2] that these are the cytoplasmic factors determining respiratory competence. Since phenotypic expression of the respiratory function of mitochondria is under nuclear control (segregational petites),[3] the synthesis of mitochondria-specific components is determined by both the nuclear and mitochondrial genomes.

The above findings, as well as the extreme limitations placed by the limited size of the mitochondrial genome in yeast (*ca.* 10^7 daltons),[1, 4] raise the question as to whether mDNA replication is independent of nDNA replication. The recent findings in synchronous cultures of yeast that nDNA replication[5] and the times of expression of several structural genes[6] are periodic during the cell cycle provides a basis for examining this relationship. If the controls for mDNA replication are independent of the controls for nDNA replication, then one might expect that the population of mDNA's would either replicate continuously or randomly over the cell cycle.

To investigate this relationship the replication of mDNA and nDNA in *Saccharomyces lactis* (*Kluyveromyces lactis*),[7] a weakly fermentative yeast, was examined. This yeast can be readily grown with organic acids as a carbon source, conditions one might expect to derepress mitochondria formation. The present paper reports our initial findings on the properties and physiological control of mDNA in *S. lactis*. Evidence is also presented to show that in this yeast mDNA replication is periodic and at an interval of the cell cycle different from that of nDNA replication.

Materials and Methods.—Organism and cultivation: Haploid parental (Y-14 and Y-123) or diploid (Y-14 × Y-123) stocks of *Saccharomyces lactis* were used. The method of isolating diploids and maintenance of stocks was described by Tingle *et al.*[8] Cells were grown in 2% succinate synthetic medium[6] (SSM) unless otherwise stated. Synchronous cultures of diploid cells in SSM were established and monitored as previously described.[6]

DNA isolation and centrifugation: DNA was isolated from yeast by the method described by Smith and Halvorson.[9] DNA solutions were centrifuged to equilibrium (17 hr at 44,770 rpm) in either cesium chloride (1.700 gm/cc; 0.01 *M* tris(hydroxymethyl)-aminomethane (Tris), pH 8.0) or cesium sulfate (1.554 gm/cc; 0.01 *M* Na borate, pH 8.5; 5×10^{-10} moles of $HgCl_2/\mu g$ DNA) at 25°C in a Beckman model E ultracentrifuge. *Escherichia coli* DNA (1.710 gm/cc in CsCl) was used as a density marker. For determinations of the mDNA/nDNA ratio the DNA was centrifuged at two different concentrations (10 μg/ml for mDNA determinations; 1 μg/ml for nDNA determinations) at the same time. Microdensitometer tracings of ultraviolet photographs were made with a Joyce-Loebl chromoscan.

Estimation of DNA content during synchronous growth: Fifty ml of a synchronous culture of diploid cells were withdrawn at intervals, filtered rapidly over a 25-mm diameter

Millipore filter disk, and washed with 50 ml cold water. The cells were suspended in 2 ml of 10% perchloric acid, incubated overnight at 4°C, and centrifuged. The cells were extracted at 70°C for 30 min with 1 ml of 10% perchloric acid. After centrifugation, the pellet was re-extracted, the perchloric acid extracts were combined, and the DNA content was determined by the method of Burton.[10]

Measurement of cytochrome content: Cells were suspended in 0.1 *M* phosphate buffer (pH 7.5) at a concentration of 20–30 mg dry weight per ml. The suspensions were placed in a 0.1-cm light path cuvette and blanked against a cuvette containing Whatman no. 1 filter paper saturated with mineral oil. After reduction with dithionate, the absorption spectrum was measured with a Cary model 15 spectrophotometer. The integral of the absorption spectrum between 520 and 630 mμ for each cell suspension was assumed to be proportional to the cytochrome content of the cells.

Experimental Results.—Characterization of satellite DNA in S. lactis: Bulk yeast DNA contains at least two components: a main band (nDNA) with a buoyant density of 1.700 gm/cc and a satellite band (mDNA) with a buoyant density of 1.685–1.687 gm/cc.[1] The only yeast showing significant deviation from this was *S. lactis*,[11] which has a "normal" main band component with a buoyant density of 1.700 gm/cc but a satellite with a buoyant density of 1.692 gm/cc in CsCl (Fig. 1*A*). The DNA preparation shows a typical absorption spectrum of nucleic acids. When this DNA is treated with DNase and snake venom phosphodiesterase, the main band as well as the satellite band disappeared (Fig. 1*D*).

Figure 1*B* also shows the tracings of DNA isolated from the mitochondria-rich fraction of *S. lactis* and from a segregational petite mutant (obtained from D. A. Herman) of the same strain. From the enrichment of the satellite DNA in the mitochondrial fraction and its reduction in the segregational petite, we conclude that the satellite band is mDNA.

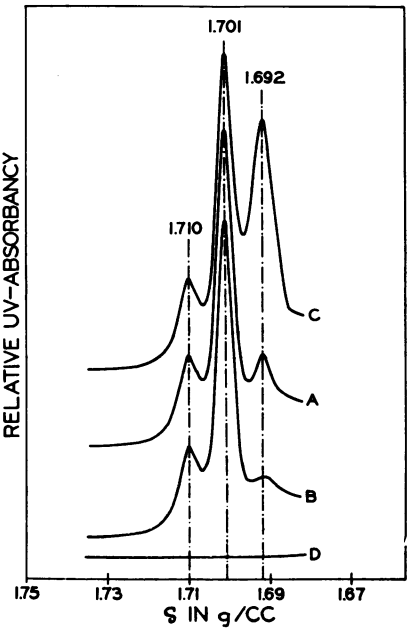

FIG. 1.—Microdensitometer tracings of ultraviolet photographs of DNA from whole cells of *S. lactis* (*A*), a segrational pettite mutant (*B*), a mitochondria-rich fraction (*C*), and bulk DNA treated with DNase (*D*), centrifuged to equilibrium in a CsCl density gradient. *Escherichia coli* DNA (1.710 gm/cc) was used as a density reference. The segregational petite was grown in 5% glycerol broth.[8]

To emphasize the difference between nDNA and mDNA, we took advantage of the finding that at low Hg^{++} concentrations the shift in the buoyant density of the HgDNA complex is proportional to the AT content.[12] The higher AT content of mDNA of *S. lactis*[11] makes it possible to separate the mercury complex of this component from nDNA in cesium sulfate (Fig. 2), enabling an accurate determination of each DNA species.

The absorbance-temperature profile of *S. lactis* DNA in saline-citrate buffer is biphasic.[11] The initial part of the heating curve has a small shoulder (T_m = 82.7°C), presumably due to the melting of mDNA. The major part of the DNA melts several degrees higher (T_m = 85.5°C). The hyperchromicity of 40 per cent is that expected of a double-stranded DNA. Based on the melting temperature and the buoyant densities, the guanine + cytosine (GC) contents of nuclear and mitochondrial DNA are 40 and 32.7 per cent, respectively. Similar determinations in *S. cerevisiae* led to an estimate of 40 per cent GC for nuclear DNA and 25 per cent GC for mitochondrial DNA.[1, 11]

Repression of mDNA and cytochrome synthesis by glucose: Since the number of mitochondria is inversely proportional to the glucose concentration during growth,[13] the amount of any intramitochondrial component might also be inversely proportional to the glucose concentration. This could be especially true in *S. lactis* which possesses a strong oxidative metabolism.

Figure 3 summarizes the effect of various carbon sources on the synthesis of mitochondrial DNA in *S. lactis*. Increasing concentrations of glucose markedly

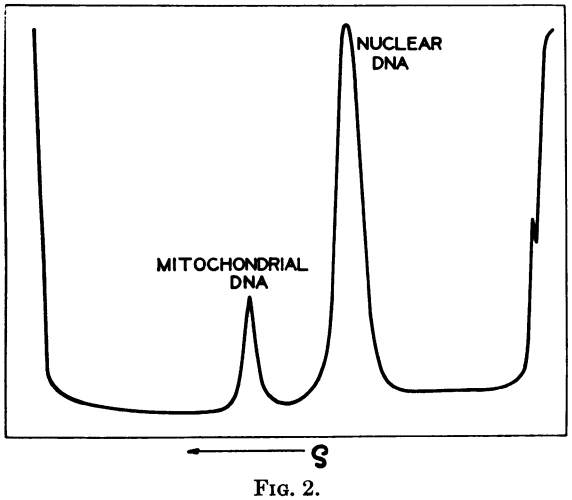

FIG. 2.

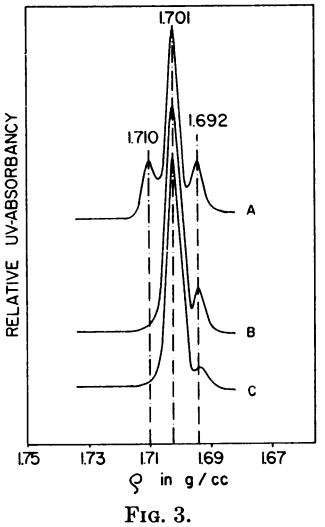

FIG. 3.

FIG. 2.—Microdensitometer tracing of *S. lactis* DNA centrifuged to equilibrium in cesium sulfate. DNA was extracted from cells and centrifuged to equilibrium (17 hr) in cesium sulfate in presence of Hg^{++} (5×10^{-10} moles/μg DNA).

FIG. 3.—Effect of carbon source on the mDNA content of *S. lactis*. Cells were grown in 1.1% succinate (*A*), 1% glucose (*B*), and 10% glucose (*C*) as carbon source, DNA was extracted and centrifuged to equilibrium in cesium chloride. *E. coli* DNA (1.710 gm/cc) was used as reference standard.

decreases the amount of this DNA below the value for cells grown in succinate. Similar repression of the synthesis of mitochondrial components by glucose has been reported in *S. cerevisiae*.[14]

Since yeast cytochromes are primarily intramitochondrial,[15] a correlation between the amount of cytochromes and mitochondrial DNA might be expected to reveal the relationship between mitochondrial function and mDNA replication. The absorption spectrum of the reduced cytochromes of *S. lactis*[11] revealed the typical bands of cytochrome $a + a_3$, cytochrome *b*, and cytochrome *c*. Figure 4 shows that there is a concomitant increase in the relative concentration of cytochromes with increase in the percentage of mDNA of cultures grown in various carbon sources.

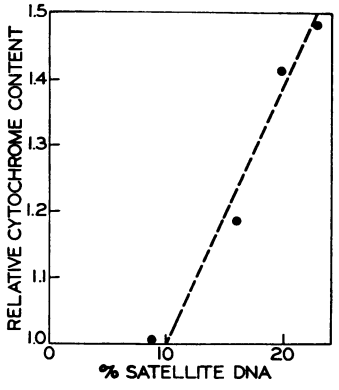

FIG. 4.—Relationship between cytochrome and mitochondrial DNA content in *S. lactis*. The relative cytochrome concentration was calculated as described in *Materials and Methods*. The values for percentage of mDNA of the total DNA from cells grown on different carbon sources were taken from Fig. 3.

Synthesis of nDNA and mDNA during the cell cycle of S. lactis: In synchronously growing cultures of *S. cerevisiae* and *S. pombe* the synthesis of DNA is periodic.[5] The onset of DNA synthesis occurs early in the cell cycle, and the total time required for a round of DNA synthesis is less than a third of the generation time. For comparison, a diploid culture of *S. lactis* (Y-14 × Y-123) was grown synchronously in 2 per cent succinate as the carbon source, and the time of total DNA synthesis during the cell cycle as well as the variations in the mitochondrial and nuclear DNA were determined. Figure 5*A* shows the results of such an experiment in which the cell number and DNA content/ml of the culture increases periodically. The onset of DNA synthesis occurs during the early part of the cell cycle following which there is a long period of no DNA synthesis. Cell division occurs at a time when no DNA synthesis occurs as previously observed in other yeast strains.[5]

To determine the pattern of mitochondrial DNA synthesis during the cell cycle, DNA was isolated at intervals from *S. lactis* growing synchronously in synthetic medium. The DNA samples were complexed with Hg^{++} and centrifuged to equilibrium in Cs_2SO_4 (see Fig. 2). The relative amounts of mitochondrial and nuclear DNA were determined from the area under each of the peaks of the densitometer tracings. Figure 5*B* shows the variation in the ratio of mitochondrial DNA to total DNA during several synchronous cycles of division. The ratio varies periodically and there are periods in the cell cycle when

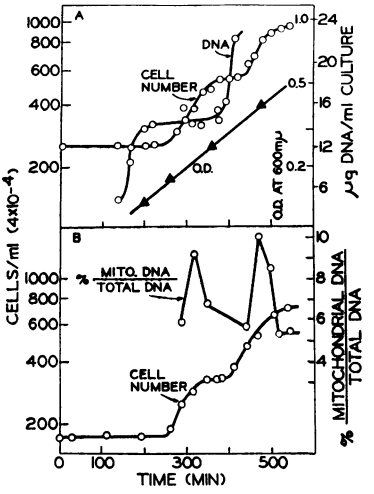

FIG. 5.—Synthesis of DNA in a synchronous diploid culture of *S. lactis* (Y-14 × Y-123).

(*A*) Total DNA was extracted from the cell samples with perchloric acid and estimated by the method described by Burton.[10]

(*B*) Variations in the ratio of mDNA to total DNA during the cell cycle of *S. lactis* (Y-14 × Y-123). One hundred-ml aliquots were removed at intervals; DNA was extracted and analyzed as described in *Materials and Methods*.

neither DNA is synthesized. The sharp increase in the ratio corresponds to the time at which mDNA is synthesized, and the decrease in the ratio corresponds to the time of nuclear DNA synthesis (Fig. 5*A*) and the dilution of mDNA by nDNA. The synthesis of mDNA is therefore discontinuous and occurs at a different interval of the cell cycle than nDNA synthesis.

Discussion.—*S. lactis* contains numerous mitochondrial particles.[16] If the population of mDNA's are replicated independently of nDNA and each other, then one would expect that their replication occurs randomly throughout the division cycle. Alternatively, mDNA synthesis could be coupled to the cell cycle by being either linked to nuclear DNA replication itself or to the periodic synthesis of an essential protein(s) during the cell cycle.

In each of these cases one would expect characteristic changes in the ratio of mDNA/total DNA during the cell cycle. If mDNA synthesis is tightly coupled to nDNA replication, then these ratios should always remain 1. On the other hand, the theoretical ratio of mDNA/total DNA should vary differently for the other two possibilities (Fig. 6). If mDNA synthesis is continuous during the cell cycle, then the ratio of mDNA/total DNA would follow the kinetics described in Figure 6*A*. After an initial decrease the ratio should increase continuously. On the other hand, if the synthesis of mDNA is periodic and occurs during a different period than that for nDNA synthesis, mDNA/total DNA ratio would follow the pattern described in Figure 6*B* and would vary periodically. The proximity in the increase and decrease in ratio would indicate the time at which each of the components is synthesized during the cell cycle.

The results reported in the present paper (Fig. 5*B*) are consistent with a discontinuous mode of mDNA synthesis in which mDNA synthesis occurs slightly before nDNA synthesis (Fig. 5*A*). These findings are in agreement with the observation in *Tetrahymena*[17] and in *Physarum*.[18] In both cases mDNA replicates at a time different from the times at which nDNA is replicated. Further evidence to support this conclusion has been obtained in several other eucaryotic organisms.[19]

The well-known dependence of DNA replication on a new round of protein synthesis[20] and the ordered transcription of the genome in *S. lactis* and other yeasts during the cell cycle[6] provide an explanation for the present results. The synchronous synthesis of the mDNA population during the cell cycle would be expected if the onset of mDNA synthesis is dependent upon the appearance of a protein coded by the nuclear genome. Since the periods of mDNA and nDNA synthesis differ, it is suggestive that this protein(s) may be specific for mDNA synthesis. The parallel between cytochrome content and mDNA content further implies that the number of mitochondrial genomes may be regulated by the nuclear genome. Whatever the basis of regulation of mDNA, the observed synchrony in mDNA replication provides an attractive experimental system with a limited genome to explore transcriptional controls in yeast.

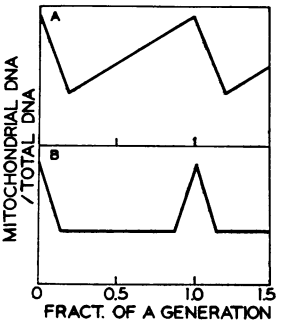

FIG. 6.—Predicted kinetics for the synthesis of mDNA during the cell cycle of *S. lactis*.
(*A*) Kinetics of continuous synthesis.
(*B*) Kinetics of discontinuous synthesis.

Summary.—*S. lactis* contains a mitochondrial DNA of buoyant density of 1.692 gm/cc in CsCl. The level of this satellite DNA can be varied in relation with the level of mitochondrial components. The replication of mitochondrial DNA is periodic during synchronous growth of the culture and occurs at a time different from that at which nuclear DNA is synthesized. There are periods in the cell cycle of *S. lactis* during which neither nuclear or mitochondrial DNA are synthesized. These results suggest the possibility that the periodic synthesis of a protein coded by the nuclear genome may be required for mitochondrial DNA synthesis.

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