THE SYNTHESIS OF MITOCHONDRIAL DNA DURING THE CELL CYCLE IN THE YEAST SACCHAROMYCES CEREVISIAE

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SUMMARY

Synthesis of mitochondrial and nuclear DNA during the growth of synchronously dividing cultures of Saccharomyces cerevisiae was followed by analytical isopycnic centrifugation in CsCl as well as by pulse labelling with radioactive adenine and determination of the rate of incorporation into each DNA component at different stages of growth. Both techniques showed that whereas nuclear DNA was synthetized discontinuously, mitochondrial DNA was replicated throughout the cell cycle. Moreover, in these cultures the rate of mitochondrial DNA synthesis continuously increased, which implies that in this organism, initiation of mitochondrial DNA replication is not restricted to one fixed point in the cell cycle.

Considerable interest attaches to the way in which the replication of mitochondrial DNA (mitDNA) is controlled in the eukaryotic cell, and one important aspect of this control concerns the timing of mitDNA synthesis in the cell cycle. Evidence from a number of systems shows that mitochondrial and nuclear DNA are not necessarily replicated simultaneously and may have quite different synthesis curves. Although nuclear DNA is almost invariably synthetized discontinuously in the eukaryotic cell cycle (i.e. during the S-period), mitDNA synthesis has been reported to be continuous for Tetrahymena[1, 2] and Physarum[3], but discontinuous (and outside the S-period) for the yeast Saccharomyces lactis[4].

The data reported here show that in the case of Saccharomyces cerevisiae synthesis of mitDNA continues throughout the cell cycle and, in the synchronised cultures used for this study, at a rate which increases with time.

MATERIALS AND METHODS

Synchronously dividing cultures[5] of Saccharomyces cerevisiae (strain NCYC 239) in a semi-defined medium[6] with 2 % glucose were incubated with aeration at 25°C and sampled at intervals for cell counting (Coulter

counter, using suitable ultrasonic treatment to separate divided pairs) and diphenylamine colorimetric analysis of total DNA[7]. Samples (ca $2x10^8$ cells) were also removed for estimation of mitDNA and nuclear DNA as described elsewhere[8]. This procedure essentially involved making detergent extracts of cells crushed in an Eaton press[9], eliminating much UV-absorbing material by centrifugation, and analysing the DNA components by analytical isopycnic centrifugation in CsCl. UV-absorption photographs of the gradients (taken with attention to the conditions for quantitative photography[10]) were scanned with a Joyce-Loebel photoelectric densitometer and the areas under the peaks of the mitochondrial and nuclear components determined planimetrically. The absolute amount of each component in the gradients was calculated with reference to measured amounts of M. lysodeikticus DNA added to the extracts just prior to centrifugation. Two dilutions of each sample were examined, differing by a factor of 3-6, the dilute one being used for estimation of the nuclear DNA, the concentrated one for the mitochondrial component.

Changes in the rate of incorporation of exogenously added adenine into the two separate DNA components were examined by pulse labelling. Culture samples of 100 ml were taken at intervals and incubated at 25° C for 30 minutes in the presence of 10 μC/ml ^{3}H-adenine (spec. act. ca 2 Ci/mM). The cells were then rapidly cooled to 4° C, harvested on a membrane filter (Millipore), resuspended and frozen in "crushing fluid"[8] prior to crushing and extraction of the DNA[8]. Preparative CsCl gradients were prepared in 5 ml buckets (Spinco SW39) by centrufiging at 33,000 rpm for 60 hrs at 20° C. The tubes were punctured and about 80 fractions per tube collected. These were diluted by adding 0.5 ml saline/EDTA (0.2 M NaCl, 0.005 M EDTA, pH 8.0), and their radioactive DNA contents assayed by scintillation counting following a conventional alkaline-digestion/acid-precipitation routine. Appropriate fractions were also examined by analytical isopycnic centrifugation in CsCl to determine the amounts of the two DNA components. This permitted unambiguous determination of the specific radioactivities of each DNA component, since it was possible to correct for any contamination of the mitochondrial satellite by nuclear DNA.

RESULTS AND DISCUSSION

The UV-absorption photographs of the analytical CsCl gradient preparations of both log phase and synchronized, resting cells (Fig. 1) show that our procedure gave good separation of the two DNA components, facilitating accurate estimation. It will also be noted that the minor dense nuclear satellite was apparently absent from the synchronized cells.

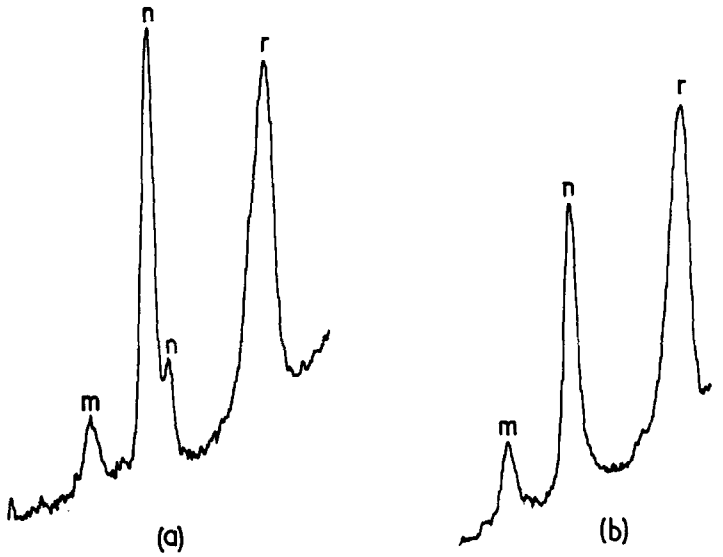

Fig. 1. Densitometer records of analytical isopycnic CsCl gradients of DNA from a) log phase cell, b) resting, synchronized cells : m, mitDNA ; n, nuclear DNA ; r, reference DNA (M. lysodeikticus, density 1.731 g/cc).

The conditions giving rise to this loss are under investigation.

The results from two typical synchronized cultures are shown in Fig. 2. The essential feature is that whereas nuclear DNA was synthetized discontinuously, in about the first quarter of the cell cycle[11], mitochondrial DNA synthesis started during the initial lag period and continued at an increasing rate throughout the subsequent cell cycles including both the S and non-S periods. The CsCl gradient technique used here gives a total DNA recovery of only 60-80%. However, this loss seems to be relatively constant for every sample ; this is shown by the reasonably close fit of the normalised ultracentrifuge data for total DNA with that obtained by diphenylamine analysis (Fig. 2a). This also rules out the possibility that the proportional recovery of the main nuclear component in the ultracentrifuge varied at different stages of the cell cycle. In the case of the mitochondrial DNA, this type of variable (and selective) recovery seems extremely unlikely, in view of the smooth curves in Fig. 2.

In any case the overall pattern of mitDNA synthesis observed in Fig. 2 was confirmed by the data presented in Fig. 3, which shows the rate of incorporation of adenine into the two components at different stages of the culture's growth. Whereas incorporation into nuclear DNA was high during the S periods and minimal at other times, incorporation into mitDNA started

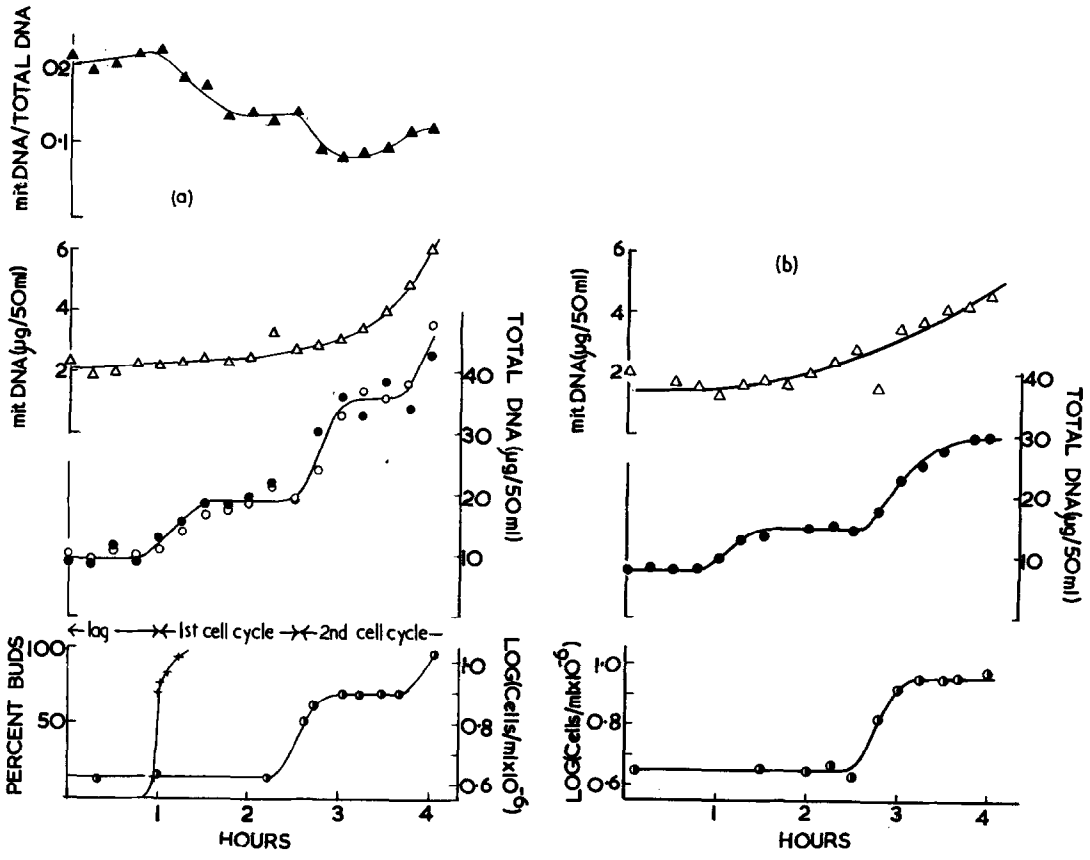

Fig. 2. Nuclear and mitochondrial DNA replication in two synchronously dividing cultures : +—+, per cent young buds : ●–●, cell counts : ●–● , total DNA by diphenylamine analysis : o–o , total DNA obtained by adding separate data for nuclear and mitochondrial DNA provided by the analytical CsCl method : △–△, mitochondrial DNA : ▲–▲, mitDNA/ total DNA. In (a) the ultracentrifuge data for total DNA have been normalised by adjusting the average of the first three points to fit that of the first three diphenylamine points. In (b) the lag phase level of mitDNA was assumed to be about 20% of the total DNA.

during the initial lag period and continued thereafter at a rate which increased in each subsequent labelling period. With the reservation that unknown changes in precursor pools may have affected the pattern observed here, it is nevertheless obvious that these data are entirely consistent with those of Fig. 2.

In interpreting these results, the possibility must be considered that the synchronizing procedure (involving feeding and starving resting cells) might have grossly distorted the "normal" pattern and timing of mitDNA synthesis. This cannot be positively ruled out, but seems unlikely

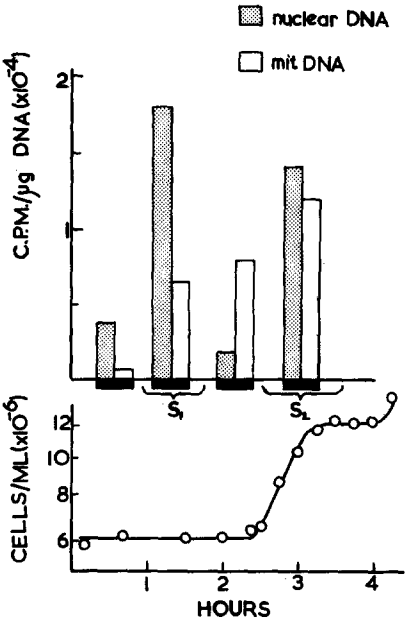

Fig. 3. Specific radioactivities of nuclear and mitochondrial DNA obtained on pulse labelling with ^{3}H-adenine at different times in a synchronously dividing culture. The blacks bars indicate the durations of the pulses, and the periods occupied by the first and second S periods (determined by separate diphenylamine analyses) are indicated.

since there is good evidence that other synthetic patterns[12], including that of nuclear DNA[11], are not thus distorted. Certainly the failure of mitDNA to double during the first cell cycle, with a consequent decline in the average mitDNA/nuclear DNA ratio (Fig. 2)+, was not an artefact of synchronization ; a similar lack of balance in the overall amount of synthesis is seen in several other parameters in this situation[12] and stems from the adjustment of the initially resting cells to the new conditions of growth in fresh medium. In any case an exactly comparable decline in mitDNA content was observed when unsynchronized resting cells were inoculated into growth medium[13]. In the latter case a compensatory increase in the relative rate of mitDNA synthesis occured after a few hours, and the start of what may be the same regulatory increase in rate can be seen towards the end of the period of observation in Fig. 2a. In this light it is clear that the increasing rate of mitDNA synthesis seen

+ This is more marked in Fig. 2a than in Fig. 2b, where in any case, synthesis of mitDNA seemed to start rather later . This illustrates the variability in this respect of different cultures.

in Figs 2 and 3 is not artefactual, though we cannot predict the shape of the synthesis curve in the case of cells growing in a balanced way. However, if as in the case of bacteria[14], the DNA polymerase travels at a constant rate along the DNA molecule, our results suggest that initiation of mitDNA replication takes place throughout the cell cycle and there is no reason to suppose that this is not also the case in the individual cells in balanced growth. However, it should be made clear that these results do not absolutely preclude the possibility that different individual cells might undergo discontinuous mitDNA replication at different random points in the cell cycle. Nor, for that matter, do they shed light on the time course of replication within the individual mitochondrion. Study of these aspects requires the development of techniques applicable to individual cells and mitochondria.

Such difficulties do not apply in the case of the strikingly different discontinuous pattern of mitDNA synthesis reported by Smith *et al.*[4] in synchronous cultures of *Saccharomyces lactis*. At first sight the difference between this yeast and ours is rather surprising and it would therefore be valuable if the observation of Smith *et al.* could be confirmed by an independent approach such as pulse labelling. However, it may be relevant that *Saccharomyces lactis*, which does not interbreed with *Sacch. cerevisiae*[15], is now considered to belong to another genus[16,17]. It may therefore be less closely related to *Saccharomyces* than hitherto supposed, and may regulate its mitDNA differently.

REFERENCES

1. Parsons, J. A., and Rustad, R. C., J. Cell Biol., 37, 683 (1968).
2. Charret, R., and André, J., J. Cell Biol., 39, 369 (1968).
3. Braun, R., and Evans, T. E., Biochim. Biophys. Acta, 182, 511 (1969).
4. Smith, D., Tauro, P., Schweizer, E., and Halvorson, H. O., Proc. Natl Acad. Sci. U.S., 60, 936 (1968).
5. Williamson, D. H., and Scopes, A. W., Nature, 193, 256 (1962).
6. Scopes, A. W., and Williamson, D. H., Exp. Cell Res., 35, 361 (1964).
7. Williamson, D. H., and Scopes, A. W., Exp. Cell Res., 20, 338 (1960).
8. Williamson, D. H., Moustacchi, E., and Fennell, D. (1971). In preparation.
9. Eaton, N. R., J. Bacteriol., 83, 1359 (1962).
10. Vinograd, J., and Hearst, J. E. In Fortschritte der Chemie organischer Naturstoffe, 22 (ed. L. Zechmeister), Springer-Verlag, Vienna, p. 372 (1962).
11. Williamson, D. H., J. Cell Biol., 25, 517 (1965).
12. Williamson, D. H. In Synchrony in Cell Division and Growth (ed. E. Zeuthen). John Wiley and Sons, Inc., New York, p. 351 (1964).
13. Williamson, D. H. In Control of Organelle Development : 24th Symp. Soc. Exp. Biol. London (ed. P. L. Miller). University Press, Cambridge, p. 247 (1970).

14. Pritchard, R. H., and Zaritsky, A., Nature, 226, 126 (1970).
15. Mortimer, R. K., and Hawthorne, C. D., Ann. Rev. Microbiol., 20, 151 (1966).
16. Wickerham, L. J., Nature, 176, 22 (1955).
17. van der Walt, J. P., Antonie van Leeuenhoek, J. Microbial Serol;, 31, 341 (1965).