INDUCTION OF RESPIRATORY INCOMPETENT MUTANTS BY UNSATURATED FATTY ACID DEPLETION IN SACCHAROMYES CEREVISIAE

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SUMMARY: Constant levels of cellular unsaturated fatty acids were obtained by growing a fatty acid desaturase mutant of Saccharomyces cerevisiae in glucose limited chemostat cultures supplemented with various concentrations of Tween 80. An increase in the frequency of cytoplasmic respiratory incompetent mutants was observed in cultures growing at low cellular levels of unsaturated fatty acids. This effect has been shown to result from an increase in the rate of mutation as the cellular unsaturated fatty acid level is decreased. The majority of induced petite mutants are ρ° (contain no mitochondrial DNA).

We have previously reported that in Saccharomyces cerevisiae the unsaturated fatty acid content of mitochondrial membranes has a profound effect on oxidative phosphorylation (1-3), mitochondrial protein synthesis (4) and a number of other membrane associated mitochondrial functions (4). This communication is concerned with the effect of alteration of cellular lipid composition on maintenance of the respiratory competent state in Saccharomyces cerevisiae.

As in the previous studies we have used the unsaturated fatty acid requiring mutant KD115 (5) to manipulate the cellular unsaturated fatty acid level.

We report our finding that growth of S. cerevisiae at low cellular unsaturated fatty acid levels leads to the induction of respiratory incompetent cytoplasmic petite mutants and that the rate of induction is determined by the degree of unsaturated fatty acid depletion.

RESULTS AND DISCUSSION

Yeast cells with low unsaturated fatty acid levels have previously been obtained by growth of KD115 (ole$^-$) in batch culture with limiting amounts of Tween 80 as a source of unsaturated fatty acids (1-3). However, under these conditions the cellular unsaturated fatty acid level changes continually during growth and the extent of growth is limited by the amount of exogenous unsaturated fatty acid supplied.

In order to study the effects of growth at constant reduced unsaturated fatty acid levels we have used glucose limited chemostat cultures of KD115 supplemented with various concentrations of Tween 80. Under these conditions a constant cellular unsaturated fatty acid level can be maintained for several generations of growth in the steady state, as illustrated in Figure 1

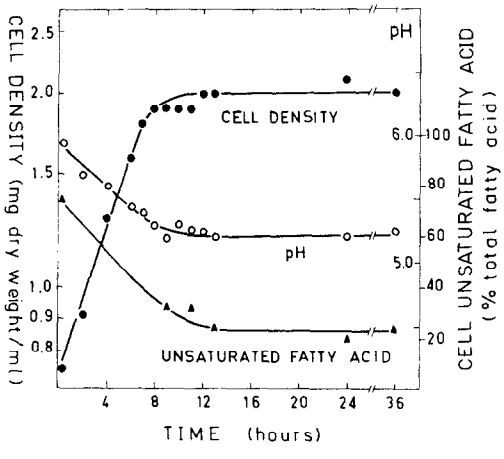

Figure 1. Maintenance of a Constant Cellular Unsaturated Fatty Acid Level by Growth of KD115 in Glucose Limited Chemostat Culture.

Saccharomyces cerevisiae KD115 was grown aerobically at 30°C in a 350 ml chemostat culture (6). The medium in the reservoir contained salts [$(NH_4)_2SO_4$ 1.2 g/l; KH_2PO_4 1 g/l; NaCl 0.5 g/l; $MgCl_2$ 0.7 g/l; $CaCl_2$ 0.1 g/l; $FeCl_3$ 0.005 g/l)] yeast extract 10 g/l, glucose 20 g/l, and Tween 80 200 µg/ml as source of unsaturated fatty acids. Samples were taken at intervals to determine the cell density (2), pH and cellular unsaturated fatty acid content. Fatty acid analysis was performed as described previously (5). Unsaturated fatty acid content is expressed as a percentage of the total fatty acid.

for a culture supplemented with 200 µg/ml Tween 80. The steady state cellular unsaturated fatty acid level can be varied from about 75%, the physiological level for aerobically grown Saccharomyces cerevisiae (2), to as low as 12% by altering the concentration of Tween 80 in the inflowing medium. The concentrations of Tween 80 used and the unsaturated fatty acid levels obtained are included in Table 1. The rate of accumulation of respiratory incompetent (petite) mutants during growth at a number of cellular unsaturated fatty acid levels are shown in Figure 2. The frequency of petite

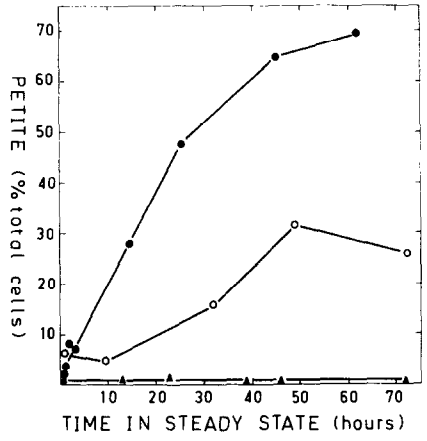

Figure 2. Induction of Petite Mutants by Unsaturated Fatty Acid Depletion.
Cells were grown in chemostats as described in Fig. 1. The media contained 10 g/l of glucose. Tween 80 supplements were 4,000, 250 and 200 µg/ml to obtain steady state cellular unsaturated fatty acid levels of 75%, 29% and 21% respectively. Samples were taken at intervals to determine the cell density, pH, unsaturated fatty acid and petite frequency. The time when the pH and cell density first reached the steady state level was taken as zero time, although the cultures show small fluctuations in pH and cell density before reaching the true steady state. All cultures were routinely checked for revertants and only cultures containing less than 0.1% OLE revertants were used. The frequency of petites was scored by plating on media containing salts (Fig. 1), yeast extract 2 g/l; glucose 1 g/l; glycerol 20 g/l; Tween 80 5 g/l and agar 20 g/l. Colonies of less than 0.5 mm diameter, after 4-5 days growth at 30°, were scored as petite. No difference was observed when the frequency of petites was scored by the tetrazolium overlay technique (7). The petite frequency in cultures growing at steady state unsaturated fatty acid levels of 75% (▲), 29% (○), and 21% (●) are shown.

mutants remained constant at approximately 1% in a culture supplemented with 5,000 µg/ml Tween 80 where the steady state cellular unsaturated fatty acid level was 75%. However, when the unsaturated fatty acid level was maintained at steady state levels of 29% or 21% a rapid increase in the frequency of petites was observed. After several generations constant levels of about 30% and 70% petites respectively were established.

The increase in the frequency of petite mutants observed during growth at reduced cellular unsaturated fatty acid levels could result from either an increase in the frequency of mutation to respiratory incompetence or from a selective advantage of petite mutants over wild type at low unsaturated fatty acid levels.

We have used a mathematical approach to resolve this question. Equations describing the accumulation of mutants in chemostat cultures of bacteria (8) have been derived assuming that the number of mutant cells in the culture and the rate of mutation are extremely small. Since these assumptions are not valid in this case, we have derived equations using no assumptions: the derivation of the equations will be published elsewhere (9). Using these equations we have calculated that the rate of increase of petite mutants observed in the culture supplemented with 200 µg/ml of Tween 80 (steady state unsaturated fatty acid level 21%) cannot be accounted for solely by selective advantage of petite over wild-type cells at low unsaturated fatty acid levels. The minimum value for the mutation rate compatible with the rate of increase in petite cells observed in this culture is 0.3 mutants/cell/generation. The maximum value for the mutation rate, in the culture supplemented with 5,000 µg/ml of Tween 80, where no increase in the frequency of petite mutants was observed, is 0.01 mutants/cell/generation. Therefore, the rate of mutation to

respiratory incompetence is at least 30 fold greater during growth of cells at an unsaturated fatty acid level of 21% than at an unsaturated fatty acid level of 75%.

The final level of petites attained in cultures growing at steady state unsaturated fatty acid levels ranging from 12% to 72% are shown in Table 1. The final frequency of petites increases as the unsaturated fatty acid level is decreased. Since the

Table 1. The Relationship Between the Steady State Cellular Unsaturated Fatty Acid Level and the Final Petite Frequency in Chemostat Cultures of KD115.

	Tween 80 Supplement (µg/ml)						
	100	250	350	500	1000	2000	5000
STEADY STATE* CELLULAR UFA (% TOTAL FA)	12	29	35	46	50	60	72
FINAL PETITE* LEVEL (% TOTAL CELLS)	80	30	15	6	2.3	0.5	0.4

* The figures used in this table are results of single typical experiments. Growth conditions were as in Figure 2.

steady state level of a mutant in a chemostat culture is a function of the rate of mutation, we propose that the rate of induction of petite cells during growth at low cellular unsaturated fatty acid levels is determined by the degree of unsaturated fatty acid depletion.

We have genetically analysed 40 petite mutants isolated from cultures grown at low unsaturated fatty acid levels and all of these were found to be cytoplasmic petite mutants.

The results presented demonstrate that alteration of cellular lipid composition leads to induction of cytoplasmic petite mutants, which implicates the involvement of at least one membrane associated process in the maintainance of yeast mitochondrial DNA. This is consistent with the hypothesis that mitochondrial DNA synthesis, replicative and/or repair, is a membrane associated process either due to an obligatory attachment of mitochondrial DNA to the mitochondrial membrane or a functional association of the DNA synthetic complex with the membrane. However, 8 of 10 induced petite mutants so far tested have been found to contain no detectable mitochondrial DNA (i.e. ρ^0) which is more consistent with a defect in replicative rather than repair synthesis. It is interesting to note that results of investigations of the effects of a number of agents and conditions on ethidium bromide mutagenesis have been interpreted as consistent with a model which assigns a key role to the inner membrane in the mutagenic process (for review see 10).

A second possibility to account for the observed defect in DNA synthesis during growth at low unsaturated fatty acid levels is that the disruption of some other process, such as maintenance of intra-mitochondrial ATP levels, has an indirect effect on mitochondrial DNA synthesis. Indeed, induction of respiratory deficient mutants has been observed when intramitochondrial ATP synthesis and transport of ATP into the mitochondria from the cytosol are inhibited simultaneously (11). Unsaturated fatty acid depletion is known to uncouple oxidative phosphorylation (1,2) and if the mitochondrial ATP transporter is similarly affected, unsaturated fatty acid depletion might result in a depletion of intra-mitochondrial ATP levels and consequently petite induction.

Further investigations to distinguish these two possibilities are currently in progress.

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