SHORT COMMUNICATION

Changes in the Lipid Composition of *Candida utilis* during the Cell Cycle

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Although contents of triacylglycerols, free sterols and esterified sterols did not alter during the 3·5 h cell cycle of *Candida utilis* NRRL Y-900 growing synchronously under glucose limitation, changes were detected in the phospholipid content. Phospholipid:sterol molar ratios were lowest in organisms sampled after 2·5 h and highest in those analysed after 0·5 h of the cycle.

INTRODUCTION

Synchronously dividing cultures of budding and fission yeasts have been extensively used to study patterns of synthesis of cell constituents during the cell cycle in eukaryotes (Mitchison, 1971). Most of the techniques used to obtain synchronously dividing cultures produce relatively small populations of organisms and this has been an impediment to studies, such as following changes in lipid composition (Ratnayake *et al.*, 1975), which require larger quantities of cell material. Dawson & Craig (1966) were the first to report on changes in lipid composition during a yeast cell cycle, when they showed that, in nitrogen-limited *Candida utilis*, oscillations occurred in the contents of the major fatty-acyl residues. Later, Penman & Duffus (1976) described a continuous and exponential pattern of accumulation of total ergosterol (free and esterified) and phospholipids during the cell cycle of *Kluyveromyces fragilis*. More recently, Čejková & Jirků (1978) claimed that cell division in a strain of *Saccharomyces cerevisiae* induced an increase in sterol content, although these workers too did not separate free and esterified sterols. The present paper reports further changes in lipid composition during the cell cycle of the strain of *C. utilis* used by Dawson & Craig (1966). By using an automatic phasing technique for obtaining synchronously dividing cultures, large crops of organisms could be analysed. The study reports for the first time the fate of all of the major classes of lipid during a yeast cell cycle.

METHODS

Experimental cultures. *Candida utilis* NRRL Y-900 (ATCC 9950) was maintained on slopes of malt extract/yeast extract/glucose/peptone/agar (Wickerham, 1951). Synchronously dividing organisms were grown using the automatic phasing technique, first described for yeasts by Dawson (1965) and since used also with bacteria (Anagnostopoulos, 1971). The phasing technique involves repeated addition of growth medium to an equal volume of continuous culture at time intervals corresponding to the doubling time

of the population. After several additions of medium, approximately 80 % of the organisms in the culture are dividing synchronously. A 3 l cyclone fermenter, with an operating volume of 900 ml, was used as described by Dawson (1968) except that the culture was halved after addition of fresh medium. The yeast was grown under conditions of glucose limitation using a medium with the following composition: glucose, 55.5 mM; $(\text{NH}_4)_2\text{SO}_4$, 18.9 mM; CaCl_2 , 0.4 mM; FeCl_3 , 200 μM ; EDTA, 200 μM ; MnCl_2 , 20 mM; CuCl_2 , 16 μM ; ZnCl_2 , 15 μM ; H_3BO_3 , 24 μM ; KI, 1.98 μM ; CoCl_2 , 1.68 μM ; and Na_2MoO_4 , 1.65 μM . Cultures were grown at 30 °C and supplied with air at the rate of 200 ml min⁻¹. Organisms were harvested by filtration on Millipore (0.45 μm) membrane filters at 0 °C. Batches of organisms were harvested at 0.5 h intervals throughout the 3.5 h cell cycle. They were washed twice with ice-cold distilled water, removed from the filter by shaking in ice-cold water and the suspension was decanted and freeze-dried.

Lipid analyses. Lipids were extracted from freeze-dried cells as described by Hossack & Rose (1976). Classes of lipid were separated by quantitative thin-layer chromatography and analysed as previously described (Hossack & Rose, 1976). Phospholipid was determined by assaying the phosphorus content of the extract, using the method of Chen *et al.* (1956). Separation of lipids and derivatives by gas-liquid chromatography (g.l.c.) was also carried out as described previously (Hossack & Rose, 1976). Peak areas on g.l.c. traces were measured, and the relative amounts of compounds were calculated by the method of Pecsok (1959). In calculating phospholipid:sterol molar ratios, an average molecular weight of 397 was assumed for sterol (ergosterol) and of 778 for phospholipid (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine).

RESULTS AND DISCUSSION

Contents of neutral lipids (triacylglycerols and esterified sterols) and free sterols showed no significant differences throughout the cell cycle. However, the phospholipid content varied throughout the cycle, and contents in organisms harvested after 2.5 and 3.0 h were significantly different from those in organisms harvested at other times (Table 1). The results were analysed statistically by treating the experiment as a two-factor experiment assuming no interaction. This assumption is reasonable since the pattern of change in phospholipid content with time was the same for each of the three batches analysed. Calculations were made of the least significant difference, at the 5 % level. The absence of variation in the contents of free and esterified sterols throughout the cycle is in agreement with the report by Penman & Duffus (1976) of a continuous and exponential increase in the ergosterol content of *K. fragilis*; in their hands, ergosterol is the only sterol detectable in this yeast (Penman & Duffus, 1974). In contrast, our results are at variance with the large differences in total sterol content reported in the cell cycle of *S. cerevisiae* (Čejková & Jirků, 1978). However, the Czech workers, although reporting analyses at only three times, described twofold differences in the total phospholipid content during the cell cycle of *S. cerevisiae*.

Nuclear magnetic and electron spin resonance studies on lipid mixtures have suggested that the presence of sterols in a phospholipid bilayer stabilizes the bilayer. Phillips and his colleagues (Phillips, 1972; Phillips & Finer, 1974) concluded that, when a sterol molecule lies alongside a phospholipid molecule with the polar group of the phospholipid juxtaposed with the hydroxyl group of the sterol, the first eight to nine methylene groups of the fatty-acyl chains on the phospholipid are constrained by the nucleus of the sterol molecule. Moreover, Rothman & Engelman (1972) have shown that the stabilizing effects of sterols are powerful even in dispersions that contain a high proportion of phospholipid to sterol. In the G2 stage in the cell cycle of *C. utilis*, where organisms contain a lower phospholipid:sterol molar ratio, nuclear division and separation have taken place and mitochondria have become distributed between mother and daughter cells. While our data do not indicate which cell membranes undergo changes in sterol content in the cell cycle, it is tempting to suggest that they occur in the plasma membrane. Conceivably, there is a need for a more rigid and stabilized plasma membrane in domains where the cross septum and bud scar are about to be formed. Another way in which the plasma membrane might be stabilized is by an increase in the proportion of saturated fatty-acyl residues in the phospholipids. However, the fatty-acyl composition of lipids from *C. utilis* remained

Table 1. *Changes in the lipid composition of Candida utilis NRRL Y-900 during the cell cycle*

Lipid	Content* [mg (mg dry wt equiv.) ⁻¹] of organisms harvested at:							
	0.0 h	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	3.5 h
Phospholipids	4.19 ± 1.33	4.14 ± 1.53	3.84 ± 0.43	4.06 ± 0.75	3.82 ± 0.55	3.10 ± 0.86	3.02 ± 0.76	4.24 ± 0.80
Triacylglycerols	0.63 ± 0.52	0.71 ± 0.22	0.94 ± 0.51	0.82 ± 0.93	0.94 ± 0.32	0.92 ± 0.93	0.81 ± 0.34	1.03 ± 1.12
Free sterols	0.27 ± 0.11	0.27 ± 0.07	0.24 ± 0.17	0.25 ± 0.12	0.25 ± 0.06	0.28 ± 0.04	0.26 ± 0.26	0.26 ± 0.04
Esterified sterols	0.01 ± 0.03	0.03 ± 0.06	0.03 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.09	0.04 ± 0.07	0.03 ± 0.02
Phospholipid:sterol molar ratio	8.16	8.51	8.46	8.17	8.40	5.73	6.03	8.20

* Values quoted are averages of triplicate analyses on each of three batches of organisms, ± 95 % confidence limits.

remarkably uniform throughout the cell cycle, the main components being residues of C_{16:0}, C_{18:1}, C_{18:2} and C_{18:3} acids. Interestingly, when *C. utilis* NRRL Y-900 was grown under conditions of nitrogen limitation, with the automatic phasing technique used in the present study, changes were observed in the fatty-acyl composition of cell lipids during the cell cycle (Dawson & Craig, 1966).

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