

Calcium uptake during the cell cycle of *Saccharomyces cerevisiae*

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Synchronous culture of the budding yeast *Saccharomyces cerevisiae* was obtained by sucrose density gradient selection with 90–100% of yeast synchronized by using the cells in the bottom. In these adult cells bud emergence is coincident with an increase in calcium uptake at 100 min of the culture, followed by a return to basal values which are maintained until the end of the first cell cycle of study. The phenothiazine derivatives, trifluoperazine and chlorpromazine inhibit bud emergence and trifluoperazine also increases calcium uptake.

Calcium Uptake Cell cycle Yeast Trifluoperazine

1. INTRODUCTION

Calcium is an important metabolic regulator in a wide variety of cell types. The relationship between cell cycle and calcium uptake has been discussed for several types of cells such as mouse fibroblasts [1,2], *Physarum polycephalum* [3] and *Tetrahymena* [4].

In systems like *Schizosaccharomyces pombe* addition of the chelating agent EDTA to the culture medium inhibits cell division, and can be used to synchronize the cells [5]; in these cells accumulation of divalent ions within the cell were an essential prerequisite for cell division [6].

In *Saccharomyces cerevisiae* initiation of budding is coincident with initiation of S phase, in which the future daughter cell appears like a discrete morphologic entity [7–10]. In this yeast the role of calcium during the cell division cycle is not well documented. This report aimed to study how calcium uptake is associated with budding in a synchronous yeast culture, and the effect of the phenothiazine derivatives trifluoperazine (TFP) and chlorpromazine (CPZ) on both phenomena.

Abbreviations: MES, 2-*N*-morpholinoethane sulfonic acid; TEA, triethanolamine; TFP, trifluoperazine; CPZ, chlorpromazine

The results show that there is an increase in calcium uptake when yeast cells start budding, followed by a return to basal values which are maintained until the end of the first cell cycle. The phenothiazine derivatives trifluoperazine and chlorpromazine inhibit bud emergence and trifluoperazine also increase calcium uptake.

2. MATERIALS AND METHODS

Bakers' yeast was obtained from commercial source and grown in liquid minimal medium (1% dextrose, 0.2% yeast extract at 30°C with shaking) for 20–24 h. The cells in exponential growth were centrifuged and washed twice with distilled water. Adult cells were isolated by sucrose density gradient selection as in [11] for the purpose of synchronizing them, then placed in fresh culture medium at 10^7 – 10^9 cells/ml. Synchronized cells were scanned throughout one cell cycle (200 min) under the microscope by observation of bud emergence as described [12]. Samples were taken every 20 min and subjected to the following procedures:

- (i) **Bud emergence:** cells were counted in a haemocytometer after appropriate dilution.
- (ii) **calcium uptake:** $100\mu\text{l}$ cells were preincubated in a 20 mM MES–TEA buffer (pH 6.0) and 100 mM dextrose; after 3 min, $^{45}\text{CaCl}_2$ ($100\mu\text{M}$)

was added and incubated for another 3 min, then cells were filtered through a Sartorius membrane (0.45 μ m pore size) and rinsed with 15 ml 20 mM CaCl_2 as in [13]. Radioactivity was measured by placing the filters in a Triton-based liquid cocktail [14] and counting in a Packard spectrometer.

Trifluoperazine and chlorpromazine (100 μ M) were added at the start of the experiment.

Chemicals used were obtained as follows: dextrose and yeast extract from Bioxon; MES, TEA and CPZ from Sigma; TFP was a gift from Dr García-Sáinz and $^{45}\text{CaCl}_2$ from NEN.

3. RESULTS

Synchronous cultures of *Saccharomyces cerevisiae* obtained by sucrose density gradient selection [11] show that between 90–100% of the cells are unbudded as observed in fig.1. These adult cells have a lag phase of 100 min in a fresh culture medium, then initiate budding. This process is coincident with an increase in calcium uptake with a maximum level at 100 min, then a return to basal values which are maintained until the end of the first cell cycle; synchrony showed by these cells was lost between the first and second cell division cycles (not shown) and our results are in agreement with the model proposed in [15] in which cell division of yeast generates cells of unequal size with daughter cells smaller than parent cells. As mentioned above,

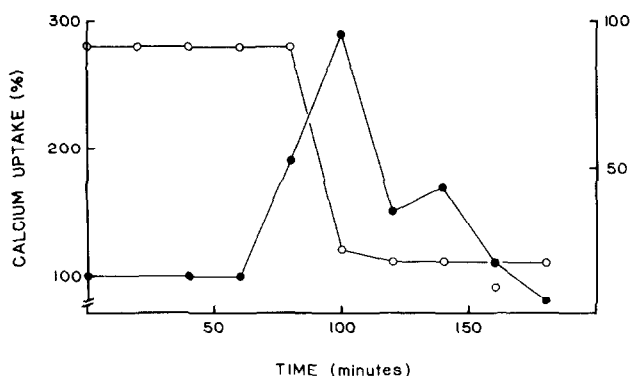


Fig.1. Calcium uptake during the cell cycle of yeast. Synchronous yeast cells were obtained as in section 2. Bud emergence (—○—) was observed under the microscope and calcium uptake (—●—) was measured as in [15]. Basal value of calcium uptake was 3000 cpm. Results are the mean of 3 separate expt.

budding and initiation of S phase are coincident in yeast [7–10] suggesting a role of calcium in this process. These events are in agreement with mastocytoma P-815 cells in which calcium uptake fluctuated during the cell cycle reaching the highest level at the middle of the S phase [16].

Fig.2 shows the effect of 100 μ M trifluoperazine on calcium uptake by synchronous cultures of the yeast *Saccharomyces cerevisiae*. As observed TFP increases calcium uptake by the cells throughout the cell cycle. The maximal effect was reached at 100 min of the culture followed by a decrease but at all times tested calcium entry was higher than in control cells. Besides these cells remain unbudded.

Fig.3 shows the effect of phenothiazine derivatives on synchronous yeasts. 100 μ M trifluoperazine and 100 μ M chlorpromazine added to the culture medium of synchronous yeast inhibit bud emergence; this inhibition is permanent both with TFP and CPZ. However, when the phenothiazines were added to the culture medium containing early budded cells both TFP and CPZ had no effect throughout the cell cycle (not shown): Using mastocytoma P-815 cells, addition of EGTA before the S phase almost completely suppressed the subsequent cell division while EGTA added at the beginning of the

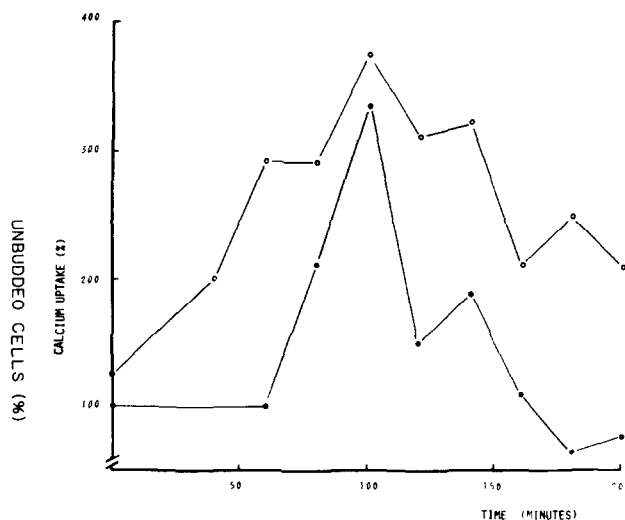


Fig.2. Effect of trifluoperazine on calcium uptake during the cell cycle of yeast. Trifluoperazine was added at a final concentration of 100 μ M. Other conditions as in fig.1. The results are the mean of 2 separate expt: (—●—) calcium uptake; (—○—) calcium uptake in the presence of TFP.

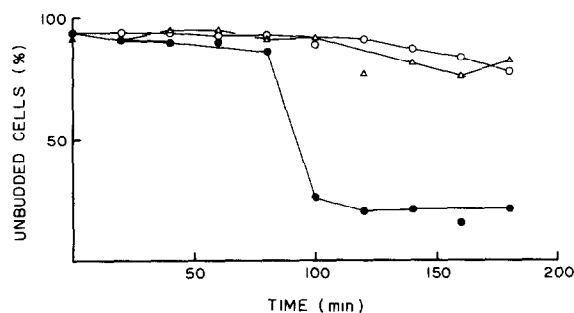


Fig.3. Effect of phenothiazine derivatives on the cell cycle of yeast. Trifluoperazine (—○—) and chlorpromazine (—△—) were added at a final concentration of 100 μ M. Other conditions as in fig.1. The results are the mean of 3 separate expt; control cells (—●—).

G2 or M phase did not affect the normal cell cycle [4].

4. DISCUSSION

The effect of calcium uptake on the cell cycle has been well explored in synchronized mammalian transformed cells [1,2], *Physarum polycephalum* [3] and *Tetrahymena* [4], but not in *Saccharomyces cerevisiae*. We have demonstrated here that synchronized yeast cells present increased calcium uptake when they start budding, then the uptake returns to basal values which are maintained until the end of the first cell cycle.

Our data (fig.1) agree with results obtained with a wide variety of types of synchronized cells [1–4] in which an increase in calcium uptake is observed in the early S phase before cells enter in mitosis. Results obtained when TFP (a calmodulin inhibitor) was present in the culture medium show an increase in calcium entry (fig.2) as compared with control at all times tested. These data suggest that calcium concentration is a 'trigger' for cell division, but after this starting point, ion levels must reach the basal levels to assure continuous budding (fig.2); if this is not attained the cells do not bud as observed in fig.3. However, when asynchronous cultures of *Tetrahymena* were studied, the authors argued against the 'trigger' role of calcium in cell division, due to the observation that increased calcium uptake is halved in the daughter cells [4]. But results obtained with synchronized yeasts disagree with this interpretation of Walker and Zeuthen [4] for *Tetrahymena* because the budding process in yeasts is a time consuming event (approximately

100 min) and the increase in calcium uptake at a specific step of the cycle, can not be explained by the fact that there are two daughter cells as it is proposed for *Tetrahymena* [4].

On the another hand, the inhibitory effect of TFP and CPZ on budding process (fig.3) suggests a possible role of calmodulin in S phase as has been pointed out in [17] in hamster ovary cells. This could also happen in this case as there is strong evidence that calmodulin is present in *Candida albicans* and *Saccharomyces cerevisiae* [18].

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