The possible functional significance of phosphatidylinositol in G_1 arrest of *Saccharomyces cerevisiae*

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Individual phospholipids were assayed in exponentially growing and G_1-arrested temperature-sensitive cell division cycle (*cdc*) mutants of *Saccharomyces cerevisiae*. It was observed that *cdc*28 cells which are known to arrest at 'start' when shifted to their non-permissive temperature, resulted in a 40% decrease in phosphatidylinositol (PI) level while the phosphatidylserine (PS) content was doubled in these cells. The reduced level of PI was restored in *cdc*4 and *cdc*7 mutants which are known to arrest past the 'start'. The increase in PS level in *cdc*28 mutant which was probably to compensate the intrinsic charging of membrane environment, was also reduced in *cdc*4 and *cdc*7 mutants. Our results demonstrate that PI may play a role in yeast cell division and growth and that the abnormalities of *cdc*28 could also be related to PI decrease.

Cell cycle *G_1 arrest* *Cell division cycle mutants* *Growth control* *Phosphatidylinositol* Saccharomyces cerevisiae

1. INTRODUCTION

The control regarding commitment to the cell division cycle of eukaryotes seems to occur before the initiation of DNA synthesis (G_1) [1]. The controlling point in *Saccharomyces cerevisiae* is termed as 'start' [1]. A cell which has passed 'start' is committed to cell division and is unable to undergo alternative developmental fates [1,2]. Evidence is accumulating to demonstrate the role of nutrient transport, enzyme activities, etc., in committing a cell to the mitotic cycle [3]. In the last few years, analysis of phospholipids during the cell cycle has come under the investigative attack to suggest that they may be involved in cell growth and division. Among various phospholipids, PI plays an important role in overall cell growth and division of eukaryotic organisms [4–6]. Using *S. cerevisiae*, we have for the first time shown the functional involvement of PI in the controlling phase (G_1) which precedes DNA synthesis. It is shown that *cdc* 28 which selectively arrests at 'start' under non-permissive conditions [2], has 40% less PI as compared to cells growing under permissive conditions. The level of PI is shown to sequentially recover after the cells have traversed the 'start'.

2. MATERIALS AND METHODS

Various *cdc* mutants, e.g., H.185.3.4 (*cdc* 28-1), H.135.1.1 (*cdc* 4-3), 4008 (*cdc* 7-4) and their wild-type strain A364A were obtained from Yeast Genetic Stock Centre (Berkeley CA). The growth conditions and maintenance of all these strains have been described in [7]. All the strains were grown in YEPD medium at 23°C except A364A which was grown and maintained at 30°C. Temperature sensitive (Ts) mutants were synchronized by shifting them to their restrictive temperatures of 38°C in the case of *cdc* 28 and *cdc* 7 and 36°C in case of *cdc* 4 cells [7]. Cell synchrony was established microscopically by seeing the terminal phenotype of individual cells and was also confirmed by DNA synthesis as has been described in [8]. DNA synthesis in Ts mutants and wild-type cells was determined by [^{3}H]uracil incorporation [8].

The method of Folch et al. [9] was used for total

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lipid extraction. Phospholipids were separated two-dimensionally by thin-layer chromatography on silica gel G plates as in [10,11].

3. RESULTS AND DISCUSSION

To explore whether phospholipids vary as a function of cell arrest in G_1, *cdc* 28 cells were arrested and compared with their counterparts grown under permissive conditions [7]. It was found that PI and PE levels were reduced by ~35–40% in these G_1-arrested cells while PS level almost doubled, with no significant variation in other phospholipids (table 1). In order to ascertain that the observed phospholipid changes in G_1-arrested cells were not simply a temperature effect, the wild-type (A364A) was subjected to similar conditions. It was observed that elevated temperature did not have significant effect on the overall phospholipid head group ratio (table 2).

It is known that three sequential gene functions are required for the initiation of DNA synthesis in *S. cerevisiae* [7]: *cdc* 28 ⟶ *cdc* 4 ⟶ *cdc* 7 ⟶ initiation of DNA synthesis. There is evidence to show that the controls regarding initiation of a new cell cycle take place in steps that precede and include the *cdc* 28 mediated step and that passing 'start' leads to commitment to the mitotic cycle

Table 1

Changes in phospholipid polar head group ratio in normally growing and G_1-arrested *Saccharomyces cerevisiae* mutants (total phospholipid, %)

cdc mutant	Condition	PI	PS	PC	PE	CL + U[a]
cdc 28	Normal	21.9	5.7	44.0	26.4	2.7
	Arrested	13.9	12.2	41.6	18.1	2.8
cdc 4	Normal	24.9	4.9	43.3	19.3	7.6
	Arrested	21.8	10.5	44.1	13.6	10.0
cdc 7	Normal	28.0	8.6	39.8	19.0	4.7
	Arrested	28.8	12.7	38.6	13.4	6.5

[a] Cardiolipin + uncharacterized

Cells were synchronized by shifting the growing cultures (23°C) to their respective non-permissive temperatures (36°C or 38°C) [7]. In all cases, 85–90% synchrony was obtained. Extracted phospholipids were separated, identified and quantitated as in [10]. All values are an average of 3–5 separate determinations

Table 2

Temperature effect on phospholipid polar head group ratio in A364A cells (total phospholipid, %)

Temp.	PI	PS	PC	PE	CL + U[a]
23°C	21.4	11.0	36.1	23.8	9.9
38°C	22.2	12.9	39.6	20.5	6.6

[a] Cardiolipin + uncharacterized

Extracted phospholipids were separated, identified and quantitated as in [10]. All values are an average of 3–5 separate determinations

[2,12]. Therefore, the fact that PI level is significantly reduced (~40%) in G_1-arrested cells, suggests that PI may be directly related to the growth and division of yeast cells. There was also ~80% increase in PS level, but it is highly unlikely that it is related to cell growth and division. PS is known to be dispensable for *S. cerevisiae* and is probably not required for functional membrane construction of this eukaryotic organism [11]. Since a cell cannot tolerate excessive intrinsic charging of membranes [13], therefore, the increase in PS level may be to compensate the overall charges which were disturbed as a result of PI and partly PE depletion. The fact that PC did not undergo any change as a result of cell arrest may be that it constitutes about half of the major phospholipids and cannot be replaced by any charged species. Cottrell et al. [14] have demonstrated stepwise increase of individual phospholipids initiating at the time of bud emergence in synchronous cultures of *S. cerevisiae*. Since the next mutant *cdc* 4, arrested at a point when the cells had emerged out of 'start' and the bud emergence had taken place [7], we tried to explore whether the decreases in PI and PE levels were restored as a result of stepwise increase of individual phospholipids, which we presumed had already begun in this mutant. Indeed we observed a partial restoration of PI and PE levels in *cdc* 4 arrested cells. On the other hand, the increase in PS level was also reduced by ~30%. This suggests that at the time of *cdc* 4 arrest, the stepwise increase of individual phospholipids had already begun, but the individual phospholipids could not be restored completely because stepwise increases may themselves be affected as a result of cell ar-

rest. This was substantiated by the next *cdc* mutant results. When *cdc* 7 was arrested, PI level was completely restored while PE and PS levels still showed some difference.

The earliest cell cycle event known to be blocked by *cdc* 28 mutation is spindle pole body (SPB) duplication which precedes both initiation of DNA synthesis and budding [15]. Piggott et al. have suggested bifunctional nature of *cdc* 28 protein [16]. They have shown that nuclear division (ND) is prevented by the *cdc* 28-1 N mutation but 'start' function seems to be unaffected [16]. Thus *cdc* 28 gene product appears to affect directly or indirectly the mitotic spindle function. It is also known that myo-inositol specifically reversed the metaphase arrest of cell division caused by colchicine in rat intestinal mucosa [17]. Since myo-inositol has no specific interaction with microtubule protein, it was postulated that PI plays a role in the cell division cycle [17]. Under the conditions defective in PI synthesis, mutant cells of *S. cerevisiae* did not undergo cell separation and thus formed cell aggregates [18]. Both evidences also suggest that PI may have some involvement in overall cell division and growth.

It is highly likely that in addition to two established functions of *cdc* 28 viz. SPB duplication and ND, it could also be involved in overall PI metabolism. The importance of PI is further substantiated by the observation that it sequentially recovers in late G_1 mutants which have passed the controlling point 'start'. It also matches well with data in [14], where they indicated stepwise increase of phospholipids at the time of bud emergence. The actual cause of PI decrease in G_1-arrested cells is not yet established, nevertheless, it appears from our results that abnormalities of *cdc* 28 could be related to PI decrease as well.

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