Coupling between phosphatidylinositol metabolism and cdc 28 gene product of *Saccharomyces cerevisiae*

On the possible mechanism of cdc 28 gene action

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Received 14 November 1983

It was shown that the decrease in phosphatidylinositol (PI) content in cdc 28 G_1-cells was due to a defect in inositol transport. This decrease in inositol transport was linked to microtubular function which was evident by the effect of a microtubular disrupting agent (colcemid) on inositol transport in stationary phase A364A cells. The involvement of PI in yeast G_1 phase was further substantiated by the observation that *o*-phenanthroline, which blocks yeast cells in G_1 phase, could inhibit inositol transport and PI levels as well. It is proposed that the regulation of PI metabolism is mediated by the gene cdc 28 and that microtubules may play a major role in the mechanism of action of this gene product.

Cell cycle *Growth control* *G_1 arrest* *Phosphatidylinositol* *Microtubule* Saccharomyces cerevisiae

1. INTRODUCTION

Similar to all eukaryotic cells, the cell division in *Saccharomyces cerevisiae* is controlled in the G_1 portion of the cell cycle [1,2]. If in the G_1 phase, the cell executes a step 'start', which is also mediated by the cdc 28 gene product, the cell is committed to the mitotic cycle [1,2], however, prior to the execution of start, yeast is capable of taking alternative developmental pathways [1,2]. We had earlier shown that the arrest of cdc 28 cells was associated with 35–40% drop in phosphatidylinositol (PI) level [3]. This decrease in PI content was shown to recover sequentially, when cells were arrested at points subsequent to start [3]. Our present results demonstrate that microtubular status when affected either by cdc 28 mutation or by some drug known to affect it, led to a decrease in the transport of inositol which ultimately could affect overall PI metabolism. It is suggested that in addition to known bifunctional nature of cdc 28 gene product [4,5], it may also be involved in PI metabolism, and that microtubules may be the potential candidates through which cdc 28 gene elicits the observed effects.

2. MATERIALS AND METHODS

All the strains, e.g., H.185 3.4 (cdc 28-1), H.135 1.1 (cdc 4-3), 4008 (cdc 7-4), and A364A, were grown in liquid synthetic media containing, per litre water, 6.7 g yeast nitrogen (w/o amino acids) (Difco), 20 g glucose; 0.02 g adenine sulphate, 0.02 g uracil, 0.03 g tyrosine, 0.02 g histidine, 0.03 g lysine, and 1 mM galactose; 0.03 g leucine was added for cdc 28 only. All the strains were grown 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 [3]. Cell synchrony was established by seeing the terminal phenotype of individual cells and was also confirmed by DNA synthesis as in [3]. *S. cerevisiae* 3059 was grown and maintained in liquid synthetic

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00145793/84/$3.00

media as in [6]. Exponentially growing *S. cerevisiae* 3059 cells were treated with *o*-phenanthroline (OP) for 3.5 h (50 μg/ml) after which 80–85% cells were arrested in G_1 phase which was further confirmed by the incorporation of [^{3}H]uracil [3].

Inositol transport was assayed essentially as in [7]. The standard assay mixture contained in a final volume of 200 μl, cells (150–200 μg protein/ml) suspended in sterile distilled water. They were incubated at the required temperatures for 10 min. [^{3}H]Inositol (280 μM, 20 μCi/ml) was added to initiate the reaction. After 10 min, the whole suspension was diluted 50-fold in chilled saline, filtered on 0.45 μm Millipore filter discs and washed thrice with chilled saline. Radioactivity retained on filters was counted in a Packard scintillation counter using a toluene based scintillation fluid.

Phospholipids were extracted, separated 2-dimensionally by thin layer chromatography on silica gel G plates, identified and quantitated as in [3,6].

3. RESULTS AND DISCUSSION

Table 1 lists the 10th-min accumulation of inositol in cdc 28, 4, 7 mutants and in the wild-type A364A cells. The level of accumulation of inositol in cdc 28 (start mutant) was the same in exponentially growing and arrested cells, however, a distinct enhancement was observed when cdc 4 and cdc 7 cells were arrested. cdc 4 and cdc 7 cells are known to arrest subsequent to start [8]. Since the cdc mutants were arrested when exposed to their respective non-permissive temperatures [3] (36°C or 38°C), and transport assays in those cells were performed at their respective non-permissive temperatures, the possibility of temperature dependent stimulation in overall inositol transport was excluded by following the transport in A364A cells at 23°C and 38°C. There was a distinct stimulation (45%) in inositol transport at 38°C. The level of inositol accumulation in cdc 28 was the same at either temperature. Keeping in view the temperature effect, it would suggest that there was a 40–45% drop in inositol uptake in cdc 28 arrested cells. The increase in inositol transport in the other two mutants, viz. cdc 4 and cdc 7 fits well with our earlier results where PI level was shown to sequentially recover [3].

Recent studies have revealed the cdc 28 gene product to be bifunctional, involved in spindle pole body (SPB) duplication [4], and nuclear division (ND) [5], thus affecting the overall mitotic spindle function. Based on our results, it appears that cdc 28 gene product could also be involved in overall PI metabolism. It has been shown that colchicine led to a decrease in PI levels [9], which could be restored by the addition of *myo*-inositol [9]. Since colchicine also inhibits microtubule assembly or mitotic spindle function, it is quite likely that both microtubular function and PI metabolism are interrelated. The above hypothesis appeared to be

Table 1

Inositol transport[b] (nmol/mg protein) in cdc 28, 4, 7 and A364A cells of *Saccharomyces cerevisiae*

Condition	cdc 28	cdc 4	cdc 7	A364A
Normal (23°C)	12.9	2.5	5.8	4.9[a]
Arrested (38°C/36°C)	11.5	7.0	15.0	9.2[a]

[a] Only a temperature effect, cell arrest is not involved
[b] All the values represent the 10th min accumulation of inositol uptake

Cells were synchronized as in [3]. In all cases, 85–90% synchrony was obtained (not shown)

Table 2

Effect of drugs (colcemid and *o*-phenanthroline) on inositol transport[c] in A364A and 3059 *S. cerevisiae* cells

Cell type	Normal	Drug treated
	(nmol/mg protein)	
A364A	4.9	2.7[a]
3059	24.9	8.8[b]

[a] Stationary phase A364A cells were incubated with colcemid (2 mM) [10] for 20 min and transport of inositol was assayed as described in table 1
[b] *o*-Phenanthroline (50 μg/ml) was added to exponentially growing 3059 cells for 3.5 h after which 80–85% synchrony was observed (not shown). Exponentially growing and G_1-arrested (OP-treated) 3059 cells were assayed for inositol transport as described in table 1
[c] All values represent the 10th min accumulation of inositol uptake

true when we tried to see the effect of a drug colcemid, which is known to act like colchicine in yeast [10] (colchicine being ineffective in yeast), on inositol transport in the wild-type A364A. It was observed that colcemid at 2 mM [10] could decrease inositol transport in A364A cells by approximately 45% (table 2). It has been shown earlier that in yeast, the transport of a phospholipid base can affect the overall biosynthesis of that phospholipid [11]. It is pertinent to mention here that drugs like colchicine have been found to inhibit the increase in amino acid transport, which is an early pre-replicative event in concanavalin A activated lymphocytes [12], and also the increase in amino acid transport and initiation of DNA synthesis in regenerating rat liver [13], thus suggesting an involvement between surface modulating assembly [14] (SMA) (comprising microtubules) and plasma membrane functions.

The involvement of PI in yeast G_1 phase was further substantiated by arresting a laboratory strain, *S. cerevisiae* 3059 by *o*-phenanthroline (OP) (since it was difficult to achieve synchrony with OP when parental strain A364A was used), which is also known to arrest cells at start [15,16]. It was observed that exposure of cells to OP (50 μg/ml) also resulted in a decrease in inositol transport (table 2) and PI levels (table 3). Therefore, our results of cdc 28 arrest and OP arrest indicate that PI metabolism and microtubular function are related.

It is thus proposed that when cdc 28 gene product is expressed, microtubules interact with *myo*-inositol permease to regulate its entry and thereby maintain PI level in the membrane (a similar phenomenon involving microtubular function and permease(s) has been observed in higher systems [12,13]). Microtubules may also affect overall PI biosynthesis [9,17]. If cdc 28 gene product is eliminated, either or both the pathways fail to function, thereby decreasing PI level which ultimately affects cell growth and division. The relationship between PI and cell growth status has been emphasized for yeast [18,19], as well as for higher systems [20,21]. While it has been pointed out that inositol could affect the rates of assembly and disassembly of microtubules [22], a later report suggested that it was of little physiological significance [23]. We suggest that instead of inositol regulating microtubular status, it is possible that microtubules by virtue of their being ubiquitous, may regulate a variety of functions, including PI metabolism. Because of its association to microtubules/mitotic spindle function, the gene cdc 28 can therefore control many processes. Moreover, start has been suggested to be the sole regulatory point in cell cycle initiation of *S. cerevisiae* [24], and thus cdc 28 gene which mediates it, could well be pleiotropic.

Table 3

Changes in phospholipid polar head group ratio in normally growing and G_1-arrested (OP-treated) cells of *S. cerevisiae* 3059

Condition	Total phospholipid %				
	PI	PS	PC	PE	CL + U[a]
S. cerevisiae (normal)	17.3	4.4	40.8	32.1	5.3
S. cerevisiae (OP-treated)	13.4	5.6	42.0	26.3	12.3

[a] Cardiolipin + uncharacterized

Exponentially growing *S. cerevisiae* 3059 cells were exposed to OP (50 μg/ml) for 3.5 h, after which 80–85% synchrony was observed. Extracted phospholipids were separated, identified and quantitated as in [3,6]. All values are an average of 3 independent determinations

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