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Activation of adenylate cyclase in *cdc*25 mutants of *Saccharomyces cerevisiae*

Luis A. Pardo*, Pedro S. Lazo and Sofía Ramos

Departamento de Biología Funcional, Universidad de Oviedo, E-33071 Oviedo, Spain

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The activation of adenylate cyclase by guanine nucleotides and 6-deoxyglucose was studied in membrane preparations from *S. cerevisiae* mutants lacking the CDC25 gene product. Adenylate cyclase from *cdc25 ts* membranes was activated by GTP and GppNHp in membranes from cells collected after glucose was exhausted from the medium. The activation was also observed in membranes from repressed cells at 2.5 mM Mg^{2+}. It is also shown that 6-deoxyglucose can activate adenylate cyclase in the absence of CDC25 gene product. The relative amount of membrane-bound adenylate cyclase was drastically reduced in *cdc25 ts* membranes when subjected to the restrictive temperature, while no significant change was observed in the wild type. These data suggest that Cdc25 might not be required in certain conditions for the guanine nucleotide exchange reaction in Ras and that it might be implicated in anchoring the Ras/adenylate cyclase system to the plasma membrane.

Adenylate cyclase; Ras protein; Cdc25; Glucose; Yeast

1. INTRODUCTION

In yeast, Ras proteins are controlling elements of the adenylate cyclase system [1–3]. Another component of the system, also with an activating role, is the CDC25 gene product. Ras proteins accomplish their biological functions through a cycle of GTP–GDP exchange and GTP hydrolysis. They are active on the GTP bound state. Hydrolysis of GTP to GDP inactivates Ras and reactivation requires the exchange of the bound GDP with GTP [4]. Cdc25 action occurs upstream of Ras proteins since mutations that hyperactivate the latter can complement CDC25 defects. It is generally accepted that Cdc25 protein acts as an activator of guanine nucleotide exchange reaction on Ras proteins. This idea is based on several genetic and biochemical data. Thus, (i) mutations that inhibit the GTPase activity in Ras proteins ($RAS2^{Val\text{-}19}$), as well as those that allow spontaneous nucleotide exchange ($Ras2^{Ile\text{-}152}$) are capable of complementing CDC25 defects [5]; (ii) there is a correlation between the GTP content of these RAS mutants and their ability to complement the lack of Cdc25; (iii) mutants in the IRA1 gene (which encodes for a RAS GTPase activating protein) also compensates Cdc25 deficiency [6].

In vitro experiments indicate that basal adenylate cyclase in *cdc25* membranes is lower than that of the wild type and so are the values obtained in the presence of guanine nucleotides [7]. Besides, the time constant of adenylate cyclase activation by GppNHp is much lower in *cdc25* mutants than in the wild type [8]. Also, Jones et al. [9], showed that extracts from strains containing high levels of Cdc25 protein promote the exchange of guanine nucleotides bound to soluble Ras protein.

Glucose is the only external nutritional signal that has been demonstrated to activate the Ras/adenylate cyclase system. Glucose signal transduction can be demonstrated both in vivo and in vitro. In vitro, glucose addition to derepressed cells causes a transient 6–10-fold increase of intracellular cAMP levels [10,11]. This is followed by a second phase in which stable cAMP levels stabilize to values 2–3-fold higher than in the absence of glucose [10–14]. Some controversy has been raised as to whether the cAMP signal is also obtained when glucose is added to cells in the logarithmic phase of growth. Thus, Eraso et al. [15] showed that cAMP levels rose when glucose was added to repressed cells. Also, when assayed in vitro, adenylate cyclase from *S. cerevisiae* SMC18 cells collected at the logarithmic phase of growth appears to be activatable by the glucose analog 6dG, though to a smaller extent than the cells collected at the stationary phase (Pardo, unpublished results). On the other hand, Argüelles et al. [16] claimed that a protein subjected to glucose repression appears to be necessary to obtain the cAMP signal.

The role of Cdc25 protein in glucose signal has been

Correspondence address: S. Ramos, Departamento de Biología Funcional, Area de Bioquímica y Biología Molecular, Universidad de Oviedo, E-33071 Oviedo, Spain. Fax: (34) (8) 510 3534.

**Present address:* Max-Planck-Institut für Biophys. Chemie, Abteilung Membranbiophysik, D-3400 Göttingen, Germany.

Abbreviations: 6dG, 6-deoxy-D-glucose; GDPβS, guanosine 5′-*O*-(2-thyodiphosphate); GppNHp, guanylyl imidodiphosphate; MES, 2-(*N*-morpholino)ethanesulfonic acid.

studied in vivo and the published results are conflicting. It has been shown that cdc25 disruption mutants have low basal cAMP levels [17] and that the addition of glucose to the cells does not cause any increase in cAMP levels [18]. However, some temperature-sensitive mutants of CDC25 show normal cAMP levels at the restrictive temperature [19] and show normal glucose-induced cAMP response [20].

In vitro, the signal of glucose can be determined as the activation of the membrane bound Ras/adenylate cyclase, and reflects the enhancement of the guanine nucleotide exchange reaction at the Ras proteins [21]. Using the in vitro approach we have now investigated the role of the CDC25 gene product on the activation of adenylate cyclase by guanine nucleotides, both in the absence and in the presence of glucose analogues. We have studied this effect in membranes derived from cells harvested in the logarithmic and the stationary phase of growth. The results indicate that membranes from *cdc25 ts* mutants retain low adenylate cyclase activity which, nevertheless, is activatable by glucose and guanine nucleotides. Altogether, the results suggest that the role of Cdc25 is by far more complex than simply acting as an activator of guanine nucleotide exchange by Ras proteins.

2. MATERIALS AND METHODS

2.1. *Materials*

Chemicals and other reagents were obtained from the following sources: Bacto-peptone and Bacto-yeast extract from Difco; [α-^{32}P]ATP (24 Ci/mmol) and [2,8-^{3}H]cAMP (31.2 Ci/mmol) from New England Nuclear; 6 deoxy-D-glucose and 3-*O*-methyl-D-glucopyranoside from Serva; GDPβS, GppNHp and β-glucuronidase arylsulfatase (Glusulase) from Boehringer-Mannheim. All the other reagents were from Sigma. All solutions were prepared in double-distilled Milli-Q water.

2.2. *Yeast strains and growth conditions*

This work was performed using the following strains of *Saccharomyces cerevisiae*:

352-5A2.1 (*MATα, ade5, lys2, his7, met10, trp1-289, ura3-52*) and 352-5A2 (*MATα, ade5, lys2, his7, met10, trp1-289, ura3-52, cdc25ts*) [7] were kindly provided by J. Daniel, Weizmann Institute, Israel.

Y294 (MATα, leu2-3, 112, ura3-52, trp1, his3) and SJ22 (MATα, leu2-3, 112, ura3-52, trp1, cdc25::HIS3 [YEp13-TPK1]) were gifts from J. Broach, Princeton University, NJ, USA.

Yeast cells were grown on YEPD medium (2% Bacto-peptone, 1% yeast extract, 2% glucose) and incubated at 30°C with shaking. Growth was monitored by determining the A_{600}, and cultures were collected when A_{600} was 0.6–1.2 for the mid-log phase (glucose repressed) cells or when A_{600} was 3.5–4.0, 5 to 7 h after glucose was completely exhausted from the medium for the stationary cells. For thermosensitive strains, growth temperature was 25°C, and when the desired growth was reached, half of the culture was quickly shifted to 34°C and then maintained at that temperature for 60 min. The rest of the culture was kept at the permissive temperature. Further processing of both types of cells was identical.

2.3. *Membrane preparations*

Crude membrane preparations were obtained as described for wild type *S cerevisiae* [21,22]. For thermosensitive strains, the same protocol was performed except that a temperature of 28°C was never exceeded. Membranes were stored in 50 mM MES/KOH buffer, pH 6.0, containing 0.1 mM $MgCl_2$, 0.1 mM EGTA, 4 mM phenylmethylsulfonyl fluoride and 10% glycerol at −70°C.

2.4. *Determination of adenylate cyclase*

Adenylate cyclase was determined as previously described [21]. Briefly, after the addition of 6dG and the guanine nucleotide at the appropriate concentrations, the membrane suspension was preincubated for 10 min at 30°C. When determining the activity of the non-regulated catalytic subunit of adenylate cyclase, the preincubation was in the absence of guanine nucleotides and in the presence of 2 mM $MnCl_2$. Afterwards, 25 μl (containing 50–100 μg of protein) of the membrane-effector mixture was diluted 1:1 with assay buffer and incubated for 30 min at 30°C. The assay temperature was also 30°C for thermosensitive strains since temperature no longer affects the behavior of mutant membrane preparations [7]. Assay buffer composition was: 100 mM MES/KOH, pH 6.0, containing 2 mM [^{3}H]cAMP (10,000 cpm/25 μl), 0.2 mM EGTA, 4 mM β-mercaptoethanol, 0.2 mg/ml bovine serum albumin, 10 mM teophylline, 40 mM phosphocreatine, 40 U/ml creatine-phosphokinase, and 0.4 mM [α-^{32}P]ATP (1–2 μCi/25 μl) plus the indicated $MgCl_2$ concentration. The reaction was stopped by addition of 0.9 ml of 6.25% trichloroacetic acid. After centrifugation, [^{32}P]cAMP was purified from the supernatant as described [23]. Protein concentration was determined by Bradford's method [24] using the commercial reagent from Bio-Rad.

2.5. *Kinetics of activation of adenylate cyclase by GppNHp*

To determine the kinetics of activation of adenylate cyclase by the non-hydrolyzable GTP analog GppNHp, the adenylate cyclase reaction was carried out essentially as above except that magnesium acetate (10 mM) was used instead of $MgCl_2$, the concentration of ATP was increased to 1 mM, and the reaction (1.2 ml) was started by addition of concentrated membranes (500–1,000 μg of protein). At the indicated times, 100 μl samples were withdrawn and processed as above.

Each kinetic experiment was replaced at least 3 times and all experimental points were used to calculate the rate constants. As previously described [8,20] the production of cAMP is adjusted to the following equation:

$$cAMP_t = V_{max}t + [(V_{max}/K_{obs})(e^{-K_{obs}} - 1)] \quad (1)$$

where $cAMP_t$ is the amount of cAMP produced in a given time t, V_{max} the apparent V_{max} of the reaction, and K_{obs} the first order equation constant which determines the pseudo lineal phase of the equation.

The slope of the apparently linear phase represent V_{max} and the interception at the time axis is a function of $1/K_{obs}$. This means that higher values of K_{obs} represent shorter delays in the activation reaction [8]. The curve and function that best fit the experimental data and the kinetic parameters of this curve were calculated using ENZFITTER computer program (Elsevier Biosoft) for a PC computer.

3. RESULTS

3.1. *Effect of guanine nucleotide on adenylate cyclase activity in* cdc25 ts *mutants*

The activation of adenylate cyclase by guanine nucleotides implicates the access of the nucleotide to its binding site in Ras proteins. We have tested the ability of guanine nucleotides to activate the adenylate cyclase present in membrane preparations obtained from *cdc25 ts* mutants subjected to the restrictive temperature for 60 min. The results are presented in Fig. 1.

As previously observed [7], both GTP and GppNHp failed to activate adenylate cyclase in membranes from

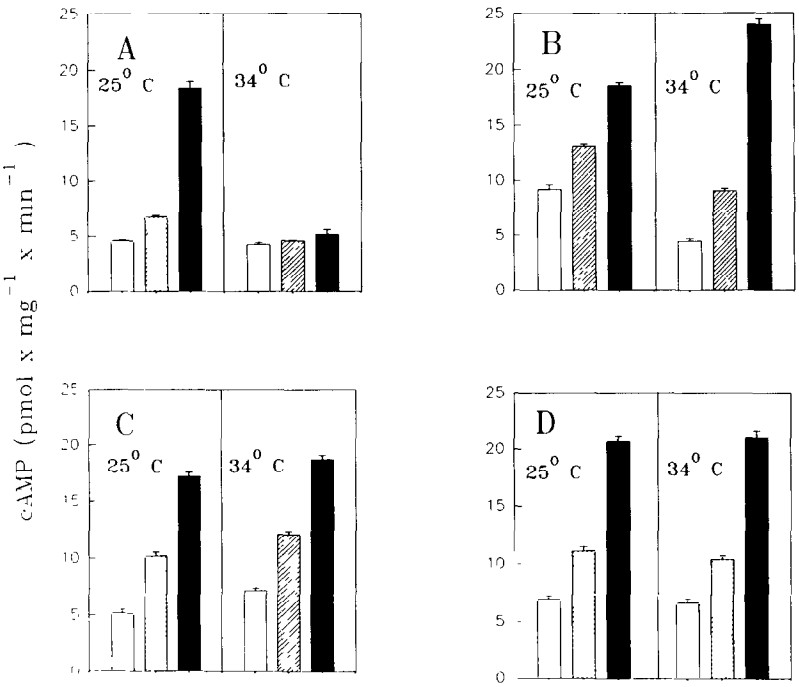

Fig. 1. Activation of adenylate cyclase by guanine nucleotides in *S. cerevisiae cdc25 ts*. *S. cerevisiae* strain 352-5A2 was grown at 25°C in YEPD medium containing 2% glucose. Cells were collected at the logarithmic phase of growth (A and B) or after glucose was exhausted from the medium (C and D). In every case membranes were prepared from control cells (25°C) and after cells had been subjected for 60 min to the restrictive temperature (34°C), as indicated. Adenylate cyclase activity was then determined in the presence of 10 mM Mg^{2+} (A and C) or in the presence of 2.5 mM Mg^{2+} (B and D). The activity in the presence of 0.1 mM GTP (hatched bars) or 0.1 mM GppNHp (closed bars) was compared to a control without guanine nucleotides (open bars).

mutant cells collected at the logarithmic phase (repressed cells). Thus, in these conditions guanine nucleotide-dependent adenylate cyclase appears to be dependent on Cdc25 function. The lack of activation by guanine nucleotides, however, was only observed when the concentration of Mg^{2+} in the assay was 10 mM (Fig. 1A). In contrast, both nucleotides activated the enzyme when the concentration of Mg^{2+} was lowered to 2.5 mM (Fig. 1B). When membranes were obtained from cells at the stationary phase of growth, regardless of the Mg^{2+} concentration used, guanine nucleotides activated adenylate cyclase (Fig. 1C,D). This activation was as effective as the one observed in the wild type membranes (not shown). These results show that, under certain experimental conditions, Cdc25 appears to be necessary for guanine nucleotide exchange. However, at low Mg^{2+} concentration in the assay or in different physiological conditions (in membranes from stationary cells) guanine nucleotide exchange is possible in the absence of Cdc25. To further test the ability of guanine nucleotides to access the binding site of Ras proteins in the absence of Cdc25 we performed similar experiments using the strain SJ22, a mutant in which the CDC25 gene has been disrupted. This mutation, which is otherwise lethal, is complemented by the presence of high copy number of the plasmid p-TPK1. This plasmid carries the gene TPK, which codes for the catalytic subunit of cAMP-dependent protein kinase. In this case we measured the activation by GTP or GppNHp in relationship to the activity in the presence of 100 μM GDPβS. To note the large difference in activity (both basal and GppNHp activated) between the membranes from repressed cells of the wild type and that of the stationary cells. This is characteristic of this strain since in the strain 352-5A2 (Fig. 1) or its wild type (not shown), the activity in both types of membranes is very similar. Also, in the strain SMC18 the activity of membranes from cells at the logarithmic phase is 50–100% higher than that of the membranes from stationary cells (Pardo, unpublished results). The basal activity in the *cdf25Δ* mutant was found to be very low (0.6 pmol/min/mg) as compared to the wild type (18.0 pmol/min/mg). This large difference was only observed when the cells were collected in the exponential phase of growth and not when membranes were obtained from stationary phase cells. The activation by GTP or GppNHp was obtained in every experimental condition and varied from 5- to 14-fold depending on the membranes used and the assay conditions (Table I). This activation is actually larger than the one obtained in the wild type membranes. These results are another strong indication that guanine nucleotides can access to their binding site even in membranes lacking functional Cdc25 protein.

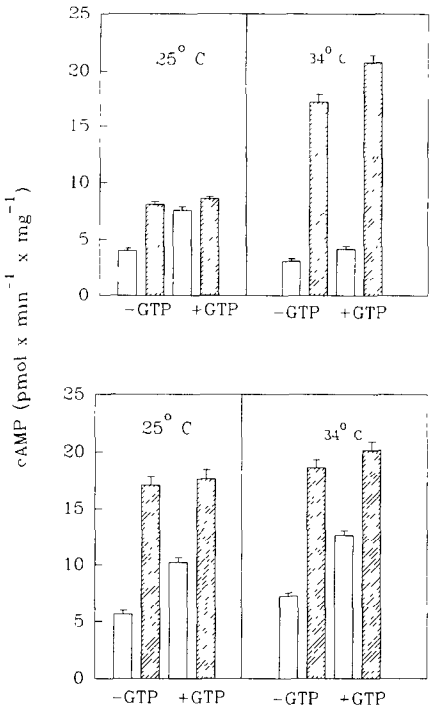

Fig. 2. Activation of adenylate cyclase by 6-deoxyglucose in *S. cerevisiae cdc25 ts*. *S. cerevisiae* strain 352-5A2 was grown as indicated in the legend of Fig. 1. Membranes were prepared from repressed cells (upper panel) or stationary cells (lower panel) in control conditions (25°C) or after the cells were subjected to the restrictive temperature (34°C), as indicated. Adenylate cyclase was activated by 100 mM 6dG (hatched bars) or 100 mM sorbitol as control (open bars) in the absence or in the presence of 0.1 mM GTP, as indicated. The concentration of Mg^{2+} in the assay was 10 mM.

3.2. *Activation of RAS/adenylate cyclase by 6-deoxyglucose in* cdc25 ts *mutants*

We have previously shown that glucose and 6dG activate the Ras/adenylate cyclase complex in vitro [21]. Thus, 6dG can mimic in vitro the physiological increase in adenylate cyclase activity induced by glucose in intact cells. Using this approach we have now tested whether this activation is dependent on Cdc25 function.

6dG was able to activate adenylate cyclase in membranes derived both from exponentially growing cells and from stationary cultures (Fig. 2). Interestingly, 6dG failed to activate adenylate cyclase in membranes from cells harvested during the exponential phase of growth and not subjected to the restrictive temperature. This agrees with our observations in other strains (Pardo et al., unpublished results) and also with observations obtained in vivo in which glucose did not raise cAMP levels when added to exponentially growing cells [16]. In membranes derived from cells which had reached the stationary phase of growth, no significant difference was observed in the behaviour of the control membranes and that of the cells subjected to the restrictive temperature. These results suggest that transduction from a putative glucose receptor to adenylate cyclase can occur in the absence of the CDC25 gene product.

3.3. *Kinetics of activation of adenylate cyclase in* cdc25 ts *mutants*

To characterize further the activation of adenylate cyclase activation by 6dG in membranes lacking Cdc25 function, the kinetics of the reaction catalyzed by adenylate cyclase was analyzed. It has been shown that the delay observed between the start of cAMP synthesis and the acquisition of linear kinetics in the presence of GppNHp reflects the rate of exchange of guanine nucleotides [8,21]. The higher the values of the activation constant K_{obs}, the faster the nucleotide exchange is. Fig. 3 shows that in the thermosensitive mutant 352-5A2, the activation of the enzyme by GppNHp was much slower after the heat treatment (B) than before (A), as previously reported [8]. The K_{obs} were 1.4×10^{-2} min^{-1} for the non-treated membranes and 10^{-4} min^{-1} for the treated ones. The presence of 6dG caused a strong shortening of the activation time on the *cdc25 ts* membranes ($K_{obs} = 1$) while slightly affecting the control mem-

Table I

Activation of adenylate cyclase by guanine nucleotides in membranes with and without Cdc25

Strain and condition	Adenylate cyclase activity (pmol/min/mg protein)					
	2.5 mM Mg^{2+} in assay			10 mM Mg^{2+} in assay		
	+GDPβS	+GppNHp	Activation (fold)	+GDPβS	+GppNHp	Activation (fold)
Wild type (Y294)						
Repressed cells	29.5	67.4	2.3	18.0	46.0	2.5
Stationary cells	1.3	3.4	2.6	2.5	4.3	1.7
cdc25Δ (SJ22)						
Repressed cells	1.2	16.3	13.6	0.6	4.3	7.2
Stationary cells	0.3	4.2	14.0	0.4	2.1	5.2

Cells from the wild type and the *cdc25* strain were grown in glucose and collected during the mid logarithmic phase (Repressed) or after glucose was exhausted (Stationary). In both cases, spheroplasts were obtained, lysed and membranes prepared as indicated in section 2. Adenylate cyclase was assayed at low and high Mg^{2+} concentrations, as indicated.

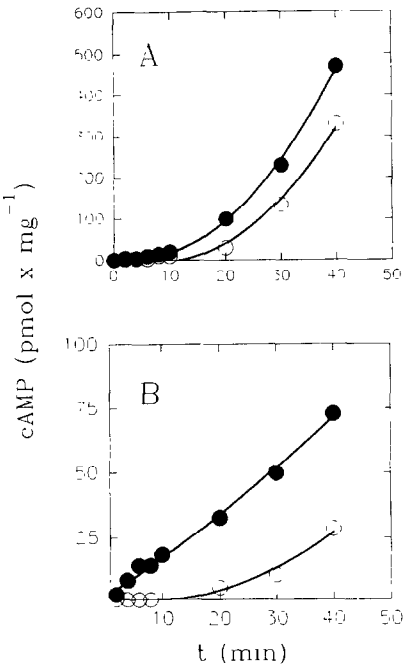

Fig. 3. Effect of 6 deoxyglucose on the kinetics of activation of adenylate cyclase by GppNHp. Membranes were prepared from repressed cells of *S. cerevisiae* strain 352-5A2 cultured at 25°C (A), or after the cells had been subjected to the restrictive temperature for 60 min (B). the kinetics of activation of adenylate cyclase by 0.1 mM GppNHp alone (open symbols) or 0.1 mM GppNHp plus 100 mM 6dG (closed symbols) was determined as described in section 2.

branes ($K_{obs} = 1.5 \times 10^{-2}$). This indicates that in the presence of an exchange enhancer, the nucleotide can easily get access to the binding site of the RAS protein. The effect of 6dG on the kinetics of adenylate cyclase was also observed in SJ22 membranes from cells obtained both at the logarithmic and the stationary phase (data not shown).

3.4. *CDC25 gene product is required for adenylate cyclase binding to the membrane*

It has been shown that *S. cerevisiae* adenylate cyclase is a peripheral membrane protein which becomes soluble in *ras1*, *ras2*, *bcy1* cells and that over expression of the gene CDC25 in these cells relocalizes adenylate cyclase to the membrane fraction [8]. From these findings a biochemical link has been suggested between Cdc25 and adenylate cyclase in the absence of Ras. Moreover, it has been suggested that the binding of adenylate cyclase to the membrane might have regulatory significance [25]. We have now determined the distribution of adenylate cyclase in cells carrying both RAS gene products, in the presence and in the absence of Cdc25. Table II shows two experiments in which the localization of adenylate cyclase was determined in membranes derived from the wild type and in the *cdc25 ts* mutant, both at the permissive and two restrictive temperatures (34°C and 37°C). Membranes were prepared from repressed cells and adenylate cyclase assayed with Mn^{2+}-ATP as substrate, which allows determination of guanine nucleotide-independent adenylate cyclase. In the wild type strain more than 90% of adenylate cyclase remained membrane bound at any of the temperatures tested. In contrast, in the *cdc25 ts* mutant, the localization of adenylate cyclase depended on the presence of Cdc25. Thus, when the cells were growing at the permissive temperature the membrane-bound adenylate cyclase was 50–60% of the total activity, which is already lower than in the wild type. When the cells were subjected to the restrictive temperatures, only 30% remained membrane bound at 34°C. When treated at 37°C, more than 90% of the activity was solubilized. These results suggest that the CDC25 gene product might be implicated in anchoring the Ras/adenylate cyclase complex to the membrane therefore providing a functional link by which the signal originating at a putative glucose receptor can be transduced to adenylate cyclase.

4. DISCUSSION

Several pieces of evidence are available indicating that the CDC25 gene product is implicated in the cAMP signaling pathway in yeast. Thus: (i) the intracellular cAMP levels are higher in the wild type than in mutants in which the CDC25 gene has been interrupted; and (ii) guanine nucleotide-dependent adenylate cyclase is lower in *cdc25* mutants than in the wild type. The results presented in this work confirm previous observations indicating that CDC25 gene product is required for guanine nucleotide exchange at the Ras proteins. However, this is restricted to certain physiological and assay con-

Table II

Effect of Cdc25 inactivation on the localization of adenylate cyclase

Phenotype	°C	Adenylate cyclase activity (pmol/min)		
		Lysate (U/ml)	Supernatant (U/ml)	% activity in pellet
Wild type	25	49.2	2.6	95
Wild type	34	31.5	2.1	93
cdc25 ts	25	21.0	8.3	61
cdc25 ts	34	19.0	12.9	32
Wild type	25	148.0	8.1	95
Wild type	37	99.0	10.0	90
cdc25 ts	25	45.0	22.0	50
cdc25 ts	37	32.5	30.5	7

Cells growing in glucose were treated at the indicated temperature for 1 h and collected. Spheroplasts were obtained, lysed and membranes prepared as indicated in section 2. Mn^{2+} (2 mM) stimulated adenylate cyclase was determined in the lysate and in the supernatant after centrifugation of the lysate for 30 min at 29,000 × *g*. *S. cerevisiae* strain 352-5A2.1 was used as wild type and strain 352-5A2 was used as *cdc25 ts*.

ditions. Thus, while Cdc25 appears essential for guanine nucleotide exchange in membranes from repressed cells assayed in 10 mM Mg^{2+}, this is no longer the case when the concentration of Mg^{2+} is lowered to 2.5 mM or when membranes from derepressed cells were used (in this latter case at any concentration of Mg^{2+} in the assay). It is known that Mg^{2+} concentration is important in determining guanine nucleotide exchange at the Ras protein, so that it becomes a free exchange in the absence of Mg^{2+}. Thus, it seems that in this particular instance, the Mg^{2+} dependence of the exchange has been relaxed so that it can occur at 2.5 mM. It should be noted, however, that at 2 mM Mg^{2+}, the exchange of nucleotides in purified Ras is very low [4,9]. The complexity of the system used in our experiments might contribute to this change in the Mg^{2+} dependence of the reaction. The fact that membranes derived from cells at the stationary phase (treated or not at the restrictive temperature) do not show any difference in guanine nucleotide-dependent adenylate cyclase, indicates that Cdc25 is no longer required in cells at the stationary phase of growth.

The results presented in Table I are apparently in conflict with those of Jones et al. [9]. They showed that cell-free extracts from the strain SJ22 do not accelerate the nucleotide exchange by purified Ras, while it is observed with that of a strain overproducing Cdc25. The fact that guanine nucleotide dependent adenylate cyclase can be demonstrated in membranes from this strain indicates that the function of Cdc25 might be influenced in a great manner by the presence of membranes. The nucleotide exchange-dependent activity is best reflected by the ratio between the activity measured in the presence of GppNHp (or GTP) and the activity measured in the presence of GDPβS. GDPβS is known to bind tightly to G proteins showing no residual activation of the enzyme. In any case, either the GppNHp/GDPβS ratio (this report) or the GTP/Mg^{2+} ratio [8,17], is shown to be at least as high in the *cdc25* mutants as in the wild type, though the total activity is always lower in the mutants.

Our results indicate that in vitro the activation caused by 6dG is independent of Cdc25 function. The fact that in *cdc25 ts* strains there is no detectable cAMP signal in vivo, while in vitro we detect adenylate cyclase activation, might be due to the fact that the assay of adenylate cyclase is a much more sensitive assay for determining signal transduction. This is especially so, considering that the amount of adenylate cyclase bound to membranes can be drastically reduced after heat treatment (Table II). Also, it is interesting that when $RAS2^{Val-19}$ was overexpressed in a cdc25 mutant, it was restored in the capacity to obtain a cAMP signal in response to glucose or dinitrophenol [26]. This is probably due to the relocalization of adenylate cyclase to the plasma membrane [8]. Moreover, in cdc25 mutants carrying also the ira1 mutation (which cooperates to maintain Ras in its active state) the cAMP signal in response to glucose can also be detected [6].

From the complementation of RAS mutations that retain GTP bound, it can be inferred that Cdc25 and Ras proteins are functionally related, but no mechanistic implications can be drawn. The results presented in this paper as well as other previously reported [8] are compatible with the concept that Cdc25 could act as an anchoring mechanism of adenylate cyclase to the membrane. The interaction between Cdc25 and adenylate cyclase should not be simple since Cdc25 appears to be important for the binding of adenylate cyclase to the membranes both in the absence [8] and in the presence (this report) of Ras proteins. In this regard it is interesting to find that in vivo the Cdc25 protein binds Ras only in its inactive (Ras-GDP) state [27]. It is conceivable that Cdc25 is needed to maintain adenylate cyclase bound to the cell membrane thus allowing the interaction Ras(GTP)-adenylate cyclase.

In summary then, although the Cdc25 protein is required for the activation of adenylate cyclase, its role as a nucleotide exchange activator can be bypassed in certain physiological and experimental conditions. Moreover, the glucose analog 6dG can activate adenylate cyclase in membranes from *cdc25 ts* mutants, suggesting that signal transduction from a glucose sensor to adenylate cyclase may occur in the absence of CDC25 gene product. There are indications that the Ras/adenylate cyclase complex might be stabilized as a membrane-bound enzyme by Cdc25, but further work will be required to clarify the exact mechanism of action of the CDC25 gene product.

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NOTE ADDED IN PROOF

After this manuscript was submitted, E. Gross et al. have published a paper [Nature 360 (1992) 762–765], in which they show that the addition of glucose to starved cells triggers the phosphorylation of Cdc25 and its relocalization to the cytoplasm. The authors suggest that this mechanism would act to reduce the steady-state level of the Cdc25-Ras complex, leading to a drop in cAMP levels. Those results are also compatible with the concept that Cdc25 acts by anchoring AC to the membrane. In this case, the AC that remains membrane bound after the addition of glucose, could still be activated by a Cdc25- independent signal transduction from the glucose sensor.