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Gpr1p, a Putative G-Protein Coupled Receptor, Regulates Glucose-Dependent Cellular cAMP Level in Yeast *Saccharomyces cerevisiae*

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How cells monitor the availability of nutrition and transduce signals is a fundamental, unanswered question. We have found that Gpr1p, a recently identified G-protein (Gpa2p) coupled receptor in yeast *Saccharomyces cerevisiae*, regulate the cellular cAMP level in response to glucose. The glucose-induced higher cAMP level found in the strain with *GPA2* in multicopy plasmid decreased by deletion of *GPR1* gene. A transient increase of cAMP in response to glucose was not observed in a Δ*gpr1* mutant strain and this defect was complemented and restored by introducing *GPR1* gene with YCp vector. Gpr1p was also required for the increase of cAMP in response to other fermentable sugars. Both membrane proximal regions o the third cytosolic loop in Gpr1p, which has been shown to be important for coupling to G-proteins, were also required for glucose-induced transient increase of cAMP. Our findings suggest that Gpr1p is part of the nutrition sensing machinery most likely acting as a receptor to monitor glucose as well as other fermentable sugars and regulate cellular cAMP levels.

In yeast *Saccharomyces cerevisiae,* Ras proteins are controlling element of cellular cAMP level. Res proteins are member of a large family of small GTP-binding proteins. They accomplish their biological functions through a cycle of GDP/GTP exchange and GTP hydrolysis. The GTP-bound form is active and hydrolysis of GTP inactivates them. The GDP/GTP exchange on Ras protein is catalyzed by Cdc25p [1][2][3] while GTP hydrolysis activity is stimulated by *IRA1* and *IRA2* gene products [4][5][6]. The activated Ras proteins stimulate adenylate cyclase to yield increased cAMP levels [7][8], and the increased cAMP level activates the protein kinase A (PKA) which display pleiotropic control through phosphorylation of proteins included transcriptional activators. This signal transduction pathway has been well characterized and designated as Ras/cAMP pathway. When glucose was added to the glucose-starved cells, a transient increase in cellular cAMP level was observed [9][10]. This glucose-induced cAMP signal was completely diminished in the Δ*ras1*, Δ*ras2*, Δ*pde2* triple mutant strain [11]. Also cells without functional *CDC25* gene were deficient in the glucose-induced cAMP increase [12]. And thus, Ras/cAMP pathway was shown to play an essential role in glucose signal transduction to regulate cellular cAMP level.

Gpa2p, a heterotrimeric G-protein α subunit was also thought to be involved in cAMP regulation in response to glucose. *GPA2* in a multicopy plasmid enhanced the rise of cAMP level in response to glucose [13][14]. Also over expression of *GPA2* suppressed the growth defect of a temperature-sensitive *ras2* mutant strain [13]. While the result that the deletion of *GPA2* gene did not affect the cAMP response made it obscure if Gpa2p is involved in this signal transduction.

Recent studies began to reveal the roles of Gpa2p in *S. cerevisiae.* It was shown that Δ*gpa2* and Δ*ras2* double mutation led to severe growth defect even in rich medium [15][16]. Also Gpa2p was shown to regulate pseudohyphal development *via* cAMP dependent mechanism [15][17]. And thus it was proposed that Gpa2p and Ras2p have partially redundant functions in a signaling pathway acting in parallel. Moreover glucose-induced cAMP signaling was eliminated in ψ*gpa2* mutant strain by pre-addition of low level of glucose [18].

Although Ras/cAMP pathway as well as Gpa2p have been shown to regulate cellular cAMP in response to glucose, how cells monitor the glucose and transmit

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signals through those pathways is remained unknown. We have cloned the *GPR1* gene encoding putative G-protein coupled receptor which physically [19] and functionally [16] interacts with Gpa2p.

Here we present the evidence that Gpr1p regulates cellular cAMP level in response to glucose as well as other fermentable sugars.

MATERIALS AND METHODS

Plasmid construction. A 5 kb *Eco* RV fragment containing *GPR1* gene was cloned into pRS413 (p*GPR1*-RS413) as described [19]. To construct the single copy and multi copy plasmid with *GPR1,* 4.2 kb of *Sac* I-*Stu* I fragment was cloned into *Sac* I-*Sma* I site of pRS416 and YEplac112 to produce p*GPR1*-RS416 and p*GPR1*-YE112, respectively. The construction of *URA3* (p*gpr1*::*URA3*) and *LEU2* (p*gpr1*::*LEU2*) disruption of *GPR1* was as described [19]. The *GPR1* deletions were made with the QuickChange kit (Stratagene) using p*GPR1*-RS416 ass a template with the following primers: for $GPR1^{d277\text{-}284}$, 5′-TTCATTACCAGTGAAAGTGACTTTAACCATAA-CGTA-3′, and its reverse complement; for $GPR1^{d610\text{-}617}$, 5′-CAAACCTACAAACAAATGAAGAATCTAAGGGCAATA-3′, and its reverse complement. The resultant constructs were designated as p$GPR1^{d277\text{-}284}$ and p$GPR1^{d610\text{-}617}$, respectively. To construct p$GPR1^{d277\text{-}284,610\text{-}617}$ double deletion, 1.9kb of *Sac* I-*Xba* I site of p$GPR1^{d610\text{-}617}$ was substituted for the corresponding position of p$GPR1^{d277\text{-}284}$.

Yeast strains and media. The *GPR1* null allele was made using p*gpr1*::*LEU2* as described [19]. Strains were grown on YPD medium containing 2% pepton, 1% yeast extract, and 2% glucose. Strains under selection were grown on SD medium containing 0.67% yeast nitrogen base without amino acids (Difco) and 2% glucose. SD medium was supplemented with auxotrophic requirements.

cAMP assay. Yeast cells were collected in the stationary phase of growth in SD selection medium, washed twice with sterile water, and suspended in 10mM MES (pH6.0) and 0.1 mM EDTA. Cells were incubated at 30°C for 2hr, then glucose or other fermentable sugars were added to a final concentration of 2%. After incubation for various periods of time, 0.5 ml aliquots were taken from the culture and transferred to 0.5 ml of 10% (wt/vol) trichloroacetic acid and flush frozen in liquid nitrogen. Each sample was defrosted and incubated at 4°C for over night, then trichloroacetic acid was removed by extraction with ethyl ether (water saturated) and subjected to free dry. cAMP content was measured using a cAMP enzyme immuno assay kit (Amersham) according to the protocol provided by the supplier. The optical density of cell suspensions was measured at 600nm and cell wet weights were calculated.

RESULTS AND DISCUSSION

Gpr1p Is Required for Glucose-Induced cAMP Response

In the yeast *Saccharomyces cerevisiae,* when glucose was added to glucose-starved cells, a transient increase of intracellular cAMP level was observed. This transient rise of cAMP is known to result from activation of the Ras/cAMP pathway. Gpa2p, a heterotrimeric Gα subunit, is also considered to regulate the cellular cAMP level in response to glucose stimulation. Although deletion of *GPA2* did not affect the glucose-induced transient increase of cAMP level, *GPA2* in multicopy plasmid enhanced the increase of cellular cAMP in response to glucose stimulation and a high level of cAMP was maintained for a long time [13]. Since *GPR1* was isolated by a two-hybrid system as a gene coding molecule which interacts with Gpa2p [19], we examined if Gpr1p regulates the cellular cAMP level in response to glucose. The *GPA2* gene in multicopy plasmid was introduced into wild type and Δ*gpr1* mutant strains and the glucose stimulated-cellular cAMP level was measured. In the wild type strain with *GPA2* in multi-

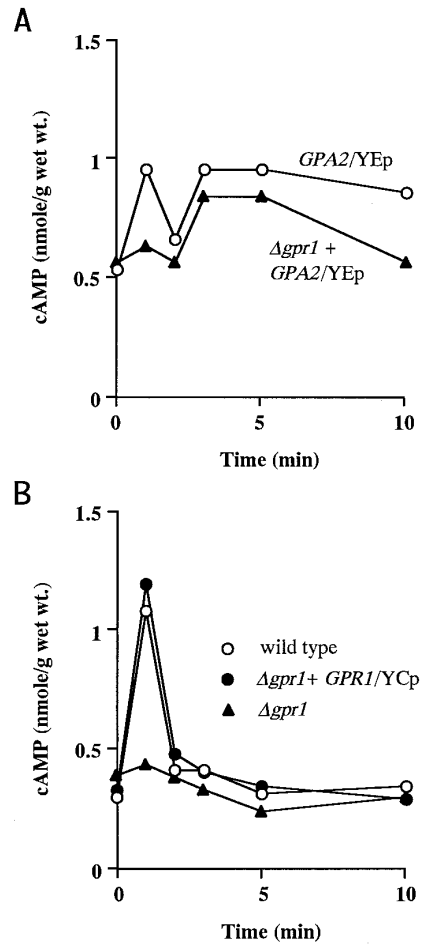

FIG. 1. Effect of *GPR1* deletion on glucose-induced increase of cAMP. Cells were grown in SD media to the stationary phase and incubated in glucose depleted conditions for 2h. Aliquots were collected at various time points before (0 min) and after (1, 2, 3. 5, 10 min) the glucose addition and subjected to cAMP assay. cAMP level was expressed as nmole/wet weight (g) of the cells calculated from the optical density of the cell suspension. Each assay shown in the same figure was done at the same time. Each value is the average from triplicated experiments. The data shown is from one of three independent experiments which gave almost the same results. (A) A wild type haploid strain (W3031A) and Δ*gpr1* mutant strain transformed with p*GPA2*-YE112, a multicopy plasmid (YEp) containing *GPA2,* were subjected to cAMP assay. (B) Δ*gpr1* mutant strain was transformed with p*GPR1*-RS413, a single copy plasmid (YCp) containing *GPR1,* and vector pRS413. A wild type strain was transformed with pRS413. These transformants were subjected to cAMP assay.

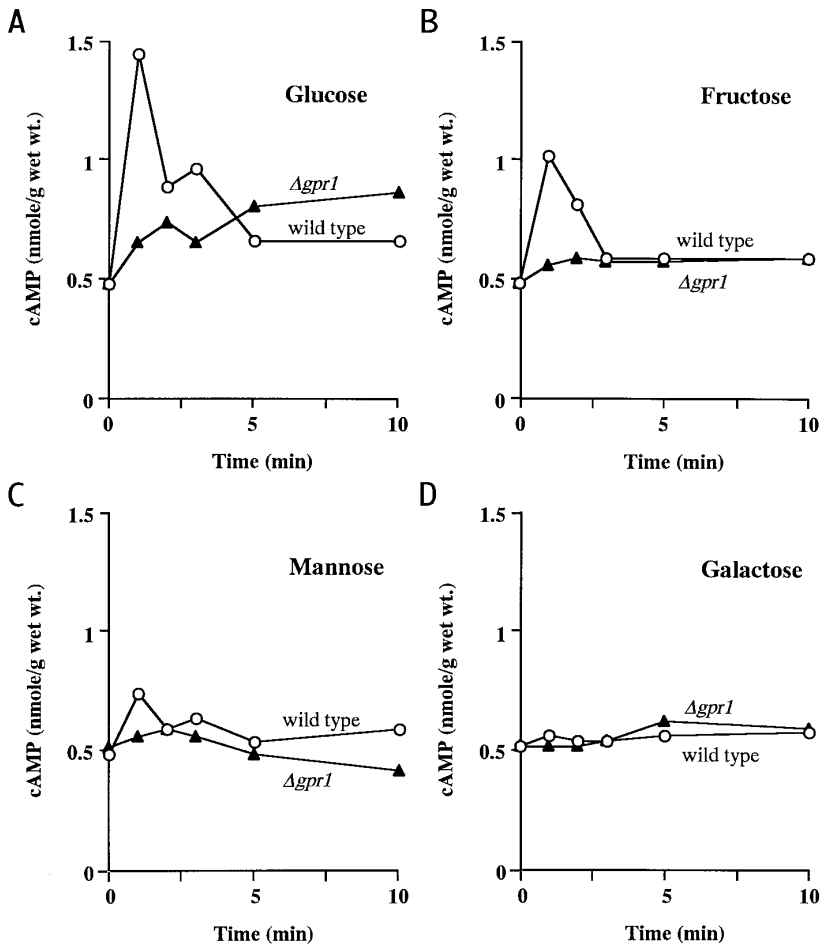

FIG. 2. Effect of *GPR1* deletion on cAMP regulation in response to various fermentable sugars. Δ*gpr1* mutant strain was transformed with p*GPR1*-RS413, a single copy plasmid (YCp) containing *GPR1*, and vector pRS413. Each transformant was grown in SD media to the stationary phase and incubated in carbon source-depleted conditions for 2h. Aliquots were collected at various time points before (0 min) and after (1, 2, 3. 5, 10 min) the addition of various sugars and subjected to cAMP assay. cAMP level was expressed as nmole/wet weight (g) of the cells calculated from the optical density of the cell suspension. All assays shown in this figure were done at the same time. Each value is the average from triplicated experiments. The data shown is from one of three independent experiments which gave almost the same results. (A) Glucose addition, (B) fructose addition, (C) mannose addition, (D) galactose addition.

copy plasmid, the cAMP level peaked at 1min in response to glucose and an increased level of cAMP was maintained for a long time as in he previous study except that the increased cAMP level was not so high in our strains (Figure 1A) [13]. While in Δ*gpr1* mutant strains with *GPA2* in multicopy plasmid, peak of cAMP at 1 min was not observed and the cAMP level after the peak decreased. Although glucose-induced increase of cAMP level was found in this mutant strain at a few minutes after the glucose addition, the increased cAMP level was lower than that of wild type strain with *GPA2* in multicopy plasmid. These results might indicate that Gpr1p is possibly involved in glucose sensing and signal transduction which regulate the cellular cAMP level *via* Gpa2p but further study is necessary.

Unexpectedly, in the Δ*gpr1* mutant strains with plasmid only, the glucose-induced transient rise of cAMP was completely absent. And this defect was complemented and restored by introducing *GPR1* gene with YCp vector (Figure 1B). Since *GPA2* deletion did not affect the glucose stimulated increase of cAMP (data not shown) [13], Gpr1p was also thought to be involved in another glucose sensing and signal transduction pathway which is independent of Gpa2p such as the Ras/cAMP pathway.

Recently, it was reported that glucose-induced cAMP increase was eliminated in Δ*gpa2* mutant strain when low level of glucose was pre-added to the cells before addition of high level of glucose [18]. In this study, we performed cAMP assay as previously reported [13], with which glucose-dependent cAMP increase has been

measured in many strains containing mutation in Ras/cAMP pathway or *GPA2*.

Gpr1p Is Also Required for the Increase of cAMP in Response to Other Fermentable Sugars

Previous results indicated that fermentable sugars other than glucose also elicit a transient increase of cAMP in yeast cells [20]. We examined if Gpr1p is also required for the increase of cAMP in response to other fermentable sugars. As previously reported, the addition of fructose or mannose to the starved cells induced a transient rise of cAMP in the wild type strain. The increase of cAMP induced by fructose (Figure 2B) and mannose (Figure 2C) was half or one fourth of that induced by glucose (Figure 2A), respectively. While, in Δ*gpr1* mutant strain, the cAMP level did not change after the addition of fermentable sugars. Galactose only slightly increased the cAMP level in the wild type strain (Figure 2D). These results indicate that fermentable sugars other than glucose are also preferred as carbon sources by yeast cells and generated signals which induce transient rise of cAMP level. Since Δ*gpr1* mutant strain did not induce transient rise of cAMP in response to glucose or other fermentable sugars, Gpr1p is part of the carbon source sensing machinery most likely acting as a receptor which recognizes the preferred carbon source and generates signals to regulate the cellular cAMP level.

The Membrane-Proximal Regions in the Third Cytosolic Loop of Gpr1p Are Also Important for Glucose-Induced Increase of Cellular cAMP

Next we examined which domain of Gpr1p plays an important role in glucose-induced transient increase of cAMP. It has been shown that conserved sequences in the membrane proximal regions of the third cytosolic loops in G-protein coupled receptors are required for coupling to the G-protein [21]. in Gpr1p, two such sequences are found in the third cytosolic loop: KRIKAQIG (277-284 aa) and KKRRAQIQ (610-617 aa). Recently it was reported that Gpr1p which lacks one of these sequences failed to complement the growth defect of a Δ*gpr1* Δ*ras2* double mutant strain [16]. To test whether these conserved sequences in the third cytosolic loop are required for glucose-induced increase of cAMP, we deleted each or both of these regions from the *GPR1* coding sequence and introduced into Δ*gpr1* mutant strain with YCp vector. Transformants were starved for glucose and the transient increase of cellular cAMP level in response to glucose stimulation was measured. *GPR1* lacking the region coding either 277-284 aa or 610-617 as residues could partially restore the transient increase of cAMP in response to glucose stimulation, while *GPR1* deleted of both

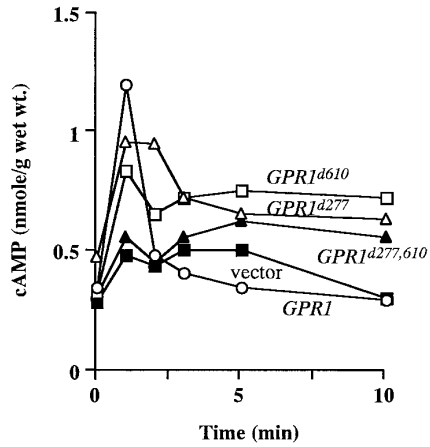

FIG. 3. Effect on cAMP regulation of deleting conserved sequences of Gpr1p which has been shown to be important for G-protein coupling. Δ*gpr1* mutant strain was transformed with p*GPR1*$^{d277\text{-}284}$, p*GPR1*$^{d610\text{-}617}$, p*GPR1*$^{d277\text{-}284,610\text{-}617}$, p*GPR1*-RS416, and vector pRS416. Each transformant was grown in SD media to the stationary phase and incubated in glucose-depleted conditions for 2h. Aliquots were collected at various time points before (0 min) and after glucose addition (1, 2, 3. 5, 10 min) and subjected to cAMP assay. cAMP level was expressed as nmole/wet weight (g) of the cells calculated from the optical density of the cell suspension. Each assay shown in this figure was done at the same time. Each value is the average from triplicated experiments. The data shown is from one of three independent experiments which gave almost the same results.

these regions could not (Figure 3). These results indicate that the regions in the third cytosolic loop shown to be important for coupling to G-protein are also required for glucose-induced increase of cellular cAMP. It was reported that the deletion in the third cytosolic region did not to affect the amount of Gpr1p, while the deletion of C-terminal region would affect the production or stability of Gpr1p [16]. Although the C-terminal region of Gpr1p was also thought to play important roles in glucose-dependent cAMP regulation, other members will be necessary to examine the role of this region.

Our observations suggest that *GPR1* is required for the transient rise of cAMP in response to glucose as well as other fermentable sugars. Although *GPR1* was isolated as a gene coding molecule which physically [19] and functionally interacts with Gpa2p [16], our results indicate that Gpr1p is also involved in another signal transduction pathway. Whether Gpr1p regulates glucose dependent cellular cAMP level through Ras/cAMP pathway or other signaling pathway remains to be elucidated.

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