Nutrient sensing systems for rapid activation of the protein kinase A pathway in yeast


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Abstract

The cAMP-protein kinase A (PKA) pathway in the yeast Saccharomyces cerevisiae controls a variety of properties that depend on the nutrient composition of the medium. High activity of the pathway occurs in the presence of rapidly fermented sugars like glucose or sucrose, but only as long as growth is maintained. Growth arrest of fermenting cells or growth on a respirative carbon source, like glycerol or ethanol, is associated with low activity of the PKA pathway. We have studied how different nutrients trigger rapid activation of the pathway. Glucose and sucrose activate cAMP synthesis through a G-protein-coupled receptor system, consisting of the GPCR Gpr1, the Gα protein Gpa2 and its RGS protein Rgs2. Glucose is also sensed intracellularly through its phosphorylation. Specific mutations in Gpr1 abolish glucose but not sucrose signalling. Activation of the PKA pathway by addition of a nitrogen source or phosphate to nitrogen- or phosphate-starved cells, respectively, is not mediated by an increase in cAMP. Activation by amino acids is triggered by the general amino acid permease Gap1, which functions as a transporter/receptor. Short truncation of the C-terminus results in constitutively activating alleles. Activation by ammonium uses the ammonium permeases Mep1 and Mep2 as receptor. Specific point mutations in Mep2 uncouple signalling from transport. Activation by phosphate is triggered a.o. by the Pho84 phosphate permease. Several mutations in Pho84 separating transport and signalling or triggering constitutive activation have been obtained.

The activity of the protein kinase A pathway is controlled by different nutrients in a concerted way

The cAMP-protein kinase A (PKA) pathway in the yeast Saccharomyces cerevisiae controls a variety of properties that depend on the nutrient composition of the medium [1–4]. When yeast cells grow with a rapidly fermented sugar like glucose or sucrose as carbon source, they display low levels of glycogen and of the storage and stress protection sugar trehalose. They also show low expression of stress response and protection genes and a low level of tolerance to various stress conditions. Fermentative growth is also associated with low cell wall resistance and low sporulation capacity. On the other hand, when yeast cells grow with respiration on non-fermentable carbon sources like glycerol or ethanol, or when they are in stationary phase they display the opposite phenotype. Mutants with an overactive cAMP-PKA pathway always display the phenotypes associated with fermentative growth. On the other hand, mutants with reduced activity of the cAMP-PKA pathway constitutively display the typical characteristics associated with respirative growth and station-

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PKA targets in phosphate-starved cells [5]. In both cases the activation does not appear to be mediated by a rise in the cAMP level although PKA activity is required. Whether this requirement is indirect or reflects activation of PKA in vivo by a signal generated directly from the nutrient-sensing system is unclear. Rapid activation of PKA targets by nitrogen sources or phosphate requires the presence of glucose or another rapidly fermented sugar. For maintenance of the effect on the PKA targets (and obviously for maintenance of growth) all essential nutrients are required. Hence, a signalling network should exist in the yeast cell that integrates information from different nutrient sensing systems into a common response that affects in a concerted way the targets of PKA [3,5].

Rapid, nutrient-induced activation of the protein kinase A pathway in nutrient-starved cells

We have been studying the nutrient sensing mechanisms involved in activation of the PKA pathway by rapidly fermented sugars, like glucose and sucrose, in respiring cells growing on glycerol, by amino acids and ammonium in cells starved for nitrogen in a glucose-containing medium and by phosphate in cells starved for phosphate in a glucose-containing medium [5]. Two mechanisms are involved in the rapid activation of cAMP synthesis by fermentable sugars: an extracellular GPCR-based mechanism that detects only glucose and sucrose, and an intracellular mechanism that is dependent on sugar phosphorylation in a way that is not well understood [4]. Because of the low \( K_m \) of sugar transport in derepressed yeast cells (about 1 mM) active glucose phosphorylation occurs at much lower glucose levels than those required for activation of Gpr1 (about 20 mM). Hence, it is possible to saturate the glucose-phosphorylation-dependent system by pre-addition of a low glucose level, triggering only a modest increase in cAMP, followed by addition of a high glucose level, which then specifically activates the GPCR system resulting in a clear spike in the cAMP level [6, 7]. Rapid activation of the PKA targets by amino acids in nitrogen-starved cells or by phosphate in phosphate-starved cells is not associated with an increase in cAMP and is still observed in strains lacking the regulatory subunit of PKA [8, 9]. However, mutations that reduce PKA activity reduce the nutrient-induced response suggesting that the response might be mediated by the catalytic subunits of PKA [9,10]. Little is known about downstream components that could link the nutrient-sensing system with PKA. Only in the case of amino acid signalling, Sch9, the yeast homologue of mammalian protein kinase B has been identified as essential for amino acid induced activation of PKA targets [11]. However, it is not required for phosphate-induced signalling [10]. Deletion of Sch9 causes a reduction in the growth rate whereas deletion of Gpr1 has no effect on the growth rate. However, double deletion of Sch9 and Gpr1 is lethal [7]. We have recently performed a screen with a mouse pancreatic \( \beta \) cell line cDNA library made in a yeast expression vector for suppression of the lethality of the \( sch9 \Delta gpr1 \Delta \) double mutant. One of the clones isolated was a putative transcription factor, called MTAC (for mammalian transcription factor activating the cAMP pathway in yeast). Further analysis showed that the factor stimulates cAMP production in yeast through the Ras2 protein and, interestingly, that it displays very strong and specific expression in pancreatic islets of mice (manuscript in preparation). It remains to be investigated whether the factor is in some way involved in glucose sensing in the pancreatic \( \beta \) and \( \alpha \) cells.

Glucose and sucrose activate through a GPCR system

Glucose and sucrose activate cAMP synthesis through a G-protein-coupled receptor system, consisting of the GPCR Gpr1, the Go protein Gpa2 and its RGS protein Rgs2 [6,7,12]. We have studied Gpr1 by SCAM (substituted cysteine accessibility method) [13]. As a result several amino acid substitutions into cysteine were identified in Gpr1 that abolish glucose but not sucrose signalling. In two of these mutant residues addition of MTSEA (methanethiosulphonate-ethyl-ammonium), which preferentially binds to cysteine in a polar environment, completely blocked the remaining activation of cAMP synthesis by sucrose. These results strongly support direct interaction of glucose and sucrose as ligands with the Gpr1 receptor. The affinity of Gpr1 for sucrose is much higher than for glucose. This is remarkable because \( S. \textit{cerevisiae} \) generally prefers glucose above sucrose as carbon source. For instance, in the presence of glucose it represses invertase so that in mixtures of glucose and sucrose, glucose is utilized first and when the glucose is exhausted and invertase derepressed, only then sucrose is utilized. The high affinity of Gpr1 for sucrose indicates that detection of low levels of sucrose is important for the survival of yeast in its natural environment. No other sugars have been found, besides glucose and sucrose, that can activate Gpr1. However, mannose has been identified as a potent antagonist of both glucose and sucrose signalling by Gpr1 [13].

Phosphorylation of low levels of glucose or fructose activates cAMP synthesis to a limited extent. Curiously, this phosphorylation-dependent activation is essential for the stimulation of cAMP synthesis by the glucose/sucrose sensing GPCR system. For instance, addition of glucose or sucrose to a yeast mutant lacking glucokinase and the two hexokinases Hxk1 and Hxk2, does not stimulate cAMP synthesis at all [14]. Addition of sucrose to an invertase-deficient strain only activates cAMP synthesis if a low level of glucose (or fructose or maltose) is added so that glucose phosphorylation is sustained [15]. This appears to be the first GPCR system where the ligand also has to be transported and converted by metabolism in order to be able to stimulate its effector. The interdependency of the GPCR system and the glucose-phosphorylation-dependent system could be related to the fact that two G-proteins act as stimulators of yeast adenylate cyclase. Gpa2 is a homologue of the classical Go proteins and is
activated by Gpr1. The Ras1 and Ras2 proteins in yeast are homologues of the mammalian Ras proteins and are required for adenylate cyclase activity [16]. They are stimulated by the upstream guanine nucleotide exchange factor Cdc25 but no upstream activator of Cdc25 has been identified that could mediate a nutrient signal to the Ras proteins. Recent work has shown that glucose addition causes a small but significant increase in the GTP content on Ras and that this increase is dependent on glucose phosphorylation [17]. Hence, glucose phosphorylation might cause a partial activation of Ras that then primes adenylate cyclase for further stimulation by the GPCR system. In this respect it has to be mentioned that the glucose-induced increase in the cAMP level is a very rapid event. Whether glucose phosphorylation is also required for stimulation of Gpr1 on a longer time scale is unclear. A hint that this might not necessarily be the case was provided by the glucose requirement for nitrogen and phosphate activation of the PKA pathway in nitrogen- and phosphate-starved cells, respectively. In this case the two glucose detection systems can apparently act separately [10].

The general amino acid permease Gap1 acts as amino acid sensor
Activation of the PKA pathway by amino acids is triggered by the general amino acid permease Gap1, which apparently functions as a transporter/receptor [18]. The first indications for such a double function of the permease were obtained with l-citrulline and D-amino acids. Low levels of l-citrulline are only transported by Gap1 and activate the PKA pathway. Transport of high levels of l-citrulline by other amino acid permeases does not activate the PKA pathway. Since D-amino acids are only transported by Gap1, also activate the PKA pathway and cannot be metabolized, involvement of Gap1 as the sensor for the amino acids became likely. Subsequent work revealed that short truncation of the C-terminus of Gap1 results in constitutively activating alleles. These alleles are dominant and they caused a high PKA phenotype for all PKA targets investigated, also under nitrogen starvation conditions and during growth with ammonium as sole nitrogen source. This indicates that transport through these alleles is not required for constitutive activation. On the other hand, introduction in such a constitutively activating allele of a mutation that abolishes transport also abolishes the overactive PKA phenotype. This indicates that the protein requires a transport-related conformational change in order to be able to constitutively activate the PKA pathway. A specific point mutation in Gap1, S391A, was also identified that had only little effect on transport of the amino acids l-citrulline and L-glutamate, but activation of the PKA target trehalase by l-glutamate was strongly reduced whereas that with l-citrulline was not affected. Such a differential effect with two amino acids further supports that Gap1 itself acts as the sensor and that the amino acids activate the receptor function of Gap1 in a similar way as ligands activate a classical receptor [18].

The Mep1 and Mep2 permeases act as ammonium sensors
Activation by ammonium apparently uses the ammonium permeases Mep1 and Mep2 as receptor (A. Van Nuland, P. Vandormael and J. Thevelein, unpublished work). Although Mep3 is a high-capacity ammonium permease it only sustains activation of trehalase very poorly. The unmetabolizable ammonium analogue, methylamine, also activates trehalase, but only when it is transported by the Mep permease and not at all when it enters the cell by diffusion. Prevention of ammonium incorporation into metabolism by inactivation of both glutamate dehydrogenase and glutamine synthase had no effect on rapid signalling. Extensive site-directed mutagenesis of Mep2 confirmed the general correlation between transport and signalling, but it also yielded several interesting and unusual alleles. For instance, mutagenesis of Asn246 to Ala abolished transport and signalling with methylamine but had no effect with ammonium. Mutagenesis of the highly conserved residues Asp339 and Asp340 to Ala abolished short-term ammonium transport and intracellular ammonium accumulation but had no effect on signalling. Remarkably, on a longer time scale ammonium transport was apparently restored to wild-type levels in the strain expressing this allele. Several Arabidopsis thaliana ammonium carriers sustained ammonium-induced trehalase activation and to varying extents ammonium transport. Interestingly they were not able to take over the requirement for Mep permeases in haploid invasive growth indicating that the function of the Mep permeases in sustaining rapid activation of the PKA pathway is different from that in haploid invasive growth (A. Van Nuland, P. Vandormael and J. Thevelein, unpublished work).

The Pho84 and Pho87 phosphate permeases act as phosphate sensors
Activation by phosphate is triggered a.o. by the Pho84 and Pho87 phosphate permeases [10]. Rapid phosphate activation is not prevented by complete inhibition of glycolysis, suggesting a metabolism-independent process, as for amino acids and ammonium. Chimaeras of phosphate carriers have been obtained that sustain normal transport but do not sustain signalling. Several point mutations in Pho84 separating transport and signalling or triggering constitutive activation have also been obtained. Conditions have been established in which a phosphate-containing compound is not transported but still activates trehalase in a phosphate carrier dependent way. This indicates that agonists can be identified that specifically activate the signalling function of transporter/receptors.

Conclusions
Nutrient control of the PKA pathway in yeast provides an excellent model system to study different types of nutrient sensing mechanisms. Under the appropriate conditions, the pathway can be activated by sugars like glucose and sucrose, by nitrogen sources like ammonium and amino acids and by
phosphate. In all cases plasma membrane based nutrient sensors are being used for nutrient-induced signalling – a GPCR system in the case of glucose and sucrose, and actively transporting permeases, transporter/receptors or 'transceptors' in the case of the other nutrients: Gap1 for amino acids, Mep1 and Mep2 for ammonium and, e.g. Pho84 for phosphate. The use of plasma membrane based nutrient sensing systems for rapid activation of the PKA pathway might be due to the short-term nature of the response. Different types of plasma membrane based nutrient sensing systems have been discovered in yeast and these findings are now being extended to other eukaryotic cells [19]. Future work will have to show whether there is a general correlation between the short-term character of nutrient responses and the use of plasma membrane components as nutrient sensors.

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