The Sch9 protein kinase in the yeast *Saccharomyces cerevisiae* controls cAPK activity and is required for nitrogen activation of the fermentable-growth-medium-induced (FGM) pathway

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In cells of the yeast *Saccharomyces cerevisiae*, trehalase activation, repression of *CTT1* (catalase), *SSA3* (Hsp70) and other STRE-controlled genes, feedback inhibition of cAMP synthesis and to some extent induction of ribosomal protein genes is controlled by the Ras-adenylate cyclase pathway and by the fermentable-growth-medium-induced pathway (FGM pathway). When derepressed cells are shifted from a non-fermentable carbon source to glucose, the Ras-adenylate cyclase pathway is transiently activated while the FGM pathway triggers a more lasting activation of the same targets when the cells become glucose-repressed. Activation of the FGM pathway is not mediated by cAMP but requires catalytic activity of cAMP-dependent protein kinase (cAPK; Tpk1, 2 or 3). This study shows that elimination of Sch9, a protein kinase with homology to the catalytic subunits of cAPK, affects all target systems in derepressed cells in a way consistent with higher activity of cAPK in *vivo*. In *vivo* measurements with trehalase and kemptide as substrates confirmed that elimination of Sch9 enhances cAPK activity about two- to threefold, in both the absence and presence of CAMP. *In vivo* it similarly affected the basal and final level but not the extent of the glucose-induced responses in derepressed cells. The reduction in growth rate caused by deletion of SCH9 is unlikely to be responsible for the increase in cAPK activity since reduction of growth rate generally leads to lower cAPK activity in yeast. On the other hand, deletion of SCH9 abolished the responses of the protein kinase A targets in glucose-repressed cells. Re-addition of nitrogen to cells starved for nitrogen in the presence of glucose failed to trigger activation of trehalase, caused strongly reduced and aberrant repression of *CTT1* and *SSA3*, and failed to induce the upshift in *RPL25* expression. From these results three conclusions can be drawn: (1) Sch9 either directly or indirectly reduces the activity of protein kinase A; (2) Sch9 is not required for glucose-induced activation of the Ras-adenylate cyclase pathway; and (3) Sch9 is required for nitrogen-induced activation of the FGM pathway. The latter indicates that Sch9 might be the target of the FGM pathway rather than cAPK itself.

Keywords: yeast, nutrient signalling, trehalase, ribosomal protein genes

**INTRODUCTION**

In micro-organisms such as the yeast *Saccharomyces cerevisiae* nutrients are the prime factors in the environment controlling growth, metabolism and development. In yeast in particular the presence in the growth medium of rapidly fermented sugars such as glucose has dramatic effects on many phenotypic properties. The
addition of glucose to cells growing on a non-fermentable carbon source or to stationary-phase cells triggers several signal transduction pathways, causing a wide variety of regulatory effects. For some of the target systems affected, more than one signalling pathway is involved. For instance, the addition of glucose triggers activation of trehalase, repression of CTT1, SSA3 and other genes with an STRE-element in their promoter, and induction of the ribosomal protein genes (for a review see Thevelein & Hohmann, 1995). These effects are caused in part by glucose-induced activation of the Ras-adenylate cyclase pathway.

In S. cerevisiae the level of cAMP is controlled by an elaborate pathway of which many components have been identified. cAMP is synthesized by adenylyl cyclase, encoded by the CYR1/CDC35 gene (Kataoka et al., 1983). The activity of the adenylyl cyclase is controlled by the Ras proteins, which belong to the family of G-proteins (Broek et al., 1985; Toda et al., 1985). The GDP/GTP ratio on the Ras proteins is controlled by the guanine nucleotide exchange proteins Cdc25 (Camonis et al., 1986; Broek et al., 1987; Jones et al., 1991) and Sdc25 (Boy-Marcotte et al., 1996) and by the GTPase-activating proteins Ira1 and Ira2 (Tanaka et al., 1990). cAMP is hydrolysed by the low-affinity phosphodiesterase encoded by PDE1 (Nikawa et al., 1987b) and the high-affinity phosphodiesterase encoded by PDE2 (Sass et al., 1986). cAMP activates cAMP-dependent protein kinase (cAPK) by binding and thereby liberating the regulatory subunits, encoded by BCY1 (Toda et al., 1987a), from the catalytic subunits, encoded by TPK1, TPK2 and TPK3 (Toda et al., 1987b). Up to now, only two triggers of the yeast Ras-adenylate cyclase pathway have been identified: glucose and related fermentable sugars (Van der Plaat, 1974; Mbonyi et al., 1988) and intracellular acidification (Thevelein et al., 1987).

The addition of glucose to derepressed yeast cells triggers a transient increase in the cAMP level resulting in a transient increase in the activity of cAPK. However, this glucose-induced cAMP increase is regulated itself by another signalling pathway, the main glucose repression pathway (Beullens et al., 1988; Arguelles et al., 1990; Dumortier et al., 1995). This pathway is known to cause repression of a large number of genes in the presence of glucose (Ronne, 1995), but is not responsible for the repression of STRE-controlled genes in a complete glucose-containing growth medium. In such a medium, the high cAPK activity induced after glucose addition is apparently maintained after the cells have become glucose-repressed by the main glucose repression pathway, since trehalase activity remains high, expression of CTT1, SSA3 and the other genes with an STRE-element in their promoter remains low and also ribosomal protein gene induction stays high for a much longer time than required to establish glucose repression (Thevelein, 1991).

The presence of a complete fermentable growth medium is essential for maintenance of this 'high-cAPK phenotype' since the absence of whatever nutrient is required for growth leads to arrest in the G1 phase of the cell cycle and the acquisition of a 'low-cAPK phenotype' similar to the phenotype of cells growing on a non-fermentable carbon source (Lillie & Pringle, 1980; Bissinger et al., 1989; Hirimburegama et al., 1992). Limitation of the growth rate on glucose by limitation of an essential nutrient leads to an intermediate phenotype. The addition of the previously lacking essential nutrient (e.g. nitrogen, phosphate or sulfate) to the cells in the presence of glucose causes rapid activation of trehalase without significant increase in the cAMP level (Thevelein, 1984; Thevelein & Beullens, 1985; Hirimburegama et al., 1992). Experiments with yeast strains lacking the regulatory subunit of cAPK have confirmed that nitrogen-induced activation of trehalase in glucose-repressed cells is not mediated by cAMP (Durnez et al., 1994). Because trehalase is always fully activated in cells containing one or more wild-type TPK genes and a deletion of the BCY1 gene, such experiments are performed in strains with two TPK genes deleted and a partially inactivating point mutation in the remaining TPK gene. Reduction of protein kinase A activity in this way reduced the extent of trehalase activation in spite of the lack of cAMP involvement (Durnez et al., 1994). Using the same strains, nutrient regulation of glycogen content, heat-shock resistance and sporulation competence (Cameron et al., 1988) and CTT1 expression (Belazzi et al., 1991) has also been demonstrated. These characteristics are known to be targets of cAPK at the transcriptional and/or posttranscriptional level. The glucose-induced upshift in ribosomal protein gene expression also depends on protein kinase A activity but is not mediated by a cAMP increase (Kraakman et al., 1993; Klein & Struhl, 1994; Griffioen et al., 1994, 1996; Neuman-Silberberg et al., 1995).

The cAMP-independent but cAPK-dependent nutrient regulation of classical cAMP pathway targets in glucose-repressed cells has led us to the suggestion that after the transient cAMP increase upon addition of glucose to derepressed cells, another pathway takes over the activation of cAPK, maintaining its high activity in a cAMP-independent way (Thevelein, 1991). Originally, we proposed the simplest model in this respect, i.e. the catalytic subunits of cAPK are directly activated by this alternative pathway. Obviously, other models are also possible, for example the catalytic subunits control the activity of another protein kinase that is activated by the new pathway and that is able to phosphorylate the same targets as cAPK. We have called this new pathway the fermentable-growth-medium-induced pathway (FGM pathway) because its activation requires a fermentable carbon source and all other nutrients required for growth (Thevelein, 1994). For activation of the FGM pathway no phosphorylation of the fermentable sugar by hexokinase 1, hexokinase 2 or glucokinase is required (Pernambuco et al., 1996). For activation of the Ras-adenylate cyclase pathway on the other hand, phosphorylation of the sugar by any one of
the three kinases is essential (Beullens et al., 1988). This supports the proposal that at least two different pathways are involved in controlling the targets affected by cAPK activity.

Other gene products have been identified whose relationship with the cAMP pathway is not so clear. One such component is the Sch9 gene product. The SCH9 gene was cloned in a screen for suppressors of the conditional growth defect of a temperature-sensitive cdc25 strain (Toda et al., 1988). SCH9 encodes a protein kinase that is homologous to the catalytic subunits of cAPK encoded by the TPK genes. Its overexpression rescues the lethality caused by deletion of the three TPK genes and causes similar phenotypic characteristics as overexpression of TPK, such as heat-shock sensitivity. Deletion of SCH9 on the other hand causes slow growth which is suppressed by elevation of cAPK activity. Therefore, the Sch9 protein kinase is believed to function in a pathway partially redundant with the cAPK pathway. However, its precise relationship to this pathway remains unclear (Toda et al., 1988). For control of ADH2 expression Sch9 was shown to act independently of cAPK (Denis & Audino, 1991). A homologue of SCH9 has been identified in Schizosaccharomyces pombe and its overexpression also rescues phenotypic defects caused by loss of protein kinase A activity (Jin et al., 1995). Deletion of cAPK is not lethal in S. pombe (Maeda et al., 1994) and this has facilitated the demonstration that in this organism a cAMP- and cAPK-independent pathway exists for glucose- and nitrogen-induced activation of trehalase (Soto et al., 1995a, b).

Here we show that deletion of SCH9 elevates cAPK activity in glucose-derepressed cells and that the Sch9 protein kinase is essential for activation of the FGM pathway by nitrogen for all targets investigated. The latter indicates that Sch9 might be the target of the FGM pathway rather than the catalytic subunits of cAPK.

**METHODS**

**Yeast strains and media.** The following *S. cerevisiae* strains were used: DC124 (MATa his4 leu2 ura3 trp1 ade8 can1), T198-8B (MATa his3 leu2 ura3 trp1 ade8 SCH9::ADE8; Toda et al., 1988), S13-3A (MATa his3 leu2 ura3 trp1 ade8 TPK1 tpk2::HIS3 tpk3::TRP1 bcy1::LEU2; Nikawa et al., 1987a), S25-31C (MATa his3 leu2 ura3 trp1 ade8 TPK1 tpk2::HIS3 tpk3::TRP1 bcy1::LEU2 sch9::ADE; Toda et al., 1988), T198-8B+YEpSCH9 (MATa his3 leu2 ura3 trp1 ade8 sch9::ADE8 + YEpSCH9; this work). Rich media used were: YPD (YPD), YPGlycerol and YPraffinose, which contained 1% (w/v) yeast extract, 2% (w/v) bactopeptone and respectively 2% (w/v) glucose, 3% (v/v) glycerol or 2% (w/v) raffinose. Yeast cells were grown into mid-exponential phase (OD600 1.5) at 30 °C. Nitrogen starvation medium was minimal synthetic medium (Sherman et al., 1986) with 4% (w/v) glucose and 0.67% (w/v) Bacto yeast nitrogen base without amino acids and without ammonium sulfate (Difco). Mid-exponential (OD600 1.5) YPD-growing yeast cells were harvested, resuspended in minimal synthetic medium and incubated with shaking for 24 h at 30 °C. Care was taken that the glucose level remained high (> 2%) throughout the 24 h of incubation.

**Incubation and extraction conditions.** Exponential-phase cells growing on YPGlycerol or YPraffinose or stationary-phase cells obtained on nitrogen-starvation glucose-containing medium were collected by centrifugation, washed twice with ice-cold 25 mM MES/KOH buffer (pH 6) and resuspended at a cell density of 25 mg ml⁻¹ either in fresh YPGlycerol or fresh glucose-containing nitrogen-starvation medium. They were pre-incubated for 30 min at 30 °C before the first sample was taken. Glucose-induced or nitrogen-source-induced activation of trehalase was triggered by addition of respectively 100 mM glucose or 10 mM L-asparagine and the amino acids for which the strain was auxotropic.

**Determination of trehalase activity and cAMP levels.** Trehalase activity in crude cell extracts was determined as described previously (Fernambuco et al., 1996). The specific activity of trehalase is expressed as nmol glucose liberated min⁻¹ (mg protein)⁻¹. cAMP levels were determined according to Thevelein et al. (1987) and expressed as nmol (g wet wt)⁻¹.

**In vitro activation of trehalase.** Activation of trehalase in cell extracts with cAMP and a protein kinase A activation mixture was carried out according to Thevelein & Beullens (1985).

**In vitro activation of cAPK with kemptide as substrate.** cAPK activity was determined in cell extracts with kemptide as substrate by using the Pierce colorimetric PKA assay kit, Spinzyme format.

**RNA extraction and Northern blot analysis.** Cells were grown under appropriate conditions and rapidly cooled by addition of ice-cold water. The cells were pelleted and washed once with ice-cold water and then stored at −70 °C. Isolation of total RNA was performed essentially as described previously (Fernambuco et al., 1996). Probes were labelled with [α-32P]dCTP and [α-32P]dATP using the High Prime kit (Bohringer Mannheim). Northern blots were made by separation of total RNA in a denaturing 5% (w/v) agarose in 50 mM boric acid, 1 mM sodium citrate, 5 mM NaOH, pH 7.5, and 1% formaldehyde. Subsequently RNA was transferred by capillary action to a Hybond-N membrane (Amersham) using 10× SSC. These blots were hybridized with 32P-labelled probe fragments of the coding region of CTT1, SSA3, HSP12 and RPL25. The blots were then analysed using phosphorimagery technology (Fuji, BAS-1000; software, PCBAS 2.0). Actin mRNA levels were used as standards.

**RESULTS**

Addition of glucose to derepressed *S. cerevisiae* cells caused activation of neutral trehalase within a few minutes (Fig. 1). In cells of an sch9 strain glucose triggered trehalase activation to a similar extent as in wild-type cells. However, the initial trehalase activity and the final level after activation were always significantly higher compared to wild-type cells (Fig. 1). In a strain constitutively high protein kinase A activity (TPK1 bcy1) trehalase activity was constantly high throughout the experiment but the level never exceeded the maximum level observed in wild-type cells after addition of glucose. When SCH9 was deleted in the same strain, trehalase activity further increased at least in the pre-incubation phase before the addition of...
glucose (Fig. 1). Apparently, deletion of SCH9 enhances — either directly or indirectly — protein kinase A activity, resulting in higher activity of trehalase.

Glucose-induced activation of trehalase in derepressed cells was preceded by a transient increase in the cAMP level (Fig. 2a). Addition of agents triggering intracellular acidification, such as 2,4-dinitrophenol, also caused a cAMP increase, but it was higher and longer-lasting than the glucose-induced increase (Fig. 2b). In an sch9 strain, cAMP increases induced by both glucose and dinitrophenol were always lower than in the wild-type strain (Fig. 2a, b). The reduced glucose-induced cAMP increase in the sch9 strain appears to contradict the enhanced trehalase activity observed in this strain (Fig. 1). However, cAMP synthesis and the glucose-induced cAMP signal are known to be downregulated by high protein kinase A activity (Nikawa et al., 1987a; Mbonyi et al., 1990). The lower cAMP increases in the sch9 strain therefore do not necessarily indicate a possible involvement of Sch9 in the control of cAMP synthesis but they are consistent with enhanced protein kinase A activity in the sch9 strain (see Discussion).

The evidence for higher cAPK activity in the sch9 strain obtained in the in vivo trehalase activation experiments was supported by in vitro measurements of cAPK activity. Trehalase was activated in cell extracts with an activation mixture and with increasing concentrations of CAMP, as described previously (Thevelein & Beullens, 1985). The maximal trehalase activity obtained after incubation of the cell extracts with the activation mixture was much higher in the sch9 strain compared to the wild-type strain both in the absence of cAMP and at all cAMP concentrations tested (Fig. 3). The difference between the activity obtained in the absence of cAMP and at the highest cAMP concentration was smaller in the sch9 strain than in the wild-type strain. It should be taken into account, however, that the initial trehalase activity present before incubation with the activation mixture was higher in the sch9 strain (Fig. 3). To avoid interference with the higher initial trehalase activity present in the sch9 strain we also measured cAPK activity in the same cell extracts with the artificial substrate kemptide. Here again we consistently found about two- to threefold higher cAPK activity in the sch9 strain compared to the wild-type strain (Fig. 4). The difference between the extent of kemptide phosphorylation obtained in the absence and presence of cAMP was smaller in the sch9 strain compared to the wild-type strain.

In stationary-phase cells obtained after nitrogen starvation of a culture on glucose-containing medium, i.e. in glucose-repressed cells, a different picture was obtained for the effect of sch9 on trehalase activation. In this case addition of a nitrogen source, for example L-asparagine, in the presence of glucose triggered rapid activation of
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In Fig. 3, in vitro activation of trehalase in a crude cell extract with different concentrations of cAMP. Cell extracts of the wild type (●) and an sch9 strain (○) were incubated for 10 min at 30 °C in the presence of a cAPK activation mixture (see Methods) and different concentrations of cAMP. At the end of the incubation period, a sample was removed for determination of trehalase activity.

Fig. 4. In vitro activation of cAPK in a crude cell extract with different concentrations of cAMP and kemptide as substrate. After incubation of the fluorescently labelled kemptide substrate in a cell extract with the cAPK activation mixture, phosphorylated substrate was separated out by specific binding to an affinity membrane and quantified by A_{360} measurements. Strains: ●, wild-type; ○, sch9.

In Fig. 5, nitrogen-induced activation of trehalase in nitrogen-starved glucose-repressed cells. L-Asparagine (10 mM) and the essential amino acids were added at time zero to cells of the following strains: ●, wild-type; ○, sch9; △, TPK1 bcY1; △, TPK1 bcY1 sch9; ■, sch9 + YEp213-SCH9.

Trehalase in wild-type cells (Hirimburegama et al., 1992; Durnez et al., 1994; Fig. 5). In the sch9 strain, trehalase activity was never induced by a nitrogen source. Initial trehalase activity was rather variable in this strain, but always higher than in the wild-type. Often, the activity dropped during the pre-incubation period (Fig. 5). This was never observed in the TPK1 bcY1 and the TPK1 bcY1 sch9 strains, where trehalase activity remained high throughout the experiment. In glucose-repressed cells there was no significant difference in trehalase activity between these two strains. When SCH9 was re-introduced in the sch9 strain on a multi-copy plasmid, nitrogen-induced trehalase activation was restored (Fig. 5). In this SCH9-overexpressing strain, both the initial activity and the extent of nitrogen-induced trehalase activation were significantly enhanced. As observed with the sch9 strain, initial trehalase activity dropped somewhat during the pre-incubation period in this SCH9 overexpressing strain (Fig. 5). When the sch9 strain was grown under the same conditions but in minimal medium rather than rich medium, nitrogen-induced trehalase activation was either absent or at least strongly reduced in all experiments (results not shown). Trehalose accumulation during nitrogen starvation on glucose-containing medium was similar in an sch9 strain and a wild-type strain (results not shown).

In addition to trehalase, we also studied the expression of CTT1, SSA3, HSP12 and RPL2, as other targets of cAPK. In yeast cells growing on a non-fermentable carbon source the expression of CTT1, SSA3 and HSP12 is elevated compared to the expression on fermentable carbon sources (Bissinger et al., 1989; Prackelt & Meacock, 1990). Addition of glucose triggers rapid disappearance of the messengers (Pernambuco et al., 1996). Consistent with the higher trehalase activity in derepressed cells of the sch9 strain (Fig. 1) we found that the initial expression level of CTT1, SSA3 and HSP12 was reduced (Fig. 6). Addition of glucose caused a further rapid reduction in the messenger level until it became nearly undetectable as in wild-type cells. In the TPK1 bcY1 and the TPK1 bcY1 sch9 strains the expression level was very low throughout the experiments (Fig. 6a), consistent with the very high trehalase
activity observed previously throughout the same experiment (Fig. 1).

The expression of ribosomal protein genes is low in wild-type cells growing on a nonfermentable carbon source and rapidly increases upon addition of glucose (Mager & Planta, 1991). This glucose-induced upshift is thought to be triggered partially by activation of cAPK (Klein & Struhl, 1994; Griffioen et al., 1994). Deletion of SCH9 did not have a significant effect on the glucose-induced upshift of RPL25 (Fig. 7). On the other hand, quantification of Northern blots showed that the expression level of RPL25 was elevated throughout the experiment in the TPK1 bcyl and the TPK1 bcyl sch9 strains (Fig. 7b). The glucose-induced upshift, however, was still present (Fig. 7a, b).

When wild-type yeast cells were starved for nitrogen on a glucose-containing medium, expression of CTT1, SSA3 and HSP12 was strongly induced (Fig. 8) while the expression of the ribosomal protein genes, for example RPL25, was very low (Fig. 9). Addition of nitrogen in the presence of glucose to such cells caused rapid disappearance of the CTT1, SSA3 and HSP12 messengers (Fig. 8) and rapid induction of RPL25 (Fig. 9). In cells of the sch9 strain the disappearance of the CTT1, SSA3 and HSP12 messengers was clearly affected compared to the wild-type strain. Initial repression was reduced and only transient. At 30 and 60 min strong signals were present for all three genes in the sch9 strain while no signals were present in the wild-type strain at these time points. However, at later times the messengers also disappeared in the sch9 strain (Fig. 8). Apparently, the disappearance is caused by both Sch9-dependent and Sch9-independent mechanisms. The induction of RPL25 was completely abolished in the sch9 strain, pointing to a central involvement of Sch9 in the pathways con-
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Fig. 7. (a) Northern blot analysis of the expression of the ribosomal protein gene RPL25 after addition of 2% glucose for 5, 10, 30, 60, 180 and 300 min to exponential-phase glycerol-grown cells of the following strains: wild-type, sch9, TPK1 bcy1 and TPK1 bcy1 sch9. Actin messenger levels are shown as control. (b) Quantification of Northern blots. Actin messenger levels were used for calibration.

Fig. 8. Northern blot analysis of the expression of CTT1 (catalase T), SSA3 (heat shock protein Hsp70) and HSP12 (heat shock protein Hsp12) after addition of a nitrogen source (10 mM L-asparagine and the essential amino acids) for 5, 10, 30, 60, 180 and 300 min in the presence of glucose to cells starved for nitrogen on glucose-containing medium. Strains: wild-type, sch9, TPK1 bcy1 and TPK1 bcy1 sch9. Actin messenger levels are shown as control.

trolling ribosomal protein induction (Fig. 9). In the TPK1 bcy1 and the TPK1 bcy1 sch9 strains expression of CTT1, SSA3 and HSP12 was very low throughout the experiment, while the level of RPL25 messenger was constitutive and somewhat higher than in the sch9 strain (Fig. 9). It has been reported previously that ribosomal protein messengers do not decrease in bcy1 mutants upon amino acid starvation elicited by aminotriazole, a competitive inhibitor of histidine biosynthesis (Klein & Struhl, 1994). In our experiments, however, the cells
were placed under complete nitrogen starvation for 24 h, which apparently caused a reduction of the ribosomal protein messengers also in \( bcy1 \) cells: the initial level in the \( bcy1 \) cells was similar or only slightly elevated compared to the wild-type strain (Fig. 9a, 0 min).

**DISCUSSION**

We have investigated a possible involvement of the Sch9 protein kinase in two nutrient signalling pathways: (1) the pathway causing glucose-induced activation of cAMP synthesis in derepressed yeast cells and its effect on different cAPK targets; and (2) nitrogen-induced activation of the same targets by the FGM pathway in glucose-repressed cells. The results obtained in the two cases were clearly different. Apparently, the Sch9 protein kinase is not required for glucose-induced activation of trehalase and glucose-induced repression of the \( CTT1 \), \( SSA3 \) and \( HSP12 \) messengers in derepressed cells. In addition, the glucose-induced upshift in ribosomal protein synthesis, which appears to be triggered partially by cAPK, was not affected by deletion of \( SCH9 \). These results are contradictory at first sight with the clear reduction in the glucose-induced cAMP signal observed in \( sch9 \) cells. However, both the trehalase activation experiments and the measurements of glucose-induced disappearance of the STRE-controlled messengers indicated an elevated basal level of cAPK activity in derepressed cells. Initial trehalase activity was clearly enhanced and expression of \( CTT1 \), \( SSA3 \) and \( HSP12 \) clearly reduced compared to wild-type cells. This elevated basal cAPK activity in the \( sch9 \) strain is probably the cause of the reduced cAMP signal observed after addition of glucose. Also the protonophore-induced cAMP increase was reduced in the \( sch9 \) strain compared to wild-type strain. It is well known that yeast mutants with enhanced cAPK activity display increased feedback inhibition of cAMP synthesis (Nikawa et al., 1987a; Mbonyi et al., 1990). We have observed that \( pde2 \) mutants have a reduced cAMP increase both with glucose and dinitrophenol while \( bcy1 \) mutants and \( pde1 pde2 \) mutants do not show an increase at all (unpublished results).

Deletion of \( SCH9 \) causes a slow-growth phenotype. This growth defect can be suppressed by high protein kinase A activity, for example by deletion of \( BCY1 \) or presence of \( RAS2^{val19} \) (Toda et al., 1988). Our finding that deletion of \( SCH9 \) itself causes partially elevated protein kinase A activity suggests that the Sch9 protein kinase might be more essential for growth than previously anticipated. If the elevation of protein kinase A activity were prevented, deletion of \( SCH9 \) might be lethal.

Up to now, the Sch9 protein kinase has been thought of as either having a function in a growth-controlling pathway partially redundant with the protein kinase A pathway or alternatively having a different function but causing the same effects as protein kinase A when
overexpressed because of a similar substrate specificity. We provide for the first time evidence that the function of Sch9 and protein kinase A might be more directly related to each other. When Sch9 is deleted the activity of the protein kinase A in derepressed cells is strongly elevated and this is supported both by in vivo measurements of protein kinase A targets and by direct in vitro measurements of its activity. Although it cannot be excluded that the reduction in the growth rate caused by deletion of SCH9 is responsible for the increase in cAPK activity, this is rather unlikely since slower growth rates in yeast generally cause a phenotype consistent with lower cAPK activity. Future work will have to show whether the effect of SCH9 deletion on cAPK activity is exerted at the transcriptional or post-transcriptional level, or at both. In addition, it appears that the extent of protein kinase A activation with increasing cAMP levels is higher in wild-type cells than in sch9 cells. If the Sch9 protein kinase directly phosphorylates protein kinase A, this would cause a stronger affinity between the catalytic and regulatory subunits of protein kinase A. Previous work has shown that prevention of phosphorylation of the catalytic subunits of protein kinase A by substitution of threonine 241 for alanine reduces the affinity of binding to the regulatory subunits (Levin et al., 1988). If such phosphorylation of the catalytic subunits is carried out by Sch9 it would explain in part the enhanced protein kinase A activity observed in vitro at the lower cAMP concentrations (Figs 3 and 4). On the other hand, the activity of protein kinase A in vitro was also elevated at the highest cAMP concentrations, where one would expect the catalytic and regulatory subunits to be completely dissociated (Figs 3 and 4). This seems to indicate that Sch9 deletion enhances the expression level of the catalytic subunits or their absolute activity. Because of the sequence and substrate similarity between Sch9 and protein kinase A, it is difficult to elucidate the physiological function of Sch9 by using strains that overexpress Sch9. The present finding that deletion of Sch9 elevates protein kinase A activity complicates the picture even further.

In glucose-derepressed cells, deletion of Sch9 appeared to affect only the basal activity of protein kinase A and not the glucose-induced responses. On the other hand, in glucose-repressed cells clear effects of Sch9 deletion on nutrient-induced regulation of protein kinase A targets were observed. There was no nitrogen-induced activation of trehalase or nitrogen induction of RPL25, while repression of CTT1, SSA3 and HSP12 was severely impaired (Figs 5, 8 and 9). We have been studying nutrient-induced effects on protein kinase A targets in glucose-repressed cells by first starving the cells on a glucose-containing medium for another essential nutrient like nitrogen, phosphate or sulfate until all cells accumulate in G0 and subsequently (with a glucose level still high enough to maintain glucose repression) adding the lacking essential nutrient to the cells. During the starvation period the cells accumulate a high level of trehalose, in spite of the continued presence of glucose in the external medium, while re-addition of the lacking essential nutrient triggers rapid (within a few minutes) activation of trehalase resulting in mobilization of trehalose (Hirimburegama et al., 1992; Durnez et al., 1994). Similar changes are observed for other protein kinase A targets (Pernambuco et al., 1996). Previous results have clearly shown that these nutrient-induced responses in the protein kinase A targets are not cAMP-mediated. The finding of a different phosphorylation requirement for the fermentable sugar in this case, as opposed to glucose-induced activation of the Ras-adenylate cyclase pathway, has supported the proposal that a different pathway is involved (Pernambuco et al., 1996). The specific requirement of Sch9 for nutrient-induced activation of the protein kinase A targets in glucose-repressed cells (Figs 5, 8 and 9) further supports the concept that a different nutrient-induced pathway controls protein kinase A or at least part of its targets in glucose-repressed cells through a mechanism, not involving glucose activation of the Ras-adenylate cyclase pathway.

We have previously called this alternative pathway the fermentable-growth-medium-induced pathway (FGM pathway) (Thevelein, 1994) and we suggested that it is able to activate the free catalytic subunits of protein kinase A in a cAMP-independent way (Thevelein, 1991). This suggestion was based first on the strong similarity between the targets affected by protein kinase A and those affected by the FGM pathway, and second on the requirement of protein kinase A activity for activation of these targets by the FGM pathway. In addition, the presence of the regulatory subunit of protein kinase A appeared to reduce the extent of activation (Durnez et al., 1994). Our present results open the possibility that Sch9 rather than protein kinase A might be the protein kinase activated by the FGM pathway and responsible for phosphorylation of the different targets. Protein kinase A activity might then be required for proper functioning of the FGM pathway or simply for expression of one or more of its intermediary components.

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