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REVIEW

Life in the midst of scarcity: adaptations to nutrient availability in *Saccharomyces cerevisiae*

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Abstract Cells of all living organisms contain complex signal transduction networks to ensure that a wide range of physiological properties are properly adapted to the environmental conditions. The fundamental concepts and individual building blocks of these signalling networks are generally well-conserved from yeast to man; yet, the central role that growth factors and hormones play in the regulation of signalling cascades in higher eukaryotes is executed by nutrients in yeast. Several nutrient-controlled pathways, which regulate cell growth and proliferation, metabolism and stress resistance, have been defined in yeast. These pathways are integrated into a signalling network, which ensures that yeast cells enter a quiescent, resting phase (G_0) to survive periods of nutrient scarceness and that they rapidly resume growth and cell proliferation when nutrient conditions become favourable again. A series of well-conserved nutrient-sensory protein kinases perform key roles in this signalling network: i.e. Snf1, PKA, Tor1 and Tor2, Sch9 and Pho85–Pho80. In this review, we provide a comprehensive overview on the current understanding of the signalling processes mediated via these kinases with a particular focus on how these individual pathways converge to signalling networks that ultimately ensure the dynamic translation of extracellular nutrient signals into appropriate physiological responses.

Keywords Nutrient sensing · Signal transduction · Yeast · TOR · PKA · Sch9

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Introduction

All living organisms have evolved complex signal transduction networks that ensure the fast and optimal adaptation of cellular metabolism to changes in the environmental conditions. Since signal transduction components and mechanisms are highly conserved among all eukaryotes, the unicellular eukaryote *Saccharomyces cerevisiae*, or budding yeast, is often used as a model organism to study cell signalling. For *S. cerevisiae* cells, the constantly fluctuating nutrient content of the environment is a key determinant of cell cycle progression and growth, stress resistance and metabolism. The nutrient-induced signalling network enables yeast both to optimally profit from rich nutrient conditions by stimulating cell proliferation and to survive periods of nutrient scarceness by inducing the entry into a quiescent, resting phase, called the stationary phase (G_0) (Winderickx et al. 2003; Roosen et al. 2004; Zaman et al. 2008). In general, a nutrient is sensed by the signalling network (i) externally, via a receptor protein in the plasma membrane, which after binding of the nutrient adopts a new conformation that activates a downstream signalling cascade, or (ii) internally, after uptake of the nutrient, possibly followed by its metabolism, thereby causing a change in its intracellular concentration which, in

turn, modulates downstream signalling (Forsberg and Ljungdahl 2001; Holsbeeks et al. 2004).

Yeast can use a wide variety of substances as a nutrient source. Nevertheless, some nutrients are preferred over others and nutrient metabolism is regulated in such a way that the preferred nutrient source is consumed first. Especially, the carbon source has a high impact on *S. cerevisiae* metabolism. In contrast to most yeast species, *S. cerevisiae* cells will, given that all other essential nutrients are present in adequate amounts, preferably ferment glucose and other rapidly fermentable sugars to ethanol and acetate, although respiration would be energetically more favourable. It is believed that this phenomenon, called the Crabtree effect, gives yeast cells a competitive advantage, as the ethanol produced during fermentation inhibits growth of other micro-organisms. When glucose becomes limiting yeast will enter the diauxic shift, during which metabolism shifts from fermentation to respiration to allow usage of ethanol and acetate, which have accumulated during the fermentative growth phase. Finally, also when these carbon sources have been exhausted, cells will enter the stationary phase (G_0). Importantly, when another essential nutrient becomes limiting before glucose, yeast cells directly enter the stationary phase without passing through all other growth phases (Fig. 1). This review will focus on the most important nutrient kinases, i.e. Snf1, PKA, Tor1 and Tor2, Sch9 and Pho85–Pho80, and clarify their roles in adaptation to the specific nutrient-induced stress conditions.

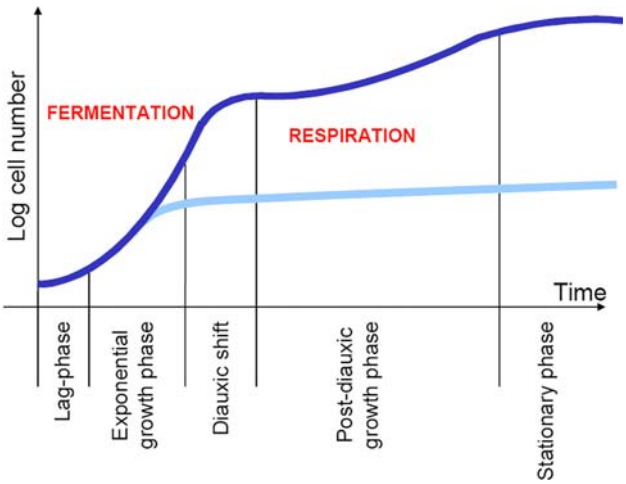

Fig. 1 Typical growth profile of a fermentative batch culture of *S. cerevisiae*. A schematic representation of the increase in cell number and cell density of a batch culture of *S. cerevisiae* inoculated in rich medium containing the rapidly fermentable sugar glucose as carbon source (*dark line*). After a short adaptive lag phase, yeast consumes glucose during the exponential fermentative growth phase. When the glucose becomes limiting, yeast cells enter the diauxic shift and shift from a fermentative to a respiratory metabolism. In the post-diauxic growth phase, the cells resume growth using ethanol, acetate and other products produced during glucose fermentation as carbon source. Finally, when these carbon sources are exhausted, the cells enter a quiescent state, the stationary phase (G_0), with the ultimate goal of surviving the starvation period. When exponentially growing yeast cells are transferred to medium containing glucose but missing an essential nutrient such as nitrogen or phosphate, they arrest growth and enter the G_0 state due to nutrient deprivation (*light line*)

Nutrient-induced signalling in *S. cerevisiae*

The main glucose repression pathway

The main glucose repression or catabolite repression pathway controls the adaptation of yeast carbon metabolism to the availability of glucose in the medium. In the presence of glucose, the central component of this pathway, the serine/threonine protein kinase Snf1 is inactivated, resulting in the transcriptional repression of genes that are not needed during fermentative growth on glucose, i.e. genes encoding for enzymes involved in gluconeogenesis, the Krebs cycle, respiration and the uptake and metabolism of alternative carbon sources (Fig. 2) (Ronne 1995; Gancedo 1998; Hedbacker and Carlson 2008).

Structure and regulation of the Snf1 kinase

The Snf1 kinase belongs to the well-conserved Snf1/AMP-activated protein kinase (AMPK) family (Woods et al. 1994; Hardie 2007). As the other members of this family, yeast Snf1 functions in a heterotrimeric complex, comprising a catalytic α subunit (Snf1), a scaffolding as well as regulatory β subunit (Gal83, Sip1 or Sip2) and a regulatory γ subunit (Snf4) (Celenza and Carlson 1989; Celenza et al. 1989; Yang et al. 1994; Jiang and Carlson 1997; McCartney et al. 2005). The Snf1 kinase complex is controlled through multiple mechanisms. When high levels of glucose are present, an auto-inhibitory interaction between the N-terminal catalytic domain and the C-terminal regulatory domain of Snf1 inactivates the protein kinase. This auto-inhibition is relieved when glucose levels drop, which correlates with an increased interaction between the γ subunit Snf4 and the Snf1 regulatory domain (Jiang and Carlson 1996).

Additionally, full activation of Snf1 requires phosphorylation of a conserved threonine residue (Thr210) in the activation loop of the Snf1 catalytic domain (Estruch et al. 1992; Wilson et al. 1996; McCartney and Schmidt 2001). Three upstream kinases, Sak1, Elm1 and Tos3, are responsible for phosphorylation of this threonine residue (Sutherland et al. 2003). These kinases appear to be functionally redundant, since only the absence of all three causes a *snf1Δ* mutant phenotype. Nevertheless, it was reported that their role in Snf1 activation is determined by the combination of the β subunit present in the kinase complex and the growth/stress conditions tested

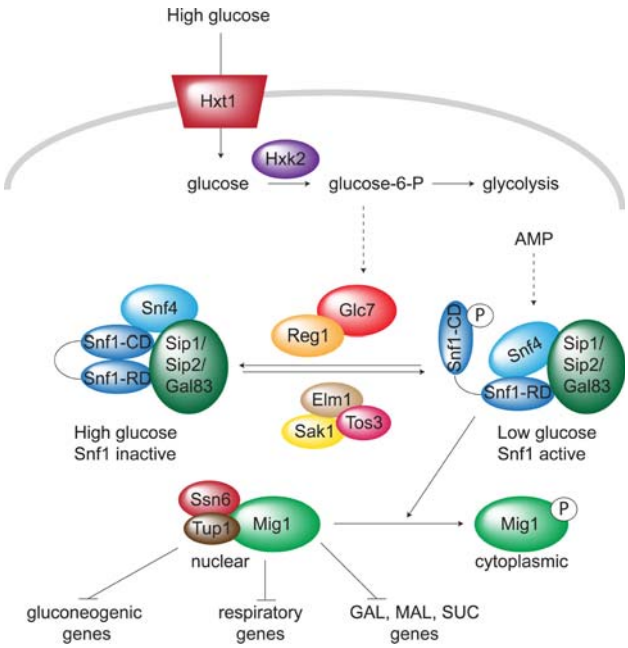

Fig. 2 The main glucose repression pathway in *S. cerevisiae*. In the presence of high levels of glucose, the Snf1 kinase complex is inactive due to an auto-inhibitory interaction between the catalytic domain (CD) and the regulatory domain (RD) of Snf1. Activation of Snf1 upon glucose exhaustion requires the phosphorylation of Thr^{210} in the activation loop of the catalytic domain of Snf1 and the binding of Snf4 to the regulatory domain of Snf1, which is necessary to lift Snf1 auto-inhibition. The phosphorylation status of the threonine residue is controlled by the upstream kinases Sak1, Elm1 and Tos3 and the Glc7–Reg1 phosphatase complex. Activated Snf1 phosphorylates Mig1, thereby stimulating the translocation of the repressor to the cytoplasm, which relieves several gene families of glucose repression. The glucose signal that controls Snf1 activity is possibly transduced via Hxk2 to Glc7–Reg1 and via a sensing of the AMP/ATP ratio by Snf4. *Arrows* and *bars* represent positive and negative interactions, respectively. *Dashed lines* represent putative or indirect interactions. See text for further details

(Hedbacker et al. 2004; Kim et al. 2005; McCartney et al. 2005). Dephosphorylation of Snf1 is mediated by the protein phosphatase complex Glc7–Reg1 (Ludin et al. 1998; Sanz et al. 2000; McCartney and Schmidt 2001). Glc7 is the catalytic subunit of the phosphatase complex, whereas Reg1 is the regulatory subunit that targets the phosphatase to Snf1 since it can bind to the catalytic domain of the protein kinase. In yeast cells, lacking a proper Glc7–Reg1 function, the Snf1 kinase complex is constitutively active resulting in loss of glucose repression (Niederacher and Entian 1991; Tung et al. 1992; Tu and Carlson 1994, 1995; Huang et al. 1996; Hong et al. 2005). However, gluconeogenic genes remain repressed in a *reg1Δ* strain, while genes involved in the utilization of alternative carbon sources are derepressed (Schuller 2003). The interaction between Glc7–Reg1 and the Snf1 kinase complex is stimulated upon glucose resupplementation, which was proposed to facilitate the fast dephosphorylation and subsequent inactivation of Snf1 by the phosphatase complex (Sanz et al. 2000). In support of such a crucial role for the phosphatase in the regulation of Snf1 activity, it was recently reported that the dephosphorylation rate of Snf1 is more subject to glucose regulation than the phosphorylation rate (Rubenstein et al. 2008). However, it also appears that the catalytic activity of the Glc7–Reg1 phosphatase itself is not controlled by glucose, but rather Snf1 itself, in such a way that the ability of the Snf1 activation loop to act as a substrate for Glc7–Reg1 is adapted to glucose availability (Rubenstein et al. 2008).

Finally, also the subcellular localization of the Snf1 kinase complex is regulated and depends on the β subunit. During growth on glucose medium, all Snf1 complexes are localized in the cytoplasm. However, upon glucose limitation, the Snf1–Sip1 complex translocates to the vacuole and the Snf1–Gal83 complex goes into the nucleus, whereas the Snf1–Sip2 complex remains in the cytoplasm (Vincent et al. 2001). Certainly, the regulation of the nucleocytoplasmic distribution of Snf1–Gal83 is important, as Snf1 has such a great impact on gene expression. Although in a *snf1Δ* mutant Gal83 exhibits a glucose-regulated nuclear accumulation, it was shown that the relocalization of the Snf1–Gal83 complex to the nucleus requires both Gal83 as well as an active Snf1 catalytic subunit (Hedbacker et al. 2004; Hedbacker and Carlson 2006). Cytoplasmic retention of inactive Snf1–Gal83 complexes is probably necessary to maintain accessibility of the complex to the Snf1-activating kinases.

Downstream targets of Snf1

The most important Snf1 target is the transcriptional repressor Mig1. In the presence of glucose, Mig1 is localized in the nucleus and inhibits the expression of many glucose-repressed genes by binding to their promoter in association with the corepressor complex Cyc8/Ssn6–Tup1 (Nehlin and Ronne 1990; Keleher et al. 1992; Lundin et al. 1994; Treitel and Carlson 1995). Glucose exhaustion induces phosphorylation of Mig1 by the activated Snf1 complex, which abolishes the interaction between Mig1 and the repressor complex, causing a relief of glucose repression (Treitel et al. 1998; Smith et al. 1999; Papamichos-Chronakis et al. 2004). Phosphorylation of Mig1 triggers at the same time its interaction with the nuclear export factor Msn5, resulting in nuclear exclusion of Mig1 (De Vit et al. 1997; De Vit and Johnston 1999).

The transcriptional activators Cat8 and Sip4 are two additional effectors of Snf1 that control the expression of gluconeogenic genes in response to glucose exhaustion (Hedges et al. 1995; Lesage et al. 1996; Randez-Gil et al. 1997). Both the expression and activity of these factors are strictly regulated. The gene encoding Cat8 is subject to

Mig1-dependent repression under high-glucose conditions (Hedges et al. 1995), and once synthesized upon glucose exhaustion, the protein must first be phosphorylated by Snf1 to obtain its transcriptional activity (Rahner et al. 1996; Randez-Gil et al. 1997). Cat8 induces transcription of *SIP4* (Vincent and Carlson 1998), and subsequent Snf1-dependent phosphorylation of Sip4 then leads to proper induction of gluconeogenic genes by both Cat8 and Sip4 (Hiesinger et al. 2001).

Apart from carbon metabolism, Snf1 affects several other processes via the regulation of transcription factors as well. In particular, the activity of Msn2/4 and Hsf1, transcriptional activators involved in general stress responses, and of Gln3, a transcriptional activator of genes induced upon nitrogen depletion, was shown to be modulated by Snf1 (Bertram et al. 2002; Mayordomo et al. 2002; Hahn and Thiele 2004). Furthermore, Snf1 does also influence transcription in a more indirect manner. As histone kinase, it phosphorylates Ser^{10} in histone H3, thereby stimulating chromatin remodelling in concert with the Gcn5 acetyltransferase, and recruiting co-activators and the TATA-binding protein to the promoter of genes like *INO1*, encoding inositol 1-phosphate synthase, and *HXT4*, which encodes one of the high-affinity glucose transporters (Lo et al. 2001; van Oevelen et al. 2006).

Transducing the glucose signal to Snf1

An important issue about the glucose repression pathway that remains unclear is how the glucose signal is exactly transduced to the Snf1 kinase complex or its regulators: the kinases Sak1, Elm1 and Tos3 and the phosphatase complex Glc7–Reg1. At the moment, two major hypotheses exist. In mammalian cells, the AMP-activated protein kinase (AMPK), homologue of Snf1, is activated by AMP that binds to the γ subunit. It was, therefore, proposed that the AMP/ATP ratio or the AMP levels in yeast also act as a signal for Snf1 activation, since they reflect the rate of glucose phosphorylation and are well correlated with Snf1 activity under a variety of growth conditions (Wilson et al. 1996). Early studies reported that Snf1 could not be activated by AMP in vitro (Mitchelhill et al. 1994; Woods et al. 1994) but, more recently, it turned out that mutations in Snf4, at sites which in AMPK contribute to AMP binding and regulation, do relieve glucose inhibition of Snf1 (Momcilovic et al. 2008).

In a second model, the role of sensor and transducer of the glucose signal is attributed to hexokinase 2 (Hxk2), which acts in the first step of glycolysis phosphorylating glucose on C6. Two other hexokinases, Hxk1 and Glk1, can also catalyse this reaction, but Hxk2 is believed to be the crucial hexokinase during growth on glucose, since Hxk1 and Glk1 are themselves subject to glucose repression (De Winde et al. 1996). It was reported that in a *hxk2Δ* mutant, the glucose repression of several genes was severely reduced (Zimmermann and Scheel 1977; Entian 1980; Entian and Zimmermann 1980), the interaction between Snf1 and Snf4 was increased (Jiang and Carlson 1996; Sanz et al. 2000) and that Snf1 still phosphorylated Mig1 in the presence of glucose (Treitel et al. 1998; Ahuatzi et al. 2007). Early reports suggested that the role of Hxk2 in glucose repression was limited to its metabolic role (Ma et al. 1989; Rose et al. 1991). More recently, however, mutants were isolated with a distinct effect on catalytic activity and glucose signalling (Hohmann et al. 1999; Kraakman et al. 1999b; Mayordomo and Sanz 2001), indicative for a more specific role of Hxk2 in glucose repression. Since it was shown that overexpression of *REG1* rescued defects in glucose repression due to deletion of *HXK2* (Sanz et al. 2000), one hypothesis states that Hxk2 exerts its regulatory role in glucose signalling via stimulation of the Glc7–Reg1 phosphatase complex. However, no direct interaction between Reg1 and Hxk2 could be detected (Sanz et al. 2000), and other findings indicate that Hxk2 interferes further downstream with the glucose repression pathway. A small portion of Hxk2 is located within the nucleus and appears to interact with the transcriptional repressor Mig1 (Herrero et al. 1998; Randez-Gil et al. 1998; Ahuatzi et al. 2004). Therefore, the main role of Hxk2 in glucose repression is possibly inhibition of the interaction between Mig1 and Snf1, thereby blocking the phosphorylation of Mig1 which would otherwise relieve glucose repression.

The cAMP-PKA pathway

The cAMP-PKA pathway plays a major role in the control of metabolism, stress resistance and proliferation, in particular in connection with the available carbon source. In response to a sudden availability of rapidly fermentable sugars, the pathway transiently induces the synthesis of cAMP to boost the activity of the cAMP-dependent protein kinase (PKA). In turn, PKA will affect several downstream targets thereby allowing cells to make the necessary adaptations for fermentative growth. These include the upregulation of glycolysis, the stimulation of cell growth and cell cycle progression, the downregulation of stress resistance and gluconeogenesis, and the mobilization of the reserve carbohydrate glycogen and the stress protectant trehalose (Thevelein et al. 2000; Santangelo 2006; Tamaki 2007; Gancedo 2008).

PKA is a hetero-tetramer composed of two catalytic subunits, redundantly encoded by *TPK1*, *TPK2* and *TPK3* and two regulatory subunits, encoded by *BCY1* (Toda et al. 1987a, b). Binding of the secondary messenger cAMP to the regulatory subunit induces the dissociation of the

hetero-tetramer and activation of the catalytic subunits (Kuret et al. 1988). PKA activity is critical for yeast since at least one of the three catalytic subunits is necessary for viability (Toda et al. 1987b). This implies as well that there is probably a large overlap in the functions of Tpk1-3, which is further consistent with the high level of protein sequence similarity between the different catalytic subunits (Toda et al. 1987b). Nevertheless, there are several examples of separate, specific functions for the different subunits, such as the induction of pseudohyphal growth, the expression control of genes involved in iron uptake or branched amino acid biosynthesis, or the regulation of mitochondrial enzymes (Robertson and Fink 1998; Pan and Heitman 1999; Robertson et al. 2000; Zhu et al. 2000; Singh et al. 2004; Chevtzoff et al. 2005; Ptacek et al. 2005). Given their central and essential role, the activity of the catalytic subunits is tightly regulated. This is best exemplified by the observation that cells lacking the regulatory subunit Bcy1 display a pleiotropic 'sick' phenotype due to hyperactivation of the Tpk subunits, which includes the inability to grow on carbon sources other than glucose, an abnormally high heat shock sensitivity, the lack of a proper G_1-arrest during nutrient starvation and defects in DNA replication and sporulation (Matsumoto et al. 1983; Toda et al. 1987a).

Regulation of the cAMP-PKA pathway

The cAMP-PKA pathway is under positive control of an intracellular glucose sensing system as well as an extracellular glucose detection system (Fig. 3). The intracellular glucose-sensing system requires the uptake and phosphorylation of glucose, but no further metabolism of the sugar (Beullens et al. 1988; Rolland et al. 2000). In *S. cerevisiae*, there are three glucose phosphorylating enzymes (Hxk1, Hxk2 and Glk1) and any of these proteins can fulfil this phosphorylation requirement to activate the cAMP-PKA pathway. It is generally believed that the intracellular glucose phosphorylation signal is further transduced to the cAMP-PKA pathway via the Ras proteins, Ras1 and Ras2, which belong to the group of small G proteins. The GTP-bound, active Ras proteins stimulate the activity of the adenylate cyclase Cyr1 (also known as Cdc35), the enzyme which catalyses the synthesis of cAMP from ATP (Casperson et al. 1983, 1985; Matsumoto et al. 1983, 1984; Kataoka et al. 1985; Toda et al. 1985; Field et al. 1988). The GDP/GTP exchange on the Ras proteins is controlled by the *g*uanine nucleotide *e*xchange *f*actors (GEF) Cdc25 and Sdc25 (Broek et al. 1987; Camonis and Jacquet 1988; Jones et al. 1991; Camus et al. 1994). The Ira proteins, Ira1 and Ira2, accelerate Ras GTPase activity by acting as *G*TPase *a*ctivating *p*roteins (GAP) and, as a result, they keep Ras in the GDP-bound, inactive state (Tanaka et al. 1989, 1990a, b, 1991). Both Ras proteins are essential to obtain a normal glucose-induced cAMP signal. Glucose addition to glucose-starved cells triggers a transient increase in the GTP loading of Ras2. This process was shown to require glucose phosphorylation (Mbonyi et al. 1988; Colombo et al. 2004). The mechanism by which glucose phosphorylation affects Ras-GTP loading remains largely to be elucidated. It is known that Cdc25 is required for the glucose-induced Ras-GTP increase and it was suggested that the intracellular levels of GTP, which quickly respond to nutrient availability, could be the metabolic signal that regulates Cdc25 activity in response to glucose (Rudoni et al. 2001; Colombo et al. 2004; Cazzaniga et al. 2008). On the other hand, it seems plausible that the Ira proteins are inhibited by the glucose signal, since an *ira1Δira2Δ* double deletion mutant displays a severe increase in Ras-GTP levels and no further increase upon glucose addition (Colombo et al. 2004).

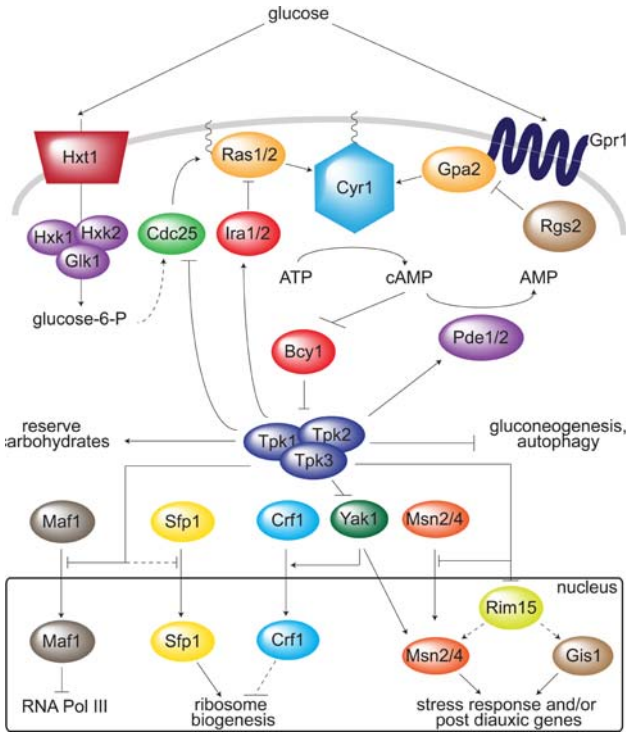

Fig. 3 The cAMP-PKA pathway in *S. cerevisiae*. Addition of glucose to glucose-starved, respiring cells triggers the rapid synthesis of cAMP and, subsequently, the activation of PKA. Glucose-induced cAMP synthesis requires two sensing systems: (i) extracellular detection of glucose via the Gpr1–Gpa2 system and (ii) intracellular detection of glucose, which requires uptake and phosphorylation of the sugar. The intracellular sensing system most probably transduces signals via the GEF protein Cdc25 and the Ras proteins. Activated PKA mediates the fast transition from respiratory to fermentative growth via the modulation of numerous downstream targets. *Arrows* and *bars* represent positive and negative interactions, respectively. *Dashed lines* represent putative or indirect interactions. See text for further details

Notably, the Ira proteins are activated by the kelch proteins (see below) (Harashima et al. 2006) and appear to be inhibited by Tfs1, a carboxypeptidase Y inhibitor, originally discovered as a dosage-dependent suppressor of a *cdc25* mutation (Robinson and Tatchell 1991; Bruun et al. 1998; Caesar and Blomberg 2004; Chautard et al. 2004). Thus, these proteins are potential transducers of the glucose signal to the Ira proteins.

Extracellular glucose detection occurs through a *G protein-coupled receptor* (GPCR) system, composed of Gpr1 and Gpa2 (Kraakman et al. 1999a). Gpr1 belongs to the *G protein-coupled seven-transmembrane receptor* (GPCR) superfamily (Yun et al. 1997; Xue et al. 1998) and Gpa2 is a member of the heterotrimeric G protein α subunit (G_α) protein family (Nakafuku et al. 1988). Addition of glucose to derepressed cells activates Gpr1, which in turn stimulates the exchange of GDP for GTP on Gpa2 (Kraakman et al. 1999a). The Gpr1–Gpa2 couple displays a rather low affinity for glucose, with a half-maximum response (EC_{50}) of 20–75 mM, depending on the genetic background tested and the experimental setup (Rolland et al. 2000; Lemaire et al. 2004). This probably ensures that the cAMP-PKA pathway is only fully activated when glucose levels are high enough to switch easily from respiration to fermentation. GTP-bound Gpa2 activates the cAMP-PKA pathway and this is most probably through stimulation of adenylate cyclase (Nakafuku et al. 1988; Kubler et al. 1997; Lorenz and Heitman 1997; Colombo et al. 1998; Rolland et al. 2000; Peeters et al. 2006). Gpa2 interacts with Rgs2, a member of the family of *regulators of G protein signalling* (RGS), that negatively regulates the Gpa2-GTP signal by stimulating the intrinsic GTPase activity of Gpa2 (Versele et al. 1999). It is unclear whether the G_α protein Gpa2 also associates with canonical G_β and G_γ subunits. A recent report suggests that Asc1 functions as the G_β subunit for Gpa2 (Zeller et al. 2007). Asc1 has the typical 7-WD domain structure of a canonical G_β protein, interacts directly with Gpa2 in a guanine nucleotide-dependent manner and inhibits Gpa2 guanine nucleotide exchange activity. In addition, Asc1 binds to adenylate cyclase and diminishes the glucose-induced production of cAMP. Another hypothesis states that the kelch-repeat proteins Krh1/Gpb2 and Krh2/Gpb1 serve as G_β subunit and Gpg1 as G_γ for Gpa2 (Harashima and Heitman 2002; Batlle et al. 2003). The kelch-repeat proteins interact with Gpa2 and contain seven kelch repeats that mimic the β propeller that is formed by seven WD-40 repeats in canonical G_β subunits. Phenotypic analysis of *gpb1Δ gpb2Δ* double deletion mutants showed that the kelch-repeat proteins act as negative regulators of PKA signalling (Harashima and Heitman 2002; Batlle et al. 2003; Lu and Hirsch 2005). Several mechanisms have been proposed to explain their effect. One report suggests that the kelch-repeat proteins inhibit Gpr1–Gpa2 coupling (Harashima and Heitman 2005). Alternatively, the kelch-repeat proteins were reported to stimulate the interaction between the catalytic and the regulatory subunits of PKA (Peeters et al. 2006). Activated, GTP-bound Gpa2 would relieve this inhibition of PKA by inhibiting the kelch-proteins, thereby bypassing adenylate cyclase to regulate PKA. Finally, it has also been found that the kelch-repeat proteins bind to a conserved C-terminal domain of the Ira proteins and stabilize them (Harashima et al. 2006). As this would decrease Ras-GTP levels, it means that the Gpr1–Gpa2 couple could possibly modulate the activity of adenylate cyclase and PKA via the Ras proteins. It should be stressed that intracellular sensing of glucose is a prerequisite for the extracellular glucose detection system to further activate cAMP synthesis (Rolland et al. 2000), but how the signals of both sensing systems are exactly integrated to control adenylate cyclase activity remains to be resolved.

A strong negative feedback mechanism ensures that the glucose-induced increase in cAMP levels and PKA activity are transient and can only be triggered in glucose-derepressed cells. PKA itself is involved in this mechanism since basal cAMP levels are dramatically increased in strains with reduced activity of the kinase (Nikawa et al. 1987a; Mbonyi et al. 1990). cAMP is hydrolyzed by the low- and high-affinity phosphodiesterases, respectively, encoded by *PDE1* and *PDE2* (Sass et al. 1986; Nikawa et al. 1987b; Wilson and Tatchell 1988). The high-affinity phosphodiesterase Pde2 appears to control basal cAMP levels, which is important to prevent undesirable PKA activity during stationary phase (Park et al. 2005). The low-affinity phosphodiesterase Pde1, however, was shown to be specifically involved in the feedback inhibition of glucose-induced cAMP signalling and is probably activated by PKA itself (Casamayor et al. 1999). The Ras proteins are also involved in the negative feedback control of the activated cAMP-PKA pathway. The glucose-induced increase of Ras-GTP loading is only transient and in a PKA-attenuated strain a severe increase in basal Ras-GTP levels was observed (Colombo et al. 2004). Whether the Ras proteins or rather one of their regulators are the targets of the feedback-inhibition mechanism is not clear. Another target for feedback regulation could be adenylate cyclase itself (Nikawa et al. 1987a). Furthermore, feedback regulation may involve other carbon source-dependent pathways as well, for instance the main glucose repression pathway, since no glucose-induced cAMP increase was observed in a *snf1Δ* mutant (Arguelles et al. 1990). Finally, it is important to note that PKA activity is further fine-tuned by modulation of its subcellular localization, phosphorylation state and abundance (Werner-Washburne et al. 1991; Griffioen et al. 2000, 2001; Schmelzle et al. 2004;

Portela and Moreno 2006). However, compared to cAMP-mediated control, this regulation of PKA appears to be less important for short-term signalling events.

Targets of cAMP-activated PKA

cAMP-activated PKA has a major impact on gene expression, which is well-illustrated by the observation that 90% of the transcriptional changes upon glucose addition to glucose-starved cells could be mimicked by artificial activation of PKA (Wang et al. 2004). Accordingly, several of the known PKA targets affect gene transcript levels, either directly or indirectly (Fig. 3). Two of those are the transcription factors Msn2 and Msn4, which mediate the transcription of the so-called *st*ress *r*esponse *e*lement (STRE)-controlled genes (Estruch and Carlson 1993; Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). STRE genes are involved in a wide variety of processes, including protection against diverse types of stress such as heat, oxidative and osmotic stress, carbohydrate metabolism and growth regulation (Mai and Breeden 1997; Moskvina et al. 1998; Smith et al. 1998; Gasch et al. 2000). Msn2 and Msn4 are inhibited by PKA and, notably, deletion of both *MSN2* and *MSN4* rescues the lethality of a *tpk null* strain (Boy-Marcotte et al. 1998; Smith et al. 1998). During growth on glucose, Msn2 and Msn4 are phosphorylated and reside in the cytosol. Upon glucose exhaustion, they are hyperphosphorylated and translocated to the nucleus, where they induce expression of the STRE-controlled genes. PKA inhibits nuclear import of Msn2/4, probably through direct phosphorylation of their nuclear localization signal (Gorner et al. 1998, 2002; Garreau et al. 2000). A second mechanism for PKA-mediated regulation of STRE-controlled gene expression involves the Ccr4-Not complex, a global transcriptional regulator that affects genes positively and negatively. It was proposed that this complex contributes to the downregulation of Msn2/4-driven transcription and this through direct interaction with Tpk2 and by modulation of the phosphorylation status of Msn2 via the Bud14-Glc7 protein phosphatase (Lenssen et al. 2002, 2005). Moreover, PKA seems to additionally inhibit the function of Msn2 and Msn4 via the protein kinases Yak1 and Rim15. Both kinases are under negative control of PKA, supposedly by direct phosphorylation, and deletion of *YAK1* or *RIM15*, like deletion of both *MSN2* and *MSN4*, can suppress the lethality caused by loss of PKA activity (Garrett and Broach 1989; Garrett et al. 1991; Reinders et al. 1998; Griffioen et al. 2001; Moriya et al. 2001; Zappacosta et al. 2002). A recent report suggests that Yak1 phosphorylates and thereby activates Msn2 through a still unknown mechanism, which apparently does not implicate the control of Msn2 subcellular localization (Lee et al. 2008a). Since the *YAK1* gene itself is induced by Msn2/4, this would generate a positive feedback loop (Smith et al. 1998). In the same study, Yak1 was also found to stimulate the activity of Hsf1, another transcriptional activator of stress response genes that was recently shown to be under negative control of PKA (Hahn et al. 2004; Ferguson et al. 2005; Lee et al. 2008a). Rim15 was initially identified as an activator of meiotic gene expression (Vidan and Mitchell 1997). Later, this protein kinase was shown to be essential for the accumulation of both the reserve carbohydrate glycogen and the stress protectant trehalose, the induction of several stress response genes, the induction of thermotolerance and proper G_1-arrest upon nutrient starvation (Reinders et al. 1998; Cameroni et al. 2004; Zhang et al. 2009). Genome-wide expression analyses confirmed that the Rim15-controlled expression program following glucose limitation at the diauxic shift is almost entirely mediated by the three transcription factors Msn2, Msn4 and Gis1 (Cameroni et al. 2004; Roosen et al. 2005). The latter is a transcription factor that induces expression of stress response genes containing so-called *p*ost *d*iauxic *s*hift (PDS) elements in their promoter regions, some of which are required during respiratory growth (Pedruzzi et al. 2000; Cameroni et al. 2004; Roosen et al. 2005; Zhang et al. 2009). Together, the above-described data indicate that PKA exerts a dual control on gene expression, first by regulation of its downstream protein kinases Rim15 and Yak1, and second by direct regulation of their presumed effectors, i.e. the transcription factors Msn2, Msn4, and likely also Gis1.

PKA activates the transcription of ribosomal protein genes as well (Herruer et al. 1987; Kraakman et al. 1993; Griffioen et al. 1994). It was reported that PKA promotes nuclear localization and binding of the transcriptional activator Sfp1 to the promoters of ribosomal protein genes (Marion et al. 2004). In addition, PKA appears to induce transcription of ribosomal protein genes also by inhibition of Yak1, which in turn is required to promote the activity of the transcriptional corepressor Crf1 (Martin et al. 2004). As will be discussed in more detail below, PKA further stimulates protein synthesis indirectly by inhibiting nuclear import of Maf1, which represses 5S rRNA and tRNAs transcription by RNA Polymerase III (Moir et al. 2006; Willis and Moir 2007).

In addition to the control of gene expression and protein synthesis, PKA directly modulates the activity of metabolic enzymes. PKA-dependent phosphorylation inhibits the activity of fructose-1,6-bisphosphatase (Fbp1) and stimulates the activity of 6-phosphofructo-2-kinase (Pfk2) and of both isoforms of pyruvate kinase (Pyk1 and Pyk2) (Gancedo et al. 1983; Rittenhouse et al. 1987; Cytrynska et al. 2001; Vaseghi et al. 2001; Portela et al. 2002; Dihazi et al. 2003). Together, these modifications result in the stimulation of glycolysis and the inhibition of

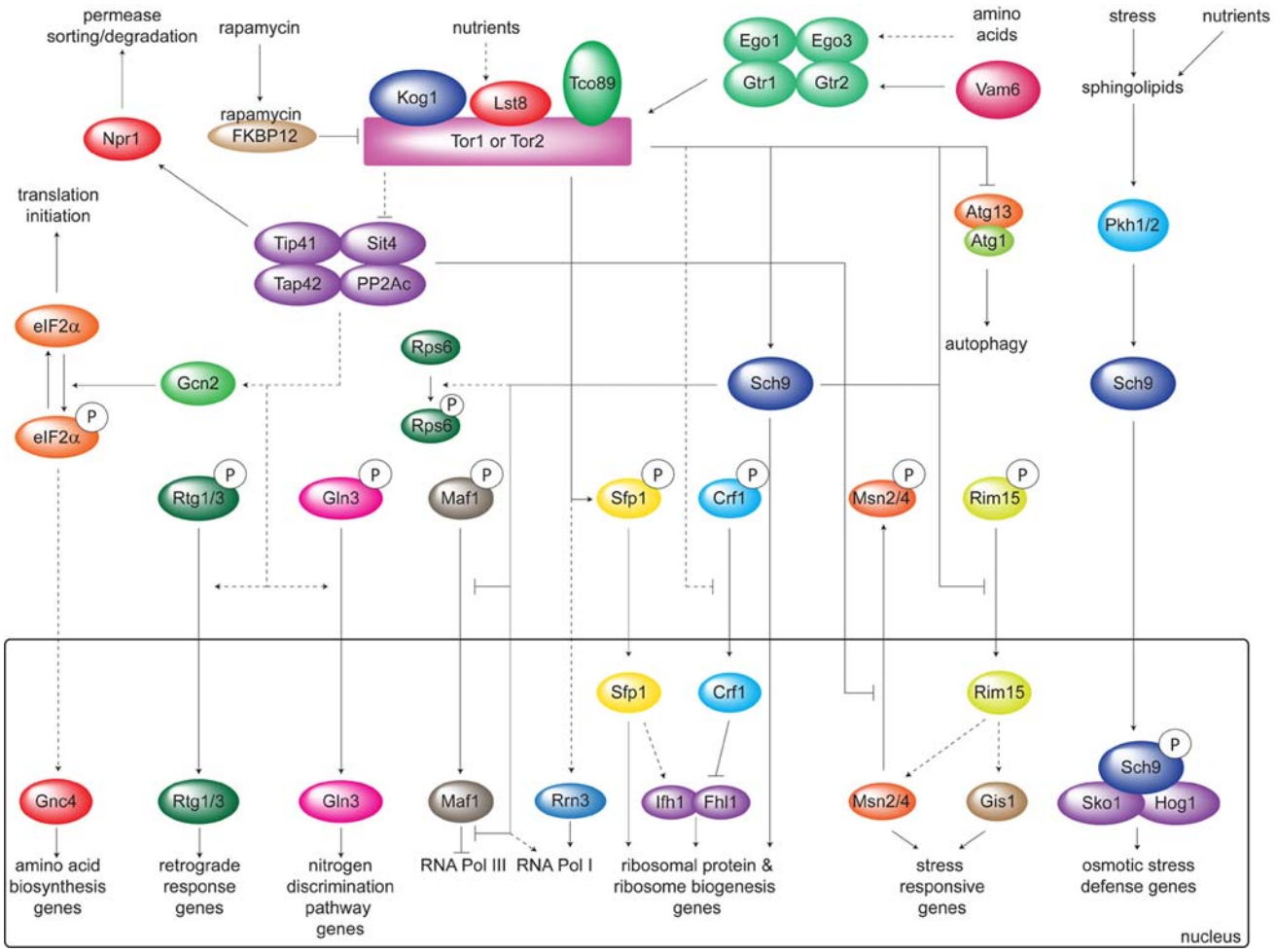

Fig. 4 The TORC1 pathway in *S. cerevisiae*. Nutrients activate TORC1, resulting in the stimulation of protein synthesis and the inhibition of stress response genes, autophagy and several pathways that allow growth on poor nitrogen sources. A major part of these processes is regulated by the rapamycin-sensitive TORC1 complex either via the Tap42-Sit4/PPA2c or the recently identified Sch9 branches. The activity of Sch9 is additionally regulated by Pkh1 and Pkh2. Note that Sch9 functions both in the cytoplasm and the nucleus. See text for further details. *Arrows* and *bars* represent positive and negative interactions, respectively. *Dashed lines* represent putative or indirect interactions

gluconeogenesis when glucose is added to glucose-starved cells. In vitro phosphorylation by PKA also modulates the activity of enzymes involved in the metabolism of trehalose (Tps1 and Nth1) and glycogen (Gsy2 and Gph1), but it remains to be established whether these enzymes are direct in vivo substrates of PKA (Uno et al. 1983; Wingender-Drissen and Becker 1983; Panek et al. 1987; Hardy and Roach 1993).

Finally, PKA is a known inhibitor of autophagy, a degradative process that recycles non-essential proteins and organelles during periods of nutrient starvation (Budovskaya et al. 2004; Schmelzle et al. 2004; Yorimitsu and Klionsky 2005). The key players involved in autophagy are the Atg proteins and three of these, i.e. Atg1, Atg13 and Atg18, contain a PKA consensus phosphorylation site. At least for Atg1, this site appears to be functional since data confirmed that PKA phosphorylation negatively controls the recruitment of Atg1 to the sites of autophagosome formation upon nutrient limitation (Budovskaya et al. 2005).

The TOR pathway

The *T*arget *O*f *R*apamycin (TOR), a highly conserved Ser/Thr protein kinase, is the central component of a major regulatory signalling network that controls cell growth in diverse eukaryotic organisms, ranging from yeast to man. The TOR proteins were first identified in yeast as the targets of the antifungal and immunosuppressive agent rapamycin, hence their name (Heitman et al. 1991). In contrast to most eukaryotes, yeast contains two TOR homologues, Tor1 and Tor2. Two functionally and structurally distinct

TOR multiprotein complexes exist: TOR complex 1 (TORC1) and TOR complex 2 (TORC2) (Zheng et al. 1995; Loewith et al. 2002); however, only TORC1 is specifically inhibited by rapamycin (Fig. 4). The addition of rapamycin induces dramatic phenotypic changes such as cell cycle arrest and entry into G_0, general downregulation of protein synthesis, accumulation of the reserve carbohydrate glycogen and the stress protectant trehalose, upregulation of stress response genes, autophagy and alterations in nitrogen and carbon metabolism (De Virgilio and Loewith 2006a, b; Rohde et al. 2008). Hence, it appears that TORC1 signalling controls the temporal aspects of cell growth in response to the quality of the available nitrogen and carbon sources. On the other hand, TORC2, which is insensitive to rapamycin and is less well-characterized in comparison to TORC1, is thought to regulate the spatial aspects of growth, such as the control of actin polarization (De Virgilio and Loewith 2006a, b; Rohde et al. 2008). Here, we will focus on TORC1, as only this complex modulates nutrient-induced signalling in response to mainly nitrogen sources and to some extent glucose.

Structural aspects and localization of the TOR protein complexes

TOR1 and *TOR2* encode two large (~280 kDa) and homologous (67% identical) proteins that belong to a family of *p*hosphatidyl*i*nositol *k*inase-related *k*inases (PIKKs) (Cafferkey et al. 1993; Kunz et al. 1993; Helliwell et al. 1994; Keith and Schreiber 1995). Despite their resemblance to lipid kinases, they are thought to function solely as Ser/Thr protein kinases. Both Tor1 as well as Tor2 can be found in the multiprotein TORC1 together with Lst8, Kog1 and Tco89. A separate pool of Tor2 also associates with Lst8, Avo1, Avo2, Avo3, Bit61 and Bit2 to form TORC2 (Loewith et al. 2002; Chen and Kaiser 2003; Wedaman et al. 2003; Reinke et al. 2004; Araki et al. 2005; Fadri et al. 2005). The precise function of these TOR-interacting proteins is not known yet. They might play a role in the binding of the TOR complexes to their substrates, be the receivers of upstream signals and/or determine the localization of the complexes. The TOR complexes are likely dimeric built on a TOR–TOR dimer (Wullschleger et al. 2005). Only TORC1 can bind FKBP12-rapamycin while in TORC2, the Tor2 FKBP12-rapamycin binding domain is probably not exposed for binding (Loewith et al. 2002), explaining why only TORC1 signalling is sensitive to rapamycin treatment. Interestingly, the constitution of TORC1 appears to be unaffected by rapamycin, implying that rapamycin does not inhibit TORC1 signalling by interfering with TORC1 stability (Loewith et al. 2002). Both TOR complexes are essential for viability, since deletion of *TOR2* (inactivation of TORC2) or deletion of both TOR1 and TOR2, or rapamycin treatment (inactivation of TORC1) are lethal to yeast (Heitman et al. 1991; Kunz et al. 1993). Deletion of *TOR1* alone, however, is not lethal, indicating that Tor1 and Tor2 have a redundant role in TORC1 signalling.

Several studies investigated the localization of TORC1 and TORC2. Various different localization patterns were observed, which is possibly a reflection of the fact that TOR signalling controls a multitude of processes. In general, the TOR complexes were found to associate with membranes ranging from the plasma membrane to the vacuolar membrane and internal membranes of the protein secretory pathway (Cardenas and Heitman 1995; Kunz et al. 2000; Wedaman et al. 2003; Aronova et al. 2007; Sturgill et al. 2008; Berchtold and Walther 2009). TORC2 appears to be predominantly localized in discrete dots at the plasma membrane (Cardenas and Heitman 1995; Kunz et al. 2000; Sturgill et al. 2008), while TORC1 is mainly found at the vacuolar membrane, which is intriguing knowing that the vacuole is a reservoir of nutrients and that TORC1 signalling is believed to be regulated by nutrients (Cardenas and Heitman 1995; Aronova et al. 2007; Sturgill et al. 2008). According to a recent study, TORC1 is also targeted to the nucleus where it induces 35S rRNA synthesis under favourable growth conditions (Li et al. 2006).

Rapamycin-sensitive signalling via TORC1

Rapamycin has proven to be a good drug for yeast researchers to study TORC1 signalling. Thanks to the fact that it rapidly and specifically inhibits TORC1, numerous proteins involved in a wide range of processes could be identified whose activity is modified by TORC1 activity. Especially, the transcriptional regulation exerted by TORC1 is well-described and many transcription factors were found to be under control of TORC1 signalling (Fig. 4).

The precise mechanism how TORC1 regulates its downstream effectors are often not well-understood. As will be discussed in the section below, several TORC1-mediated processes involve the protein kinase Sch9 (Urban et al. 2007). Others appear to be regulated via the PP2A and the PP2A-related protein phosphatases. These phosphatases consist of heteromeric protein complexes (Duvel and Broach 2004). The PP2A holoenzyme contains one of the two redundant catalytic subunits (PP2Ac), Pph21, Pph22 (Sneddon et al. 1990; Ronne et al. 1991), a scaffolding subunit, Tpd3 (van Zyl et al. 1992), and one of the two regulatory subunits, Cdc55 or Rts1 (Healy et al. 1991; Zhao et al. 1997). The PP2A-related phosphatase is mainly found as a complex between the catalytic subunit, Sit4 (Arndt et al. 1989), and one of the four regulatory subunits,

Sap4, Sap155, Sap185 and Sap190 (Luke et al. 1996). TORC1 controls the activity of these phosphatases via Tap42. When Tap42 is phosphorylated by TORC1, it will compete for binding the catalytic subunits of the phosphatases leading to the exclusion of other subunits of the phosphatase holoenzymes (Di Como and Arndt 1996; Jiang and Broach 1999). Thereby, TORC1 stimulates the formation of a Tap42-associated phosphatase complex that further includes either one of the regulatory proteins Rrd1 or Rrd2, both of which are known to confer phosphotyrosyl phosphatase activity to the catalytic phosphatase subunits in vitro (Zabrocki et al. 2002; Zheng and Jiang 2005). Tap42 as well as Rrd1 and Rrd2 may redirect the substrate specificity of the catalytic phosphatase subunits, and as such, it is not surprising that the proteins have been attributed both inhibitory as well as activatory roles, dependent on the substrate being studied (Van Hoof et al. 2001; Cherkasova and Hinnebusch 2003; Duvel et al. 2003; Duvel and Broach 2004). In actively growing cells, the Tap42-associated phosphatase complexes reside mainly at membranes where they associate with TORC1. Rapamycin treatment or nitrogen starvation abrogates the TORC1 association and releases the Tap42-associated phosphatase complex into the cytosol (Yan et al. 2006). Once cytoplasmic, this complex then slowly dissociates, presumably concomitant with the dephosphorylation of Tap42 (Zheng and Jiang 2005; Yan et al. 2006). Several studies revealed an important role for yet another player in TORC1-dependent regulation of PP2Ac and Sit4, i.e. Tip41. This protein was initially identified as an inhibitor that could specifically interact with dephosphorylated Tap42 (Jacinto et al. 2001). However, more recent data suggest that both Tip41 and Tap42 cooperate in determining the substrate specificity of PP2Ac and Sit4, and that both proteins may fulfil essentially a similar function in TORC1 signalling (Duvel and Broach 2004; Santhanam et al. 2004; Kuepfer et al. 2007).

One of the first described examples where TORC1 signalling involves the regulation of PP2A and the PP2A-like phosphatase Sit4 is the control of nitrogen metabolism. Yeast cells adapt their metabolism to the available nitrogen sources via the *n*itrogen *c*atabolite *r*epression pathway (NCR) also known as the *n*itrogen *d*iscrimination *p*athway (NDP) (Magasanik and Kaiser 2002). This pathway ensures that genes encoding proteins required for the usage of poor nitrogen sources are repressed when rich nitrogen sources, such as glutamine, are present in sufficient quantities. Four GATA family zinc-finger transcription factors are involved in the transcriptional control exerted by the NDP: two activators, Gln3 and Gat1, and two repressors, Dal80 and Gzf3 (Minehart and Magasanik 1991; Coffman et al. 1995, 1996, 1997; Cooper 2002). TORC1 inhibits transcription of NDP genes by controlling Gln3 and Gat1 function (Beck et al. 1999; Cardenas et al. 1999; Hardwick et al. 1999; Shamji et al. 2000). Normally, during growth on rich nitrogen sources, Gln3 is phosphorylated and sequestered in the cytoplasm through its binding with the cytoplasmic anchor protein Ure2. Rapamycin treatment, however, rapidly triggers, in a Sit4-dependent manner, the dephosphorylation of Gln3, dissociation from Ure2 and entry into the nucleus, where Gln3 can exert its transcriptional activator function on NDP genes (Beck et al. 1999). Thus, it appears that TORC1 through inhibition of the Tap42-Sit4 phosphatase complex promotes Gln3 phosphorylation, which inhibits Gln3 activity by stimulating its association with Ure2. Nevertheless, recent results indicate that PP2A phosphatase activity is also necessary for Gln3 nuclear import upon rapamycin treatment, although the mechanistic details remain elusive (Tate et al. 2009). Additionally, TORC1 promotes via an unknown mechanism the phosphorylation of Ure2 and this might further modulate the interaction between Gln3–Ure2 (Cardenas et al. 1999; Hardwick et al. 1999). Rapamycin treatment also triggers nuclear import of Gat1 (Beck et al. 1999). How TORC1 regulates this process is unclear, yet it appears to be different from TORC1-dependent Gln3 control and does not involve Ure2 or Sit4 (Kuruvilla et al. 2001; Crespo et al. 2002; Georis et al. 2008).

TORC1 further negatively controls the *g*eneral *a*mino *a*cid *c*ontrol (GAAC) pathway (Hinnebusch 2005). The central component of this pathway is Gcn4, a transcription factor important for activating transcription of genes needed for amino acid biosynthesis in response to amino acid starvation (Natarajan et al. 2001). The pathway is induced by uncharged tRNAs, which presumably activate the kinase Gcn2. In turn, Gcn2 phosphorylates the α subunit of eIF2 and although this results in a reduction of the general translation initiation, it specifically stimulates the translation of *GCN4* mRNA (Dever et al. 1992). TORC1 inhibits Gcn2 activity by promoting its phosphorylation at Ser^{577}. This occurs indirectly and involves the inhibition of the PP2A-like phosphatase Sit4 via Tap42. As such, TORC1 enhances translation initiation and antagonizes *GCN4* mRNA translation (Valenzuela et al. 2001; Cherkasova and Hinnebusch 2003; Kubota et al. 2003; Rohde et al. 2004). Note that *GCN4* is also a target of the NDP (Godard et al. 2007), suggesting that TORC1, via inhibition of NDP gene expression, also inhibits *GCN4* transcription.

A third pathway that is involved in nitrogen metabolism and that is subject to TORC1 control is the *ret*rograde response pathway (RTG). Apart from having other functions, this pathway induces the expression of genes whose products are required for the biosynthesis of α-ketoglutarate as precursor for glutamate synthesis in cells grown on poor nitrogen sources as well as in respiration-deficient cells (Liu and Butow 2006). Expression of these genes

requires the transcriptional activators Rtg1 and Rtg3. TORC1 controls the cytoplasmic sequestration of these factors through phosphorylation of Mks1, which thereby forms a complex with the 14-3-3 proteins Bmh1/2 to provide the cytoplasmic anchor for Rtg1 and Rtg3. Rapamycin treatment induces dephosphorylation of Mks1 and causes disassembly of the complex. This directs Mks1 to bind to the positive regulator of the pathway, i.e. Rtg2, thereby relieving the cytoplasmic sequestration and promoting nuclear translocation of Rtg1 and Rtg3 and the induction of the target genes of RTG pathway (Komeili et al. 2000; Dilova et al. 2002, 2004; Tate et al. 2002; Liu et al. 2003). Genome-wide expression analysis revealed that Tap42 is probably also involved in this regulation mechanism (Duvel et al. 2003).

Finally, TORC1 also appears to control the turnover of several amino acid permeases. Depending on the quality and quantity of the nitrogen sources in the medium, yeast cells activate a different set of amino acid permeases. Under nutrient rich conditions, the so-called constitutive permeases, such as the high-affinity tryptophan permease Tat2, are targeted to the plasma membrane, whereas the nitrogen-responsive permeases, such as the general amino acid permease Gap1, are sorted to the vacuole for degradation (Roberg et al. 1997; Beck et al. 1999). During periods of nitrogen limitation, opposite sorting occurs and Gap1 is allowed to reach the plasma membrane, while Tat2 is endocytosed and delivered to the vacuole (Roberg et al. 1997; Beck et al. 1999). The protein kinase Npr1 plays a major role in the sorting of these two classes of permeases. Npr1 is required for stabilization of Gap1 at the plasma membrane and induces the degradation of Tat2, possibly by regulating their ubiquitination (Schmidt et al. 1998; Springael and Andre 1998; De Craene et al. 2001; Helliwell et al. 2001; Soetens et al. 2001; Springael et al. 2002). TORC1 activity, through control of the Tap42–Sit4 phosphatase complex, promotes the phosphorylation of Npr1 (Schmidt et al. 1998; Jacinto et al. 2001; Gander et al. 2008). Presumably, this phosphorylation inhibits Npr1 which would promote Tat2 stabilization and Gap1 degradation. Accordingly, it was found that rapamycin induces Tat2 targeting to the vacuole and that this process depends on Npr1 (Schmidt et al. 1998; Beck et al. 1999). For Gap1, it was initially reported that rapamycin-induced inhibition of TORC1 did not affect its sorting (Chen and Kaiser 2002). More recently, however, it was shown that mutants affected in Lst8 display vacuolar targeting of Gap1 under conditions where the permease should normally be sorted to the plasma membrane. Lst8 is a component of TORC1 and TORC2 and, consistently, the impairment of TOR signalling by treatment with low, sublethal doses of rapamycin triggers a similar missorting of Gap1. As both mutation of Lst8 and rapamycin-induced impairment of TOR signalling causes a significant increase in intracellular amino acid pools, it was suggested that this increase could act as signal that directs the vacuolar sorting of Gap1 (Chen and Kaiser 2003).

Apart from its controlling function on nitrogen metabolism, TORC1 has a major regulatory role in protein synthesis as it promotes expression of the rRNA and the *r*ibosomal *p*roteins (RP) genes as well as of the so-called *ri*bosome *bio*genesis (Ribi) regulon (Jorgensen et al. 2004). This regulon contains ~236 genes encoding for proteins that are also needed for an efficient functioning of the translation apparatus, including translation factors, rRNA and RP modifying and processing enzymes, tRNA synthetases and subunits of RNA polymerases I and III. 35S rRNA, the precursor of 5.8S, 18S and 25S rRNA, is transcribed by RNA polymerase I (Planta 1997). TORC1 positively affects the interaction between Rrn3 and RNA polymerase I, which is necessary for proper recruitment of the polymerase to the 35S rDNA promoter (Claypool et al. 2004). However, recent data suggest that TORC1 promotes recruitment of the RNA polymerase I to the *rDNA* locus in a Rrn3-independent way, via a signalling route that requires the TORC1-effector protein kinase Sch9 (see below) (Huber et al. 2009). Tor1 itself binds to the 35S and 5S rDNA chromatin under favourable nutrient conditions and this seems to be essential for the synthesis of 35S and 5S rRNA via, respectively, RNA polymerase I and III (Li et al. 2006). In addition, the Tor1 association with 5S rDNA chromatin is also required for TORC1 to stimulate the expression of tRNAs by RNA polymerase III (Wei et al. 2009). This regulation of RNA polymerase III expression involves, at least in part, the inhibition of Maf1, a repressor of RNA polymerase III transcription that is also inhibited by PKA (Moir et al. 2006; Oficjalska-Pham et al. 2006; Roberts et al. 2006; Wei et al. 2009). Apparently, 5S rDNA-associated TORC1 phosphorylates Maf1, thereby inhibiting the nucleoplasm-to-nucleolus translocation of Maf1 and the concomitant binding of Maf1 with RNA polymerase III-transcribed genes (Wei et al. 2009). Interestingly, most recent data suggest that TORC1 mediates phosphorylation of Maf1 indirectly via Sch9 (Huber et al. 2009).

Concerning the regulation of RP gene transcription by RNA polymerase II, it was found that TORC1 promotes complex formation between Fhl1, a forkhead-like transcription factor that binds to the promoter of RP genes, and its co-activator Ifh1 (Lee et al. 2002; Martin et al. 2004; Schawalder et al. 2004; Wade et al. 2004; Rudra et al. 2005). This Fhl1–Ifh1 complex then promotes the expression of RP genes. Depending on the genetic background, Fhl1 can also act alone or in complex with the corepressor Crf1, but then it mediates repression of the RP genes (Martin et al. 2004; Zhao et al. 2006). How TORC1 exactly

interferes with Fhl1 complex formation and activity is unclear. It was suggested that Yak1 phosphorylates Crf1 to promote its nuclear entry and that TORC1, possibly via PKA, inhibits Yak1 (Martin et al. 2004). When TORC1 is active, Crf1 would then remain in the cytoplasm and the Fhl1–Ifh1 complex could promote RP expression, whereas under less favourable conditions, Yak1-activated Crf1 enters the nucleus and displaces Ifh1 from Fhl1 to repress RP expression. Note, however, that it is generally believed that TORC1 and PKA operate in parallel and therefore the hypothesis that TORC1 activates the PKA pathway needs further testing. Furthermore, the role of Crf1 as corepressor of RP gene expression appears to depend on the genetic background of the strains under study (Zhao et al. 2006) and other mechanisms of TORC1-dependent control of the Fhl1 complex have been suggested. One such mechanism seems to involve the stress- and nutrient-sensitive transcription factor Sfp1. TORC1 regulates, probably via direct phosphorylation, the nucleocytoplasmic distribution and the promoter binding of Sfp1. In exponentially growing cells, Sfp1 is localized in the nucleus where it promotes both RP as well as Ribi gene expression, but upon TORC1 inactivation Sfp1 translocates to the cytoplasm (Jorgensen et al. 2004; Marion et al. 2004; Lempiainen et al. 2009). Sfp1, in turn, appears to affect the localization of the Fhl1–Ifh1 complex. In the absence of nutrients or Sfp1, both Fhl1 and Ifh1 remain nuclear but relocalize to the nucleolar regions, which occurs concomitant with reduced RP gene transcription (Jorgensen et al. 2004). In the nucleolus, Fhl1 and Ifh1 were proposed to have additional functions, such as repressing rRNA transcription, which would provide an interesting link between rRNA and RP synthesis. Different studies indicated that Sch9 should be involved in the regulation of RP and Ribi gene expression and it was proposed that this protein kinase could be the effector allowing for TORC1 control independent of Fhl1 and Sfp1 (Crauwels et al. 1997a; Jorgensen et al. 2004; Roosen et al. 2005; see also below). Another protein involved in TORC1-mediated transcriptional control of ribosome biosynthesis is Hmo1. This protein is a member of the HMG protein family that encompasses architectural proteins that bind to DNA with low sequence specificity. Hmo1 associates with RP gene promoters and with the rDNA region and this association requires TORC1 activity (Hall et al. 2006; Berger et al. 2007). By regulating Hmo1, TORC1 probably ensures that rRNA and RP expression are well-coordinated.

TORC1 also regulates protein synthesis at the post-transcriptional level. It was recently shown that TORC1 controls the nucleocytoplasmic shuttling of Dim2 and Rrp12, two 40S ribosome synthesis factors that are involved in ribosome assembly and the nucleocytoplasmic translocation of pre-ribosomes (Vanrobays et al. 2008). Furthermore, TORC1 was found to be essential for translation initiation (Barbet et al. 1996). Based on the fact that deletion of *CDC33*, encoding for the translation initiation factor eIF4E, results in a similar phenotype as observed when TORC1 is inactivated, it was proposed that TORC1 might control translation initiation via eIF4E (Barbet et al. 1996; Danaie et al. 1999). In addition, TORC1 was shown to have a positive effect on the stability of translation initiation factor eIF4G that binds to eIF4E (Berset et al. 1998). A third translation initiation factor that is regulated by TORC1 is eIF2. However, as mentioned above, the regulation of eIF2 by TORC1 is indirect and mediated through the kinase Gcn2, of which the phosphorylation status is controlled via the Tap42 effector branch. Phosphorylated Gcn2 in turn prevents phosphorylation of the α subunit of eIF2 and thereby the inhibition of translation initiation (Cherkasova and Hinnebusch 2003; Kubota et al. 2003; Hinnebusch 2005).

Next, TORC1 exerts a major impact on the transcription of stress response genes. Here, TORC1 has a dual control. On the one hand, it prevents nuclear translocation of the protein kinase Rim15, thereby ensuring that this kinase is maintained inactive through PKA-mediated phosphorylation (Pedruzzi et al. 2003; Urban et al. 2007; Wanke et al. 2008). Since this control involves Sch9, it will be discussed in more detail in the section below. On the other hand, TORC1 inhibits the transcription of stress-responsive genes via a Rim15-independent, but Tap42–PP2A-dependent route, thereby promoting the phosphorylation and cytoplasmic retention of Msn2 (Beck and Hall 1999; Duvel et al. 2003; Santhanam et al. 2004). It is important to note that the latter is independent of the PKA-mediated phosphorylation of the Msn2/4 nuclear localization signal (Gorner et al. 1998, 2002; Garreau et al. 2000; Santhanam et al. 2004).

Finally, TORC1 is also a known negative regulator of autophagy (Chang et al. 2009). TORC1 activity controls the phosphorylation status of Atg13. When TORC1 is active, Atg13 is hyperphosphorylated, whereas rapamycin addition induces a rapid dephosphorylation of Atg13 (Kamada et al. 2000). The latter apparently stimulates the affinity of Atg13 for Atg1 and promotes Atg1–Atg13 complex formation which is a requirement for autophagy (Funakoshi et al. 1997; Kamada et al. 2000). It is possible that PP2A is involved in TORC1-dependent regulation of Atg13 phosphorylation, since it was recently shown that autophagy is negatively regulated by the Tap42-PP2A pathway (Yorimitsu et al. 2009).

Recently, a label-free quantitative phosphoproteomic screen revealed more than 100 novel TORC1-dependent phosphorylation events, which were only partially dependent on the two known direct downstream effectors of TORC1, i.e. Sch9 and Tap42. This suggests the existence of additional but yet unidentified direct targets of TORC1

(Huber et al. 2009). The authors described possible new links between TORC1 and filamentous growth through the Ksp1 protein kinase (Bharucha et al. 2008) and between TORC1 and the DNA damage response through Pin4, a protein involved in G2/M progression, and Rph1, a histone demethylase (Kim et al. 2002; Pike et al. 2004). Furthermore, they described additional links between TORC1 and ribosome biogenesis through regulation of Stb3, Dot6 and Tod6, three transcriptional repressors of *ribi* gene expression (Liko et al. 2007; Badis et al. 2008; Zhu et al. 2009).

Rapamycin-insensitive signalling via TORC2

TORC2 signalling is less well-characterized than TORC1 signalling due to the absence of a rapamycin equivalent for TORC2. The *C*ell *W*all *I*ntegrity (CWI) pathway that regulates cell wall synthesis and actin polarization was the first pathway that could be connected to TORC2 signalling (Schmidt et al. 1996; Levin 2005). Overexpression of components of the CWI pathway could restore viability and actin polarization defects of mutants compromised in their TORC2 function (Schmidt et al. 1996; Bickle et al. 1998; Helliwell et al. 1998). These results suggest that TORC2 functions upstream or in parallel to the CWI pathway to regulate actin polarization. Additionally, it was found that the protein kinases, Ypk1 and Ypk2, and the PH domain proteins, Slm1 and Slm2, which also play a role in actin polarization, are downstream targets of TORC2 signalling (Audhya et al. 2004; Fadri et al. 2005; Kamada et al. 2005). Interestingly, Ypk1/2 and Slm1/2 appear to be upstream regulators of the CWI pathway and might, therefore, provide a mechanistic link between TORC2 and the CWI pathway (Schmelzle et al. 2002; Ho et al. 2008). Since both Ypk1/2 and Slm1/2 were also shown to be regulated by sphingolipids, these proteins also couple TORC2 to sphingolipid metabolism and signalling (Aronova et al. 2008).

How is TOR signalling regulated?

Rapamycin treatment, transfer of yeast cells from good- to poor-quality carbon or nitrogen sources, or starvation for carbon or nitrogen all elicit very similar responses indicating that TORC1 is regulated by the abundance and/or quality of the available carbon and nitrogen source. While it is still largely unknown which metabolite(s) may regulate TORC1, glutamine appears to play a particularly important role in TORC1 activation (Crespo et al. 2002). Notably, glutamine can be readily converted to α-ketoglutarate (for use in the TCA cycle) or serve as a precursor for the biosynthesis of other amino acids, nucleotides and nitrogen containing molecules (such as NAD^+). It is, therefore, not only a key intermediate in nitrogen metabolism, but also an important indicator of the cell's general nutrient status (Magasanik and Kaiser 2002). In line with the idea that glutamine acts upstream of TORC1, glutamine starvation phenocopies the effects of TORC1 inactivation inasmuch as it causes nuclear localization and activation of Gln3 and Rtg1/Rtg3 (Crespo et al. 2002; Butow and Avadhani 2004). Nevertheless, since other TORC1 readouts, such as for instance the subcellular distribution of Msn2, remain unaffected by glutamine starvation, TORC1 is likely to respond to additional nutrients and to elicit nutrient-specific responses. Understanding of how nutrients (including amino acids such as glutamine) are sensed and how this information is transmitted to TORC1 still remains one of the major challenges in the TORC1 field.

In this context, the vacuolar membrane-associated EGO (*ex*it from rapamycin-induced *gro*wth arrest) protein complex (EGOC) (Dubouloz et al. 2005), which consists of Ego1/Meh1/Gse2, Ego3/Nir1/Slm4/Gse1, Gtr1, and Gtr2, has been proposed to function as a critical hub that directly relays an amino acid signal to TORC1 (De Virgilio and Loewith 2006a, b; Gao and Kaiser 2006; Piper 2006). This initial idea has recently been bolstered by the finding that the EGOC subunit Gtr1, which is homologous to mammalian Rag GTPases, directly interacts with and activates TORC1 in an amino acid-sensitive and nucleotide-dependent manner (Binda et al. 2009). Accordingly, expression of a constitutively active (GTP-bound) $Gtr1^{GTP}$, which interacted strongly with TORC1, rendered TORC1 partially resistant to leucine deprivation, while expression of a growth inhibitory GDP-bound $Gtr1^{GDP}$ caused constitutively low TORC1 activity. In line with this proposed model in yeast, two complementary studies in *Drosophila* and mammalian cells have also reported that the conserved Rag GTPases act as upstream regulators of TORC1 and play important roles in coupling amino acid-derived signals to TORC1 (Kim et al. 2008; Sancak et al. 2008).

Interestingly, a genome-wide synthetic genetic interaction screen revealed that *tor1Δ* cells were particularly sick, or not viable, in the absence of individual subunits of either of two protein complexes, namely the EGO complex and the *ho*motypic fusion and vacuole *p*rotein *s*orting (HOPS/class C-Vps) complex (Zurita-Martinez et al. 2007). These and additional genetic data indicated that the class C-Vps/HOPS complex may, like EGOC, directly or indirectly control TORC1 signalling in response to amino acids. Notably, the HOPS complex is thought to facilitate the transition from tethering to trans-SNARE pairing during fusion at the vacuole in part by nucleotide exchange on the GTPase Ypt7, which is exerted by the HOPS complex subunit Vam6 (Wurmser et al. 2000). Intriguingly, recent genetic and biochemical data indicate that Vam6 may in fact control TORC1 function rather directly by regulating the nucleotide-binding status of the EGOC subunit Gtr1

(Binda et al. 2009). This suggests that Vam6 may actually integrate amino acid signals to coordinate the control of TORC1 activity and vacuolar fusion events. While the discovery of the EGOC as an activator of TORC1 signalling represents an important step in deciphering the molecular events that signal nutrient availability to TORC1, an exciting question that remains to be addressed is how amino acid availability is sensed and communicated to Vam6 and/or the EGOC.

In addition to the abundance and/or quality of nutrients, TORC1 activity also appears to be sensitive to stress cues, but the mechanisms by which these cues are sensed and how this is communicated to TORC1 are presently unknown (De Virgilio and Loewith 2006a). Finally, the Golgi Ca^{2+}/Mn^{2+} ATPase Pmr1 has recently also been implicated in TORC1 regulation (Devasahayam et al. 2006). Accordingly, loss of Pmr1 increased rapamycin resistance, while addition of Mn^{2+} to the growth medium restored rapamycin sensitivity to *pmr1Δ* cells. Together with genetic epistasis analyses that placed Pmr1 upstream of TORC1, it was suggested that manganese within the Golgi lumen might, via a yet unidentified mechanism, inhibit TORC1 activity (Devasahayam et al. 2007).

The upstream signals that regulate TORC2 remain currently elusive, but may possibly include nutrients and/or diverse stresses. Given the importance of cell polarization during mitosis (Pruyne and Bretscher 2000a, b), an interesting additional or alternative model is that the cell cycle machinery may impinge on TORC2.

The protein kinase Sch9

As indicated above, the serine/threonine protein kinase Sch9 plays a central role in nutrient-mediated signalling. This kinase was originally identified through a screen aiming to isolate multi-copy suppressors of the growth defect at high temperatures of a cAMP-PKA signalling-deficient *cdc25^{ts}* strain (Toda et al. 1988). It was then also shown that the typical phenotypes associated with the loss of Sch9 activity, such as slow growth, reduced cell size and small colony formation, can all be suppressed by overactivation of the PKA pathway, an observation that was subsequently confirmed in several studies (Toda et al. 1988; Hartley et al. 1994; Jorgensen et al. 2002). Later, it was reported that the deletion of *GPR1*, *GPA2* or *RAS2*, which all encode proteins involved in the upstream activation of the cAMP-PKA pathway, results in a synthetic growth defect when combined with the deletion of *SCH9* (Kraakman et al. 1999b; Lorenz et al. 2000). Thus, there appears to be an intimate relationship between Sch9 and PKA as both kinases seem to have overlapping functions. Subsequently, Sch9 was reported to be essential for the proper nutritional regulation of PKA-dependent phenotypes in glucose-repressed cells, such as the activation of trehalase, the repression of stress response genes and the induction of ribosomal protein genes (Crauwels et al. 1997a). These findings led to the concept of the so-called *F*ermentable *G*rowth *M*edium (FGM) pathway that is believed to ensure the maintenance of PKA-dependent phenotypes of yeast cells growing on medium containing a rapidly fermented sugar and all essential nutrients, after the transient activation of the cAMP-PKA pathway that occurs during lag phase when cells prepare themselves for fermentative growth (Thevelein 1994; Crauwels et al. 1997a). As the FGM pathway appeared to operate independent of cAMP, it was postulated that Sch9 could control the activity of PKA at the level of the free catalytic Tpk subunits (Thevelein 1994; Crauwels et al. 1997a). More recently, however, it was found that PKA and Sch9 act in parallel (Roosen et al. 2005). For instance, both kinases have an additive effect for the expression of proteins required for nucleotide metabolism but they exert opposite effects on the expression on proteins involved in detoxification or proteolysis (Roosen et al. 2005). In addition, they independently determine the sensitivity of yeast cells for the ATP-analogue 1NM-PP1 as illustrated by the observation that a mutant with combined analogue-sensitive *sch9^{as} tpk1^{as} tpk2^{as} tpk3^{as}* alleles is more sensitive than the single *sch9^{as}* mutant or the *tpk1^{as} tpk2^{as} tpk3^{as}* strain (Yorimitsu et al. 2007; Huber et al. 2009; Lee et al. 2009).

Sch9 is homologous to the mammalian protein kinase B (PKB/Akt). Alignment of Sch9 and PKB revealed 44% identity and 68% similarity over 397 residues and showed that the homology between Sch9 and PKB is most pronounced at the catalytic domain and the C-terminus (Geyskens et al. 2001). Furthermore, Sch9 and PKB are not only structurally, but also functionally related since expression of PKB rescues the slow growth and small colony phenotype of a *sch9Δ* deletion mutant (Geyskens et al. 2001). PKB was originally discovered as an oncogene and is involved in the regulation of cell survival, cell cycle progression and metabolism. The PKB kinase contains a *p*leckstrin *h*omology (PH) domain which binds to the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 is produced by phosphorylation of phosphatidylinositol-4,5-bisphosphate via the phosphoinositide-3-kinase PI3K, which is activated by receptor tyrosine kinases, such as the insulin receptor. After binding to PIP3 via its PH domain, PKB is localized at the plasma membrane where it is phosphorylated, and thereby activated, by the phosphoinositide-dependent kinase PDK1, which also contains a PH domain, and by mammalian TORC2 (Fayard et al. 2005; Corradetti and Guan 2006). Yeast contains two PDK1 orthologs, Pkh1 and Pkh2, which, however, contain no PH domain and are apparently activated by sphingolipids rather than by phosphoinositides (Casamayor et al.

1999; Friant et al. 2001; Liu et al. 2005a, b). Sch9 also contains a PDK1 consensus site and it was found that phosphorylation of this site by Pkh1/2 activated Sch9 in vitro (Liu et al. 2005a; Urban et al. 2007). Moreover, phosphorylation of the PDK1 site appears to be essential for Sch9 to function in vivo for the control of cell growth control and stress resistance (Roelants et al. 2004). To date, there are no data that show that Sch9 is a target of the rapamycin-insensitive TORC2. In contrast, Sch9 is directly phosphorylated by TORC1 on at least five residues in the C-terminal half and these phosphorylation events are critical for its catalytic activity (Urban et al. 2007). As already described above and illustrated in Fig. 4, the phosphorylation of Sch9 allows TORC1 to prevent the induction of typical G_0 traits as well as to stimulate ribosomal biogenesis and translation initiation (Urban et al. 2007; Huber et al. 2009). A recent report indicates that TORC1-dependent phosphorylation of Sch9 is subject to feedback control since it is negatively regulated by Sfp1, a transcriptional activator of ribosomal protein and *ribi* gene expression, which itself is activated by TORC1 (Lempiainen et al. 2009). Note that in addition to the Pkh- and TORC1-dependent phosphorylation sites, Sch9 appears to be phosphorylated at many additional residues, indicating that yet unidentified protein kinases may control Sch9 function (Urban et al. 2007). Like TORC1, Sch9 is predominantly localized at the vacuolar membrane (Jorgensen et al. 2004; Urban et al. 2007). In this context, it should be mentioned that Sch9 possesses no PH domain but instead contains a calcium-dependent C2 domain at its N-terminus. While the role of this domain for Sch9 function remains to be determined, it may possibly serve to regulate the subcellular localization of Sch9, as C2 domains can bind phospholipids and are known to serve as membrane-targeting domains in other proteins (Lemmon 2008). A fraction of the Sch9 pool appears also to be localized in the nucleus as co-immunoprecipitation analysis showed that Sch9 is recruited to the chromatin of osmostress-responsive genes (Pascual-Ahuir and Proft 2007).

One major role of Sch9 is to regulate translation in function of nutrient availability and the growth potential. As such, Sch9 controls the expression of RP genes and of the Ribi regulon, by interfering with the transcriptional processes conducted by the RNA polymerases I, II and III (Crauwels et al. 1997a, b; Jorgensen et al. 2004; Roosen et al. 2005; Urban et al. 2007; Smets et al. 2008; Huber et al. 2009). Concerning RNA polymerase I transcription, Sch9 appears to be required to maintain the optimal activity of the polymerase, presumably by promoting the recruitment of the catalytic subunit Rpa190 to the rDNA locus. In addition, Sch9 is essential for the proper processing of the 35S transcript into the 25S, 18S and 5.8S rRNA and at least one component of the processome, i.e. Rps6, was shown to be phosphorylated by Sch9. The latter led to the conclusion that Sch9 should be considered as the orthologue of the mammalian kinase S6K1, rather than being the yeast counterpart of PKB (Urban et al. 2007; Huber et al. 2009). Possibly, Sch9 may still combine the functions of S6K and PKB and thus represent the ancestor from which both kinases have evolved. For processes mediated by the RNA polymerase II, Sch9 phosphorylates and inhibits the activity of the transcriptional repressors Stb3 and Dot6/Tod6, which, respectively, bind the RRPE and PAC elements in the promoters of *ribi* genes (Huber et al. 2009). For RNA polymerase III-dependent transcription, the downstream target of Sch9 is Maf1, the repressor that is also regulated by PKA and TORC1. Here, Sch9 was first reported to phosphorylate Maf1 at a subset of the sites that are also recognized by PKA (Lee et al. 2009). Most recently, it was shown that Sch9 phosphorylates Maf1 at seven distinct sites in vitro and that it thereby can not only partially inhibit nuclear accumulation of Maf1 after rapamycin treatment, but also completely block the repressive association between Maf1 and the RNA Pol III subunit Rpc82 (Huber et al. 2009). In addition to ribosome biogenesis, Sch9 controls the expression of different tRNA synthetases, proteins involved in amino acid metabolism as well as translation initiation and elongation factors (Crauwels et al. 1997b; Roosen et al. 2005) and recent evidence suggests that Sch9 also controls the phosphorylation status of eIF2α (Urban et al. 2007). Hence, Sch9 functions as a central coordinator of protein synthesis and this may explain why *sch9Δ* cells are characterized by a small cell size, since, indeed, the translational capacity of a cell is tightly coupled to the size threshold at which cells commit to cell division (Jorgensen et al. 2002, 2004).

Several studies implicated Sch9 in the regulation of the cellular response to stress. Best studied is its role in the regulation of the protein kinase Rim15, which in turn controls the expression of stress-responsive genes through the transcriptional activators Gis1 and Msn2/4 that, respectively, bind to the PDS and STRE elements (Crauwels et al. 1997a; Pedruzzi et al. 2003; Cameroni et al. 2004; Roosen et al. 2005). Sch9 phosphorylates Rim15 at Ser^{1061} in vitro and this phosphorylation event is necessary for normal cytoplasmic retention of Rim15 (Pedruzzi et al. 2003; Wanke et al. 2008). Mechanistically, phosphorylated Ser^{1061} in Rim15, together with phosphorylated Thr^{1075} by the Pho85–Pho80 kinase complex (see also below), may allow Rim15 to engage in binding the two monomeric subunits within a single 14-3-3 (Bmh2) protein dimer (Wanke et al. 2005, 2008) and thereby guarantee optimal sequestration of Rim15 in the cytoplasm (Fig. 5). As expected, the regulation of Rim15 localization by Sch9 was also found to be dependent on TORC1 signalling as rapamycin treatment causes dephosphorylation

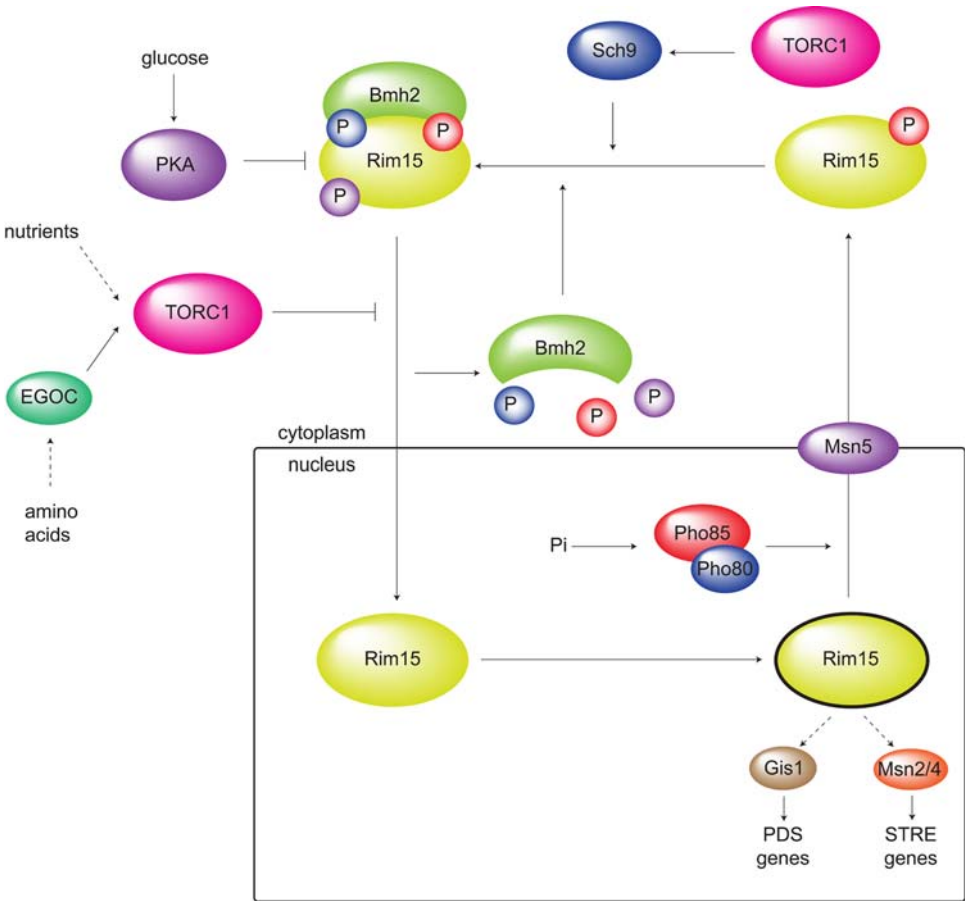

Fig. 5 The Rim15 protein kinase acts as a nutritional integrator in *S. cerevisiae*. Rim15 is regulated by at least four nutrient signalling protein kinases. In response to glucose, PKA directly phosphorylates and thereby inactivates the kinase activity of Rim15. Active TORC1 and Sch9 promote cytoplasmic retention of Rim15. Notably, TORC1 favours phosphorylation of Rim15 at both Ser^{1061} (via activation of Sch9) and Thr^{1075} (via inhibiting a yet unidentified protein phosphatase) to promote the association of Rim15 with a 14-3-3/Bmh2-protein dimer for its optimal sequestration in the cytoplasm. Inactivation of TORC1 and Sch9 results in the nuclear translocation of Rim15 where this kinase controls the expression of Msn2/4- and Gis1-dependent genes. Rim15 is subject to autophosphorylation, which apparently stimulates nuclear export. Also Pho85–Pho80, which phosphorylates Rim15 at Thr^{1075}, controls the nuclear export of Rim15. *PDS* post-diauxic shift, *STRE* stress-responsive element

of the 14-3-3-binding site and enforces nuclear accumulation of Rim15 (Pedruzzi et al. 2003; Wanke et al. 2005; Urban et al. 2007). In addition to control via Rim15, TORC1 and Sch9 independently regulate the effectors Msn2/4 and Gis1. As mentioned above, TORC1 controls the phosphorylation and cytoplasmic retention of Msn2 via the Tap42-PP2A-dependent route (Beck and Hall 1999; Duvel et al. 2003; Santhanam et al. 2004). Sch9, on the other hand, is essential to maintain the Gis1 activity, which explains why Sch9 and TORC1 exert essentially opposed effects for the expression of the PDS genes at the diauxic shift (Roosen et al. 2005; Smets et al. 2008). How Sch9 stimulates Gis1 is not known, but it is tempting to speculate that it may involve a function of Sch9 that was only recently uncovered. Indeed, for the induction of osmostress-responsive genes, Sch9 appears to act as a chromatin-associated transcriptional activator since it is recruited to the promoter region of these genes when yeast is exposed to osmotic stress (Pascual-Ahuir and Proft 2007). This recruitment is independent of TORC1 but requires Sko1, a transcriptional activator of osmostress genes, and Hog1, a *m*itogen-*a*ctivated *p*rotein *k*inase (MAPK) that is part of osmoregulatory signalling cascade. In vitro experiments demonstrated that Sch9 interacts with both Sko1 and Hog1 and that Sch9 is able to phosphorylate Sko1 (Hohmann et al. 2007; Pascual-Ahuir and Proft 2007). Moreover, a recent genome-wide transcriptional analysis revealed other genes that require Sch9 for their optimal expression, while being negatively regulated by TORC1 (Smets et al. 2008). These include several genes encoding mitochondrial functions, such as proteins involved in the tricarboxylic acid cycle, the fatty acid metabolism as well as mitochondrial ribosomal proteins. The same study also showed that Sch9 acts independent of TORC1 to reduce the basal expression of NDP target genes, such as *GAP1*, which encodes the general amino acid permease, as well as genes regulated by GAAC pathway and its central transcription factor Gcn4.

Finally, the role of Sch9 for the induction of autophagy was studied in more detail. These studies revealed that autophagy is induced in yeast cells upon the simultaneous inactivation of Sch9 and PKA (Yorimitsu et al. 2007). This induction required the Atg1 kinase complex and was only in part dependent on the presence of Rim15 and Msn2/4. Interestingly, neither Rim15 nor Msn2/4 seemed to be essential for rapamycin-induced autophagy and the

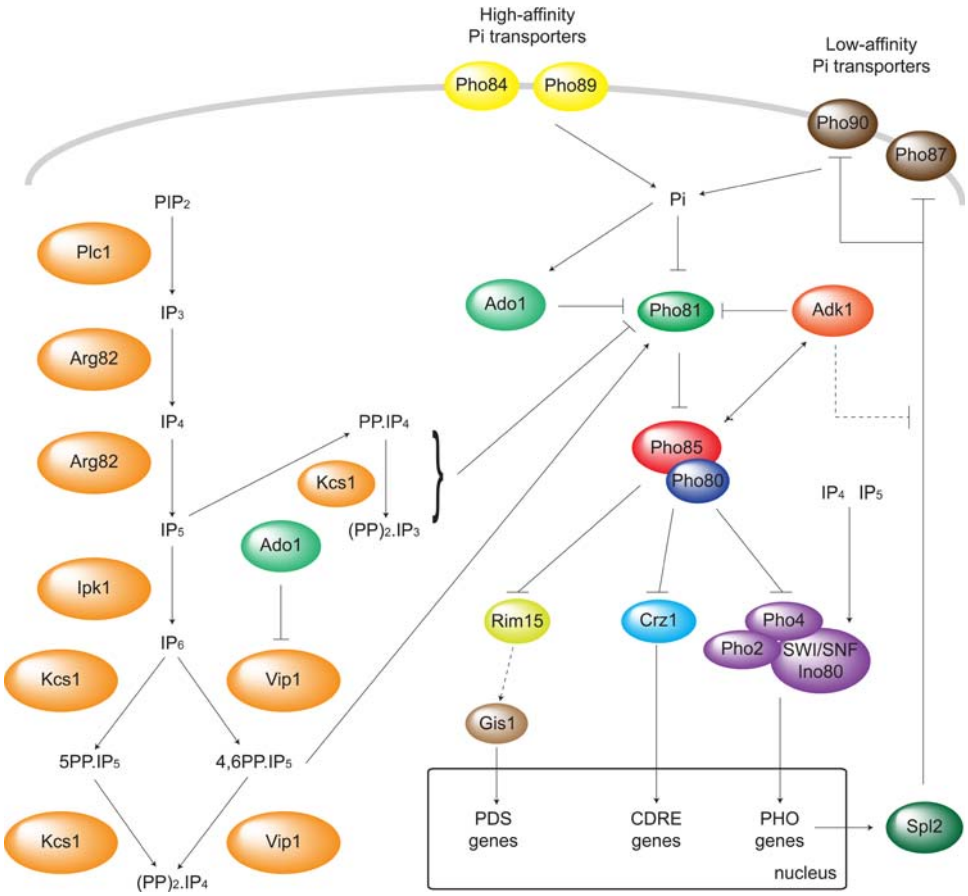

Fig. 6 Central role of Pho85–Pho80 in Pi-signalling and Pi-dependent stress responses in *S. cerevisiae*. Activation of the PHO pathway in response to Pi-limiting conditions requires inhibition of Pho85–Pho80 by Pho81 and subsequent transcription of the PHO genes via interaction of Pho2 and Pho4. Inositol phosphate metabolism and adenosine nucleotide metabolism both influence Pi-dependent responses on different levels. Additionally, Pho85–Pho80 also negatively regulates Rim15 and Crz1. *Dashed lines* represent putative or indirect interactions. See text for further details. *PDS* post-diauxic shift, *CDRE* calcineurin-dependent response element

combined inactivation of Sch9 and PKA appeared to additively stimulate autophagy, indicating that also for autophagy Sch9 and PKA act, at least in part, in parallel to the TORC1 pathways (Yorimitsu et al. 2007).

The Pho85–Pho80 kinase complex

Pho85 is a cyclin-dependent kinase (CDK) in *S. cerevisiae* with distinct functions in several pathways, which is confirmed by the pleiotropic phenotype of a *pho85Δ* strain. Deletion of *PHO85* results in slow growth with a G_1-delay on rich medium and a severe growth defect on poor carbon and nitrogen sources (Lee et al. 2000). More specifically, mutant *pho85Δ* cells display a background-dependent hyperaccumulation of glycogen (Timblin et al. 1996; Lee et al. 2000), morphology and polarity defects (Measday et al. 1997; Tennyson et al. 1998), constitutive expression of phosphate-responsive or so-called PHO genes, CWI defects and hypersensitivity to stress conditions (Huang et al. 2002), sporulation defects (Gilliquet and Berben 1993), aberrant expression profiles during the diauxic shift (Nishizawa et al. 2004) and a hyperinduction of nutrient starvation-induced autophagy (Wang et al. 2001). Consistent with its multiple functions, Pho85 can interact with ten different cyclins that can be divided into two different subfamilies according to their sequence similarities (Measday et al. 1997). The first so-called Pcl1,2 subfamily includes the cyclins Pcl1, Pcl2, Pcl5, Pcl9 and Clg1. The second subfamily is denoted as the Pho80 subfamily and includes Pcl6, Pcl7, Pcl8, Pcl10 and Pho80. The interaction of Pho85 with its corresponding cyclin is essential for its activity and to confer substrate specificity. In this review, we will focus on Pho80, which is the major cyclin involved in phosphate metabolism and regulation of proper entry into G_0 under phosphate starving conditions. For the other cyclins, we refer to a recent review (Huang et al. 2007a).

The role of Pho85–Pho80 kinase complex in phosphate metabolism and phosphate signalling

The Pho85–Pho80 kinase complex plays a central role in the PHO pathway, which allows cells to properly respond to changes in extra- and/or intracellular phosphate levels, as outlined in Fig. 6. Pho85–Pho80 controls the localization of the Pho4 transcription factor, which is essential for induction of the PHO genes (O'Neill et al. 1996; Ogawa et al. 2000), leading to optimized phosphate acquisition (see further). Under phosphate limiting conditions, the

low-phosphate signal activates the PHO pathway by triggering activation of the CDK-inhibitor (CKI) Pho81 (Lenburg and O'Shea 1996). In turn, the active Pho81 maintains the Pho85–Pho80 kinase complex in its inactive form and thereby Pho81 prevents that the kinase complex would hyperphosphorylate Pho4 (O'Neill et al. 1996). Under these conditions, Pho4 can interact with its nuclear import factor Pse1 and translocate into the nucleus, where it activates the transcription of the PHO genes (Kaffman et al. 1998b). However, transcriptional activation occurs only in the presence of the transcription cofactor Pho2, which binds to a Pho4 dimer to form a stabilized heterotrimeric protein complex (Magbanua et al. 1997; Shao et al. 1998). Pho2 must be phosphorylated at Ser^{230} in order to bind unphosphorylated dimeric Pho4 and it appears that this phosphorylation is also regulated by phosphate availability (Xia and Ao 1999; Liu et al. 2000). The proteins that control phosphorylation of Pho2 under physiological conditions have not been identified, but it is known that the Cdc28 is able to phosphorylate Pho2 in vitro (Liu et al. 2000). Note that the function of Pho2 is not restricted to phosphate signalling, as this cofactor is also involved in mating-type switching, through its interaction with Swi5 (Bhoite and Stillman 1998; Bhoite et al. 2002), as well as in one-carbon metabolism and especially adenine synthesis, through interaction with Bas1 (Zhang et al. 1997; Bhoite et al. 2002; Subramanian et al. 2005). The latter is of particular interest since it provides a link between phosphate signalling and adenylic nucleotide synthesis as will be discussed below.

When phosphate is abundant, Pho81 is inactivated and as a result the Pho85–Pho80 kinase complex becomes active, which can now phosphorylate Pho4 resulting in the disassembly of the Pho4–Pho2 heterotrimeric protein complex and the repression of the PHO genes. Phosphorylated Pho4 then interacts with the export factor Msn5 to be excluded from the nucleus (Kaffman et al. 1998a). The first step in activation or repression of the PHO pathway thus occurs via modulation of the activity of the Pho81 CKI. Pho81 is constitutively localized in the nucleus where it is bound to the Pho80 cyclin under high- and low-Pi conditions. Similarly, Pho81 is able to inhibit the Pho85–Pcl7 kinase complex in response to low-phosphate levels via direct interaction with the Pcl7 cyclin (Lee et al. 2000). The Pho81 protein consists of 1,179 amino acids, but only the so-called minimum domain, located between the amino acid 644 to 723, is essential for inhibition of Pho85–Pho80 towards Pho4 (Huang et al. 2001). Other domains of Pho81 have multiple functions towards non-Pho4 targets of the Pho85–Pho80 kinase or inhibit other Pho85–cyclin complexes (Huang et al. 2007b; Swinnen et al. 2005). Since Pho81 always interacts with the Pho80 cyclin, but only inhibits the Pho85–Pho80 kinase under phosphate limiting conditions, Pho81 may be regulated by post-translational or allosteric activations. Recent evidence shows that inositol polyphosphate species play a particular role in this regulation. More specifically, isoform synthesized by Vip1 was found to bind non-covalently with Pho80–Pho85–Pho81, thereby inducing additional interactions between Pho81 and Pho80–Pho85 that prevent substrates from accessing the kinase active site (Lee et al. 2007; Lee et al. 2008b). Intriguingly, under phosphate starvation conditions, Pho4-dependent antisense and intragenic RNA transcription in the *KCS1* locus induces downregulation of the Kcs1 activity, leading to a positive feedback loop for activation of the PHO genes (Nishizawa et al. 2008a).

Activation of the PHO pathway results in increased expression or breakdown of proteins involved in the uptake and storage of phosphate (Ogawa et al. 2000). For the uptake of phosphate, the regulation occurs both by Pho4-dependent transcriptional and post-transcriptional processes. *S. cerevisiae* encodes for two different phosphate transporter systems: a low-affinity system consisting of Pho87, Pho90 and Pho91 and a high-affinity system containing Pho84 and Pho89. Under phosphate limiting conditions, genes encoding for high-affinity phosphate transporters, i.e. *PHO84* and *PHO89*, are induced. In contrast, the expression of the genes encoding the low-affinity system is independent of Pho4 and phosphate availability (Auesukaree et al. 2003). Nonetheless, Pho4 may control the low-affinity system via the induction of Spl2 (Wykoff et al. 2007). Spl2, which was originally discovered as a multi-copy suppressor of *plc1Δ* (Flick and Thorner 1998), appears to limit the phosphate-uptake velocity of Pho87 and Pho90 in vivo through its interaction with the SPX domain of these low-affinity phosphate transporters (Hurlimann et al. 2009). This provides yet another feedback loop for the control of the PHO pathway. Thus, while the low-affinity phosphate transporters Pho87 and Pho90 are active at the plasma membrane under high-phosphate conditions, the high-affinity system takes over the phosphate uptake function of Pho87 and Pho90 under phosphate limiting conditions. The activity of Pho91 was shown to be independent of Pho4 and Spl2 (Wykoff et al. 2007) and Pho91 was identified as a vacuolar phosphate transporter essential for proper polyphosphate accumulation (Hurlimann et al. 2007). For storage of phosphate, the products of the *PHM1* to *PHM5* genes are important. These proteins are involved in orthophosphate and polyphosphate accumulation and their expression is induced under phosphate limiting conditions (Ogawa et al. 2000; Auesukaree et al. 2004). This may seem to be contradictory since polyphosphate is consumed under phosphate limiting conditions. However, the increased intracellular phosphate levels obtained by high-affinity phosphate uptake have to be stored as polyphosphate in the vacuole to avoid negative

feedback, in particular to the Pho84 transporter, sustaining in this way a high rate of phosphate uptake under phosphate limiting conditions. In addition, several repressible acid phosphatases, encoded by *PHO5*, *PHO11* and *PHO12* (rAPases) as well as the repressible alkaline phosphatase *PHO8* (rALPases), are induced (Persson et al. 2003). The acid phosphatases are secreted into the periplasmic space where they act on a multitude of phospho-ester substrates, while the alkaline phosphatase Pho8, localized at the vacuole, acts on other phosphate-containing substrates to liberate free phosphate (Klionsky and Emr 1989). Genes involved in catabolism of alternative phosphorous sources (*GIT1*, *GDE1* and *HOR2*) are also induced under phosphate limiting conditions (Ogawa et al. 2000; Almaguer et al. 2003). Finally, *PHO81* is itself induced under these conditions, comprising a third positive feedback loop for constitutive activation of the PHO pathway. Several other PHO genes undergo Pho4-dependent induction under low-phosphate conditions (Ogawa et al. 2000), but for most of them no clear function in phosphate metabolism or phosphate signalling has been identified. Recently, ChIP-on-ChIP analysis revealed 18 novel PHO-type genes with no apparent functional relationship to phosphate metabolism (Nishizawa et al. 2008a). Since most of them have already been identified as upregulated during nitrogen starvation and entry into stationary phase, it can be concluded that Pho4 has a possible role in a cross-talk between phosphate starvation and other nutrient starvation-induced stress conditions.

Apart from the main function of Pho4 to induce the PHO genes under phosphate limiting conditions, this transcription factor has been shown to be involved in other cellular processes. Two major phenotypes of a *pho85Δ* strain are the absence of growth on poor nutrient sources (Lee et al. 2000) and vacuolar dysfunction (Huang et al. 2002) and involvement of Pho4 appears to play a role in both processes. The additional deletion of *PHO4* can rescue the growth defects of a *pho85Δ* strain on non-fermentable carbon sources and proline as sole nitrogen source (Nishizawa et al. 1999; Popova Iu et al. 2000). However, this phenotype is not only dependent on the Pho80 cyclin but also seems to involve the cyclins Pcl6 and Pcl7 (Lee et al. 2000), though a possible link between Pcl6 and Pcl7 and Pho4 was never investigated. Secondly, a *pho85Δ* and a *pho80Δ* strain show hypersensitivity to vacuolar stress conditions, such as high osmolarity, salt stress and elevated Ca^{2+} levels, and this is due to severe abnormalities in vacuolar structure and function (Cohen et al. 1999; Huang et al. 2002). These vacuolar phenotypes are completely suppressed by additional deletion of *PHO4* or *VTC4* (*PHM3*), a Pho4-activated gene involved in polyphosphate metabolism as discussed above (Ogawa et al. 2000). Pho4-dependent hyperinduction of Vtc4 thus leads to vacuolar dysfunction and sensitivity to various environmental stress conditions. Interestingly, the ChIP-on-Chip analysis, mentioned above, revealed phosphate independent but Pho4-dependent transcription of several genes, providing additional evidence that a nuclear pool of Pho4 exists that can bind to a promoter and control transcription under high phosphate conditions (Nishizawa et al. 2008a). In this way, Pho4 was shown to act as a transcriptional repressor as it negatively regulates the transcription of *CIS3*, *YPS3* and *SNZ1* (Nishizawa et al. 2008a, b). Additionally, a role for Pho4 in G_1-arrest caused by DNA damage has also been reported (Wysocki et al. 2006).

The role of the Pho85–Pho80 kinase complex in phosphate starvation-induced stress responses

Inorganic phosphate is an essential nutrient for all organisms required for biosynthesis of nucleotides, phospholipids and metabolites, making it an important messenger to signal a growth limiting metabolic state and reduced developmental capacities of the cell. Similar to glucose or nitrogen starvation conditions, the depletion of phosphorous sources forces yeast cells to enter the quiescent G_0-state (Swinnen et al. 2006).

Thorough investigation of phosphate starvation-induced stress responses uncovered a central role for the Pho81 CKI in the activation of Rim15, the kinase that is essential for the accumulation of the reserve carbohydrate glycogen and the stress protectant trehalose and the expression of stress-responsive genes under glucose starvation conditions (Reinders et al. 1998; Cameroni et al. 2004; Roosen et al. 2005). Indeed, in a *pho81Δ* mutant strain, like in a *rim15Δ* strain, the accumulation of trehalose and the expression of PDS genes, like *SSA3* and *GRE1*, are clearly delayed and reduced under phosphate starving conditions, while an effect on the expression of STRE genes appears less obvious (Swinnen et al. 2005). Interestingly, deletion of *PHO85* reverted all of the observed phenotypes in a *pho81Δ* strain, but deletion of *PHO80*, while fully reverting the defect in PDS-driven gene expression, only partially reverted the trehalose accumulation defect in a *pho81Δ* strain. This indicates that additional Pho85-associated cyclins are required to maintain proper trehalose levels. Note that the regulation of PDS genes particularly requires the protein kinase Sch9, and, therefore, it may not come as a surprise that also this kinase plays a role in controlling these genes in response to phosphate availability, which is further reflected by the fact that the combined deletion of *PHO85* and *SCH9* is synthetic lethal (Swinnen et al. 2005). As discussed above, Pho81 gets activated when phosphate becomes limiting and this results in nuclear translocation of Pho4 and the activation of the PHO genes. For this process, the minimum domain of

Pho81 is sufficient (Huang et al. 2001). In contrast, the control of Rim15-dependent phenotypes requires full-length Pho81, providing evidence that Pho81 is able to discriminate between different effectors of the same Pho85–Pho80 kinase (Swinnen et al. 2005). The molecular mechanism underlying this discrimination is still largely elusive, but several phosphorylation sites on Pho81 (Knight et al. 2004) and an involvement of inositol polyphosphates (Lee et al. 2008b) could be essential.

Meanwhile, it is known that Pho85–Pho80 promotes the nuclear exclusion of Rim15 when phosphate is abundantly available (Wanke et al. 2005). This is mediated by Pho80–Pho85-dependent phosphorylation of Rim15 on Thr^{1075}, which favours the association of Rim15 with the 14-3-3 protein Bmh2 in the cytoplasm (Wanke et al. 2005). Conversely, TORC1 inactivation triggers (likely due to the activation of protein phosphatases) dephosphorylation of Rim15 at Thr^{1075}, thereby favouring accumulation of Rim15 in the nucleus. Since Pho85–Pho80 is mainly localized within the nucleus and TORC1 is predominantly associated with the vacuolar membrane (Kaffman et al. 1998a; Sturgill et al. 2008), Pho85–Pho80 and TORC1 may act on different pools of Rim15. Interestingly, the intrinsic protein kinase activity of Rim15 is also required for its nuclear export (Wanke et al. 2005).

The role of the Pho85–Pho80 kinase complex in Ca^{2+} signalling and alkaline stress response

The third in vivo effector of the Pho85–Pho80 kinase is the *C*alcineurin *R*esponsive *Z*inc finger transcription factor 1, Crz1. This protein was identified via a synthetic dosage lethality screen in a *pho85Δ* and a *pho80Δ* strain and localization and phosphorylation studies confirmed that Pho85–Pho80 promotes nuclear exclusion of Crz1 via direct phosphorylation (Sopko et al. 2006). Crz1 is known to be activated via dephosphorylation by the serine/threonine protein phosphatase Calcineurin, consisting of two catalytic subunits Cna1 and Cna2 and a regulatory subunit Cnb1, in response to ion stress involving Li^+, Na^+, Mn^{2+} and Ca^{2+}. Activation of Calcineurin promotes nuclear translocation of Crz1 via interaction with its import factor Nmd5 (Polizotto and Cyert 2001), thereby inducing transcriptional activation of the "*c*alcineurin-*d*ependent *r*esponse *e*lement" (CDRE) genes such as the Na^+-ATPase *ENA1*/*PMR2*, the Ca^{2+}-ATPases *PMC1* and *PMR1* and the catalytic subunit of the *β*-1-3 glucan synthase *FKS2* (Matheos et al. 1997; Stathopoulos and Cyert 1997; Stathopoulos-Gerontides et al. 1999). Ion stress responses are often transient and after adaptation the subsequent nuclear export of Crz1 occurs via interaction with its export factor Msn5 (Boustany and Cyert 2002). Additional fine-tuning of the Crz1-dependent response occurs via regulation by two other kinases. Hrr25, a yeast casein kinase type 1 (CK1) homologue, stimulates nuclear exclusion of Crz1 (Kafadar et al. 2003), while PKA inhibits nuclear import of Crz1 (Kafadar and Cyert 2004), both of which occur through direct phosphorylation.

A physiological role for the regulation of Crz1 by the Pho85–Pho80 kinase remains elusive. Possibly, Pho85–Pho80 regulates expression of the high-affinity phosphate transporter Pho89 under alkaline stress response (Serrano et al. 2002). It was shown that increasing the extracellular pH to 7.6 leads to induction of *PHO84* and *PHO89*, encoding the high-affinity phosphate transporters, and *PHM1*, *PHM2* and *PHM3*, encoding the vacuolar polyphosphate synthases. Further investigation revealed a strict Pho4-dependent induction of *PHO84*, while full induction of *PHO89* required both Pho4 and Crz1. Under phosphate starving conditions, however, induction of *PHO89* is only Pho4-dependent (Swinnen et al. 2005), providing evidence for a Calcineurin-dependent response under alkaline conditions, which is absent in a low-phosphate environment. Specific domains of Pho81 may inhibit the Pho85–Pho80 kinase towards Crz1 and Pho4, which can lead to full activation of *PHO89* in combination with the activation of Calcineurin under alkaline pH conditions.

Connections between the PHO pathway, the purine pathway and inositol phosphate metabolism

In yeast, the amount of phosphate in nucleotides resembles the free intracellular orthophosphate concentration, making a co-ordinated regulation of phosphate metabolism and nucleotide synthesis feasible (Gauthier et al. 2008). Furthermore, mutants lacking the genes that encode the adenylate kinase Adk1, catalysing the interconversion of AMP and ATP to ADP (Konrad 1988), or the adenosine kinase Ado1, required for the synthesis of AMP from adenosine and ATP (Lecoq et al. 2001), display a Pho81-dependent nuclear translocation of Pho4 and, for instance, constitutive expression of the secreted acid phosphatase Pho5, even under conditions of high extracellular phosphate levels (Auesukaree et al. 2005; Huang and O'Shea 2005; Gauthier et al. 2008). In addition, the expression of PHO genes, i.e. *PHO84*, appears to be responsive to extracellular adenine (Gauthier et al. 2008). This interconnection of the control of phosphate metabolism and adenylic nucleotides is further reflected in a common requirement of the Pho2 transcriptional cofactor. Indeed, apart from playing a crucial role in the control of phosphate homeostasis, Pho2 together with the transcription factor Bas1 co-regulates the expression of the so-called ADE regulon that includes genes involved in the de novo synthesis of purines (Daignan-Fornier and Fink 1992). Consistently, a recent transcriptome analysis revealed that the

metabolic intermediate AICAR (5′-phosphoribosyl-5-amino-4-imidazole carboxamide), which was previously identified as a transcriptional regulator of the purine pathway genes (Rebora et al. 2001; Rebora et al. 2005), also mediates the expression of several PHO genes (Pinson et al. 2009). These authors reported that Pho4 and Bas1 compete for Pho2 binding and that AICAR stimulates the interaction between either Pho4 or Bas1 with Pho2.

As AICAR is known to stimulate the activity of the AMP-dependent protein kinase in mammalian cells, it was analysed whether the list of AICAR-responsive genes also includes known Snf1 targets. However, no such genes were found, suggesting that regulation of genes targeted by Pho2 does not involve AICAR activation of Snf1 (Pinson et al. 2009).

Several studies also established a link between phosphate signalling and inositol phosphate metabolism. Strains deleted for *PLC1*, *ARG82* and *KCS1* were found to exhibit constitutive expression and activity secreted phosphatases such as Pho5. This phenotype is dependent on a functional Pho81 and corresponds with the inability of these deletion mutants to synthesize $PP.IP_4$ and $(PP)_2.IP_3$ (Auesukaree et al. 2005). Both $PP.IP_4$ and $(PP)_2.IP_3$ can apparently inhibit Pho81 and thereby repress the PHO genes under high-phosphate conditions. In contrast, full induction of the PHO genes under phosphate-limiting conditions requires IP_4 and IP_5. These molecules appear to modulate the activity of the SWI/SNF and INO80 chromatin remodelling complexes, thereby affecting the induction of transcription of some phosphate-responsive genes (Steger et al. 2003). Thus, inositol polyphosphates and inositol pyrophosphates modulate the expression of the PHO genes at different levels and in opposite ways.

Conclusion: integration of nutrient-induced responses

For both unicellular and multicellular organisms, nutrients are the essential building blocks to make the necessary cellular components and metabolites. As outlined above, nutrients fulfil also regulatory functions since they act as triggers for several signalling pathways. Initial experimental work started from the concept of linear pathways acting in parallel, but along the road this concept changed towards a nutrient-dependent signalling network with extensive cross-talk between pathways at different levels. Furthermore, it is now clear that pathways converge on common effectors. As a consequence, one should bear in mind that the phenotype observed when changing a particular nutritional stimulus, or when the flow through a specific pathway is genetically modified, reflects the action of a signalling network and thus that the propagation of a signal can be enhanced, blocked or redirected dependent on the overall nutrient availability and metabolic status of the yeast cell. Therefore, it is not surprising that central signalling components can exert additive or opposed effects dependent on the target being studied. Perhaps a good way to picture signalling is by the principle of ‘gating’, which was first reported by Iyengar in 1996 (Iyengar 1996; Jordan and Iyengar 1998). He described that cAMP-activated PKA acts as a gatekeeper to control the flow through several signal transduction cascades in mammalian cells. This phenomenon has been recognized in yeast as well (Roosen et al. 2005). Although both papers concentrated on the gatekeeper function of PKA, it obviously is also applicable to other central kinases and phosphatases operating in a signalling network.

Our insight into the constitution of the nutrient signalling network has been advanced tremendously ever since the whole yeast genome was fully sequenced and new methods became available to study signalling events on a genome-wide scale. Despite all the efforts, there are still crucial gaps to be filled. For instance, we do not know how the intracellular and extracellular glucose sensing systems connect to each other to control the activity of adenylate cyclase and PKA. Neither do we know how nutrients are being sensed to drive the activation of TORC1 and Sch9. One theme that is starting to emerge is the fine-tuning of signalling by intracellular metabolic intermediates, such as the influence of inositol phosphates and intermediates formed by the purine biosynthesis pathway on phosphate signalling. Without doubt there is still a lot to be discovered, certainly when it comes to analysis of the concerted action of different nutritional stimuli. Hence, it can be expected that novel interconnection between individual pathways will be found in the coming years.

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