REVIEW ARTICLE

Nutrient sensing and signaling in the yeast *Saccharomyces cerevisiae*

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Abstract

The yeast *Saccharomyces cerevisiae* has been a favorite organism for pioneering studies on nutrient-sensing and signaling mechanisms. Many specific nutrient responses have been elucidated in great detail. This has led to important new concepts and insight into nutrient-controlled cellular regulation. Major highlights include the central role of the Snf1 protein kinase in the glucose repression pathway, galactose induction, the discovery of a G-protein-coupled receptor system, and role of Ras in glucose-induced cAMP signaling, the role of the protein synthesis initiation machinery in general control of nitrogen metabolism, the cyclin-controlled protein kinase Pho85 in phosphate regulation, nitrogen catabolite repression and the nitrogen-sensing target of rapamycin pathway, and the discovery of transporter-like proteins acting as nutrient sensors. In addition, a number of cellular targets, like carbohydrate stores, stress tolerance, and ribosomal gene expression, are controlled by the presence of multiple nutrients. The protein kinase A signaling pathway plays a major role in this general nutrient response. It has led to the discovery of nutrient transceptors (transporter receptors) as nutrient sensors. Major shortcomings in our knowledge are the relationship between rapid and steady-state nutrient signaling, the role of metabolic intermediates in intracellular nutrient sensing, and the identity of the nutrient sensors controlling cellular growth.

Introduction

Nutrients do not only provide energy and building blocks to cells and organisms, but also exert crucial regulatory roles. Most attention in that respect has been paid to phenomena of specific nutrient regulation, probably inspired by classical examples like the lac operon (Saier *et al.*, 1996) and the stringent response (Chatterji & Ojha, 2001) in *Escherichia coli*. In the yeast *Saccharomyces cerevisiae*, the glucose repression pathway raised a lot of interest because of its involvement in controlling ethanol fermentation, an exquisite characteristic of this species, which also has great industrial importance (Carlson, 1999). Subsequently, other nutrient regulation pathways started to be tackled, nitrogen catabolite repression (NCR; Hofman-Bang, 1999) and general amino acid control (GAAC; Niederberger *et al.*, 1981), phosphate regulation (Sabbagh, 2013), and regulation by other nutrients like sulfate (Thomas & Surdin-Kerjan, 1997), metal ions (Cyert & Philpott, 2013), and vitamins (Hohmann & Meacock, 1998). All these pathways are used to control the response to starvation and replenishment of a specific nutrient, and this biological phenomenon was the starting point for elucidating the pathways involved in more detail. Other nutrient signaling pathways were discovered from different angles. The Ras-cAMP pathway was discovered in an effort to elucidate the signaling function of the oncogenic mammalian Ras protein using yeast as a model system (Powers *et al.*, 1984). Parallel research had been focusing on glucose regulation of storage carbohydrate levels through the cAMP-PKA pathway (Thevelein, 1984). These two independent research lines merged and Ras was found to mediate glycolysis-dependent intracellular glucose sensing for the activation of

cAMP synthesis, in concert with a G-protein-coupled receptor (GPCR) system for extracellular glucose sensing (Thevelein & de Winde, 1999). Research on the first putative glucose transporter gene cloned in yeast surprisingly led to the discovery that the protein was unable to transport and rather acted as a glucose sensor for glucose-induced upregulation of regular glucose transporters (Ozcan *et al.*, 1996). This paved the way for the discovery of a similar amino acid sensor (Didion *et al.*, 1998; Iraqui *et al.*, 1999a, b; Klasson *et al.*, 1999) and firmly established the concept of transporter-like proteins being used as sensors for the nutrient they likely once transported previously in evolution. Discovery of the nitrogen-sensing target of rapamycin (TOR) pathway was based on the discovery of TOR itself as a target of the inhibitor rapamycin, which forced yeast cells into a stationary phase resembling proliferation arrest caused by nitrogen starvation (Zaragoza *et al.*, 1998).

Gradually, cellular targets were discovered that responded to multiple nutrients rather than to a single nutrient. The first and most logical target was nutrient regulation of ribosomal gene expression. Because the ribosome content of yeast is tightly connected to the growth rate (Mager & Planta, 1991), it could be predicted that starvation and replenishment for any single essential nutrient would affect ribosomal RNA and protein gene expression. A second, more surprising set of targets were the systems controlled by the protein kinase A (PKA) pathway. During the growth on glucose, these targets indicate that the PKA pathway must be highly active, as opposed to growth on nonfermentable carbon sources, in which PKA activity should be low. Surprisingly, however, starvation for a single essential nutrient on glucose medium also downregulates all PKA targets in conjunction with entrance into stationary phase, indicating that in a fermentable medium all essential nutrients somehow control the PKA pathway and that the capacity for a high fermentative specific growth rate rather than glucose availability is the main controlling factor of *in vivo* PKA activity (Thevelein *et al.*, 2000). Research on nutrient activation of ribosomal protein (RP) gene expression (Griffioen *et al.*, 1996) and activation of the PKA pathway (Thevelein *et al.*, 2000) has led to the common conclusion that short-term and long-term nutrient signaling phenomena have different requirements. This has also been found for glucose signaling in the main glucose repression pathway (De Winde *et al.*, 1996) and recently also for the galactose induction pathway (Abramczyk *et al.*, 2012), suggesting that it may be a general characteristic of nutrient signaling phenomena.

The difference between short- and long-term nutrient signaling brings us to the specific problems associated with studies on nutrient sensing. As opposed to classical primary messengers, like hormones, pheromones, and growth factors, which tend to have a single receptor system to convey their presence to the cellular machinery, nutrients often seem to act in multiple ways. Nutrients are metabolized and for many, but not all, nutrient signaling phenomena, at least partial metabolism of the nutrient is required. This has often been taken as an indication that sensing of the nutrient occurred through one of its metabolites or metabolite-converting enzymes inside the cell. However, it does not exclude a parallel system of extracellular and intracellular sensing, in which the two systems may be more or less interdependent. Sensing through intracellular metabolites acting as second or 'metabolic messengers' is obviously difficult to study because it requires the technical possibility of carrying out precise genetic modifications at single and multiple reactions in metabolic pathways, as well as the determination of all relevant metabolic intermediates. Interference with metabolism can easily cause many side effects and is often lethal in central metabolic pathways, which further complicates experimental design and interpretation of results. As will become clear from this review, the best-characterized nutrient-sensing proteins in yeast are receptors, transporter-like sensors, and transporter receptors in the plasma membrane that sense the presence of nutrients in the extracellular medium. This specific localization has greatly facilitated the identification of these nutrient-sensing proteins, as well as the demonstration that they directly interact with the nutrients as ligands.

This review has been organized in two main sections. First, we discuss specific nutrient signaling pathways. These are pathways that respond to the presence or absence of a specific nutrient or a class of related nutrients and serve to regulate mainly the uptake and metabolism of this category of nutrient. Second, we discuss general nutrient signaling pathways. These are pathways that are affected by the absence or presence of multiple types of nutrients. They serve to regulate physiological properties and developmental traits, such as growth and cell cycle control, stress tolerance, storage compound levels, aging, and pseudohyphal and invasive growth. The difference can be illustrated by the response to iron and zinc starvation. The former will specifically trigger induction of the high-affinity iron transporter, while the latter will specifically trigger induction of the high-affinity zinc transporter. These are specific nutrient responses triggered by a specific pathway for each nutrient. On the other hand, upon starvation for iron or zinc, the cells also show a common response: The cellular growth rate drops, and the cells arrest in the G1 phase of the cell cycle and accumulate stress tolerance protectants. This is a general nutrient response. When the starved cells are replenished with iron or zinc, there are again specific and general

responses. The iron and zinc transporters are endocytosed upon the addition of iron and zinc, respectively, which is a specific nutrient response because iron will not trigger endocytosis of the zinc transporter and vice versa. On the other hand, in both cases, ribosomal gene expression is induced to allow the start-up of growth. This is a general nutrient response. The review focuses mainly on the actual nutrient-sensing mechanisms and signaling pathways, while the downstream targets and physiological outputs of the signaling pathways are mentioned more succinctly.

Specific nutrient signaling pathways: triggered by a single type of nutrient

Snf1 protein kinase signaling in the glucose repression pathway

Role of Snf1 in carbon regulation

Saccharomyces cerevisiae preferentially uses glucose as a fermentable source of carbon and energy (Fig. 1). When this sugar or related rapidly fermented sugars, like fructose or mannose, are present, a complex regulatory network is activated that downregulates many components involved in transport and metabolism of alternative carbon sources, as well as respiratory function (most recently reviewed by Broach 2012). These adaptations are mainly dependent on the AMP-activated kinase (AMPK)/sucrose nonfermenting 1 protein, Snf1. Snf1, first identified in the budding yeast by Celenza & Carlson (1984), is the founding member of the SNF1/AMPK family of protein kinases, which is highly conserved in eukaryotes and required for energy homeostasis in mammals, plants, and fungi (Hedbacker & Carlson, 2006). AMPKs have a central role in sensing and responding to conditions in which energy reserves are depleted, by promoting both generation and preservation of energy (Hardie, 2011).

In *S. cerevisiae*, Snf1 mainly responds to declining levels of glucose, by promoting respiratory metabolism, glycogen accumulation, gluconeogenesis, autophagy, glyoxylate cycle, peroxisome biogenesis and, ultimately, aging (Ashrafi *et al.*, 2000; Lin *et al.*, 2003; Hedbacker & Carlson, 2008; Usaite *et al.*, 2009). In addition, it regulates acetyl CoA homeostasis and histone acetylation to increase fitness and stress resistance (Zhang *et al.*, 2013a). On the other hand, activated Snf1 represses anabolic processes, such as fatty acid and amino acid biosynthesis, through the inactivation of acetyl CoA carboxylase and several transcription factors, and the transcription factor Gcn4, respectively (Woods *et al.*, 1994; Honigberg & Lee, 1998; Ashrafi *et al.*, 2000; Kuchin *et al.*, 2002; Shirra *et al.*, 2008; Chumnanpuen *et al.*, 2012).

Composition of the Snf1 complex

Snf1 works as part of a heterotrimeric protein complex [here referred to as SNF1; structure solved by Amodeo (Amodeo *et al.*, 2007)] composed of Snf1 as catalytic kinase subunit, a γ-like regulatory subunit, Snf4, and a β-subunit, encoded by *GAL83*, *SIP1,* or *SIP2* (Jiang & Carlson, 1997). Snf1 has an N-terminal kinase domain and a C-terminal autoinhibitory domain. Snf4 binds to the C-terminus of Snf1 to alleviate Snf1 from autoinhibition (Celenza *et al.*, 1989; Leech *et al.*, 2003; Momcilovic *et al.*, 2008). In mammalian cells, binding of AMP to the γ-subunit releases the catalytic domain from the autoinhibitory domain (Chen *et al.*, 2009). However, Snf4-mediated protection of active Snf1 kinase in yeast seems to take place through allosteric interaction of other nucleotides, like ADP, with Snf4 (Mayer *et al.*, 2011). Snf4 has two ADP-binding sites able to bind ATP, AMP, and ADP with varying strengths, in which NADH can compete for the stronger site. Upon glucose depletion and increase in ADP levels, ADP binds to the weaker site inducing a conformational change in Snf4 that protects active Snf1 (Wilson *et al.*, 1996; Mayer *et al.*, 2011). On the other hand, association with each β-subunit determines differential substrate and upstream kinase specificity as well as the localization of the different SNF1 subcomplexes (Schmidt & McCartney, 2000; Vincent *et al.*, 2001). At high glucose levels, the subcomplexes are cytosolically located, regardless of the β-subunit. Upon glucose depletion, Sip1-containing SNF1 locates at the vacuolar membrane, Gal83-containing SNF1 at the nucleus, and Sip2-containing SNF1 at the cytosol (Vincent *et al.*, 2001; Hedbacker *et al.*, 2004a, b; Hedbacker & Carlson, 2006).

Regulation of Snf1 activity

Activation of Snf1 is generally associated with increased phosphorylation of Thr210, located within its activation loop. Thr210 phosphorylation happens in response to glucose limitation, for which the physiological role is well understood, but it is also stimulated by other stress conditions including high salinity, alkaline pH, oxidative stress, nitrogen starvation, and conditions causing the inactivation of Tor kinases (Nath *et al.*, 2003; Sutherland *et al.*, 2003; Orlova *et al.*, 2006; Hong & Carlson, 2007; Zhang *et al.*, 2011b; Perez-Sampietro *et al.*, 2013). Phosphorylation of this residue is dependent on the activity of the three partially redundant upstream kinases Sak1, Tos3, and Elm1 (Hong *et al.*, 2003; Nath *et al.*, 2003; Sutherland *et al.*, 2003) and counteracted by the

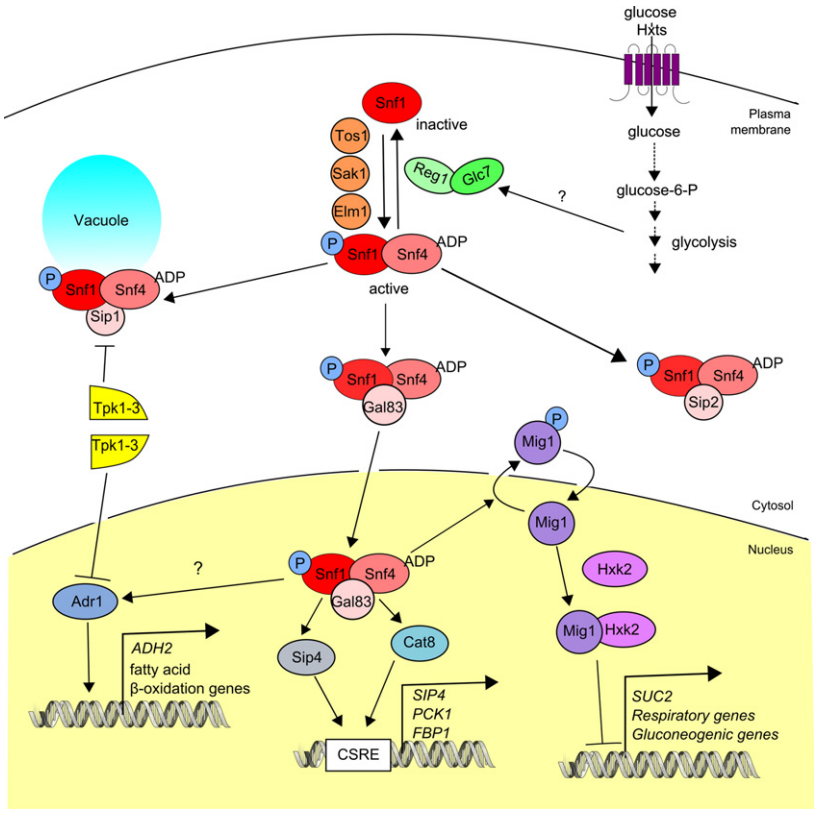


Fig. 1. The Snf1 protein kinase as a central player in the main glucose repression pathway. The Snf1 protein kinase orchestrates glucose repression of alternative carbon source utilization, and genes involved in respiration and gluconeogenesis. The Snf1 heterotrimeric complex consists of the catalytic subunit Snf1, the stimulatory subunit, Snf4, and one of the three β-subunits: Gal83, Sip1, or Sip2. Snf1 is active in phosphorylated form and the phosphorylation is performed by the three upstream protein kinases Sak1, Tos3, and Elm1, while the phosphatase Glc7 in conjunction with its regulatory subunit Reg1 is responsible for its dephosphorylation. The actual glucose-sensing mechanism, in which Hxk2 appears to play an important role, possibly activates the Glc7-Reg1 protein phosphatase to trigger dephosphorylation of Snf1. In its active form, Snf1-Snf4 binds to each of the three β-subunits, acquiring differential specificity for localization and target phosphorylation. Upon glucose exhaustion, a major role is played by the Snf1–Gal83 complex, which enters the nucleus to trigger derepression. This is accomplished by activation of the transcription factors Adr1, Sip4, and Cat8 and inactivation of Mig1 by dislodging its interaction with Hxk2 and promoting its cytosolic localization by phosphorylation. This leads to the expression of a wide range of carbon source-responsive element (CSRE) containing genes involved in the use of alternative carbon sources, gluconeogenesis, ethanol, and fatty acid metabolism. Metabolic reactions are depicted by dotted arrows; regulatory and signaling interactions by full arrows.

Reg1-Glc7 protein phosphatase 1, PP1 (Tu & Carlson, 1995). Recent research has also identified a possible role for the type 2A-like protein phosphatase, Sit4, and the Ptc1 protein phosphatase 2C, in Thr210 dephosphorylation (Ruiz *et al.*, 2011, 2013). Of the three upstream kinases, Sak1 (Snf1-activating kinase 1) plays a major role in Snf1–Gal83 complex activation and the $Snf1^{+}$ phenotype. While the other kinases complement the function of Sak1 in its absence, the deletion of only *ELM1* or *TOS3* has little effect on Snf1 activity (Hedbacker *et al.*, 2004a, b; Kim *et al.*, 2005; McCartney *et al.*, 2005). Interestingly, the three upstream kinases also seem to play additional roles in glucose regulation. Recent work has shown that Sak1, Tos3, and Elm1 also phosphorylate Gpa1, the Gα subunit of the heterotrimeric G protein of the pheromone signaling pathway, upon glucose limitation, resulting in reduced pheromone signaling and mating efficiency. This may explain why glucose-growing cells show superior mating efficiency compared to cells grown on respiratory carbon sources. Moreover, Reg1 was also found to dephosphorylate Gpa1 to maintain a strong mating response in the presence of glucose (Clement *et al.*, 2013).

There is also a preference for specific substrates and upstream kinases depending on the β-subunit in the Snf1 oligomeric complex (Hedbacker *et al.*, 2004a, b; McCartney *et al.*, 2005). Gal83 is the most important isoform not only for growth on nonfermentable carbon sources but also for the regulation of sterol biosynthesis under glucose-limiting conditions. Sip2, but not Sip1, can

partially take over its function when *GAL83* is deleted. Sip1, on the other hand, seems sufficient for specific processes, such as the regulation of nitrogen metabolism and meiosis (Zhang *et al.*, 2010). When only Sip2 is present, Tos3 and Elm1 cannot support the induction of invertase activity encoded by *SUC2*. Furthermore, Elm1 is much less effective in supporting induction of invertase when only Sip1 is present compared to the presence of Gal83 in the complex. The Gal83 isoform of the Snf1 complex can be activated by all three upstream kinases, but there is a clear preference for Sak1 over the other two kinases and deletion of Sak1 also leads to cytoplasmic retention of Gal83 upon glucose depletion (Hedbacker *et al.*, 2004a, b).

Glucose regulation of Snf1 activity

Until recently, little was known about the signaling event (s) leading to the activation of SNF1. Although phosphorylation of Thr210 in Snf1 is crucial for its activation, the activity of the upstream kinases remains unaffected by the addition of glucose, suggesting an alternative pathway for glucose regulation (Hong *et al.*, 2005; Rubenstein *et al.*, 2008). Phosphorylation of a truncated form of Snf1 (1–309), unable to interact with Snf4 (γ) and the β-subunits, or of a wild-type Snf1 in cells lacking Snf4 (γ) and β-subunits, still increased in response to glucose limitation, indicating that activation can happen independently of these regulatory subunits (Ruiz *et al.*, 2011). This is difficult to reconcile with the previous hypothesis that Snf4 exerts its regulation by limiting access of the phosphatases to Thr210 (Mayer *et al.*, 2011). An alternative hypothesis is that Snf1 is constitutively phosphorylated by the upstream kinases and that the changes in its phosphorylation level as a function of glucose availability are due to changes in the activity or recruitment of the Reg1-Glc7 phosphatase. However, Reg1-Glc7 activity also seems unaffected by changes in glucose levels (Rubenstein *et al.*, 2008). A recent report suggests that adenylate ligand binding to Snf4 and to the active site of Snf1 could trigger a conformational change, rendering Snf1 in the complex more resistant to phosphatase activity (Chandrashekarappa *et al.*, 2013). As a result, the phosphorylation of Thr210 and activation of SNF1 would be enhanced even in the presence of high glucose levels. This, along with results showing that particular alterations in different parts of the SNF1 heterotrimeric complex result in increased phosphorylation of Thr20 and activation of SNF1 even in the presence of high glucose, indicates that a proper conformation of the SNF1 complex is crucial for its activity, at least for the maintenance of the inactive state during growth on high glucose, independent of the phosphorylation level of Thr210 (Momcilovic *et al.*, 2008). In this respect, the glycogen-binding domains in the β-subunits, which are required for interaction with Snf4 (Momcilovic *et al.*, 2008), are known to be important for conferring glucose repression activity to the complex (Ruiz *et al.*, 2011). In addition to glucose-induced dephosphorylation of Thr210 in Snf1, glucose-induced SUMOylation of K549 in Snf1 has also been reported to downregulate SNF1 activity by the inhibition of its catalytic function and by directing Snf1 kinase for degradation (Simpson-Lavy & Johnston, 2013).

Recent findings highlight the possibility that glucose activation of the cAMP-PKA pathway may play a role in the inactivation of Snf1 by dephosphorylation in the presence of glucose. Early work already indicated the loss of glucose repression in yeast strains with strongly reduced PKA activity (Mbonyi *et al.*, 1990). More recent work has shown that Sak1 and Tos3 contain putative PKA phosphorylation sites and that cells lacking the Ras GTPase-activating proteins (GAPs), Ira1/2, or the regulatory PKA subunit, Bcy1, exhibited reduced activation of the Snf1 pathway upon glucose exhaustion, whereas Snf1 activity and derepression in the presence of glucose was elevated in cells lacking the G-protein-coupled glucose receptor, Gpr1 (Barrett *et al.*, 2012). Other recent work revealed that Glc7 activity dramatically increases within 1 min after the addition of glucose and that this post-translational activation depends on glucose activation of the cAMP-PKA pathway. Deletion of the Reg1 or Shp1 regulatory subunit abolishes both glucose-induced activation of PP1 and glucose repression, supporting a correlation between both phenomena (Castermans *et al.*, 2012).

Regulation of target genes by Snf1

Active Snf1 uses different mechanisms to regulate the expression of a variety of target genes. Derepression of genes involved in the metabolism of alternative carbon sources and induction of filamentous growth during glucose limitation are mediated by Snf1-dependent inactivation of the transcriptional repressor, Mig1/2 (Treitel *et al.*, 1998; Karunanithi & Cullen, 2012), reviewed by Hahn & Young (2011). Mig1/2 functions as transcriptional repressor in association with Hxk2. Phosphorylation of Mig1 and Hxk2 by Snf1 prevents their nuclear localization and thus prevents access to the target genes (Ahuatzi *et al.*, 2004; Fernandez-Garcia *et al.*, 2012). Hxk2 interaction with Mig1 under high glucose conditions might prevent the phosphorylation of Mig1 by Snf1 (Pelaez *et al.*, 2010). Snf1 also activates transcription factors such as Cat8, Sip4, and Rsd2, responsible for the induction of gluconeogenesis genes (Vincent & Carlson, 1999; Roth *et al.*, 2004). It regulates stress-response genes through phosphorylation of transcription factors such as

Hsf1 and Msn2 (Sanz, 2003; Hahn & Thiele, 2004; De Wever *et al.*, 2005). Snf1-dependent activation of Adr1, for the induction of genes involved in the β-oxidation of fatty acids and ethanol metabolism, is counteracted by binding of the yeast 14-3-3 proteins to the phosphorylated Ser230 in the Adr1 regulatory domain (Parua *et al.*, 2010; Ratnakumar & Young, 2010; Braun *et al.*, 2013). Besides the regulation of transcription factors, Snf1 also controls the expression of genes through chromatin remodeling. During glucose starvation, Snf1 phosphorylates the histone H3 and aids in the recruitment of the SAGA complex, resulting in acetylation of histone H3 and subsequent recruitment of RNA polymerase II to the promoters of *HXT2*, *HXT4* and *INO1*, encoding two hexose transporters and inositol 3-phosphate synthase, respectively (Lo *et al.*, 2001; Abate *et al.*, 2012; Young *et al.*, 2012). The 14-3-3 proteins could also have an Snf1-dependent role in maintaining promoter nucleosomes in a hypoacetylated state by preventing the Snf1-dependent histone hyperacetylation that promotes nucleosome mobility and Adr1 binding because they have been shown to interact with both histone acetylases and deacetylases (Lottersberger *et al.*, 2007; Braun *et al.*, 2013).

Snf1 homologs in other organisms

Yeast Snf1 has conserved homologs in both mammals and plants, the AMPK and Snf-related kinase 1 (SnRK1), respectively (reviewed by Polge & Thomas, 2007). As in yeast, they play an important role in controlling energy homeostasis during nutrient stress conditions. These kinases are not only functionally but also structurally conserved, consisting of heterotrimeric complexes similar to SNF1 in yeast. The mammalian AMPK complex is emerging as a major signal transduction hub, with an important role in the nutritional regulation of gene expression and whole-body energy metabolism. In humans, AMPK regulates lipid and glucose metabolism and has been implicated in metabolic disorders, such as diabetes, obesity, cardiovascular disease, and cancer (Shackelford & Shaw, 2009).

The *GAL* gene switch and the bifunctional role of Gal1 and its paralog Gal3

Regulation of galactose metabolism genes

In order to be metabolized, galactose must be converted into the glycolytic intermediate, glucose-6-phosphate (Fig. 2). This is carried out by the highly conserved Leloir pathway (Kew & Douglas, 1976). In *S. cerevisiae,* most of the genes encoding components of this pathway and the galactose transporter (*GAL1*, *GAL10*, *GAL7,* and *GAL2*) are clustered in the *GAL* regulon (reviewed by Rubio-Texeira, 2005; Campbell *et al.*, 2008; Sellick *et al.*, 2008). Expression of the *GAL* genes is tightly regulated by the type of carbon source. In the presence of glucose, they are strongly repressed, while in nonfermentable carbon sources, they show a basal level of expression and are poised for induction by galactose. In the absence of glucose, galactose promotes one of the strongest inductions known for eukaryotic genes, close to a 1000-fold increase in expression for some of the structural genes (Lohr & Lopez, 1995). The dramatic transition from the highly repressed to the fully induced state, generally coined the 'galactose switch', has been the subject of intensive research in the past two decades, constituting one of the best understood paradigms for eukaryotic transcriptional regulation.

Regulatory proteins of *GAL* gene expression

Switches in *GAL* expression in response to environmental cues are mainly governed through the interplay of three major regulatory proteins, two activators, and one repressor, encoded by *GAL3*, *GAL4*, and *GAL80* (Bhat & Murthy, 2001; Bhat & Iyer, 2009). Induction of the *GAL* genes by galactose is dependent in first instance on the transcriptional activator Gal4 that operates through an upstream activating sequence (UAS_{GAL}) present in their promoters. The number of UAS_{GAL} sites and their relative affinity for Gal4 vary among the *GAL* genes leading to differential levels of induction (Lohr & Lopez, 1995). In the presence of galactose, Gal4, bound as homodimer to its UAS_{GAL} sites, recruits the transcriptional machinery composed of at least three protein complexes known as SAGA, TFIID, and mediator (reviewed by Traven *et al.*, 2006). This action is impaired by the interaction of Gal4 with its repressor, Gal80. The more UAS_{GAL} sites present in the promoter, the tighter Gal80 dimers are able to complex with Gal4 dimers, impairing transcription to increasing extents. *GAL80* expression is also dependent on Gal4, so that the activator induces an autogenous feedback inhibition loop in response to galactose. Although *GAL4* expression is not activated by galactose, it is like the other *GAL* genes responsive to glucose repression via activation of the Snf1-controlled, Mig1/2 repressors. Relief from glucose repression is mediated in part by Snf1-dependent phosphorylation leading to Mig1/2 nuclear export and subsequent degradation by the proteasome (Trumbly, 1992; Lim *et al.*, 2011). A third player within the *GAL* regulon, Gal3, relieves Gal4 from Gal80 inhibition. Interaction of Gal3 with Gal80 releases Gal4 so that it can induce the *GAL* genes. How exactly this happens is still a matter of controversy. Structural analysis of Gal80, in complex with either Gal3

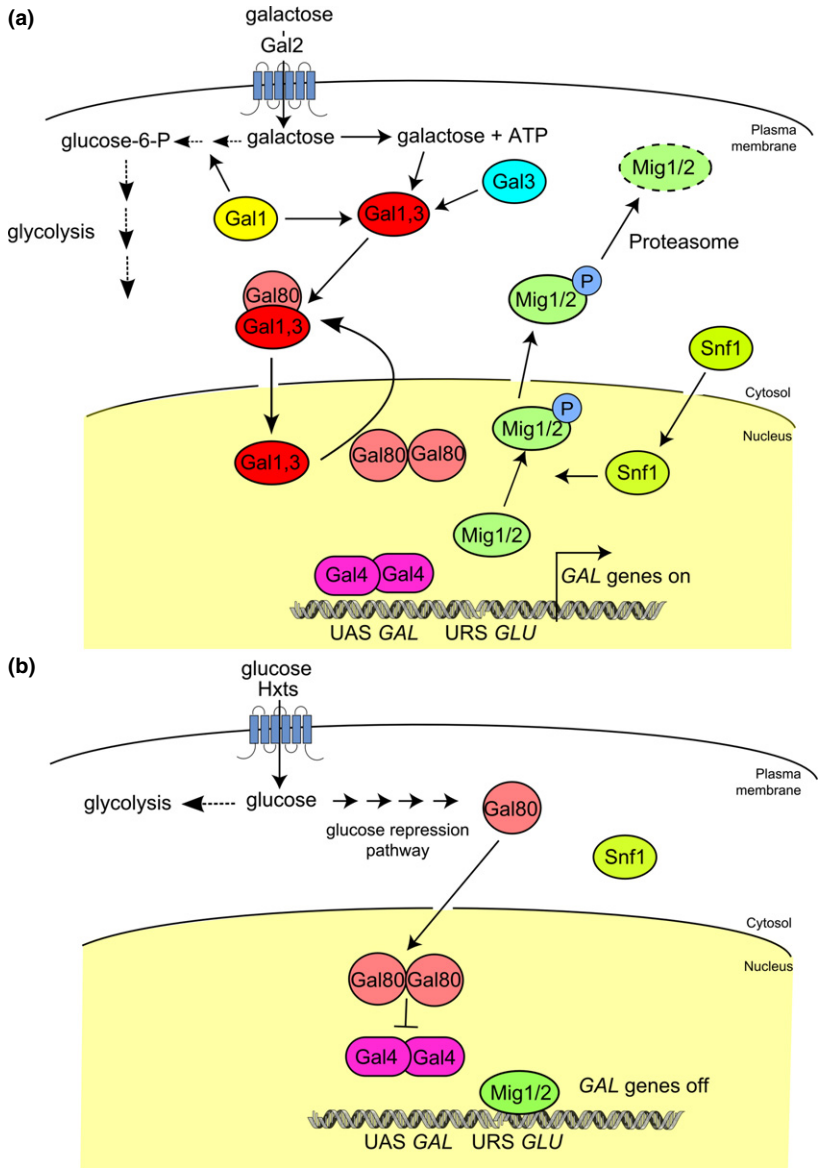


Fig. 2. Induction of the *GAL* regulon for galactose utilization. (a) In the absence of glucose and presence of galactose, the *GAL* genes are induced. Galactose enters the cells via its transporter, Gal2, which is present at a low basal level under this condition. Trace amounts of the intracellular sensor protein, Gal3, bind galactose and ATP in the cytosol, which promotes binding of Gal3 to the *GAL*-specific transcriptional repressor, Gal80. This prevents the accumulation of Gal80 in the nucleus, which reduces its inhibition of the transcriptional activator Gal4. A tripartite interaction between Gal3, Gal80, and Gal4 may also occur in the nucleus to facilitate Gal4 release from Gal-80-mediated inhibition. In later stages of galactose induction, the bifunctional protein Gal1 replaces Gal3 in its signaling role. The Snf1 protein kinase complex, which is active under this condition, phosphorylates the Mig1/2 repressor proteins, which causes their dissociation from upstream repressor sequences (URS_{GLU}) and subsequent export to the cytosol. Gal4 activation facilitates the association of chromatin remodeling complexes and the basal transcriptional machinery leading to induction of the *GAL* genes. (b) In the presence of glucose (irrespective of the absence or presence of galactose), expression of the *GAL* genes is repressed. Glucose enters the cells via the multiple hexose transporters (HXT). Once the levels of intracellular glucose increase, Gal80 is relieved from inhibition by Gal1,3 and enters the nucleus where it inhibits Gal4. Glucose also causes inactivation of the Snf1 protein kinase, which favors Mig1/2 nuclear import and thus downregulation of the *GAL* genes by these transcriptional repressors. Metabolic reactions are depicted by dotted arrows; regulatory and signaling interactions by full arrows.

or Gal4, has revealed the importance of allosteric interactions with small molecules for the regulation of these complexes. The Gal3–Gal80 interaction is only possible in the presence of galactose and ATP, both bound to Gal3 (Lavy *et al.*, 2012). NAD^+ facilitates Gal80 binding to Gal4, while $NADP^+$ destabilizes this interaction and

appears to be the initial trigger for Gal4 activation (Kumar *et al.*, 2008; Li *et al.*, 2010).

The Gal3 and Gal1 paralogs

One of the most peculiar characteristics of the regulation of *GAL* genes lies in the origin of *GAL3*. Gal3 shows 92% similarity and 72% sequence identity to the galactokinase Gal1, but lacks galactokinase activity (Thoden *et al.*, 2005; Diep *et al.*, 2006). Unlike Gal3, Gal1 is not sufficiently expressed in the absence of galactose to serve as an inducer (Tsuyumu & Adams, 1974; Broach, 1979; Bhat *et al.*, 1990; Hittinger & Carroll, 2007). However, when Gal1 is expressed from a surrogate promoter, it can substitute for Gal3 in activation of the *GAL* genes, even when its galactokinase activity is abolished (Bhat & Hopper, 1992). Insertion of serine and alanine (Gal3-SA) within one of the Gal3 galactokinase homology motifs suffices to restore its galactokinase activity (Platt *et al.*, 2000). Conversely, a D62A substitution in Gal1 abolishes its ability to phosphorylate galactose, while the corresponding amino acid substitution in wild-type Gal3 impairs its capacity to induce *GAL* gene expression in response to galactose (Sellick & Reece, 2006). *GAL1* and *GAL3* are paralogs that arose from a single bifunctional ancestral gene, still present in the closely related yeast, *Kluyveromyces lactis* (Hittinger & Carroll, 2007; Hsu *et al.*, 2012). *GAL1* and *GAL3* have not only undergone specialization in their coding sequence but also in their promoters: While *GAL1* has maintained four Gal4-binding sites, only one remains in *GAL3* (Hittinger & Carroll, 2007). This not only causes lower basal and induced levels of *GAL3* but also lowers repressibility compared to *GAL1*. Duplication of the ancestral bifunctional gene may have allowed the resolution of an adaptive conflict between the transcriptional regulation of the two gene functions: Although possessing more Gal4-binding sites leading to higher galactose-driven induction of the galactokinase would be desirable, the presence of a single bifunctional gene in the ecological niche of *S. cerevisiae*, where galactose is present more rarely than glucose, would face the disadvantage of being more tightly repressed not allowing the highly sensitive early adaptation to galactose mediated by Gal3. On the other hand, high expression of the galactokinase before galactose levels are high enough to cause full induction could be deleterious because of the accumulation of galactose-1-phosphate to toxic levels. Changes in promoter structure have thus allowed constitutively low levels of expression of *GAL3* for better galactose sensing and a tight coordination between *GAL1* expression and the galactose concentration to prevent metabolic toxicity (Conant & Wolfe, 2008). Interestingly, recent work has shown that the Gal3 protein may be involved solely in the short-term response to galactose, being replaced by Gal1 in the complex with Gal4 and Gal80 for continued expression of the *GAL* genes (Abramczyk *et al.*, 2012). Similar observations on different mechanisms being involved in short-term and long-term adaptation have also been made for other nutrient responses.

Other aspects of GAL regulation

Cells that have been previously exposed to galactose and then switched to glucose can adapt more rapidly when returned to galactose (Kundu *et al.*, 2007). This phenomenon seems to occur at the epigenetic level and was initially thought to result from nucleosome repositioning. More recently, it has been shown that, although nucleosomes are indeed repositioned upon galactose activation, this 'memory effect' rather results from residual levels of Gal1 protein, which remain significant for up to seven generations during the growth in glucose medium. The 'memory effect' ensures a faster re-activation of the *GAL* regulon upon switch to galactose medium due to the residual Gal3-like sensor activity of Gal1 still present in the cells (Zacharioudakis *et al.*, 2007).

Transcriptional complexes such as the mediator are able to orchestrate their own recruitment to the *GAL* promoter and act upstream of their recruiter, Gal4, in response to Snf1-conveyed signals, by controlling the E3 ligase SCF^{Mdm30}-mediated ubiquitination and subsequent proteasomal degradation of Gal80 (Ang *et al.*, 2012). Gal4 monoubiquitination, initially thought to destabilize this transcription factor, seems to serve instead as protective mechanism against the promoter-stripping proteasome ATPases (Archer *et al.*, 2008).

Galactose regulation is also connected to other regulatory pathways. Adaptive evolution for faster growth on galactose resulted in mutants in the Ras2 protein, showing to different extents a higher specific growth rate on galactose and a higher specific galactose uptake rate (Hong *et al.*, 2011; Hong & Nielsen, 2012). Subsequent work showed that the Ras2 mutations also caused a reduction in glucose utilization, causing a tradeoff for the improved galactose utilization (Hong & Nielsen, 2013).

A GPCR system and Ras in glucose-induced cAMP signaling

Two glucose-sensing systems for cAMP signaling

In budding yeast, an important part of extracellular glucose sensing and signaling is mediated by the cAMP-PKA pathway, which in response to the presence of glucose represses stress tolerance and adaptation mechanisms and stimulates fermentation and cell proliferation (reviewed

by Thevelein & de Winde, 1999; Santangelo, 2006; Smets *et al.*, 2010; Fig. 3). It is important to emphasize that the activity of the PKA pathway is also strongly influenced by all other nutrients essential for yeast growth (see further). A dual glucose-sensing system is involved in the activation of the cAMP-PKA pathway: On the one hand, extracellular glucose sensing occurs through the GPCR system composed of Gpr1 and its associated Gα protein, Gpa2, and on the other hand, an intracellular system dependent on glucose uptake and hexokinase-mediated phosphorylation that activates in some unknown way the Ras proteins (Rolland *et al.*, 2000). Hence, yeast adenylate cyclase (AC) is controlled by two G proteins that each mediate one branch of a glucose-sensing pathway.

The glucose-sensing GPCR system

The GPCR system was discovered by the merging of different research lines. First, the Gα protein Gpa2 was discovered based on the sequence similarity with its mammalian counterpart, the Gα subunit of the heterotrimeric G protein. Overexpression of *GPA2* enhanced cAMP levels, but a *gpa2*Δ strain still showed a glucose-induced cAMP signal, making its precise function unclear (Nakafuku *et al.*, 1988; Papasavvas *et al.*, 1992). Subsequent work showed that Gpa2 was involved as signal transmitter in glucose-induced cAMP signaling, while the Ras proteins mediated the activation of cAMP synthesis by intracellular acidification, which is a potent stimulator of intracellular cAMP levels in yeast (Colombo *et al.*, 1998). The G-protein-coupled receptor Gpr1 was discovered in a two-hybrid screen with Gpa2 as a probe (Xue *et al.*, 1998) and in a screen for mutants showing delayed glucose-induced loss of heat tolerance (Kraakman *et al.*, 1999). An issue that has for a long time confused elucidation of the mechanisms underlying glucose-induced cAMP signaling is that the GPCR system that senses extracellular glucose is unable to activate AC if the latter is not made responsive by the activation of the Ras proteins (Rolland *et al.*, 2000). Because the activation of the Ras proteins requires glucose transport and phosphorylation (Colombo *et al.*, 2004), the sensing of extracellular

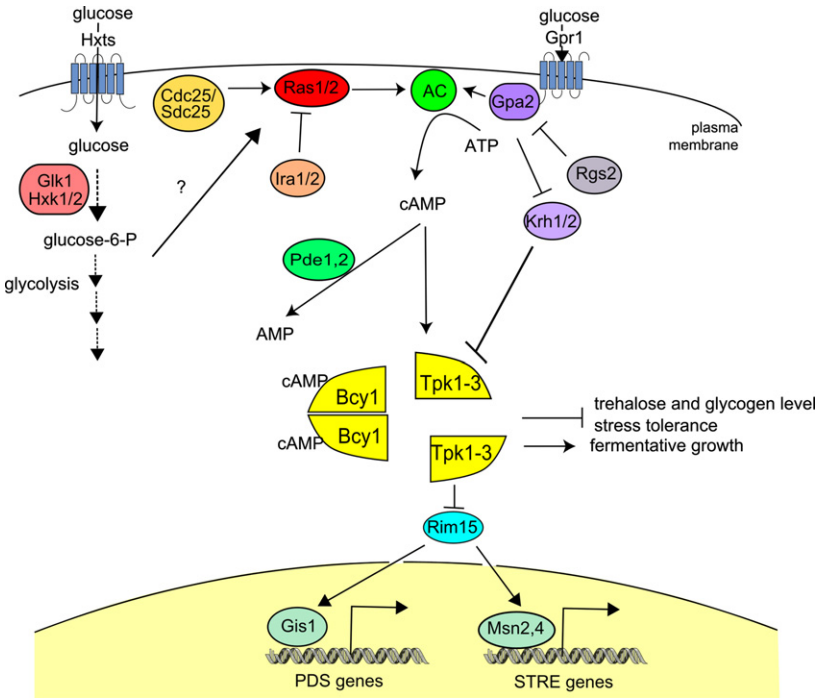


Fig. 3. Glucose activation of the cAMP-PKA pathway. AC is activated by glucose through two different G-protein-coupled systems. The Gpr1-Gpa2-Rgs2 GPCR system senses extracellular glucose, while the Cdc25,Sdc25-Ras1,2-Ira1,Ira2 system senses intracellular glucose through glucose catabolism in glycolysis in a way that is not yet understood. The glucose-sensing GPCR, Gpr1, and the Cdc25,Sdc25 proteins stimulate guanine nucleotide exchange on Gpa2 and Ras1,2, respectively. Rgs2 and Ira1,2 act as GAPs on Gpa2 and Ras1,2, respectively. cAMP binds to the Bcy1 regulatory subunits of PKA causing dissociation and activation of the catalytic subunits, Tpk1-3. The Krh1,2 kelch repeat proteins mediate a cAMP-independent pathway triggered by the glucose-sensing GPCR system for direct activation of PKA, by lowering the affinity between catalytic and regulatory subunits. Metabolic reactions are depicted by dotted arrows; regulatory and signaling interactions by full arrows.

glucose by the GPCR system is actually dependent on intracellular conversion of glucose in metabolism. Requirement of uptake and metabolism of a GPCR ligand for its extracellular sensing by the GPCR system is highly unusual. Glucose-induced cAMP signaling was shown to depend on glucose phosphorylation a long time ago, and this seemed to contradict for a long time the involvement of a glucose receptor system (Beullens *et al.*, 1988). The mechanism by which glucose catabolism activates the Ras proteins is not understood. One possibility is that one or more intermediates of glycolysis function as allosteric activators of the Ras proteins, acting directly or through one of the Ras regulatory proteins.

The Ras protein system

As opposed to the GPCR part of the network, deletion of components of the Ras part is lethal, as is deletion of AC or PKA. All Ras proteins are members of a eukaryotic subfamily of small GTPases involved mainly in cellular signal transduction. In yeast, Ras activates AC, which is encoded by the *CYR1* gene (Kataoka *et al.*, 1985). Double deletion of *RAS1* and *RAS2* is lethal, and just like the deletion of AC, this lethality can be rescued by mutations in the Bcy1 regulatory subunit of PKA, which make PKA activity independent of cAMP (reviewed by Broach & Deschenes, 1990). Mammalian Ras can also suppress the lethality caused by the loss of yeast Ras (reviewed by Tamanoi, 2011). The activity of Ras proteins depends on GDP/GTP exchange by a guanine nucleotide-exchange factor (GEF) and regulation of its intrinsic GTPase activity by a GAP (Broach & Deschenes, 1990). In the case of yeast Ras, Cdc25 and its homolog Sdc25 act as GEFs, while Ira1 and 2 function as GAPs (Broek *et al.*, 1987; Tanaka *et al.*, 1990; Boy-Marcotte *et al.*, 1996). Ras proteins contain C-terminal features that determine their tethering to membranes (Kato *et al.*, 1992). Recent localization studies have shown that Ras, Ira, and Cdc25 as well as some of their downstream effectors, for example the AC, Cyr1, are not only localized at the plasma membrane but also associated with internal membranes, in compartments such as the ER, mitochondria, and nucleus (Belotti *et al.*, 2011, 2012; Dong & Bai, 2011; Broggi *et al.*, 2013). The relative distribution between plasma membrane and internal membranes is dependent on the carbon source and the activity and localization of other components of the cAMP-PKA pathway, for example Gpr1-Gpa2 or PKA catalytic subunits (see below). Glycolytic enzymes such as Hxk2 also seem to play an important role in the localization of active Ras (Broggi *et al.*, 2013). Compartmentalization thus provides an additional layer of regulation to the Ras signaling system.

Protein kinase A

PKA is a heterotetrameric protein comprising two catalytic and two regulatory subunits, the former encoded by the *TPK1-3* genes and the latter by the *BCY1* gene (Toda *et al.*, 1987a, b). Binding of cAMP to the regulatory subunits causes their dissociation from the catalytic subunits, resulting in the activation of PKA. cAMP is degraded to AMP by the low- and high-affinity phosphodiesterases, Pde1 and Pde2, respectively (Sass *et al.*, 1986; Nikawa *et al.*, 1987). PKA establishes a negative feedback loop by regulating the activity of Pde proteins. For example, Pde1 is activated by PKA-dependent phosphorylation (Ma *et al.*, 1999). PKA also regulates the localization and protein concentration of Pde2 (Hu *et al.*, 2010). Other targets for the PKA feedback inhibition mechanism have been proposed, but up to now it has not been possible to mimic the very high cAMP levels observed in yeast strains with attenuated PKA activity by the inactivation of one or more phosphorylation sites in such putative target proteins (reviewed by Vandamme *et al.*, 2012).

Actin remodeling and Ras function

Production of cAMP is also influenced by binding of GTP-bound Ras to the adenylyl cyclase-associated (Srv2p/CAP) proteins (Gerst *et al.*, 1991). Srv2p/CAP exhibits both adenylyl cyclase-activating and actin-binding and regulatory functions. Actin remodeling events are needed to downregulate Ras2, in order to prevent constitutive activation of cAMP production in stationary phase, which otherwise results in improper activation of PKA leading to elevated levels of ROS and apoptosis (Gourlay & Ayscough, 2006). The inappropriate activation of one of the yeast PKA catalytic subunits, Tpk3, is sufficient to commit cells to an apoptotic death through transcriptional changes that promote the production of dysfunctional, ROS-producing mitochondria (Leadsham & Gourlay, 2010). Loss of the Whi2 protein causes actin-mediated apoptosis as a result of inappropriate Ras-cAMP-PKA activity in stationary-phase cells, and this is due to deficient targeting of Ras2 to the vacuole for proteolysis (Leadsham *et al.*, 2009). Hxk2 also plays a role in this process because its loss causes mislocalization of Ras to mitochondria again resulting in apoptosis (Amigoni *et al.*, 2013).

Gpa2 and the Krh AC bypass pathway

As mentioned above, the Gα protein, Gpa2, stimulates AC in response to glucose stimulation of the Gpr1 receptor (Colombo *et al.*, 1998; Kraakman *et al.*, 1999).

The intrinsic GTPase activity of Gpa2 is stimulated by the Rgs2 protein, which thus acts as an inhibitor (Versele *et al.*, 1999). An intriguing finding in the yeast cAMP-PKA pathway was that Gpa2 functions without a genuine β- and γ-subunit (Harashima & Heitman, 2002; Batlle *et al.*, 2003; Hoffman, 2005; Peeters *et al.*, 2006). Several proposals have been made for an alternative subunit, such as Asc1, but a clear role for this protein still needs to be defined (Zeller *et al.*, 2007). A controversial proposal was made for the kelch repeat proteins, Krh1 and Krh2, which were also called Gpb2 and Gpb1, referring to a possible role as Gβ-subunit for Gpa2 (Harashima & Heitman, 2002). Later work, however, showed that these proteins function in an AC bypass pathway, allowing direct activation of PKA by activated Gpa2 (Lu & Hirsch, 2005; Peeters *et al.*, 2006). The kelch repeat proteins directly bind to the catalytic subunits of PKA and thereby stimulate the association of the catalytic and regulatory subunits of PKA, lowering PKA activity. Their inactivation thus reduces the amount of cAMP required to activate PKA, creating a mechanism for the activation of PKA without change in the cAMP level or for synergistic stimulation of PKA activation after an increase in the cAMP concentration. Further work has shown that Krh1,2 affect both the abundance and phosphorylation state of Bcy1, such that its levels increase upon glucose limitation in a Krh-dependent manner. PKA establishes a negative feedback loop by phosphorylation of Bcy1 in Ser145, which targets Bcy1 for degradation unless it is protected by Krh proteins (Budhwar *et al.*, 2010, 2011).

The Sch9 protein kinase

Recent research has reported that the yeast ortholog of mammalian PKB/Akt and TOCR1 effector, Sch9 protein kinase, inhibits PKA activity by regulating the localization and phosphorylation of Bcy1 (Zhang *et al.*, 2011a). Sch9 seems to have both PKA-related and unrelated roles not only in glucose signaling but also as an activator of ribosome biogenesis, translation initiation, cell size control, and division in response to general nutrient availability (see further; Roosen *et al.*, 2005; Smets *et al.*, 2008). Activity of PKA and Sch9 is also modulated by additional kinases. For example, it has been shown that they are phosphorylated by Pkh1, 2, and 3, the yeast orthologs of mammalian 3-phosphoinositide-dependent protein kinase 1 (PDK1; Casamayor *et al.*, 1999). Phosphorylation of Sch9 by these kinases appears to be required for its activity, and mutagenesis of the PDK1 site in the catalytic Tpk1 subunit interferes with binding to the regulatory Bcy1 subunit and thus with cAMP dependency (Voordeckers *et al.*, 2011; Haesendonckx *et al.*, 2012). This regulation establishes a link between sphingolipid signaling pathways and PKA.

PKA target systems

PKA affects a wide variety of targets in yeast cells. In general, it acts positively on properties that are associated with rapid fermentative growth (e.g. rate of fermentation and growth) and acts negatively on properties associated with slow, respirative growth or stationary phase (e.g. accumulation of carbohydrate stores, stress tolerance, and other stationary-phase characteristics; Thevelein & de Winde, 1999). To achieve this outcome, PKA directly phosphorylates cytosolic enzymes, for example trehalase (Schepers *et al.*, 2012), phosphofructokinase 2 (Dihazi *et al.*, 2003), pyruvate kinase (Portela *et al.*, 2002), and fructose-1,6-bisphosphatase (Rittenhouse *et al.*, 1987), and regulates gene expression at the transcriptional level. An important intermediate component in PKA-mediated regulation of gene expression is the Rim15 protein kinase (Cameroni *et al.*, 2004). PKA phosphorylates and inhibits Rim15, which is itself a positive regulator of three major transcription factors that activate postdiauxic growth and stress-response gene expression. Shift from growth on glucose to growth on ethanol following the diauxic shift induces a set of genes, containing a postdiauxic shift element in their promoter, through the transcription factor Gis1, which is stimulated by Rim15 (Pedruzzi *et al.*, 2000). A wide array of stress-response and tolerance genes, containing an STRE element in their promoter, which are expressed during respirative growth and in stationary phase, are induced by the Msn2,4 transcription factors (Martinez-Pastor *et al.*, 1996). These are also stimulated by Rim15. PKA also directly regulates Msn2,4 by controlling their nuclear localization (Gorner *et al.*, 1998).

Intracellular nitrogen sensing for activation of the TOR pathway

The TORC1 and TORC2 complexes

The structurally and functionally conserved TOR pathway has for a long time been suggested to play a role in the regulation of cell growth and many related properties by nutrient availability (Fig. 4). However, no clear mechanisms have been identified by which the TOR pathway would detect extracellular nutrients and the more recent work suggests that the TOR proteins rather sense intracellular nitrogen availability, in particular mobilization of nitrogen reserves from the vacuole/lysosome. From yeast to humans, inactivation of TOR affects multiple processes involved in cell metabolism, growth, and longevity (most

recently reviewed by Kim & Guan, 2011; Loewith & Hall, 2011). Quantitative phosphoproteomics revealed that mammalian TOR controls the phosphorylation of 335 proteins (Robitaille *et al.*, 2013). *Saccharomyces cerevisiae* differs from almost all other eukaryotes by possessing two *TOR* genes instead of one (Helliwell *et al.*, 1994). Tor1 and Tor2 are 282 kDa in size, 67% identical and also highly similar in sequence to the mammalian TOR protein (37%). They are also the founding members of the family of phosphatidylinositol protein kinases (or phosphatidyl inositol 3′ kinase-related kinases, PIKK; Keith & Schreiber, 1995). Although they contain a catalytic domain resembling that of lipid kinases (PI3K and PI4K), no PIKK has shown lipid kinase activity. All Tor proteins have the same essential features: From N- to C-terminus, they contain the HEAT (Huntington, elongation factor 3, regulatory subunit A of PP2A, TOR1) repeats, the FAT (FRAP, ATM, TTRAP) domain, the FRB (FKBP12-rapamycin-binding) domain, the kinase domain, and the FATC (FAT C-terminus) domain (Schmelzle & Hall, 2000). The HEAT repeats are the binding region for subunits of the TOR complexes (Wullschleger *et al.*, 2005). The central and C-terminal FAT domains are conserved in PIKK (Dames *et al.*, 2005). The FRB domain is responsible for binding to FKBP (FK506 binding protein)-rapamycin (Loewith & Hall, 2011). Tor proteins act in complex with different protein subsets, which provides functional versatility (Helliwell *et al.*, 1994). TOR complex 1 (TORC1) consists of either Tor1 or Tor2, associated with Kog1, Lst8, and Tco89 (Loewith *et al.*, 2002; Wedaman *et al.*, 2003; Reinke *et al.*, 2004). TORC1 is rapamycin sensitive, and its inactivation affects protein synthesis, ribosome biogenesis, transcription, cell cycle, meiosis, nutrient uptake, and autophagy. TORC2 complex contains exclusively Tor2, associated with the subunits Avo1-3, Bit61, and Lst8 (Loewith *et al.*, 2002; Wedaman *et al.*, 2003; Reinke *et al.*, 2004). TORC2 is rapamycin insensitive and affects actin cytoskeleton organization, endocytosis, lipid synthesis, and cell survival. The mechanisms by which the immunosuppressant lipid macrolide rapamycin inhibits TORC1, but not TORC2, are now starting to be understood. Rapamycin hijacks the cytosolic peptidyl-prolyl cis-trans isomerase, also known as immunophilin, FKBP12, or its yeast homolog, Fpr1 (FK506-binding protein 12; Schreiber, 1991). This Fpr1 association with rapamycin causes Fpr1 to interact with TOR resulting in its inhibition. But FKBP-rapamycin can only bind TORC1, apparently because in TORC2 the FRB domain, to which it binds, is protected by Avo1 (Loewith *et al.*, 2002; Wullschleger *et al.*, 2005). This lack of interaction between Fpr1-rapamycin and TORC2 accounts for the previously observed insensitivity of TORC2 to rapamycin.

TORC1 functions at the lysosome/vacuole membrane

In the last few years, research in both mammalian cells and yeast has contributed to a better understanding of the mechanisms that lead to the activation of TORC1. Increasing evidence points toward the lysosome/vacuole limiting membrane as the main localization for the activation of TORC1 signaling (Kunz *et al.*, 2000; Urban *et al.*, 2007; Sturgill *et al.*, 2008; Binda *et al.*, 2009). This localization and its direct regulation by interaction with other lysosome/vacuole membrane-localized complexes has led to an emergent model by which TORC1 would respond to changes in intracellular amino acid availability determined by v-ATPase-dependent export of vacuolar amino acids to the cytosol (Zoncu *et al.*, 2011). Such changes would be communicated to TORC1 by specific lysosome/vacuole protein complexes.

In yeast, activity of TORC1 is regulated at the vacuolar membrane through the interplay of four proteins: Ego1, Ego3, Gtr1, and Gtr2, which together form the EGO complex, EGOC (Dubouloz *et al.*, 2005). Gtr1 and Gtr2 are Ras-family GTPases represented in metazoans by the orthologous Rag GTPases, Rag A-D (Sancak *et al.*, 2008; Efeyan *et al.*, 2012). Ego1 and 3 are functional homologs of the Ragulator complex in vertebrates (Kogan *et al.*, 2010; Sancak *et al.*, 2010). Ego1 is N-terminally myristoylated and palmitoylated, tethering EGOC to the vacuolar membrane (Dubouloz *et al.*, 2005; Binda *et al.*, 2009; Zhang *et al.*, 2012). The function of Ego3 in the complex is still unclear but it is known to form homodimers that, like the C-terminal domains of Gtr1 and Gtr2, are structurally similar to members of the Roadblock/LC7 superfamily of proteins, a conserved family of dynein-associated proteins (Kogan *et al.*, 2010; Zhang *et al.*, 2012). In yeast, GTP-bound Gtr1 in heterodimeric complex with GDP-bound Gtr2 stimulates TORC1 in response to amino acids, similar to what is observed in vertebrates (Kim *et al.*, 2008; Sancak *et al.*, 2008). In spite of their homology, differences have been observed between mammalian and yeast cells in the mechanism of action of Rag/Gtr. In mammalian cells, for example, Rag GTPases do not directly activate TORC1, but trigger TORC1 relocalization from the cytoplasm to the lysosomal limiting membrane for further activation via the GTPase, Rheb (Sancak *et al.*, 2008, 2010; Efeyan *et al.*, 2012). In contrast, *S. cerevisiae* TORC1 seems to remain associated with the vacuolar limiting membrane regardless of the amino acid levels (Binda *et al.*, 2009). The EGO complex can either regulate TORC1 positively or negatively via physical interaction with TORC1 subunits. Gtr1, particularly when bound to GTP, physically interacts with Tco89 and

Fig. 4. Role of TORC1 in the NCR and RTG pathways. (a) Preferred nitrogen sources for yeast are these that can easily be converted into glutamate (Glu) and glutamine (Gln), major precursors for amino acid biosynthesis. Their presence in the medium results in increased levels of intracellular glutamate and glutamine. This causes repression of genes involved in the metabolism of less preferred nitrogen sources, nitrogen catabolite repression (NCR). This transcriptional repression is achieved mainly by hyperphosphorylation of Ure2 and Gln3, causing their association and preventing nuclear localization of the transcription factor Gln3. The Gat1 transcription factor is regulated in a similar way. High glutamine levels as well as other amino acids stimulate the vacuolar/endosome membrane-located, EGO complex. This complex is composed of the two Ras-like GTPases, Gtr1, Gtr2, and the Ragulator-like, Ego3 and Ego1. Activation of EGO is stimulated by GTP-bound Gtr1 and GDP-bound Gtr2. GTP loading of Gtr1 is stimulated by the guanine nucleotide-exchange factor (GEF) activities of Vam6/Vps39 and the L-Leu-tRNA synthetase. SEACAT prevents GAP activity of SEACIT on Gtr1. Activated EGO stimulates in turn the vacuolar membrane-associated fraction of the TORC1 complex. TORC1 also phosphorylates Sch9 and Tap42, the latter leading to the inhibition of several protein phosphatases (PPA2, Sit4, etc.). As a result, the protein phosphatases can no longer dephosphorylate the Ure2 complexes with Gln3 and Gat1, reinforcing their hyperphosphorylation. Synthesis of glutamine and glutamate occurring via anaplerotic reactions shared with the TCA cycle is also downregulated. In this case, TORC1 phosphorylated Mks1 bound to Bmh1,2 proteins prevents nuclear localization of the RTG transcription factors, Rtg1 and Rtg3. TORC1-dependent phosphorylation of Npr1 causes Npr1 inactivation, which in a yet not completely understood manner increases plasma membrane stabilization of specific AAPs like Tat2, while stimulating endocytosis of the alternative general AAP, Gap1. (b) Under poor nitrogen conditions, intracellular glutamate and glutamine levels drop. GAPs like the SEACIT increase GDP loading of Gtr1, which inactivates the EGO complex. An inactive EGO complex can no longer stimulate TORC1, which leads to release into the cytosol and activation of Tap42–protein phosphatase complexes. They reduce phosphorylation of Ure2, Gln3, and Gat1 causing nuclear localization of the latter two and subsequent stimulation of NCR gene expression. The phosphatases also dephosphorylate Mks1, which then complexes with Rtg2. This allows Rtg1,3 nuclear localization resulting in stimulation of the expression of *RTG* genes, sustaining amino acid biosynthesis through the synthesis of glutamate and glutamine. The phosphatases also dephosphorylate Npr1, which then phosphorylates the Rsp5-associated arrestins Bul1 and Bul2 provoking their association with Bmh1/2 proteins, which in turn leads to the stabilization of Gap1 at the plasma membrane. Metabolic reactions are depicted by dotted arrows; regulatory and signaling interactions by full arrows.

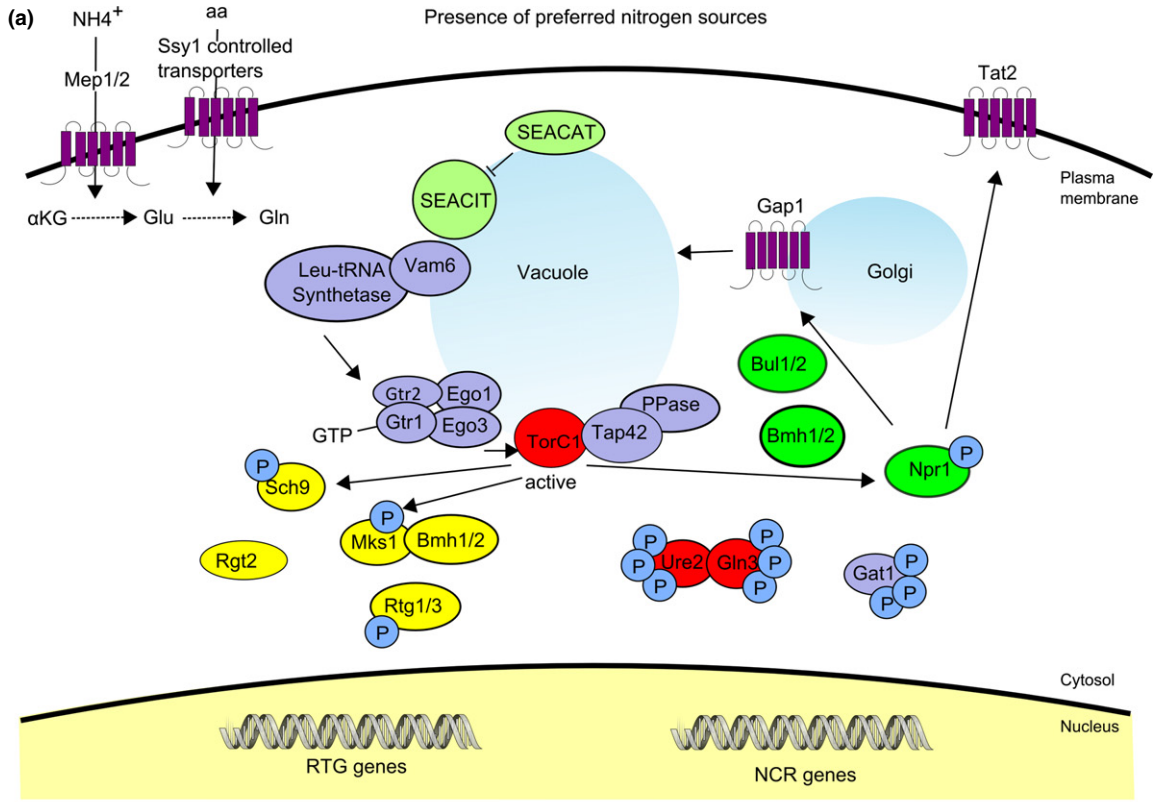
Kog1, and this interaction is reduced by leucine starvation (Binda *et al.*, 2009).

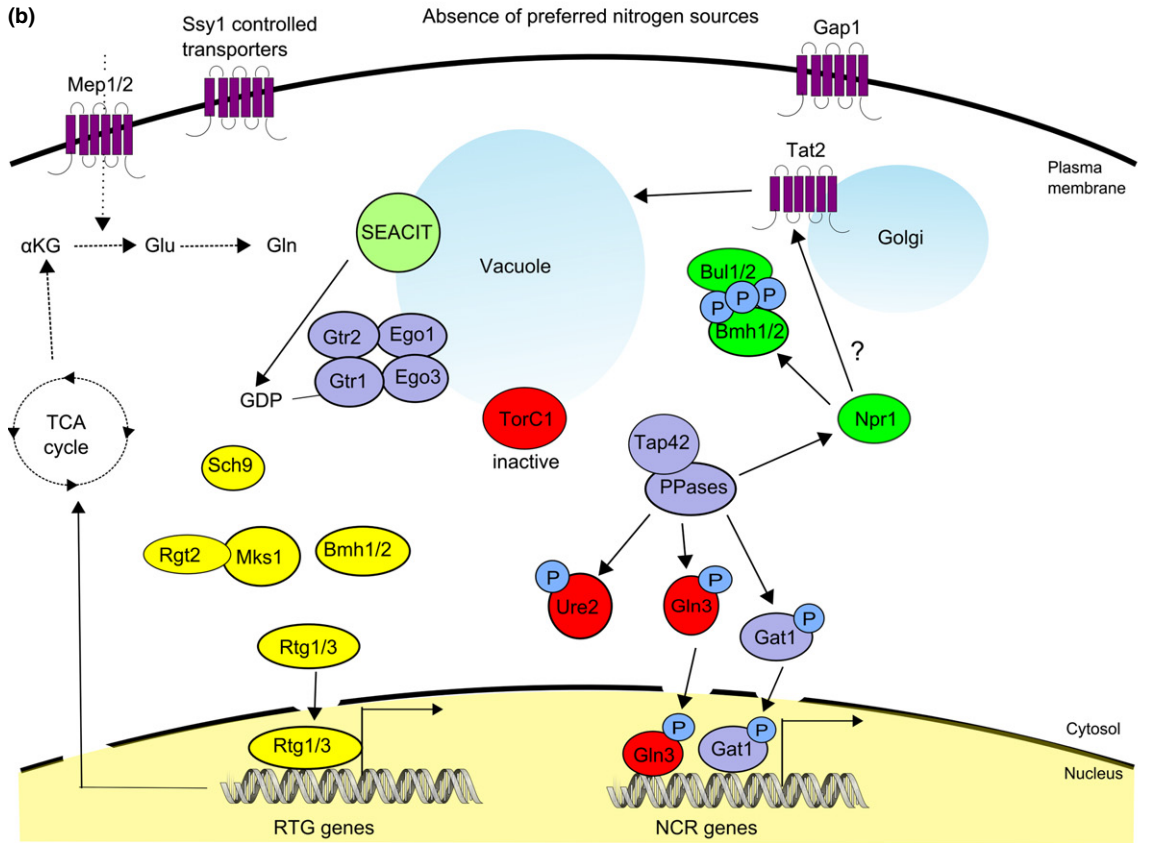
Amino acid-sensing mechanisms

Several mechanisms have been found by which the EGO complex could sense intracellular amino acids. In metazoans, the change in guanine nucleotide-binding status of the Rag GTPase heterodimer is a key step and is stimulated by all 20 amino acids, among which branched-chain amino acids, like leucine, seem to be the strongest effectors (Kim *et al.*, 2008; Sancak *et al.*, 2008). In yeast, one possible candidate proposed to be sensitive to amino acid changes is Vam6/Vps39, the guanine nucleotide-exchange factor (GEF) of Gtr1, the yeast homolog of the Rag GTPase (Binda *et al.*, 2009). Another recently found mechanism involves the L-leucyl-tRNA synthetase (LRS). Leucine binding to LRS has been proposed to stimulate GAP activity in LRS toward mammalian RagD, whereas in yeast Leu binding to LRS seems to promote instead an LRS-dependent GEF-like activity toward Gtr1 (Bonfils *et al.*, 2012; Han *et al.*, 2012). Most recently, the intervention of another lysosome/vacuolar membrane-associated complex, Iml1–Npr2–Npr3, or SEACIT subcomplex (for SEAC subcomplex Inhibiting TORC1 signaling), has also been unveiled (Panchaud *et al.*, 2013a, b). Leucine deprivation triggers Npr2- and Npr3-dependent, Iml1 transient interaction with Gtr1. Iml1 can in this way exert its GAP function on Gtr1, thus inhibiting TORC1 function. The GAP activity of SEACIT is also conserved, as the orthologous complex in *Drosophila* and human cells, known as GATOR1, also acts as a GAP toward RagA and RagB (Bar-Peled *et al.*, 2013). Also recently, a role of the octameric vacuolar Seh1-associated complex (SEAC) has been identified in inhibiting SEACIT activity. SEAC contains the vacuolar membrane-associated proteins Sea2, Sea3, Sea4, Seh1, and Sec13, which are orthologs of the mammalian and *Drosophila* GATOR2 complex proteins. With the exception of Sec13, GATOR2 proteins have been found to display an inhibitory function of GATOR 1 (Bar-Peled *et al.*, 2013). Similarly in yeast, the SEAC subcomplex has now been shown to play a role in inhibiting SEACIT-mediated TORC1 inhibition (Panchaud *et al.*, 2013a, b). For this reason, this subcomplex has recently been coined under the name of subcomplex SEACAT (or SEAC subcomplex Activating TORC1 signaling). These findings indicate that multiple mechanisms are involved in the sensing of amino acid levels at the lysosome/vacuole membrane in order to coordinate a proper TORC1 response.

The Sch9 effector pathway

TORC1 exerts most of its control via two major effector branches: the mammalian S6 kinase (S6K) ortholog, AGC kinase Sch9, and the Tap42–PPase complex (Loewith & Hall, 2011; Broach, 2012). Through these two proximal effectors, TORC1 modulates distal readouts to positively regulate ribosome biogenesis and translation and to inhibit stress responses that are incompatible with growth





and are typically induced in quiescent cells (De Virgilio, 2012). Yeast TORC1 phosphorylates serine and threonine residues in the C-terminus of Sch9, and this phosphorylation is necessary for Sch9 activity and is used as a fast read-out for TORC1 activation (Urban *et al.*, 2007). Sch9 is rapidly dephosphorylated not only in response to rapamycin but also in response to carbon, nitrogen, phosphate, or specific amino acid starvation (Urban *et al.*, 2007; Binda *et al.*, 2009). As we will comment in other sections, Sch9 functions in coordination with other nutritional sensor pathways, beyond TORC1. One of the main TORC1-regulated roles of Sch9 is to modulate translation and cell size attained before cell division (Jorgensen *et al.*, 2002, 2004). Sch9 also mediates TORC1-dependent control in the regulation of cellular response to stress and entry into the G0 phase, via phosphorylation and subsequent cytosolic sequestration of Rim15, which hampers the activation of stress genes by Gis1, and Msn2/4 (Wanke *et al.*, 2008). Although it is mainly localized at the vacuolar limiting membrane, consistent with its TORC1-related function, Sch9 is also associated with chromatin where it could exert more direct effects in the control of transcription factors like Gis1 (Pascual-Ahuir & Proft, 2007). This role seems to take place independently from TORC1. Other genes subjected to TORC1 negative regulation via Sch9 are involved in mitochondrial function (Smets *et al.*, 2010), sphingolipid homeostasis and signaling (Swinnen *et al.*, 2013, 2014; most recently reviewed by Huang *et al.*, 2013), autophagy and longevity (Sampaio-Marques *et al.*, 2011). Recent work in the latter field has shown that abrogation of the conserved TOR, Ras/cAMP-dependent PKA and/or Sch9 proteins, all negative regulators of autophagy, promotes longevity (Swinnen *et al.*, 2013, 2014). In this respect, both TOR and PKA regulation converge on Sch9 to control autophagy not only at the level of Atg (autophagy-related gene) proteins but also through the regulation of ceramide synthesis (Huang *et al.*, 2013). Inhibiting the synthesis of pro-aging sphingolipids, including ceramides, has a positive effect on longevity by promoting autophagy (Hernandez-Corbacho *et al.*, 2011; Swinnen *et al.*, 2013, 2014).

The Tap42-PPase effector pathway

The PP2A phosphatases consist of heterotrimeric complexes, which contain the scaffolding subunit, Tpd3 (van Zyl *et al.*, 1992), the catalytic subunit, which can be one of three redundant proteins (Pph21, Pph22, or Pph3), and the regulatory subunit, which may be Cdc55 or Rts1 (Healy *et al.*, 1991; Zhao *et al.*, 1997). The PP2A-related protein phosphatase complexes are composed of Sit4 or Ppg1, accompanied by one of four regulatory subunits (Sap4, Sap155, Sap185, and Sap190; Luke *et al.*, 1996). When TORC1 is active, the essential downstream regulatory protein, Tap42, is phosphorylated and associates with phosphatase complexes along with either one of the regulatory proteins Rrd1 or Rrd2 (Di Como & Arndt, 1996; Jiang & Broach, 1999). Tap42-associated complexes reside mainly in membranes, where they interact with TORC1 (Kunz *et al.*, 2000; Aronova *et al.*, 2007). Rapamycin treatment or nitrogen starvation releases these complexes to the cytosol, concomitant with dephosphorylation of Tap42 (Yan *et al.*, 2006). This activates the phosphatases, which in turn allows the expression of nitrogen catabolite repressed genes and stress-response genes (see next section, and Shamji *et al.*, 2000; Duvel *et al.*, 2003). Tip41 cooperates with Tap42 in a similar regulation of the activity of PP2A-related phosphatase complexes containing Sit4 (Jacinto *et al.*, 2001; Santhanam *et al.*, 2004). In addition to causing Sch9 downregulation and release of phosphatases from Tap42/Tap41 inhibition, rapamycin and nutrient starvation activate the cell wall integrity (CWI) pathway, a major stress-response pathway in yeast that controls actin polarization and cell wall expansion in response to various stress conditions, including heat, caffeine, nutrient starvation, cell wall damage, and actin perturbation (Fuchs & Mylonakis, 2009). Rho1, a member of the Rho family of small GTPases, is the core sensor component of the CWI pathway (Ozaki *et al.*, 1996; Philip & Levin, 2001). It has recently been found that, upon activation by stress factors, Rho1 impinges on TORC1 and PP2A activities by directly binding to the TORC1-specific subunit Kog1, which results in downregulation of TORC1 activity and disruption of its membrane association. This binding also triggers the release and activation of the Tap42-2A phosphatase (Yan *et al.*, 2012). PP2A phosphatases are also regulated by methylation. This mode of regulation has been recently found to play a role in the regulation of SEACIT complex activity by PP2A (Sutter *et al.*, 2013). In particular, in response to increasing levels of *S*-adenosylmethionine, which in turn depend on methionine levels, Ppm1 methyltransferase-dependent methylation of PP2A catalytic subunit takes place, which in turn promotes Npr2 dephosphorylation. This inhibition of SEACIT prevents the inactivation of TORC1 and entrance in autophagy upon switching of cells from rich to synthetic medium without nitrogen starvation.

Nitrogen sensing via the NCR and retrograde pathways

Components of the NCR pathway

In the presence of preferred nitrogen sources, that is, nitrogen compounds that can be easily converted into the main amino acid precursors, ammonia, glutamate, and

glutamine, yeast activates the NCR pathway (Fig. 4). It represses the expression of genes involved in the use of alternative, less preferred nitrogen sources, such as proline, urea, allantoin, GABA. The expression of NCR genes results from the interplay of four transcription factors, two activators, Gln3 and Gat1/Nil1, and two repressors, Gzf3/Nil2/Deh2 and Dal80/Uga3, which bind to GATA sequences in the promoters (Cooper, 2002; Magasanik & Kaiser, 2002). With the exception of Gln3, the expression of the transcription factors is also subjected to NCR control, such that without Gln3 no derepression of NCR genes can take place (Mitchell & Magasanik, 1984). Gat1-dependent activation of NCR depends on Gln3 activating Gat1 expression, whereas Gln3-dependent activation can promote transcription in the absence of the other transcription factors (Georis *et al.*, 2009). Both transcription factors have overlapping but also specific effects on NCR genes (Kuruvilla *et al.*, 2001). Aside from the four major regulators, each pathway specific for a particular nitrogen source depends on the expression of additional transcription factors binding to a separate cognate sequence; for example, Aro80 activates the transcription of genes involved in the utilization of aromatic amino acids (Iraqui *et al.*, 1999a, b), and Dal81 positively regulates the genes involved in the metabolism of GABA, urea, arginine, and allantoin (Coornaert *et al.*, 1991). Recent work has shown that Gln3 and Gat1 act in many cases directly *in cis* with these specific transcription factors to coordinate the expression of NCR genes (Cardillo *et al.*, 2012; Lee & Hahn, 2013).

Regulation of Gln3

Because Gln3 is not subjected to NCR control but is constitutively expressed, post-transcriptional mechanisms must take place to prevent the activation of NCR genes in the presence of preferred nitrogen sources. Gln3 has a total of 146 Ser/Thr putative phosphorylation sites, and its phosphorylation state determines its exclusion from the nucleus (Rai *et al.*, 2013). Additionally, under optimal nitrogen conditions, Gln3 is found sequestered in a cytosolic complex with the preprion protein, Ure2 (Blinder *et al.*, 1996). Gln3 and Gat1 phosphorylation/dephosphorylation and shuttling in/out the nucleus seem to respond separately to TORC1 and nitrogen limitation. Rapamycin treatment causes Gat1 and to a lesser extent Gln3 nuclear localization independently of TORC1-controlled phosphatase activity (Georis *et al.*, 2011). In contrast, nitrogen limitation such as growth on proline or addition of the glutamine synthetase inhibitor, methionine sulfoximine (Msx), causes Gln3 but not Gat1 nuclear localization. Changes in Gln3 phosphorylation also do not seem to correlate with the activity of a single pathway upstream of TORC1. For example, Gln3 phosphorylation is reduced upon rapamycin addition but not under nitrogen limitation, while both conditions inhibit TORC1 activity. Inactivation of TORC1 can affect Gln3 phosphorylation status via activation of the Tap42–PP2A and Tap42–Sit4 complexes (Beck & Hall, 1999). Gln3 is peripherally associated with membranes, and this localization may facilitate its control by TORC1-dependent phosphorylation/dephosphorylation events (Puria *et al.*, 2008). Additional evidence for nitrogen limitation acting in parallel rather than as part of the TORC1-mediated control of NCR genes includes the fact that rapamycin cannot activate NCR when Tap42 is inactivated, while Tap42 inactivation has no effect on the response to nitrogen limitation (Duvel *et al.*, 2003). Modifications in Gln3 that abolished its ability to associate with Tor1 and diminished its cytosolic sequestration in nitrogen-rich medium, abolished the Gln3 localization response to rapamycin but did not compromise its response to nitrogen limitation (Rai *et al.*, 2013). Furthermore, changes in Ure2 phosphorylation also seem to control Gln3 cytosolic localization. Ure2 is active as homodimer and the monomers contain an N-terminal (1–93) Gln/Asn-rich preprion domain and a C-terminal (94–354) nitrogen regulatory region required for cytosolic interaction with Gln3 (Feller *et al.*, 2013). Within the C-terminal region, a small stretch (267–298) protrudes from the globular shape of the monomer, forming a flexible domain known as αcap. Alterations in this region abolished rapamycin-dependent nuclear localization of Gln3 and to a lesser extent, Gat1, but had no effect on Gln3 and Gat1 responses to nitrogen limitation (Feller *et al.*, 2013). Ure2 is dephosphorylated in response to rapamycin and mutations in the αcap prevented this dephosphorylation, which was independent from the PP2A and Sit4 phosphatases. In contrast, Ure2 phosphorylation levels were barely modified in response to nitrogen limitation. Hence, nitrogen cues seem to control NCR gene expression via at least two parallel signaling branches, only one of them conveying TORC1-dependent signals. In further support for this observation, a very recent study has analyzed the effect of the five different conditions regularly used to affect the regulation of NCR gene expression: nitrogen starvation, Msx addition, nitrogen limitation, rapamycin addition, and leucine starvation (Tate & Cooper, 2013). This study has demonstrated that Sit4 and PP2A are not required in all cases to trigger Gln3 nuclear localization. Leucine starvation or treatment with leucyl-tRNA synthetase inhibitors also did not elicit increased nuclear Gln3 levels. As result of this work, the authors have proposed that rather than a single TORC1-dependent pathway, different pathways are involved in NCR regulation, which converge in a common regulatory branch where

glutamine and/or a related metabolite act as the metabolic signal.

Control of nitrogen uptake

One important level of NCR regulation takes place through control of the uptake of alternative nitrogen sources. This occurs both at the transcriptional and at post-transcriptional level. Under nutrient-rich conditions, SPS (Ssy1-Ptr3-Ssy5)-controlled expression of amino acid permeases (AAPs) specific for different amino acid subsets takes place, whereas in the presence of poorer nitrogen sources or total absence of nitrogen, expression of most of these permeases is replaced by expression of the general AAP, Gap1 (Ljungdahl & Daignan-Fornier, 2012). The TORC1-responsive kinase Npr1 is responsible for the stabilization of Gap1 at the plasma membrane and vacuolar sorting of specific AAPs like Tat2 in nitrogen-derepressed conditions (Schmidt *et al.*, 1998; Springael & Andre, 1998). This effect does not seem to occur by Npr1-dependent direct phosphorylation of the permease, at least in the case of Gap1. Increasing evidence has shown instead that Npr1 controls endocytosis of the permeases by phosphorylation of ubiquitin ligase adaptors, also known as arrestins, for example Aly1,2, Bul1,2 (O'Donnell *et al.*, 2010; MacGurn *et al.*, 2011; Merhi & Andre, 2012). It is known that inactivation of TORC1 leads to Tap42-Sit4-dependent dephosphorylation of Npr1 (Schmidt *et al.*, 1998; Jacinto *et al.*, 2001; Gander *et al.*, 2008). Most recent work links Tap42-Sit4-dependent Npr1 dephosphorylation to its activation and subsequent phosphorylation of arrestin-like Bul proteins, which in turn inhibits endocytosis of Gap1 under nitrogen limitation (Merhi & Andre, 2012). Although being an attractive model fitting with several observations, this model still fails to explain how under similar conditions, Bul1,2-dependent vacuolar sorting of Tat2 can take place (Abe & Iida, 2003). It also fails to explain why Gap1 is endocytosed in response to addition of external amino acids to nitrogen-starved cells, a condition in which TORC1 is supposedly inactive. One argument would be that amino acids are quickly incorporated raising intracellular glutamine pools, which in turn would activate TORC1, but this still cannot explain how nonmetabolizable nitrogen sources entering through Gap1 can also trigger endocytosis (G. Van Zeebroeck, M. Rubio-Texeira, J. Schothorst, J. M. Thevelein, manuscript in preparation). These data indicate that just like it has been shown for transcriptional regulation, further mechanisms regulating permease sorting must exist besides the TORC1-mediated branch. These other mechanisms likely exert control not only at the level of Npr1 and arrestin but at the level of other proteins yet to be identified.

The retrograde signaling pathway

The expression of NCR genes is strongly influenced by changes in the intracellular pools of amino acids. Nitrogen regulatory pathways assess these internal pools through sensing of ammonia, glutamate (Glu), and glutamine (Gln), main precursors for amino acid biosynthesis (Chen & Kaiser, 2002; Butow & Avadhani, 2004; Liu & Butow, 2006). Synthesis of Glu and Gln depends on the activity of tricarboxylic acid cycle (TCA) enzymes involved in the production of α-ketoglutarate. Gdh1 uses α-ketoglutarate and ammonia to synthesize Glu, and Gln1 uses ammonia and glutamate to synthesize Gln. While the expression of Gdh1 and Gln1 is mainly dependent on Gln3, the expression of enzymes of the TCA cycle devoted to the synthesis of α-ketoglutarate from oxaloacetate is instead controlled through what is known as the retrograde (RTG) signaling pathway (reviewed by Butow & Avadhani, 2004). Expression of RTG genes becomes determined by the interplay between four positive regulatory factors, Rtg1-3 and Grr1, and four negative regulatory factors, Mks1, Lst8, Bmh1, and Bmh2. Nuclear localization of Rtg1 and 3 transcriptional activators is, similarly to Gln3 and Gat1, negatively regulated by phosphorylation changes of themselves and of their negative regulator Mks1, which determine their cytosolic sequestration. Highly phosphorylated Mks1 complexes with 14-3-3 proteins Bmh1 and 2 and in this form prevents nuclear localization of Rtg1 and 3. Mks1 becomes inactivated by interaction with Rtg2. Release of Mks1 from Bmh1,2 ultimately causes its ubiquitination by Grr1 and subsequent degradation (Zaman *et al.*, 2008).

Although rapamycin inhibition of TORC1 activates the RTG pathway, the effect of TORC1 on nutrient regulation of the RTG pathway seems rather indirect, because for example, cells grown on glutamate do not activate the RTG pathway in response to rapamycin (Dilova *et al.*, 2002, 2004). Nevertheless, TORC1 has a clearly negative effect on the pathway via its WD-40 subunit, Lst8 (Loewith *et al.*, 2002; Chen & Kaiser, 2003). Mutant *lst8* alleles were initially identified that caused diverted targeting of Gap1 to the vacuole under nutritional conditions in which the transceptor should normally be sorted to the plasma membrane (Roberg *et al.*, 1997). Further analysis of these alleles by (Chen & Kaiser, 2003) revealed that the missorting of Gap1 in *lst8-1* mutants is in fact due to activation of the RTG pathway and a resulting increase in total intracellular amino acid levels, the latter serving as a signal for Gap1 sorting to the vacuole. Lst8 seems to act on the RTG pathway at two different levels, one upstream and the other downstream of Rtg2 (Liu *et al.*, 2001; Chen & Kaiser, 2003). The former is believed to involve a role of Lst8 in controlling the activity or assembly of the SPS

amino acid-sensing system, affecting the ability of the cells to sense external glutamate (Forsberg & Ljungdahl, 2001).

Role of the protein synthesis initiation machinery in general control of nitrogen metabolism

The function of GAAC

When yeast cells are starved for one or more amino acids, the GAAC pathway is activated, which results in global inhibition of translation initiation and preferential expression of the transcription factor Gcn4, which in turn activates a set of 57 genes, mainly involved in amino acid biosynthesis, nitrogen utilization, signaling, and gene expression (Hinnebusch, 1986; Natarajan *et al.*, 2001; Staschke *et al.*, 2010; Fig. 5). Rapid inhibition of protein synthesis via activation of the GAAC pathway is similarly triggered by a variety of stresses (e.g. glucose or nitrogen limitation, presence of less preferred nitrogen sources, heat, salt, metal, and oxidative stress, etc.), in which cells have to readapt translation to the synthesis of specific factors involved in preservation of energy and protection from stress. Because stress conditions reduce the specific growth rate, they could indirectly activate the GAAC pathway (reviewed by Simpson & Ashe, 2012).

Role of protein synthesis initiation

How the GAAC pathway manages to make this important switch is well understood at present (reviewed by Hinnebusch, 2005 and by Simpson & Ashe, 2012). Under optimal growth conditions, the eukaryotic translation initiation factor 2 (eIF2) occurs mainly in its GTP-bound active form, in which it initiates the formation of the ternary complex (TC). The TC consists of eIF2, GTP, and the charged methionyl initiator tRNA. Once formed, the TC recruits the 40S small ribosomal subunit, along with other eIFs, to form the 43S preinitiation complex (PIC). After its binding to mRNA, PIC scans the mRNA in search for an AUG start codon. Once found, the 60S ribosomal subunit binds, after which translation can begin (Kimball, 1999; Jackson *et al.*, 2010). Under amino acid starvation, levels of uncharged tRNA increase, activating phosphorylation of the α-subunit of eIF2 on Ser51 by its kinase, Gcn2 (Dever *et al.*, 1992; Gomez *et al.*, 2002; Dey *et al.*, 2011). In particular, Gcn2 contains an autoinhibited kinase domain that is allosterically activated by binding of uncharged tRNA to an adjacent histidyl-tRNA synthetase-like domain (Wek *et al.*, 1989; Dong *et al.*, 2000; Qiu *et al.*, 2001). Phosphorylated eIF2α exhibits enhanced affinity for the GTP-GDP exchange factor (GEF) eIF2B, competitively inhibiting the rate of nucleotide exchange, which in turn results in a reduced rate of TC formation (Dever *et al.*, 1993; Hinnebusch, 2005).

Regulation of *GCN4* messenger translation

Diminished levels of TC reduce the efficiency of scanning ribosomes to reinitiate translation of the bulk of the messenger RNAs. Paradoxically, this specifically increases the extent of *GCN4* translation. This gene encodes Gcn4 (General control nonderepressible 4 protein), a basic leucine zipper transcriptional factor required for the activation of GAAC-responsive genes via binding to specific UAS_{GCRE} sequences (GA(C/G)TCA) present in their promoter (Hinnebusch, 2005). *GCN4* is transcribed to an mRNA with four short open reading frames (microORFs) in the 5′-untranslated region (UTR). When a scanning TC-bound 40S ribosomal subunit finds the first initiator codon in μORF1, GTP in the TC is hydrolyzed to GDP, releasing eIF2-GDP, after which the 60S ribosome is recruited and translation of this ORF proceeds. When it terminates, the 40S subunit continues scanning further but its chances to reinitiate translation depend on the availability of free TC. In optimal nutritional conditions (noninducing conditions), reinitiation of translation after the first ORF is relatively efficient because high levels of TC are available. This ultimately decreases the chances of translation of the downstream full-length *GCN4* ORF. Under amino acid starvation, however, the levels of TC are low and strongly reduce this efficiency, which allows the scanning 40S ribosomal subunit to finally reach the distantly located position at which the *GCN4* ORF begins, before it binds a new TC (Mueller & Hinnebusch, 1986; Dever *et al.*, 1995).

Connection with TOR

Recent research has highlighted the strong connection between GAAC and other central regulatory pathways involved in nutritional sensing and control of cell growth and division, such as the intracellular amino acid-sensing TORC1 pathway (Cherkasova & Hinnebusch, 2003; Staschke *et al.*, 2010). On the one hand, it has been found that activation of Gcn2 is influenced by TORC1. Recent evidence supports a model by which TORC1 indirectly facilitates inhibition of Gcn2 via phosphorylation of its Ser577 by a yet unidentified protein kinase. Rapamycin has been suggested to release TORC1-dependent TAP42-mediated inhibition of the type 2A-related protein phosphatase, Sit4, which in turns dephosphorylates Gcn2, resulting in enhanced eIF2α phosphorylation (Cherkasova & Hinnebusch, 2003). Gcn2 activation thus seems to

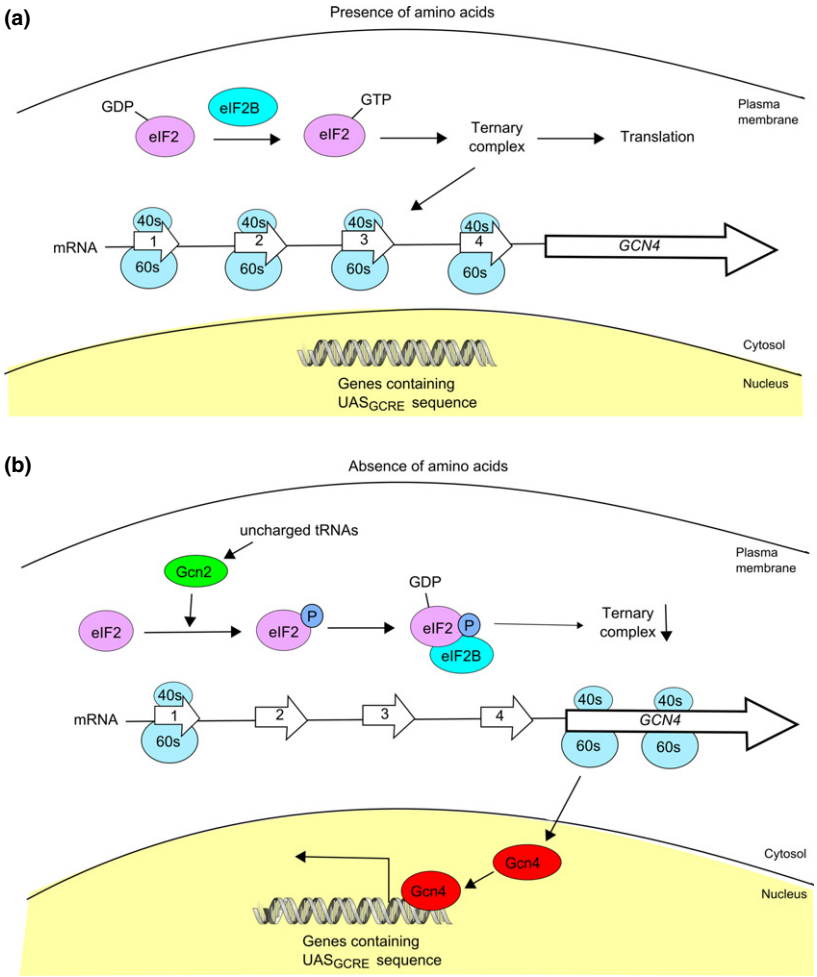


Fig. 5. The GAAC pathway. (a) In the presence of amino acids, eukaryotic translation initiation factor, eIF2, is mainly in the GTP-bound state as a result of stimulation by its GEF, eIF2B. GTP-bound eIF2 forms a TC with initiator Met-tRNA. TC along with the 40S ribosomal unit scans mRNA and recruits the 60S ribosomal unit to form the functional ribosome. The latter starts translating mRNA into protein once it encounters the start codon. This is also true for the *GCN4* mRNA, but the ORF is preceded by multiple μORFs, which largely prevent the ribosomes from reaching the *GCN4* ORF. Hence, under these conditions, production of the Gcn4 transcription factor is very limited. (b) Under amino acid starvation, the levels of uncharged tRNA increase, which activates the Gcn2 protein kinase. This enhances the level of phosphorylated eIF2, which as a result binds too tightly to eIF2B, preventing the stimulation of the exchange of GDP for GTP on eIF2. Hence, eIF2 is largely in the GDP-bound state and the level of TC drops, which causes a strong reduction in the level of general protein synthesis. However, paradoxically, the μORFs in front of the *GCN4* ORF are now largely read through by the ribosomes because of the lack of TC to initiate translation. As a result, the ribosomes are now able to reach the main *GCN4* ORF, causing its translation into Gcn4 protein. The enhanced level of the Gcn4 transcription factor stimulates the expression of genes involved in amino acid biosynthesis, resulting in a strong increase in the endogenous synthesis of amino acids when amino acids are absent in the medium.

result from the combined effect of Gcn2 release from TOR inhibition and the progressive increase in uncharged tRNA levels. The timing of these two events suggests a sequential contribution to GAAC activation, because release from TORC1-mediated inhibition occurs faster than accumulation of uncharged tRNAs (Staschke *et al.*, 2010). The separate contribution of both effects to Gcn2 activation is also clear when considering that histidine starvation (induced by the potent inhibitor of histidine biosynthesis, 3-aminotriazole or 3-AT) and other stress conditions that increase eIF2α phosphorylation do not reduce Ser577 phosphorylation levels in Gcn2 (Cherkasova & Hinnebusch, 2003). Presumably, the inhibitory effect of Ser577 phosphorylation in Gcn2 can be overcome by sufficiently high levels of uncharged tRNA (Garcia-Barrio *et al.*, 2002). Gcn4 has been found to act in conjunction with other transcription factors, such as the TORC1 and NCR-regulated GATA transcription factor, Gln3 (see further sections, and Valenzuela *et al.*, 2001). Combined deletion of Gcn4 and Gln3 causes high

rapamycin resistance and a similar number of genes depend on Gcn4 or Gln3 for their induction by rapamycin treatment (Staschke *et al.*, 2010).

Connection with Snf1

Snf1 also regulates the GAAC pathway in response to glucose levels (Shirra *et al.*, 2008). Glucose depletion causes rapid inhibition of translation initiation, in particular by downregulation of genes involved in ribosome biogenesis (Simpson & Ashe, 2012). Under these conditions, Snf1 also inhibits Gcn4 either at the level of its translation or at the level of its role in gene transcription, a conclusion supported by the fact that inactivation of Snf1 leads to unexpected induction of Gcn4-dependent genes (Shirra *et al.*, 2008). Conversely, under amino acid limitation in the presence of glucose, Snf1 collaborates with other mechanisms to activate Gcn2. Snf1 promotes the formation of phosphorylated eIF2α by stimulating the function of Gcn2 during histidine starvation of glucose-grown cells. Thus, eliminating Snf1 or mutating its activation loop lowers Gcn2 kinase activity, reducing the autophosphorylation of Thr882 in the Gcn2 activation loop, and decreases eIF2α-P levels in starved cells (Cherkasova *et al.*, 2010). Conversely, in amino acid-replete, but glucose-limited cells, uncharged tRNAs are at low levels and Gcn2 activity/phosphorylated Thr882 is low. Under these conditions, Snf1 does not stimulate Gcn2 function but rather inhibits Gcn2 by either promoting Ser577-P formation and/or by inhibiting its dephosphorylation by Sit4 and Glc7 (Shirra *et al.*, 2008).

Connection with the actin cytoskeleton

Additional levels of GAAC regulation are also being progressively unveiled. One other mechanism involves Yih1, which competes with Gcn2 for Gcn1 binding, thus inhibiting Gcn2. Yih1 binds free G-actin, which in turns frees Gcn2 from its inhibition. In areas where actin is mainly polymerized, free Yih1 may sequester Gcn1, thus preventing Gcn2 activation. This additional layer of regulation is in agreement with a previously suggested role of the cytoskeleton in the regulation of protein synthesis (Sattlegger *et al.*, 2011).

Cyclin-controlled Pho85 in phosphate regulation

Cellular function of Pho85

Yeast Pho85 is a cyclin-dependent kinase (Cdk), involved in the regulation of phosphate metabolism in function of external phosphate availability, but also in a broad spectrum of other cellular processes, including the sensing of other environmental changes and cell cycle control (Huang *et al.*, 2007a, b; Fig. 6). Downstream targets of Pho85 include the Pho4 transcription factor controlling expression of the *PHO* genes, the Gcn4 transcription factor controlling amino acid biosynthesis genes, the cyclin Cln3 involved in cell cycle control, and the Rim15 protein kinase involved in different nutrient-regulated pathways, controlling among others the activity of the transcriptional activators, Msn2 and Msn4. To perform its different functions, Pho85 associates with and is activated by 10 different cyclins, which can be divided into two distinct subfamilies. The Pho80 subfamily (including Pho80, Pcl6, Pcl7, Pcl8, and Pcl10) is mainly associated with the regulation of phosphate metabolism and sensing of environmental changes and the Pcl1,2 subfamily (consisting of Pcl1, Pcl2, Pcl5, Pcl9, and Cgl1) is predominantly associated with cell cycle regulation (Measday *et al.*, 1997).

Role of Pho85 in phosphate regulation

One of the best studied functions of Pho85 is its role in the response to phosphate limitation, which consists of two distinct downstream pathways, targeting either Pho4 or Cln3 (Lenburg & O'Shea, 1996; Persson *et al.*, 2003). In the presence of high external phosphate levels, Pho4 is phosphorylated in a manner dependent on the Pho85–Pho80 complex after which Pho4 is excluded from the nucleus. Because Pho4 is a transcriptional activator, the *PHO* genes are not expressed under this condition. Upon phosphate limitation, Pho85–Pho80 complex activity is inhibited, allowing Pho4 to enter the nucleus and induce the expression of *PHO* and *VTC* genes (Kaffman *et al.*, 1994; O'Neill *et al.*, 1996). These include *PHO3* and *PHO5*, which encode secreted phosphatases that can now scavenge for phosphate in the medium by hydrolyzing phosphate-containing compounds. The actual mechanism by which phosphate limitation is sensed remains unclear, although intracellular phosphate levels as well as inositol pyrophosphate, IP_7, appear to play a role (Auesukaree *et al.*, 2004; Lee *et al.*, 2008). The IP_7 levels are upregulated upon phosphate limitation, resulting in the inhibition of Pho80–Pho85 complex activity. This effect is mediated through the CDK-like inhibitor, Pho81, a positive regulator of the PHO pathway (Lenburg & O'Shea, 1996). Pho81 constitutively interacts with the Pho85–Pho80 complex, but only inhibits this complex, thus preventing Pho4 hyperphosphorylation, in the presence of elevated levels of IP_7 (O'Neill *et al.*, 1996; Lee *et al.*, 2008). The binding of IP_7 by Pho81 is thought to reversibly change the conformation of Pho81, inducing additional interactions between Pho81 and the Pho85–Pho80 complex, which prevent the kinase activity of the complex to access

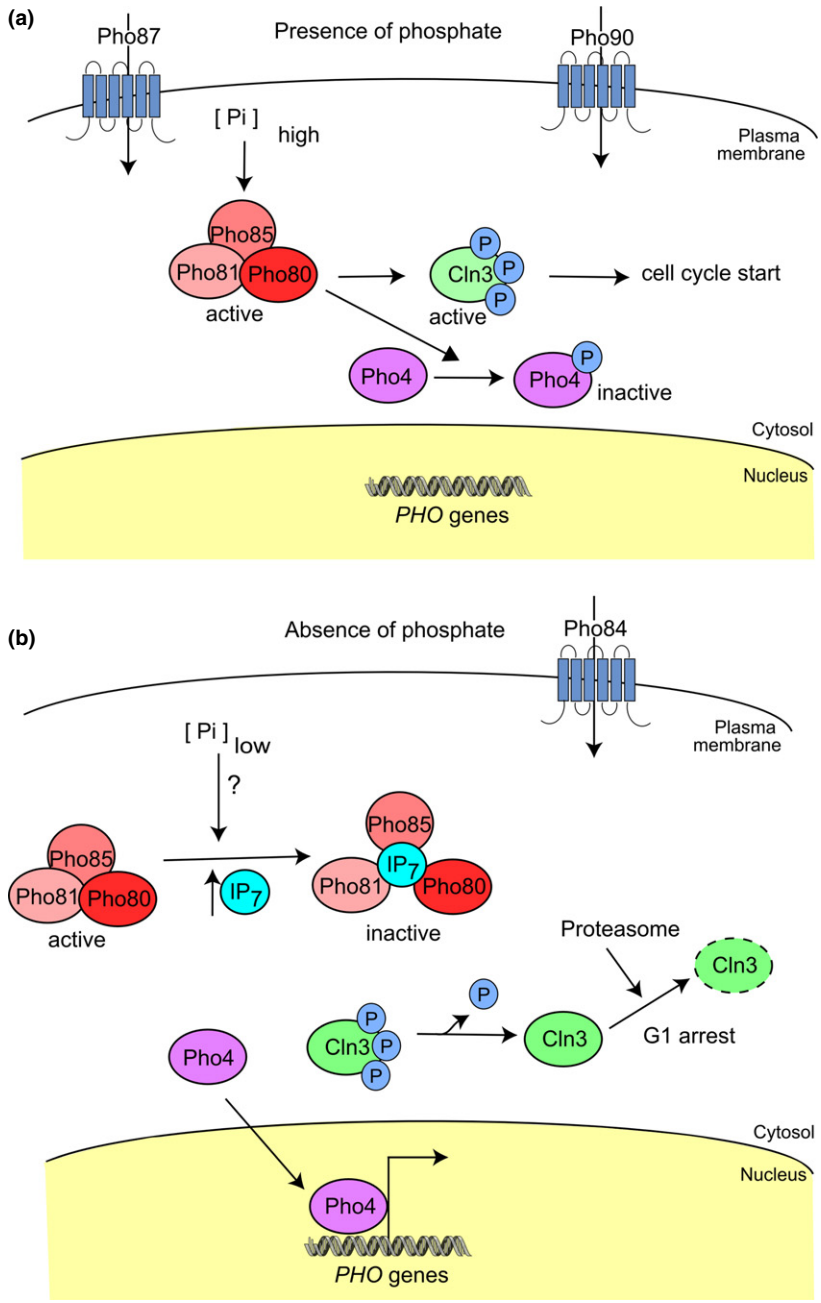


Fig. 6. Central role of Pho85 in phosphate regulation of the PHO pathway. (a) When present in high levels, external phosphate is imported by the low-affinity phosphate carriers Pho87 and Pho90, which raises the intracellular phosphate level. This activates the Pho85 complex, which phosphorylates the transcriptional activator Pho4, causing its sequestration in the cytosol. As a result, the *PHO* genes, encoding, for example, the secreted phosphatases Pho3 and Pho5, are not expressed. Active Pho85 also phosphorylates cyclin Cln3, which aids in progression over start in G_1 of the cell cycle. (b) Upon intracellular phosphate limitation, inositol pyrophosphate (IP7) levels increase. IP7 promotes Pho81-dependent inhibition of the Pho85–cyclin Pho80 complex. This inhibition results in dephosphorylation of Pho4, which then migrates to the nucleus where it activates transcription of the *PHO* and *VTC* genes. Inactivation of the Pho85 complex also dephosphorylates Cln3, which is then degraded by the proteasome leading to cell cycle arrest.

Pho4 (Lee *et al.*, 2008). Although there are some data suggesting that the increase in the IP_7 level upon phosphate limitation is mediated through the regulation of its metabolic enzymes, either by Kcs1 inhibition (Nishizawa *et al.*, 2008) or through an increase in Vip1 activity (Ljungdahl & Daignan-Fornier, 2012), the actual

phosphate-sensing mechanism triggering the increase remains to be identified. In conclusion, the current data support a model in which Pho81 acts as a mediator of intracellular phosphate sensing, by detecting the phosphate limitation-induced increase in the IP_7 level.

Sensing of extracellular phosphate

In addition to the intracellular phosphate sensing, it has been hypothesized that Pho85 might also be influenced by a sensor for extracellular phosphate, similar to glucose sensing for activation of the cAMP-PKA pathway, in which also sensing systems for intracellular and extracellular glucose are involved. This hypothesis is supported by the finding that mutations in the three low-affinity phosphate transporters, Pho87 and Pho90 in the plasma membrane and Pho91 in the vacuolar membrane, cause the induction of phosphate-regulated genes in a manner that is independent of the intracellular phosphate concentration (Pinson *et al.*, 2004; Hurlimann *et al.*, 2007). For a long time, it was believed that Pho84 could possibly fulfill this role, but other data appear more in agreement with Pho84 affecting Pho85 activity only through its capacity of importing phosphate into the cell (Wykoff & O'Shea, 2001; Samyn *et al.*, 2012). In this regard, it is noteworthy that the low-affinity transporters contain an SPX domain in their N-terminus, which is also found in other proteins with important regulatory roles in Pi homeostasis in yeast, including Pho81 and the proteins of the vacuolar transporter chaperone (VTC) complex involved in vacuolar polyphosphate homeostasis (Secco *et al.*, 2012). This domain allows negative regulation of the low-affinity transporters by the low-phosphate-induced Spl2 protein (Wykoff *et al.*, 2007). Whether an SPX-dependent mechanism would be involved in extracellular Pi sensing is still unclear.

Exclusivity of high- and low-affinity phosphate uptake

The existence of a complex feedback mechanism supporting reciprocal switching between low- and high-affinity phosphate transporters has recently been demonstrated (Wykoff *et al.*, 2007; Secco *et al.*, 2012). When internal phosphate is low, the PHO pathway is activated, Pho4 is dephosphorylated, and stimulates expression of Pho84, resulting in higher phosphate uptake, generating a negative feedback loop. At the same time, a low internal phosphate level activates Spl2, which inhibits phosphate uptake by Pho87 and Pho90 (Wykoff *et al.*, 2007), thus creating a positive feedback mechanism. Because the signaling of these two feedback mechanisms is mediated by the PHO pathway, the low- and high-affinity phosphate transporters are mutually exclusive and only one type can function at the same time (Wykoff *et al.*, 2007).

Connections with other parts of metabolism

Pho85 interaction with alternative cyclins provides a high level of promiscuity for its regulatory functions. For example, with its cyclin Pcl5, Pho85 is known to be involved in the regulation of Gcn4 stability (Shemer *et al.*, 2002; Bomeke *et al.*, 2006; Streckfuss-Bomeke *et al.*, 2009). Upon amino acid starvation, the Pho85–Pcl5 complex is disrupted due to apparent Pho85-induced self-catalyzed phosphorylation and subsequent degradation of Pcl5 (Aviram *et al.*, 2008). This disruption causes in turn a loss of Pho85 activity, resulting in a lack of phosphorylation and subsequent stabilization of Gcn4. While the Pho85-mediated downstream responses to amino acid starvation are well understood, how the starvation is sensed and results in loss of Pcl5 stability remains to be elucidated.

Pho85 also plays an important role in lipid metabolism. The Pho85–Pho80 complex phosphorylates Pah1, a phosphatidate phosphatase (PAP), which catalyzes the penultimate step in the synthesis of triacylglycerol. This phosphorylation reduces Pah1 activity and its interaction with liposomes and in this way affects lipid homeostasis (Choi *et al.*, 2012). PKA also phosphorylates Pah1 in conjunction with Pho85–Pho80, resulting in inhibited PAP activity (Su *et al.*, 2012). Recently, it has also been shown that phosphate availability stimulates progression over start in G_1 of the cell cycle through Pho85–Pho80-induced Cln3 stabilization (Menoyo *et al.*, 2013). Upon phosphate limitation, Pho85-mediated Cln3 phosphorylation is abolished, resulting in the degradation of Cln3 and subsequent G_1 arrest. Also here, the upstream phosphate-sensing mechanism remains unclear. Interestingly, Truman *et al.* (2012) showed that the Cln3 abundance pattern is also regulated by Pho85 upon nitrogen limitation. Pho85 can directly phosphorylate the Ssa1 chaperone, a process likely supported by the cyclins, Clg1 and Pcl2. This phosphorylation is thought to trigger a displacement of the Ssa1 co-chaperone Ydj1, allowing Ssa1 to directly interact with Cln3, promoting its degradation. Thus, through different mechanisms, phosphate and nitrogen limitations regulate cell cycle progression over start by controlling Cln3 abundance through Pho85 activity (Valk & Loog, 2013).

Conservation in mammalian cells

Interestingly, the whole concept of regulation by cyclin-dependent kinases has been highly conserved from yeast to mammals. It has been shown that *S. cerevisiae* Pho85

and mammalian CDK5 are functional homologs, able to complement each others' functions *in vivo* (Huang *et al.*, 1999). Furthermore, multiple regulatory components of the Pho85 pathway show high conservation in both structure and function between *S. cerevisiae* and mammals, allowing the use of *S. cerevisiae* homologs to study the function and regulation of its mammalian counterparts (Huang *et al.*, 2007a, b). A striking example of this similarity in regulation has recently been reported for the involvement of Pho85-dependent phosphorylation of the Ssa1 chaperone in cell cycle regulation (Truman *et al.*, 2012). This regulation mechanism can be extrapolated directly to human cells, where the human HSP70 homolog of yeast Ssa1 is similarly regulated by CDK activity and as such affects Cln3 cyclin levels to regulate cell cycle progression.

Glucose and amino acid sensing by transporter-like proteins in the plasma membrane

Transporter-like nutrient sensors

Expression of the glucose and amino acid transporters in yeast is regulated by a special class of plasma membrane nutrient carrier homologs, which have gained a nutrient-sensing function and lost their transport function (Fig. 7). Hence, they function as pure nutrient sensors. The glucose sensors, Snf3 and Rgt2, are responsible for the induction of genes encoding hexose transporters, or *HXT* genes (Ozcan *et al.*, 1998) and Ssy1, a protein forming part of the SPS sensor system, is responsible for amino acid induction of genes encoding specific AAPs (Didion *et al.*, 1998). Both Snf3/Rgt2 and Ssy1 have high sequence similarity with either glucose or amino acid transporters; however, they have lost during evolution the capacity to transport any substrate (Didion *et al.*, 1998; Ozcan *et al.*, 1998). Although both glucose- and amino acid-sensing pathways are functionally and mechanistically different, they share certain elements, such as the requirement for the casein kinases Yck1,2 and the ubiquitin ligase SCF^{Grr1} for downstream signaling (Spielewoy *et al.*, 2004).

Glucose sensing by Snf3 and Rgt2

Yeast expresses multiple Hxt transporters with variable affinities for glucose and other hexoses (most recently reviewed by Horak, 2013). The expression of the glucose transporters is fine-tuned by the interplay between the Snf3-Rgt2 regulatory complex, the Snf1-Mig1 glucose repression pathway, and the Ras-cAMP pathway (Ozcan *et al.*, 1996). Although Snf3 and Rgt2 show 60% homology, Snf3 is a sensor for low glucose concentrations, while Rgt2 is a sensor for high glucose concentrations. Both sensors have an unusually long C-terminal tail through which they interact with the downstream regulatory proteins (Moriya & Johnston, 2004). *HXT* expression is largely regulated by Rgt1, a member of the Gal4 family of transcription factors that contains a Zn2Cys6 binuclear cluster DNA-binding domain (Ozcan & Johnston, 1995; Ozcan *et al.*, 1996; Kim *et al.*, 2003; Kim, 2009). Under glucose-limiting conditions, Rgt1 recruits the general corepressor complex Ssn6-Tup1 to the *HXT* promoters (Ozcan & Johnston, 1995; Kim *et al.*, 2003). Rgt1 does this in combination with the corepressors Mth1 and Std1 (Tomas-Cobos & Sanz, 2002; Lakshmanan *et al.*, 2003; Polish *et al.*, 2005). Glucose binding to the plasma membrane Snf3-Rgt2 sensors recruits the corepressors, Mth1 and Std1, to the plasma membrane where they are subsequently phosphorylated by the type I casein kinases Yck1,2 (Moriya & Johnston, 2004). This phosphorylation targets their Grr1-dependent ubiquitination and subsequent degradation by the proteasome (Spielewoy *et al.*, 2004). This degradation exposes the Rgt1 transcriptional repressor to phosphorylation by PKA, which releases it from its repressive upstream binding sites and switches it into a transcriptional activator, leading to derepression of the *HXT* genes (Palomino *et al.*, 2006; Jouandot *et al.*, 2011). Although phosphorylation on one of the four PKA sites of Rgt1 is sufficient for the induction of transcription, all sites need to be phosphorylated before total release of the DNA-binding site takes place. Even though Mth1 and Std1 are paralogs, they have different functions mainly due to differential transcriptional regulation (Sabina & Johnston, 2009). *STD1* expression is induced by glucose via Snf3-Rgt2 signaling, while *MTH1* expression is repressed by the Snf1 glucose repression pathway (Kaniak *et al.*, 2004; Kim *et al.*, 2006). Recent observations of combined haploinsufficiency showed that this system is extremely sensitive and responsive to subtle changes in corepressor levels (Dietzel *et al.*, 2012). The system conserves energy by maintaining just enough corepressor for repression of the target genes, as well as enabling rapid induction of expression in the presence of glucose. Rgt1 activity is not only influenced by the Ras-cAMP/PKA pathway via Tpk3-dependent phosphorylation but also by Snf1 (Palomino *et al.*, 2006; Zaman *et al.*, 2009). Snf1 affects *HXT* expression via repression of Mig1,2, which in turn plays additional roles in glucose positive and negative feedback loops by repressing the *STD1*, *MTH1*, *SNF3,* and *HXT* genes (Kaniak *et al.*, 2004).

Amino acid sensing by the Ssy1-Ptr3-Ssy5 system

Another well-characterized nontransporting nutrient sensor in *S. cerevisiae* is the amino acid sensor Ssy1 (Andre,

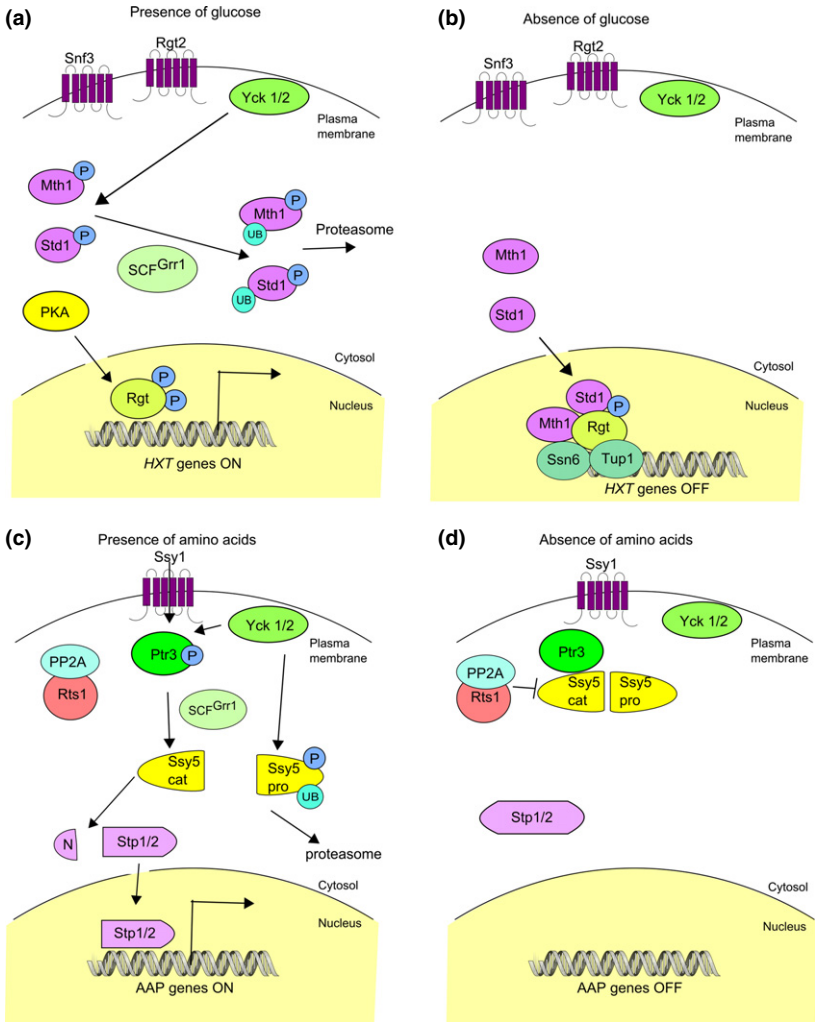


Fig. 7. Nutrient sensing by transporter-like plasma membrane sensors. *The Snf3-Rgt2 glucose-sensing pathway*. (a) Glucose binding to the Snf3/Rgt2 sensors recruits Mth1 and Std1 to the plasma membrane, where they are phosphorylated by Yck1,2. This phosphorylation targets them for ubiquitination by Grr1 and degradation by the proteasome, exposing Rgt1 to phosphorylation by PKA. This turns Rgt1 into a transcriptional activator for the expression of *HXT* (hexose transporter) genes. (b) In absence of glucose, Yck1,2 fail to phosphorylate Mth1 and Std1, which are no longer degraded and enter the nucleus to repress the expression of *HXT* genes. *The SPS amino acid-sensing pathway*. (c) In the presence of external amino acids, the amino acids bind to Ssy1, causing recruitment of the Yck1,2 protein kinases. They hyperphosphorylate Ptr3 and the Ssy5 prodomain, making it sensitive to ubiquitination by Grr1, after which it is broken down by the proteasome. Subsequently, the enhanced Ssy5 protease activity toward Stp1,2 leads to the removal of the N-terminal part of Stp1,2, which enables it to enter the nucleus and activate transcription of the target AAP genes. (d) In the absence of extracellular amino acids, phosphorylation of Ssy5 is counteracted by the phosphatase PP2A and its subunit Rts1, which keeps Ssy5 inactive. This prevents migration of Stp1,2 from the cytosol to the nucleus, and the expression of AAP genes is thus kept down.

1995). Although it can be classified as a member of the AAP family based on sequence similarity, it does not show detectable transport activity with any amino acid and differs strongly from the other members in possessing a long N-terminal extension. This region is essential for the sensor function and is highly conserved in putative *SSY1* homologs in other yeast species (Klasson *et al.*, 1999; Souciet *et al.*, 2000). Together with Ptr3 and Ssy5, Ssy1 forms the plasma membrane SPS (Ssy1-Ptr3-Ssy5) amino acid-sensing system, which induces transcription of a set of regular amino acid transporter genes as well as genes involved in amino acid metabolism in response to extracellular amino acids (Ljungdahl, 2009). The discovery of hyper- and hyporesponsive mutant alleles of *SSY1* strongly suggests that the extracellular amino acid must directly bind to the transporter homolog in order to activate the SPS system (Gaber *et al.*, 2003; Poulsen *et al.*, 2008). The downstream effectors of this pathway

are the transcription factors Stp1,2, which are synthesized as latent factors and activated by endoproteolytic cleavage, triggered by extracellular amino acid sensing, and catalyzed by the endoprotease, Ssy5 (de Boer *et al.*, 2000; Andréasson & Ljungdahl, 2002; Abdel-Sater *et al.*, 2004). Ssy5 is a chymotrypsin-like endoprotease with an inhibitory pro-domain and a catalytic domain. The pro-domain is autocatalytically cleaved from the catalytic domain but remains noncovalenty attached to it, forming an inactive protease complex that binds Stp1,2 precursors (Andreasson *et al.*, 2006). The pro-domain thus functions as an inhibitory subunit of the SPS sensor, because Stp1,2 are processed only after amino acid-induced signals cause their dissociation from the inhibitory pro-domain. (Pfirrmann *et al.*, 2010). Key steps in amino acid-induced Ssy5 activation include amino acid-induced conformational changes and phosphorylation-induced ubiquitination by the combined activity of the casein kinases, Yck1,2 and the SCF^{Grr1} ubiquitin ligase complex, the latter targeting the pro-domain for proteasomal degradation (Abdel-Sater *et al.*, 2004, 2011; Omnus *et al.*, 2011). Activation of Ssy5 requires Ptr3. Recent research has revealed that Ptr3 functions as an adaptor, coupling Ssy1 to Yck1,2, resulting in hyperphosphorylation of both Ptr3 and Ssy5 (Omnus & Ljungdahl, 2013). Yck1,2-mediated phosphorylation of Ssy5 is counteracted by the Rts1 regulatory subunit of the PP2A phosphatase, counteracting Ssy5 activation in the absence of amino acid induction (Eckert-Boulet *et al.*, 2006; Liu *et al.*, 2008; Omnus & Ljungdahl, 2013).

The processed forms of Stp1,2 are efficiently translocated into the nucleus where they activate the expression of SPS sensor-regulated genes. The N-terminal regulatory domain of the Stp1,2 transcription factor precursors functions as a nuclear exclusion determinant, such that its presence limits their entrance in the nucleus. In the absence of extracellular amino acids, the inner nuclear membrane-located Asi proteins (Asi1-Asi2-Asi3) also prevent any full-length Stp1,2 transcription factors that escaped cytoplasmic retention from derepressing the SPS-regulated genes, by restricting their access to promoters (Boban *et al.*, 2006; Zargari *et al.*, 2007). The transcription factor Dal81/Uga35, on the other hand, has a synergistic role on the induced expression of the SPS-regulated genes, by facilitating the binding of processed Stp1,2 to the promoters (Abdel-Sater *et al.*, 2004; Boban & Ljungdahl, 2007).

Although Stp1 and Stp2 have redundant functions, recent research provides evidence that they have functionally diverged as can be inferred from their differential regulation and localization pattern (Wielemans *et al.*, 2010; Tumusiime *et al.*, 2011). E2 ubiquitin-conjugating enzyme Cdc34 is required for the degradation of both full-length and processed Stp1, but not Stp2. Full-length Stp1 is localized both in the cytoplasm and at the cell periphery, whereas full-length Stp2 is localized only in the cytoplasm. Stp2 is the primary factor involved in basal activation of target gene expression. The SPS amino acid-sensing pathway and the TOR pathway are also somehow interconnected at the level of Stp regulation. Inactivation of TORC1 by rapamycin results in Sit4-dependent degradation of Stp1 and disappearance of Stp1 from the nucleus (Shin *et al.*, 2009). Additional sensing pathways also impinge on the control of SPS amino acid sensing and the expression of AAPs. For example, through the control of glutamine transporters Gnp1 and Dip5, the SPS sensor has a PKA-dependent role in regulating *FLO11*-dependent invasive growth and biofilm formation in *S. cerevisiae* (Torbensen *et al.*, 2012). The amino acid SPS sensor system is conserved in other organisms such as the pathogenic yeast, *Candida albicans*, in which it also plays an important role in nitrogen source-dependent virulence (Brega *et al.*, 2004; Martinez & Ljungdahl, 2005; Davis *et al.*, 2011).

Trehalose-6-phosphate: from allosteric regulator in yeast to signaling molecule in plants

Trehalose is a disaccharide, formed by the linkage of two glucose molecules as an α-α-1-1-glycoside, which serves both as a storage carbohydrate and as a stress protectant in yeast and other fungi (Fig. 8). The intermediate of trehalose biosynthesis, trehalose-6-phosphate, was found to exert a crucial inhibitory control over the influx of glucose into glycolysis at the level of hexokinase-mediated glucose phosphorylation. Trehalose-6-phosphate is a potent allosteric inhibitor of Hxk1 and Hxk2 (Blazquez *et al.*, 1993), which functionally resembles the well-known product inhibition of mammalian hexokinase by glucose-6-phosphate (Wilson, 2003). Inactivation of Tps1 causes deregulation of glycolysis, with hyperaccumulation of all intermediates upstream of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and depletion of all downstream intermediates, as well as the absence of glucose-induced signaling phenomena (Bell *et al.*, 1992; Van Aelst *et al.*, 1993). Strains with inactive Tps1 cannot grow on glucose, but grow on galactose and nonfermentable carbon sources, of which metabolism does not involve hexokinase. Because most glucose-sensing mechanisms in yeast depend in some way on glucose phosphorylation, the *TPS1*-encoded trehalose-6-phosphate synthase sits at the onset of multiple glucose signaling pathways (Thevelein & Hohmann, 1995). The glucose growth and signaling defects of the *tps1* mutant

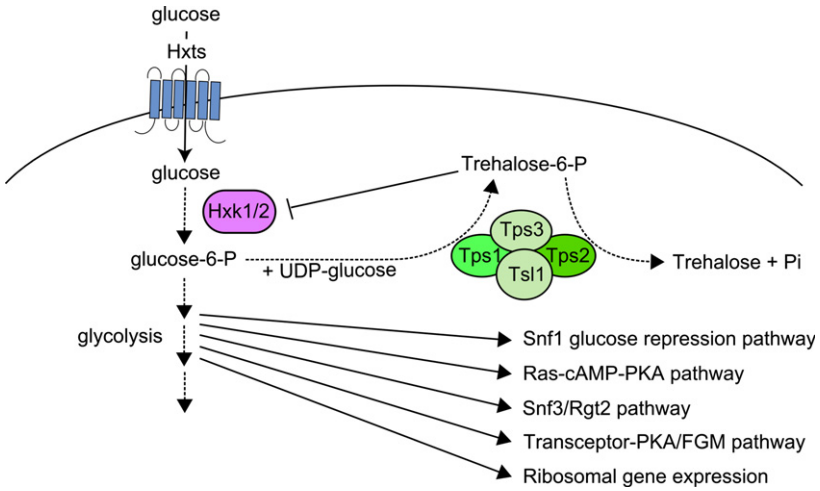


Fig. 8. Function of trehalose-6-phosphate as allosteric regulator of Hxk2 activity. Glucose is taken up by the hexose transporters (Hxt) and subsequently phosphorylated predominantly by hexokinase I (Hxk1) and II (Hxk2) to glucose-6-P, which is then further converted in glycolysis. Glucose-6-P and UDP-glucose are converted to trehalose-6-phosphate by the Tps1 enzyme and further to trehalose by the Tps2 enzyme in the trehalose synthase complex, which also contains the regulatory subunits Tps3 and Tsl1. Trehalose-6-phosphate is a potent allosteric inhibitor of Hxk1 and Hxk2, causing feedback inhibition on the influx of glucose into glycolysis. Although the precise mechanisms are generally unclear, the early steps of glucose catabolism are in some way important for the activation of most glucose signaling pathways. Metabolic reactions are depicted by dotted arrows; regulatory and signaling interactions by full arrows.

are suppressed by deletion of the most active hexokinase, Hxk2 (Hohmann *et al.*, 1993). Tps1 appears to reside both in the trehalose synthase complex and as a free protein (Bell *et al.*, 1998). Glucose addition causes a strong, transient increase in the level of trehalose-6-phosphate to levels much higher than would be expected from the inhibition constant of hexokinase (Hohmann *et al.*, 1996). There are several other indications that the absence of trehalose-6-phosphate inhibition of hexokinase alone is not sufficient to explain the glucose-induced defects in the *tps1* mutant (Blazquez & Gancedo, 1994; Hohmann *et al.*, 1996; Bonini *et al.*, 2000, 2003; Walther *et al.*, 2013), but whether and how the Tps1 protein itself could also play a direct role in control of glucose influx and/or glucose signaling remains unclear (Thevelein & Hohmann, 1995). A remarkable outcome of the research on yeast Tps1 has been the discovery that trehalose-6-phosphate functions as a genuine signaling molecule in plants, in spite of the fact that most plants, as opposed to bacteria and fungi, do not accumulate trehalose. *Arabidopsis* homologs of yeast *TPS1* can complement the growth defect of the yeast *tps1*Δ mutant on glucose (Blazquez *et al.*, 1998; Chary *et al.*, 2008). As opposed to yeast, no direct inhibition of plant hexokinases by trehalose-6-phosphate has been detected. However, as in yeast, trehalose-6-phosphate also seems to serve in plants as a signal for the availability of high sugar levels. It regulates sugar allocation between source and sink tissues in connection with plant growth and development (O'Hara *et al.*, 2012).

General nutrient signaling pathways: triggered by multiple types of nutrients

Nutrient sensing for control of cellular growth

Essential nutrients are required for provision of energy and building blocks for the growth of cells. There are indications from nutrient control of cell cycle progression that such nutrients must be sensed by the cells, because depletion of cells for a single essential nutrient is well known to cause cell cycle arrest in the G_1 phase and entrance into G_0 (Hartwell, 1974). A cellular parameter that shows a close correlation with cell growth is ribosome content (Mager & Planta, 1991; Kraakman *et al.*, 1993) and it is not a surprise that depletion of cells for any single nutrient downregulates synthesis of ribosomal components, both ribosomal RNA and RPs. Re-addition of the missing nutrient triggers a rapid increase in ribosomal RNA and protein expression, and the mechanisms underlying this process have been studied in great detail because of its importance for nutrient control of cell growth. However, in spite of the detailed information available on the downstream targets of nutrient regulation of ribosomal RNA and protein

expression, the most upstream mechanisms involved in the actual sensing of the nutrients largely remain unclear.

Fermentative growth and PKA activity

In yeast, a remarkable correlation has been documented extensively between the control of targets of the PKA pathway and the arrest and induction of fermentative growth, that is, growth on glucose or another rapidly fermented sugar (Thevelein & de Winde, 1999). The level of glycogen and trehalose, the stress tolerance of the cells, the strength of the cell wall, and the expression of stress-response genes are all downregulated when cells grow exponentially on a fermentable sugar, whereas starvation for a single nutrient in the same fermentative medium causes cell cycle arrest and entrance into stationary phase and at the same time upregulation of all these PKA targets. This indicates that PKA activity is somehow closely coregulated with fermentative growth. On nonfermentable carbon sources, this relationship is either absent or not very clear, because the cells already display low activity of the PKA pathway, when growing on a nonfermentable carbon source. These observations have therefore led to investigation of a second pathway that is apparently controlled by all essential nutrients of yeast cells, in addition to the pathway involved in nutrient control of cell growth. Whether there is a mechanistic connection between nutrient control of the PKA pathway and control of fermentative growth remains to be elucidated. It also remains unclear whether other pathways, processes, or cellular traits may be regulated in a similar way by the whole array of essential nutrients that is required to sustain the growth of cells. At this moment, no such other phenomena have been clearly documented. One could expect that processes that are directly or indirectly connected to the entrance and exit of stationary phase upon depletion and re-addition of an essential nutrient may be good candidates (Gray *et al.*, 2004). An interesting example is autophagy, which is also induced by nutrient starvation in addition to other environmental cues (Levine & Klionsky, 2004; Reggiori & Klionsky, 2013).

Multiple nutrient signaling through transceptor activation of the PKA pathway

Nutrient activation of PKA during start-up of fermentative growth

When yeast cells have been starved on a fermentative medium for a single essential nutrient and have entered stationary phase, they display a range of properties indicating low activity of PKA *in vivo* (Fig. 9). When the missing nutrient is added again and the cells exit stationary phase, these phenotypes are rapidly reversed, indicating that PKA activity in the cells increases to the high level typical for fermentatively growing cells (Thevelein & de Winde, 1999). Interestingly, one of the earliest readouts indicating the activation of PKA is the enzyme trehalase that undergoes a 5- to 10-fold phosphorylation-induced increase in activity within just a few minutes after the addition of the missing nutrient (Hirimburegama *et al.*, 1992). This process is purely post-translational and has been observed after re-addition of amino acids or ammonium to nitrogen-starved cells, phosphate to phosphate-starved cells (Hirimburegama *et al.*, 1992; Schepers *et al.*, 2012), and sulfate to sulfate-starved cells (Hirimburegama *et al.*, 1992; H. N. Kankipati, M. Rubio-Texeira, D. Castermans, J. M. Thevelein unpublished results). It is dependent on the presence of glucose, and as opposed to glucose activation of PKA targets, like trehalase, it is not mediated by an increase in the cAMP level (Hirimburegama *et al.*, 1992). This type of nutrient activation of trehalase is still observed in cells lacking the regulatory subunit of PKA, which provides strong evidence that it must be mediated by another regulatory mechanism (Durnez *et al.*, 1994; Giots *et al.*, 2003). Although the presence of glucose is detected by the same dual glucose-sensing system activating the Ras-cAMP pathway, both systems can act independently of each other in this case (Rolland *et al.*, 2000; Donaton *et al.*, 2003; Giots *et al.*, 2003). Because of the different regulation compared to the Ras-cAMP pathway and the combined requirement for a fermentable carbon source and a complete growth medium, the signaling pathway involved has been named the fermentable growth medium-induced or FGM pathway (Thevelein, 1994).

Transceptors as nutrient sensors

Investigation of the nutrient-sensing mechanism involved in this rapid 'FGM signaling' phenomenon has led to the identification of transporters that display in addition to their transport function also a receptor function for activation of the PKA pathway by their substrate. We have proposed the name of 'transceptors' for proteins with a double function as transporter and receptor (Holsbeeks *et al.*, 2004). Hence, as opposed to the Snf3, Rgt2 and Ssy1 transporter homologs serving as nutrient sensors, the transceptors have retained their normal transport activity. A common feature for the transceptors identified up to now is that they are all strongly induced in cells starved for their substrate, while other transporters, which do not serve as transceptors, are downregulated. In nitrogen-starved cells, Gap1 is

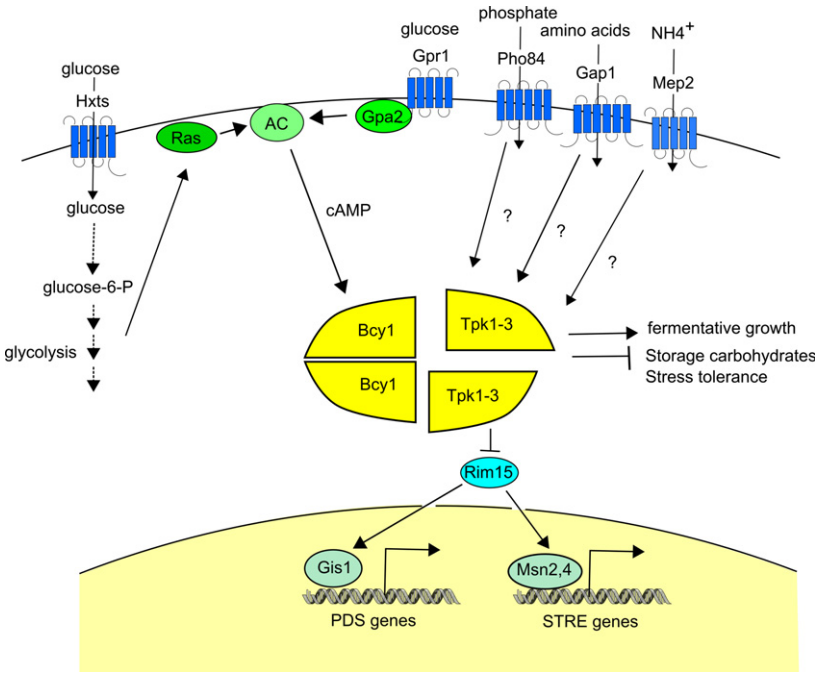


Fig. 9. Activation of the PKA pathway in the presence of glucose by different essential nutrients through multiple transceptors. Glucose-fermenting cells of *Saccharomyces cerevisiae* that are starved for another essential nutrient, like nitrogen or phosphate, enter stationary phase and develop a low-PKA phenotype, that is, accumulation of trehalose and glycogen, acquirement of high stress tolerance, and downregulation of stress-responsive genes. Re-addition of nitrogen or phosphate triggers rapid activation of PKA targets, which is not mediated by cAMP as a second messenger. The nutrient sensors or 'transceptors' involved are specific transporters that were induced in the starvation period and that act as nutrient receptors for activation of the PKA pathway. Gap1 senses amino acids, Mep2 senses ammonium, and Pho84 senses phosphate in appropriately starved cells. The presence of glucose is essential for nutrient transceptor activation. It can be detected either by the GPCR (Gpr1-Gpa2) system for extracellular glucose sensing or by the Ras system, which is activated by intracellular glucose catabolism. Metabolic reactions are depicted by dotted arrows; regulatory and signaling interactions by full arrows.

strongly induced and has long been considered a 'scavenger' AAP. Re-addition of an amino acid triggers rapid downregulation of Gap1 by endocytosis and sorting to the vacuole (Magasanik & Kaiser, 2002; Lauwers *et al.*, 2010). Investigation of amino acid-induced activation of PKA targets like trehalase in nitrogen-starved cells revealed that Gap1 acts as an amino acid receptor for activation of the PKA pathway (Donaton *et al.*, 2003). Similar work indicated that Mep2 and Mep1 function as transceptors for ammonium activation of the PKA pathway in nitrogen-starved cells (Van Nuland *et al.*, 2006). Phosphate-induced activation in phosphate-starved cells is mediated by Pho84 (and to a lesser extent by Pho87; Giots *et al.*, 2003). Pho84 is also downregulated by phosphate-induced endocytosis and sorting to the vacuole (Lundh *et al.*, 2009). The same is true for the Sul1,2 transporters, which have been implicated as transceptors for sulfate activation of the PKA pathway in sulfate-starved cells (Kankipati *et al.*, unpublished results). The transceptor function of these transporters has raised the question whether the rapid downregulation by endocytosis and the complex intracellular trafficking control of these proteins may serve to prevent overstimulation and interconnected regulation, respectively, of the PKA signaling pathway, a feature that is well known for classical receptors (Kriel *et al.*, 2011).

Nontransported agonists of transceptor signaling

Screening of analogues of the transceptor substrates has revealed nontransported agonists of the signaling function of the transceptor for Gap1 (Van Zeebroeck *et al.*, 2009), Pho84 (Popova *et al.*, 2010), and Sul1,2 (Kankipati *et al.*, unpublished results). This has provided a strong new argument for the signaling function of the transceptors and has also shown that transport through the transceptor is not required to trigger signaling. Moreover, recent work using these substrate analogues has revealed that some of these compounds can also trigger endocytosis of the transceptor, showing that transport is not required for the induction of endocytosis (Van Zeebroeck *et al.*, unpublished results). This

allows us to conclude that, apart from their transport capacity, transceptors may function in a very similar way as classical receptors. This is further supported by the finding that the Gap1 transceptor maintains the capacity to signal to the PKA pathway after its endocytic internalization to endosomes, a feature that has also been demonstrated for classical receptors (Rubio-Texeira *et al.*, 2012).

Conversion of transceptors into nutrient sensors

Several of the transceptors activating the PKA pathway are proton-coupled symporters. The finding that the transceptors do not require transport for signaling has led to the idea of mutagenizing putative proton-binding sites in order to inactivate transport while maintaining signaling. This has now been successfully accomplished for the Pho84 phosphate transceptor (Samyn *et al.*, 2012) and the Sul1,2 sulfate transceptors (Kankipati *et al.*, unpublished results). Identification of amino acid residues in co-transporters has been difficult because inactivation of transport by site-directed mutagenesis can have other causes than abolishing binding of the co-transported ion. Hence, the signaling function of the transceptors provides a new interesting read-out for assessing maintenance of proper membrane integration and general functionality of mutagenized co-transporters that function as transceptors.

Transceptor signaling pathway to PKA

Although little is known about the specific downstream components that link the transceptors to PKA in the FGM pathway, requirement of the Sch9 kinase for amino acid and ammonium activation in nitrogen-starved cells has been demonstrated (Crauwels *et al.*, 1997). However, absence of Sch9 does not prevent phosphate-induced activation, indicating that this is not a general requirement for transceptor signaling (Giots *et al.*, 2003).

Transceptors in other organisms

The mechanism of nutrient sensing by transceptors seems to be conserved because permeases for sugars, amino acids, ammonium, nitrate, and sulfate have also been described to function as transceptors in other organisms. Examples are the amino acid transporters PATH in *Drosophila* (Goberdhan *et al.*, 2005), SNAT2 in mammalian cells (Hyde *et al.*, 2007), the mammalian GLUT2 glucose transporter (Stolarczyk *et al.*, 2010), the nitrate transporter NRT1 in plants (Gojon *et al.*, 2011), and the *Arabidopsis thaliana* SULTR1;2 sulfate transporters (Zhang *et al.*, 2013b).

Multiple nutrient control of ribosomal gene expression

Ribosome content and growth rate

The importance of nutrient control of ribosome synthesis is evidenced by the crucial role of ribosomes in supporting cellular proliferation and the massive size of their task in sustaining growth of the cells (Fig. 10). Ribosomes are the protein factories of the cell. The number of ribosomes and the rate of translation at each ribosome determine the rate of cellular mass accumulation. They also play a crucial role in cell proliferation by allowing cells to reach the critical size needed to initiate cell division. Exponentially growing *S. cerevisiae* cells contain an average of 200 000 ribosomes and around 2000 new ribosomes must be produced every minute to maintain a doubling time of 100 min (Warner, 1999). Each ribosome contains not $<$ 79 RPs encoded by 138 RP genes, and four rRNA molecules, the 5S rRNA, and the three other derived from the 35S pre-rRNA, all four encoded by the chromosome XII-located rDNA locus. Synthesis of all these components in equimolar amounts has to be precisely up- or downregulated together. Ribosome biogenesis represents nearly 60% of the total cell transcription, and RP mRNA transcription represents approximately 50% of the total RNA Pol II transcription initiation events. As a result, ribosome biogenesis is one of the most energetically expensive processes in cells. Sensing of the quality and quantity of nutrients therefore plays an important role in the regulation of ribosome biogenesis in order to obtain a growth rate optimally adjusted to the available nutritional conditions (Rudra & Warner, 2004; Zaman *et al.*, 2008).

Nutrient control of Pol I

Regulated ribosome biogenesis requires the coordinated activities of all three RNA polymerases: Pol I for rRNA, Pol II for RP genes, and Pol III for tRNA and small nuclear RNA (5S RNA) synthesis (reviewed by Lempiainen & Shore, 2009). In most conditions, several signaling pathways such as PKA and TORC1 are coordinated to regulate RNA polymerases, which influence the transcription of ribosomal genes (Lippman & Broach, 2009). Formation of Pol I initiation complexes at the 35S ribosomal DNA promoter depends on its tight association with Rrn3, which constitutes a major limiting step in 35S rRNA transcription (Yamamoto *et al.*, 1996). Glucose availability may affect Rrn3 levels through PKA (Broach, 2012). Signaling cascades trigger dephosphorylation and phosphorylation of Pol I and Rrn3 resulting in either enhanced or reduced formation of Pol I–Rrn3 complexes at the rDNA promoter (Fath *et al.*, 2001; Claypool *et al.*,

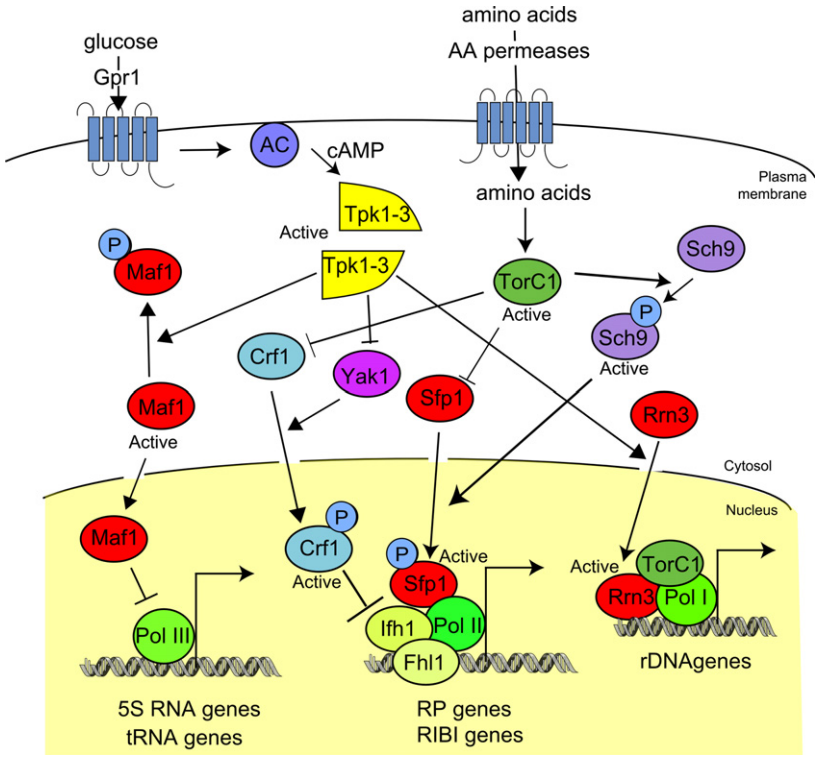


Fig. 10. Nutrient control of ribosomal gene expression. Ribosomal gene expression involves RNA polymerase (Pol) I for expression of rRNA, Pol II for expression of RPs and ribosome biogenesis (RiBi) factors, and Pol III for tRNA and small nuclear (5S) RNA. Different nutrient-sensing pathways are involved. Glucose sensing by Gpr1 activates the cAMP-PKA pathway, which stimulates ribosome biogenesis through effects on the three polymerases. The presence of preferred nitrogen sources impinges on this pathway via TORC1 and its downstream kinase Sch9. Pol I-dependent expression is positively regulated via interaction of Rrn3 with Pol I. TORC1 stabilizes this interaction. Glucose may stimulate Pol I-dependent transcription through PKA control of the level of Rrn3. Pol II-dependent expression is positively regulated by Sfp1 and Fhl1. Upon nutrient deprivation, the Crf1 inhibitor competes with the co-activator Ifh1 for binding to Fhl1, which causes inactivation of the latter. TORC1 prevents Crf1 nuclear shuttling, whereas Yak1 favors it. Sfp1 stimulates Pol II-dependent expression and is positively regulated by TorC1 via Sch9. Pol III-dependent expression is negatively regulated by Maf1. PKA phosphorylates Maf1, which prevents entrance of Maf1 into the nucleus and thus allows Pol III-directed gene expression.

2004; Gerber *et al.*, 2008; Goetze *et al.*, 2010). Dephosphorylation of yeast Rrn3 seems to favor its binding to Pol I, whereas certain types of phosphorylation prevent Pol I binding. For example, recent research in which the crystal structure of Rrn3 has been analyzed has revealed the presence of a surface serine patch in Rrn3 (Blattner *et al.*, 2011). During normal cell growth, the Rrn3 serine patch is not phosphorylated, enabling Rrn3 to bind Pol I, resulting in stable Pol I recruitment to rDNA and efficient transcription. During stress, phosphorylation of the serine patch impairs Rrn3 interaction with Pol I and its recruitment to rDNA, downregulating Pol I transcription, ribosome production, and cell growth. Presence of glucose is known to increase the expression of Rrn3, whereas Tor activity has a role in stabilizing the Rrn3–Pol I complex (Claypool *et al.*, 2004). In cells grown to stationary phase or treated with rapamycin, the amounts of Pol I–Rrn3p complexes are significantly reduced (Milkereit & Tschochner, 1998; Claypool *et al.*, 2004). TOR inactivation also leads to proteasome-dependent degradation of Rrn3, which further contributes to the strong reduction in initiation competent Pol I–Rrn3 complexes affecting yeast rRNA gene transcription (Philippi *et al.*, 2010).

Nutrient control of Pol II

Pol II-dependent RP and RIBI gene transcription is regulated both by repression and by activation. The RP and RIBI promoters contain RRPE and PAC motifs, which constitute binding sites for the transcriptional repressors Stb3, Dot6, and Tod6. Repression by these transcription factors is counteracted by mechanisms dependent on both the glucose-responsive PKA pathway and the TOR pathway (Huber *et al.*, 2009; Lippman & Broach, 2009). Stb3 is restricted to the nucleus in quiescent cells and is immediately released into the cytoplasm after glucose repletion, whereas Tor/Sch9 inhibition causes its nuclear accumulation (Liko *et al.*, 2010). Inhibition of TORC1/

Sch9 causes dephosphorylation of the three transcriptional repressors allowing their binding to RRPE and PAC motifs (Huber *et al.*, 2009, 2011). Once bound to the promoters, they recruit RPD3L histone deacetylase complex, which causes transcriptional repression. The recruitment of the RPD3L at RP and RIBI promoters is also increased upon TORC1 inhibition by other mechanisms including the release of the Esa1 histone acetyltransferase (Rohde & Cardenas, 2003; Humphrey *et al.*, 2004). Activation of these promoters is influenced by Rap1 and the forkhead-like transcription factor, Fhl1. Rap1 maintain nucleosome-free regions, which then facilitates access of transcriptional activators to the promoters, and this role is not affected by nutrient deprivation (Yu & Morse, 1999). In fact, both Rap1 and Fhl1 can be found constitutively bound to RP and RIBI promoters. However, Fhl1 activity is subjected to nutrient regulation. Nutrients influence Pol I activity by regulating the localization of two Fhl1-binding cofactors, the activator Ifh1 and the repressor, Crf1 (Martin *et al.*, 2004). In optimal growth conditions, TORC1 retains Crf1 in a dephosphorylated state in the cytoplasm, whereas upon nutrient deprivation the PKA-responsive antagonist, Yak1, phosphorylates Crf1 provoking its nuclear translocation. In the nucleus, Crf1 competes with Ifh1 to bind Fhl1, which then represses its function. Another important Pol II transcription factor subjected to nutrient regulation is the split finger factor Sfp1. Deletion of *SFP1* causes defects in expression, processing, and localization of RPs, which in turn results in slow growth and very small cell volume (Jorgensen *et al.*, 2002, 2004; Marion *et al.*, 2004). Nutrient depletion causes Sfp1 cytoplasmic binding to the Rab escort protein, Mrs6 (Singh & Tyers, 2009). Under optimal growth conditions, it localizes to the nucleus upon previous binding and phosphorylation by TORC1 (Lempiainen & Shore, 2009). Although both Sfp1 and Sch9 mediate a TORC1 positive regulation of RIBI and RP genes, interaction of Sfp1 with TORC1 reduces Sch9 phosphorylation, resulting in a negative feedback loop (Lempiainen & Shore, 2009). Repression of these genes upon nutrient limitation is also enhanced via Blm10-dependent proteasome degradation of Sfp1 (Lopez *et al.*, 2011).

Nutrient control of Pol III

RNA polymerase III transcription in yeast is negatively regulated by Maf1, a repressor highly conserved in eukaryotes (reviewed by Boguta, 2013). Maf1-mediated repression is activated by rapamycin, starvation, nutrient depletion, and a variety of stress conditions. Maf1 is a protein with multiple phosphorylation sites. It is dephosphorylated mainly by protein phosphatase 4 (PP4), and in the dephosphorylated state, it represses Pol III transcription (Oficjalska-Pham *et al.*, 2006; Oler & Cairns, 2012). It is phosphorylated by different kinases in response to different signaling pathways and the phosphorylation states determine both its export to the nucleus and its ability to inhibit Pol III. Four main protein kinases have been described to act on Maf1, PKA, Sch9, Tor, and casein kinase 2 (CK2). PKA-mediated phosphorylation of Maf1 prevents its shuttling to the nucleus and thus downregulates Pol III activity (Moir *et al.*, 2006). TORC1 affects Maf1 activity indirectly by phosphorylating its substrate protein kinase, Sch9. Maf1 is phosphorylated by Sch9 also counteracting its migration into the nucleus and inhibition of Pol III (Huber *et al.*, 2009; Lee *et al.*, 2009). Finally, Maf1 phosphorylation by CK2 takes place directly on the promoters and helps to release Maf1 from chromatin, releasing Pol III from inhibition (Graczyk *et al.*, 2011; Moir *et al.*, 2012).

Nutrient-sensing mechanisms

In summary, quite detailed information is available on the downstream processes involved in ribosomal biosynthesis and their regulation by signaling pathways. Up to now, however, little information has been gained on nutrient-sensing mechanisms specifically involved in the control of ribosomal biogenesis. Maybe such mechanisms do not exist and nutrient regulation of ribosomal biogenesis is mainly carried out by an interplay of the different specific nutrient signaling pathways that have been identified up to now. The only pathway that seems from a physiological viewpoint able to regulate ribosomal biogenesis as a function of the availability of all essential nutrients is the FGM pathway. Future research will have to show whether the FGM pathway is directly connected or involved in nutrient control of cell growth. Many ribosomal components are conserved from yeast to humans, and ribosomal biogenesis as a function of nutrient conditions is likely important in all cell types. Hence, the studies in yeast provide an important model to understand nutrient control of eukaryotic cell growth in general.

General conclusions and outlook

Nutrient regulation of metabolic and other physiological properties has been a very active area of research in the model yeast *S. cerevisiae*. Most attention has focused on specific nutrient regulatory pathways, in which a nutrient regulates its own transport and metabolism. For these nutrient signaling pathways, most information has been gained in general on the components of the signal transduction pathway and on the regulation of the

downstream targets, for instance the transcription factors controlling the expression of target genes. The actual nutrient-sensing mechanisms have been more difficult to identify, with galactose induction being a notable exception. The discovery of several types of plasma membrane nutrient sensors, including a GPCR, several transporter-like sensors, and multiple transceptors, has firmly established yeast as the leading model organism in the field of cellular nutrient sensing. A major challenge for the future is the elucidation of the molecular mechanisms involved in nutrient responses that at least partially depend on metabolism of the nutrient. These mechanisms are much more difficult to identify because of the complex nature of metabolism and the many side effects caused by genetic modification of metabolic pathways. It can be predicted easily that there must be many more allosteric interactions between metabolic intermediates and components of signaling pathways than what is currently known. A major mechanism likely to be identified soon is that involved in activation of the Ras proteins by one or more intermediates of glucose catabolism. Because of the importance of Ras in cancer induction in mammalian cells and the well-known overactive glycolysis in cancer cells, that is, the Warburg effect, elucidation of this mechanism may have major consequences for our general understanding of the connection between glycolysis and control of cellular proliferation. The TOR pathway has long been considered as the main integrator of multiple nutrient signals for cellular growth control. However, more and more evidence indicates that the TOR pathway is primarily a specific nitrogen-sensing pathway, with a main role in coordinating the availability of extracellular nitrogen with that of intracellular nitrogen reserves and with its effect on cellular growth being one of the multiple outcomes of this function. Hence, a major challenge for the future remains to be the identification of the nutrient sensors that regulate cellular growth. In this respect, it is important to realize that all essential nutrients, the macronutrients providing carbon, nitrogen, phosphorus, and sulfur, as well as the micronutrients like metal ions and vitamins, have a decisive effect on cellular growth control and hence should all be sensed in some way to exert this function. A specific mechanism may exist for the regulation of cellular growth by each nutrient, but alternatively, a common principle may be involved in sensing all essential nutrients for cellular growth control and these nutrient sensors may interact much more directly with the protein synthesis machinery than previously anticipated. At present, nutrient control of bulk protein synthesis remains vague and the true relevance of the few controls identified remains ill-defined. Another gap in our understanding is the link between initial nutrient responses and long-term adaptation to the same nutrient. At present, we know that in the nutrient responses for which it has been investigated, the two processes have different requirements, but how the rapid response proceeds to the long-term response at the molecular level is unknown. The powerful genomic and proteomic technologies currently available have led to rapid progress in identifying the scope of signal transduction pathway targets. Most of this information, however, has been obtained with gene deletion or overexpression strains, or using small-molecule inhibitors that completely inactivate the target protein. This raises the question to what extent the very many changes in target genes or proteins usually detected are physiologically relevant. Another outcome of these studies has been that the signaling pathways investigated affect targets in other parts of metabolism than previously considered. Here too, the true physiological relevance of the widened scope remains to be determined.

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Authors' contribution

M.C., J.S. and H.N.K contributed equally to this work.

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