Genetics and Regulation of Glycogen and Trehalose Metabolism in *Saccharomyces cerevisiae*

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Abstract Glycogen and trehalose are two important glucose stores of the yeast *Saccharomyces cerevisiae*, and the content of which varies strongly and rapidly in response to changing environmental conditions. Although the metabolic pathways of these two glucose stores have been studied for decades, recent biochemical and molecular studies have unraveled unexpected metabolic features, such as the ability to accumulate glycogen in the absence of glycogenin, the demonstration that acid trehalase encoded by *ATH1* is localized at the cell surface instead of the vacuole and

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allows cells to grow on trehalose. It is also clearly demonstrated that glycogen and trehalose pathways are subject to hierarchical control dependent on major nutrient-sensing protein kinases, namely TOR, PKA, Snf1 kinase homologous to mammalian AMP-activated protein kinase (AMPK), Pho85p, and the energy sensor Pas kinase. The sophisticated control mechanisms highlight the importance of these two glucose stores in the context of growth and cell cycle of the yeast.

1 Introduction

The budding yeast *Saccharomyces cerevisiae* accumulates two types of glucose stores, glycogen and trehalose. Glycogen is a high molecular mass branched polysaccharide of linear α-(1,4)-glucosyl chains with α-(1,6)-linkages, whereas trehalose is a nonreducing disaccharide composed of two α-(1,1)-linked glucose molecules. The content of these two glucose stores varies strongly and rapidly in response to changing environmental conditions, which emphasizes their role as energy and carbon resources in yeast cells. Detailed biochemical and molecular studies over the past 10 years have shown that both glycogen and trehalose metabolic pathways are subject to hierarchical control dependent on major nutrient-sensing protein kinases, namely TOR, PKA, Snf1 kinase homologous to mammalian AMP-activated protein kinase (AMPK), Pho85p, and the energy sensor Pas kinase. This chapter provides an overview of the genetic and metabolic control of storage carbohydrate metabolism and discusses these mechanisms in the context of growth and cell cycle of the yeast *S. cerevisiae*. For enzymatic systems participating in glycogen or trehalose metabolism, readers can refer earlier reviews (Lomako et al. 2004; Gancedo and Flores 2004; Parrou et al. 2005).

2 Metabolic Pathways

This section describes pathways and regulation of glucose storage in the yeast *Saccharomyces cerevisiae*. It includes new and unexpected features underlined using combined approaches of genomics, genetics, and proteomics by researchers over the last 10 years.

2.1 The Glycogen Metabolic Pathway

Glycogen is a highly branched polysaccharide of linear α-(1, 4)-glucosyl chains with α-(1, 6)-linkages. Each linear chain has an average length of 13 glucose units and contains two branching points by means of α-(1, 6) glycosidic bonds (Fig. 1). This structural organization results in a spherical shape of the glycogen molecule

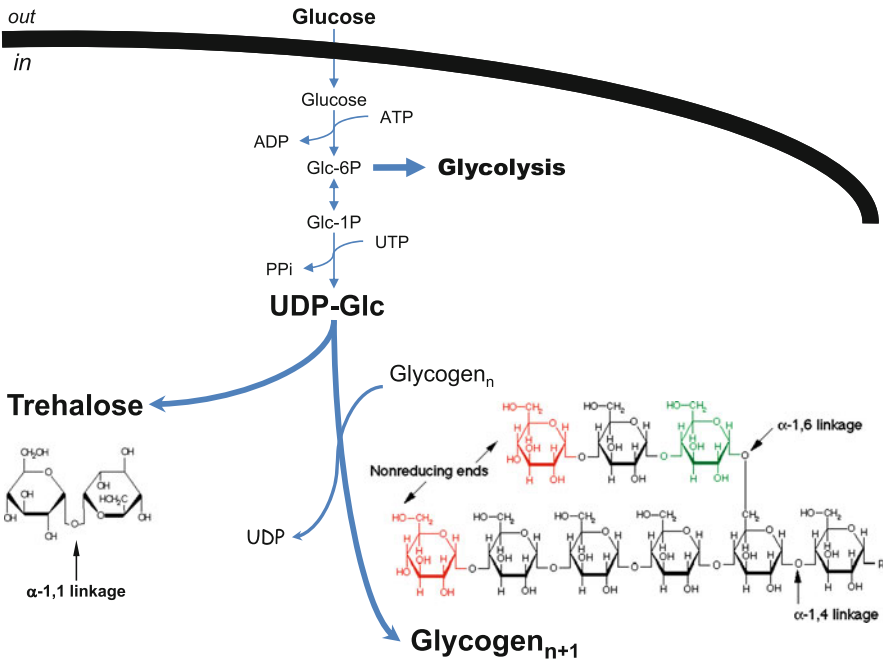

Fig. 1 Structures of glycogen and trehalose and their metabolic routes from glucose in the yeast *S. cerevisiae*

reaching a molecular mass $>10^6$ Da (Melendez et al. 1999). As it is the case for the production of any polymers, the formation of glycogen requires enzymes for initiation, elongation, and ramification. The initiation step is carried out by a protein denoted "glycogenin" which preferentially uses UDP-Glc but can accept CDP-Glc or TDP-Glc as substrates (Alonso et al. 1995a) to produce a short α-(1,4)-glucosyl chain covalently attached to a tyrosine residue by autoglucosylation activity. This initiation step is specific for all eukaryotic cells and has not been identified in bacteria in which the glycogen synthase is responsible for both initiation and elongation (Ugalde et al. 2003). In yeast, glycogenin is encoded by two genes, *GLG1* and *GLG2* (Cheng et al. 1995; Cheng et al. 1995). However, loss of function of these genes did not result in a complete glycogen deficiency but in a stochastic accumulation of glycogen particles in some individual colonies (Torija et al. 2005). In addition, the occurrence of these glycogen positive *glg1 glg2* mutant colonies is strongly enhanced by the presence of a hyperactive glycogen synthase or upon deletion of *TPS1*, encoding a subunit of the trehalose synthase complex. Altogether, these results contradict previous claims that glycogenin is essential for glycogen biogenesis in eukaryotic cells (Lomako et al. 2004) and favor the idea that the initiation step can take place using alternative primers whose synthesis and/or distribution may be controlled by epigenetic silencing (Torija et al. 2005). On the other hand, overproduction of glycogenin does not lead to hyperaccumulation of

glycogen (Cheng et al. 1995), indicating that these proteins are likely reiteratively used in the glycogen synthesis process. Details of this reiteration process remain to be demonstrated.

The elongation step is catalyzed by glycogen synthase that operates by the successive addition of α-1,4-linked glucose residues to the nonreducing end of glycogen, using UDP-Glc as the donor substrate. The yeast *S. cerevisiae* contains two genes, *GSY1* and *GSY2*, encoding two glycogen synthases (Fig. 2) that are 80% identical at the protein level and share 50% similarity with the mammalian muscle enzyme. Gsy2p was shown to be the predominant glycogen synthase as indicated by a 90% reduction in both enzyme activity and glycogen levels in a *gsy2Δ* mutant growing on glucose (Farkas et al. 1991; Farkas et al. 1990). However, under other conditions such as during growth on galactose, Gsy1p appears to be as important as Gsy2p in glycogen accumulation (JL Parrou & J François, personal communication).

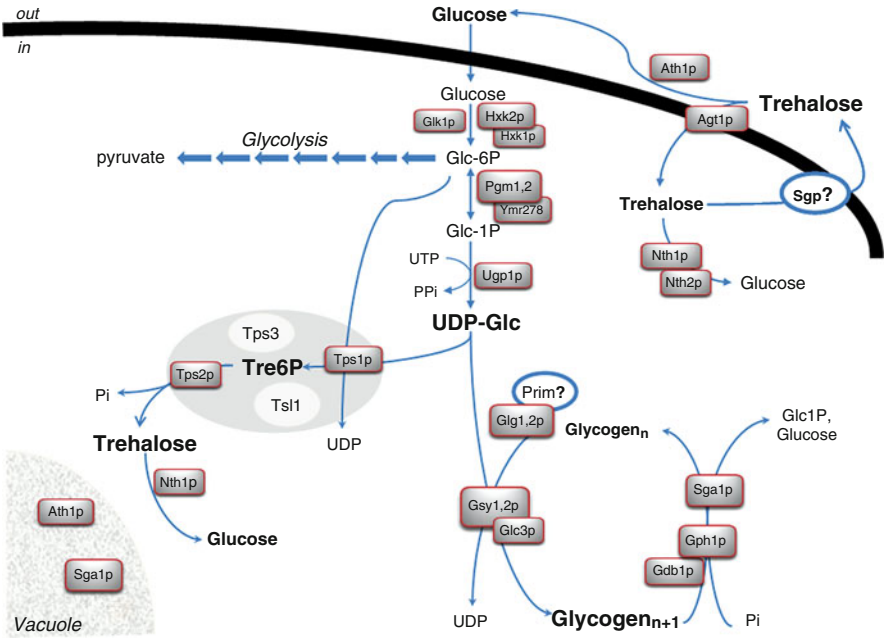

Fig. 2 A schematic illustration of the metabolic pathways for glycogen and trehalose biosynthesis and biodegradation and for trehalose assimilation in the yeast *S. cerevisiae*. Putative or yet uncharacterized proteins in the pathways are outlined. Abbreviation of the enzyme name: Hxk2p, hexokinase II; Hxk1p, hexokinase I; Glk1p, glucokinase; Pgm1,2p, phosphoglucose mutase isoform 1 and 2; Ymr278p, homologous to phosphoglucomutase; Ugp1p, uridylylglucose pyrophosphorylase; Glg1,2p, glycogenin isoform 1 & 2; Gsy1,2p, glycogen synthase isoform 1 & 2; Glc3p, glycogen branching enzyme; Gph1p, glycogen phosphorylase; Gdb1p, glycogen debranching enzyme; Sga1p, amylo (1 → 4), (1 → 6) glucosidase; Ath1p, acid trehalase or extracellular trehalase; Agt1p, α-methylglucose transporter; Nth1, 2p, neutral trehalase isoform 1 & 2; Sgp1p, putative sugar permease; Tps1p, trehalose-6-phosphate synthase; Tps2p; trehalose-6-P phosphatase; Tps3p & Tsl1p, regulatory subunit of the trehalose synthase complex

Elongation of glucosyl chains in glycogen is followed by the branching step catalyzed by an amylo α-(1,4), α-(1,6)-transglucosidase (branching enzyme) encoded by *GLC3* (Thon et al. 1992). This enzyme transfers a terminal stretch of seven glucose residues from the linear α-(1, 4) glucosyl chain to another linear chain making an α-(1, 6) bond between glucosyl units deeper in the molecule.

Like in mammals, metabolic regulation of glycogen synthesis in yeast is brought about through allosteric control of glycogen synthase by Glc6P and by reversible covalent phosphorylation. The nonphosphorylated, highly active form of glycogen synthase is insensitive to Glc6P, whereas the phosphorylated, less active form is highly dependent on the presence of the sugar phosphate. Thus, measurement of glycogen synthase activity in the absence and in the presence of Glc6P gives a direct value of the ratio between these two interconvertible forms (Francois and Hers 1988; Pederson et al. 2000). Mutagenesis studies in yeast (Pederson et al. 2004; Pederson et al. 2000) identified two conserved Arg clusters (Arg579/580/582/583 and Arg586/587/588/591) that are part of the allosteric control site for Glc6P. Refinement of the crystal structures corresponding to the basal activity state and glucose-6-phosphate activated state of yeast glycogen synthase-2 allowed showing that the enzyme is assembled into an unusual tetramer by an insertion unique to the eukaryotic enzymes, and this subunit interface is rearranged by the binding of glucose-6-phosphate, which frees the active site cleft and facilitates catalysis. Two arginine residues at positions 583 and 587 are shown to be responsible for the enzyme's response to control by Glc6P, while the other Arg residues are implicated in the phosphorylation response of Gsy2p (Baskaran et al. 2010). Glycogen synthase possesses three phosphorylation sites (Ser-650, Ser-654, and Thr-667) at its C-terminus, which is in accordance with a maximum of 3 moles phosphate/mol protein incorporated in the purified inactive glycogen synthase (Peng et al. 1990) and with the observation that the removal of the C-terminus by mild proteolysis results in a fully active, Glc6P-insensitive form of Gsy2p (Hardy and Roach 1993). The cyclin-dependent kinase Pho85 and the PAS kinase encoded by *PSK1* and *PSK2* (Rutter et al. 2002) are the two so far identified kinases that can directly phosphorylate Gsy2p *in vitro* and *in vivo* (Huang et al. 1998; Rutter et al. 2002). The cAMP-dependent protein kinase (PKA) is not effective on this enzyme (Hardy and Roach 1993) in spite of the fact that several mutants impaired in the PKA activity exhibit strong alteration of their glycogen content. The action of Pho85p on Gsy2p requires association of Pho85p with its cyclin partners Pcl8p and Pcl10p. This protein complex facilitates phosphorylation of Gsy2p at Ser-654 and Thr-667 (Wilson et al. 1999) (Fig. 2), and failure of this phosphorylation event results in a hyperactive glycogen synthase and higher glycogen content of the cells (Wang et al. 2001; Timblin et al. 1996). On the other hand, the PAS kinase only phosphorylates the Ser-654 *in vivo*. This phosphorylation is physiologically relevant since a mutant defective in this kinase has a higher Glc6P-dependent glycogen synthase activity (Rutter et al. 2002). The reversibility of a phosphorylation event is ensured by protein phosphatases, which remove the covalently bound phosphate from Ser/Thr. On Gsy2p, this task is mainly taken over by type I Ser/Thr protein phosphatase encoded by *GLC7*, which is targeted to the glycogen synthase by a specific targeting

subunit encoded by *GAC1* (Francois et al. 1992; Skroch Stuart et al. 1994). A tripartite interaction of Gac1p with Glc7p and Gsy2p has been demonstrated, and these interactions are necessary for a productive and complete dephosphorylation of glycogen synthase (Wu et al. 2001). In addition to Gac1p, two proteins encoded by *PIG1* and *PIG2* isolated by two-hybrid screen with Gsy2p as the bait (Cheng et al. 1997) may participate in the control of glycogen synthesis since a *gac1 pig1 pig2* triple mutant shows a more severe glycogen defect than a *gac1* single mutant, whereas a *pig1 pig2* double mutant does not show any glycogen defect (Cheng et al. 1997; J. Francois, unpublished data). Besides the major role of the type I Ser/Thr protein phosphatase, type 2A protein phosphatase has been shown to exert a minor effect on glycogen synthase, but this control is likely to take place at the transcriptional level (Posas et al. 1993) (see below).

In summary, the activity of glycogen synthase is controlled by the dynamic equilibrium between the active, nonphosphorylated form and the less active, phosphorylated form of the enzyme. Whether the active or less active form of glycogen synthase is more abundant in the cells depends on the relative activities of kinases and phosphatases that are acting on Gsy2p. In addition to its role as an allosteric activator of glycogen synthase, Glc6P likely orchestrates the transition between the different phosphorylation states of Gsy2p by stimulating dephosphorylation and inhibiting phosphorylation of the enzyme (Francois and Hers 1988; Pederson et al. 2004; Baskaran et al. 2010). Therefore, one can expect that any condition leading to dramatic changes in Glc6P should have a direct impact on glycogen synthesis (Fig. 3). This hypothesis is actually supported by mutants defective in phosphoglucose isomerase (*pgi1*), in mutant with a reduced activity of the glycolytic 6-phosphofructokinase (*pfk2*) as they contain both higher Glc6P and higher glycogen levels than the wild type on glucose (Corominas et al. 1992; Huang et al. 1997), as well as in *tps1* mutant defective in trehalose synthesis that also exhibit very high levels of hexose monophosphates (J François, Th Walter, and JL Parrou, unpublished results).

The biodegradation of glycogen in yeast occurs in the cytosol by the sequential actions of glycogen phosphorylase and glycogen debranching enzymes encoded by *GPH1* (Hwang et al. 1989) and *GDB1* (Teste et al. 2000), respectively, which degrade glycogen to glucose-1-P and glucose (Fig. 2). Like in mammals, the yeast glycogen phosphorylase (Gph1p) is activated by phosphorylation, and this phosphorylation occurs on a single threonine residue (Thr^{31}) of the protein (Lin et al. 1995). Since Gsy2p and Gph1p exist as interconvertible forms in the cells, the balance of the two forms depends upon the stringent of the relative activity of the kinases and phosphatases. Unlike for glycogen synthase, there is no technical means to determine the proportion of the two Gph1p forms *in vivo*, although it is feasible with the mammalian cells for which the dephosphorylated, inactive form is highly sensitive (and stimulated by) to AMP (Fletterick et al. 1986). In addition, the protein kinases and protein phosphatases implicated in the regulation of yeast Gph1p regulation have not yet been fully understood. Recent data indicated the implication of the Pho85-Pcl6p/Pcl7p complex in controlling the phosphorylation state of Gph1p. However, this implication was not direct but mediated through the

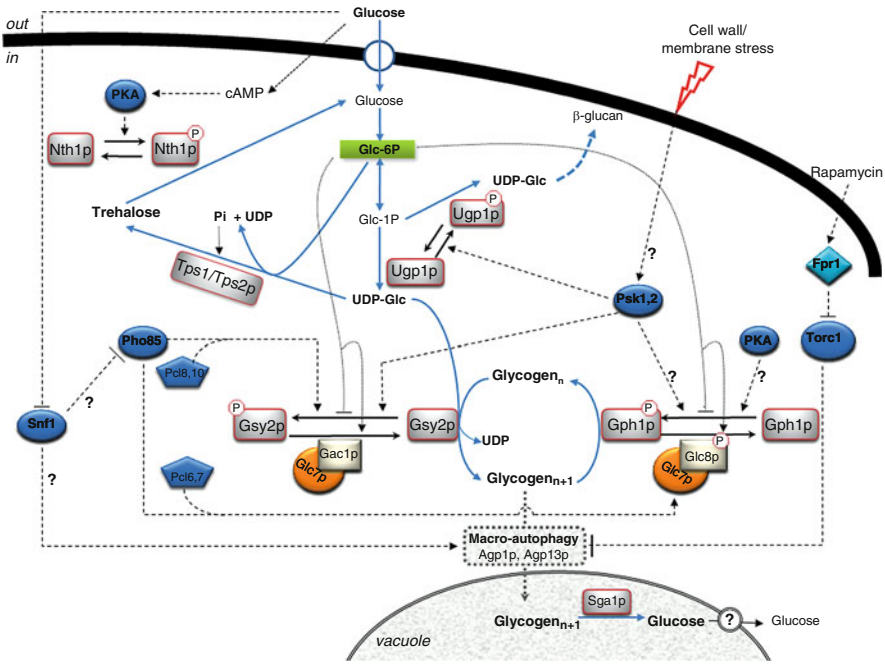

Fig. 3 Scheme of the posttranslational (metabolic) control of glycogen and trehalose in the yeast *S. cerevisiae*. *Question marks* indicate mechanisms of actions either uncharacterized or uncertain. See text for detailed explanations

phosphorylation of Glc8p. This latter protein interacts with the protein phosphatase Glc7p to form a Glc7–Glc8p complex, which in turn dephosphorylates and hence inactivates Gph1p (Wilson et al. 2005). Therefore, and contrary to expectation, the effect of Pho85p is also to inactivate Gph1p as in the same time this kinase inactivates Gsy2p by direct phosphorylation. This result merits further investigation since it contradicts the fact that two enzymes are controlled by an on/off mechanism, posing that glycogen synthase be active when glycogen phosphorylase is inactive and vice versa (Francois and Parrou 2001). The PKA has been reported to phosphorylate Gph1p *in vitro* (Lin et al. 1995) but has not been verified *in vivo*. On the other hand, the crystal structure analysis of phosphorylated and nonphosphorylated Gph1p bound to Glc6P revealed that this metabolite serves as a dephosphorylation facilitator by modifying the accessibility of the phosphorylation site to protein phosphatases (Lin et al. 1996). This finding supports the role of Glc6P as a major effector controlling glycogen phosphorylase activity *in vivo* (Fig. 3).

The other mechanism for glycogen breakdown involves an amylo (1,4), (1,6) glucosidase encoded by *SGA1* that releases glucose as the final product (Colonna and Magee 1978; Clancy et al. 1982). Initially thought to be expressed only during sporulation (Clancy et al. 1982; Chu et al. 1998), *SGA1* has now been found to be induced in late stationary phase or under starvation conditions (Teste et al. 2009).

The encoded protein is localized in vacuoles and serves to hydrolyze glycogen particles that have been imported into vacuole by the autophagy process during growth on glucose. Thus, the vacuolar glycogen pool is protected from degradation by the cytosolic glycogen phosphorylase and takes place only under extreme growth conditions (Wang et al. 2001). The observation that impairment of the vesicular trafficking or of the vacuole formation resulted in hypoaccumulation of glycogen in cells at the stationary phase or under starved conditions argues in favor of this model (Wilson et al. 2002b).

It should be pointed out that glycogen has been recognized as the first biological fractal structure at the molecular level. Fractal objects are complex structures built by an iterative process, which is the case for the glycogen molecule (Alonso et al. 1995b). To successfully produce this fractal structure, the following rules have to be obeyed: (i) the branching activity must be in excess over the synthase such that a new branch is made when it is physically possible, (ii) the growth of glycogen must be favored in the inner growing chains to avoid excessive external growth, and (iii) glycogen phosphorylase should exhibit activities even during the biosynthesis of the polymer in order to correct any mistake under the abovementioned conditions (Melendez et al. 1999). While two out of the three conditions have received experimental evidence (Wilson et al. 2004), the role of glycogen phosphorylase in the synthesis of the fractal glycogen structure remains to be proved.

2.2 The Trehalose Metabolic Pathway

Trehalose is a disaccharide made of two glucose units linked by an α-1 → 1 bond (Fig. 1). The metabolic pathways for synthesis, mobilization, and assimilation of this disaccharide are depicted in Fig. 2. At least five different biosynthetic pathways are known for trehalose synthesis (Avonce et al. 2006). The most widely distributed pathway in nature, present in fungi, consists of two consecutive enzymatic reactions employing a trehalose-6-phosphate-synthase (TPS) enzyme, producing the intermediate trehalose-6-phosphate (Tre6P), and a Tre6p-phosphatase (TPP) enzyme. In filamentous fungi and yeasts, the two activities are borne on a single protein complex, whereas in bacteria, they exist as two separated entities. A recent evolutionary study on trehalose biosynthesis genes provided evidence that the formation of bifunctional protein complexes took place already in some group of bacteria and archea, but the physiological consequence of this protein fusion is still unclear (Avonce et al. 2010). In *S. cerevisiae*, the TPS/TPP complex is encoded by *TPS1* and *TPS2*, respectively, and contains two additional subunits encoded by *TPS3* and *TSL1* that are apparently not present in other fungal TPS/TTP (Kwon et al. 2003; Avonce et al. 2006). These two subunits show high degree of similarity and may function as stabilizer of the complex as suggested by the fact that a *tps3 tsl1* double mutant has a reduced TPS activity and trehalose content (Reinders et al. 1997; Bell et al. 1998). The loss of *TPS1* not only abolishes the synthesis of trehalose but also causes several other metabolic disorders that will be detailed below. Also, the

deletion of *TPS2* results in a temperature-sensitive growth phenotype, which has been attributed to an excessive accumulation of Tre6P since a suppressor of this phenotype was found to be *PMU1* encoding a putative phosphomutase. Overexpression of *PMU1* reduced levels of Tre6P and converted it into yet uncharacterized intermediates (Elliott et al. 1996). In contrast to enzymes of the glycogen pathway, the TPS/TPP is not the subject of reversible phosphorylation. The Tps1p subunit is highly sensitive to inhibition by Pi, which acts as a noncompetitive inhibitor to both Glc6P and UDP-Glc ($K_i \sim 2$ mM) (Vandercammen et al. 1989; Londesborough and Vuorio 1993), whereas Tps2p requires the presence of Pi for full activity. On the other hand, Fru6P acts as an allosteric effector, reducing the Km for Glc6P from around 5 to 1.5 mM. Taking into account these enzymatic data, the *in vivo* rate of Tre6P synthesis is actually largely determined by the availability of Glc6P and UDP-Glc as substrates and by levels of its main allosteric effectors, Fru6P and Pi (Vandercammen et al. 1989; Londesborough and Vuorio 1993), besides the fact that the TPS complex is also subject to repression by glucose (Neves et al. 1991; Winderickx et al. 1996). This may thus explain the rapid accumulation of Tre6P that takes place upon glucose addition to respiring yeast cultures as under this condition, there is a transitory increase of Glc6P and Fru6P, accompanied by a drop of Pi triggers which leads to imbalance of Tps1p and Tps2p activity (Walther et al. 2010). However, trehalose accumulation during stationary phase is not accompanied by a noticeable increase of Tre6P (J. François, unpublished data), which indicates that both trehalose 6-P synthase and phosphatase functioned at the same rate.

In the yeast *S. cerevisiae*, two types of trehalase, distinct in their optimal pH and localization, can hydrolyze trehalose into glucose. *NTH1* encodes a cytosolic trehalase that is optimally active at neutral pH with a relatively high K_m (5–35 mM) for trehalose (Londesborough and Varimo 1984; App and Holzer 1989). A relevant regulatory property of Nth1p is to be activated by phosphorylation. To date, the PKA is the sole protein kinase that has been reported to directly phosphorylate this protein (Fig. 3). Interestingly, the Nth1p harbors eight putative PKA-dependent phosphorylation sites (Wera et al. 1999), but only Ser^{21} and Ser^{23} have been shown to be phosphorylated *in vivo* (Ficarro et al. 2002). In addition, complete activation of Nth1p requires the binding with the 14-3-3 protein encoded by *BMH1*/*BMH2* on phosphorylated Ser^{21} (Panni et al. 2008). Yeast possesses a second functional trehalase encoded by *NTH2* that is 77% identical to Nth1p (Jules et al. 2008). Little is known about the kinetic properties and regulation of the second trehalase, except that it has been implicated in trehalose mobilization in late stationary phase of growth on glucose or upon growth recovery from heat and saline stress (Nwaka et al. 1995; Jules et al. 2008; Garre and Matallana 2009).

Another hydrolase acting on trehalose is encoded by *ATH1*. Strong experimental evidence shows that this trehalase has a dual localization, both at the cell surface and in the vacuole. However, only the cell-surface localized enzyme was found to be active and able to hydrolyse extracellular trehalose (Jules et al. 2004; He et al. 2009). Hence, this localization can account for its requirement for the growth on trehalose as a sole carbon source (Nwaka et al. 1996; Jules et al. 2004) (Fig. 2).

The cell-surface localization of Ath1p is likely mediated by the classical secretion "Sec" pathway, despite the fact that the protein does not harbor any secretion signal (He et al. 2009); S.He and JL Parrou, unpublished data), whereas the delivery of Ath1p to the vacuole follows the multivesicular body pathway (MVB) (Huang et al. 2007). The function of this vacuolar-localized Ath1p is unknown. Since constraining the enzyme into vacuolar impairs the growth on trehalose, no evidence for vacuolar import of trehalose by the autophagy process can be done as it has been shown for glycogen (Jules et al. 2008) (Fig. 2). As a result, it is proposed to replace the terms of neutral and acid trehalase by "cytosolic" and "extracellular" trehalases as they are more adequate to describe the localization and the function of these two enzyme forms (Parrou et al. 2005).

As mentioned above, trehalose can be assimilated as an exogenous carbon source by several fungi, including the yeast *S. cerevisiae* (Parrou et al. 2005). In addition to the Ath1-dependent pathway (Fig. 2), a second route that couples the high-affinity trehalose H^+-symporter encoded by *AGT1* (Plourde-Owobi et al. 1999) with the neutral trehalase encoded by *NTH1* can facilitate cell growth on trehalose (Jules et al. 2004). However, this second pathway is not functional in *mal*$^-$ strain because *AGT1* expression is dependent upon the *MAL* system (Han et al. 1995) or is weakly effective even in Mal$^+$ strain since Agt1p rapidly loses activity during growth on trehalose (Jules et al. 2004). It is noteworthy that the growth on trehalose is strictly respiratory (Jules et al. 2005) and thus subject to the so-called Kluyver effect, i.e., the inability to ferment a sugar even under anaerobic conditions (Fukuhara 2003). This effect is likely due to the rate-limiting activity of Ath1p since the growth rate can be increased to a maximum of threefold by overexpression of *ATH1* (Jules et al. 2005; He et al. 2009). However, no further increase in growth rate could be obtained even after a 20-fold increase in the expression of Ath1p, suggesting that other limiting steps may exist that prevent cells to ferment trehalose.

2.3 UDP-Glucose Partitioning

UDP-glucose is a donor of glucose units at the crossroads between several pathways, including glycogen and trehalose, cell wall β-glucan, and glycosylation of proteins. The production of UDP-Glc is catalyzed by UDP-glucose pyrophosphorylase encoded by *UGP1* (Daran et al. 1995). Significant reduction of UDP-Glc levels by reducing the activity of Ugp1p was accompanied by a significant decrease in glycogen and trehalose production, whereas levels of cell-wall β-glucan were slightly altered, raising the hypothesis that UDP-glucose could be channeled toward the synthesis of β-glucan (Daran et al. 1997). A partitioning of glucose toward β-glucan and away from glycogen (Smith and Rutter 2007) demonstrated that Ugp1p is phosphorylated on Ser^{11} by the PAS kinase with the consequence that the phosphorylated enzyme is targeted to the cell periphery to favor glucan synthesis, while its catalytic activity is not affected (Fig. 3). Therefore, the inability to phosphorylate Ugp1p or the deletion of *PSK1* and *PSK2* leads to elevation of

glycogen and renders the cells hypersensitive to cell wall perturbing agents likely because of a reduction of β-glucan.

3 Nutrients, Stress, and Growth Control of Glycogen and Trehalose

It is well established that levels of glycogen and trehalose in yeast cells vary significantly according to growth, nutrients, and stress conditions (e.g., osmotic, saline, and heat shock (Francois and Parrou 2001)). These variations are accounted largely to the main nutrient-sensing pathways PKA, TOR, and SNF1 (Fig. 4). As recently illustrated by microarrays analyses of starved cells challenged with nutrient repletion (Slattery et al. 2008), the transcription response, which is strongly repressive for the glycogen and trehalose-related genes, is largely dependent on the cAMP/PKA pathway. At present, this repressive effect exerted by the PKA is explained by at least three modes of action. First, and likely the most effective mechanism, is to restrict Msn2/4p in the cytosol, which is facilitated by the PKA-dependent phosphorylation

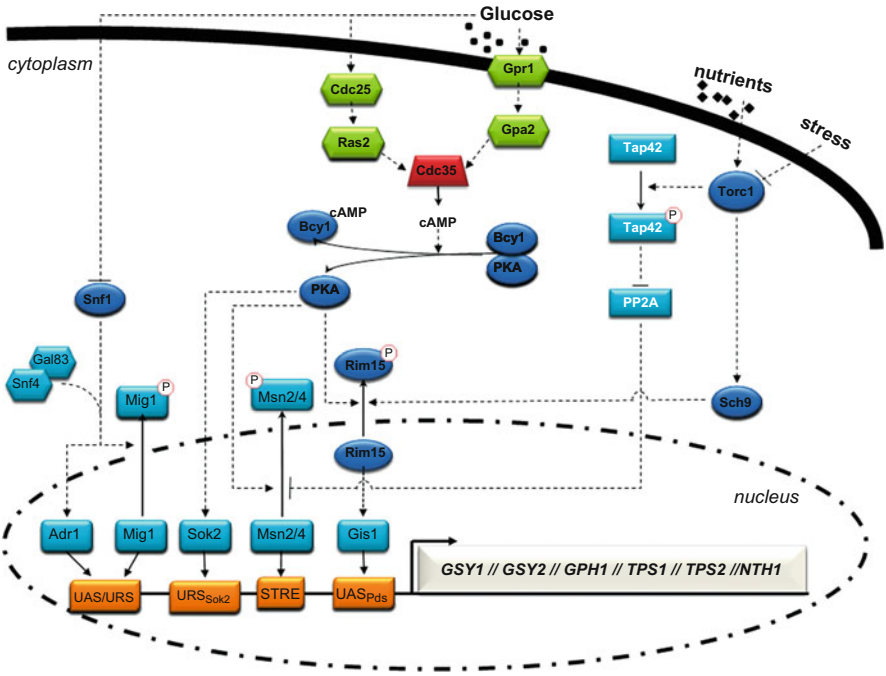

Fig. 4 A schematic illustration of the transcriptional control of glycogen and trehalose by the nutrient-sensing pathways dependent on the PKA, TORC1, and SNF1 kinases in the yeast *S. cerevisiae*. Genes given as targets in these pathways are those encoding principal enzymes in the synthesis and degradation of the two glucose stores. See text for detailed explanations

of this protein (Gorner et al. 2002). Consequently, this restriction prevents the transcription activation of glycogen and trehalose-related genes (to which can be included *PGM2* and *UGP1*, two genes required for the production of UDP-Glc) that normally takes place by DNA binding of Msn2/4p to the STRE elements (CCCCT) present in several copies in the promoter of these genes (Ni and LaPorte 1995; Parrou et al. 1999b; Winderickx et al. 1996; Parrou et al. 1999a; Sunnarborg et al. 2001; Zahringer et al. 2000). The second mechanism implicates the transcriptional repressor Sok2p (Ward et al. 1995) since all important glycogen and trehalose-related genes harbor a consensus motif for *SOK2* in their promoter region. It was reported that overexpression of *SOK2* reduced expression of *GAC1* (Ward et al. 1995); J. François, unpublished data), whereas *SOK2* deletion partially released *GSY2* repression in a mutant with a high PKA activity (Enjalbert et al. 2004). A third pathway by which the PKA exerts its repressing effect is through the blockage of the Rim15-Gis1p cascade. The latter pathway mediates its effects through an upstream activating sequence (UAS_{PDS}) that is present in most of the glycogen and trehalose-related genes. However, this cascade is only operative in stationary phase cells or when cells enter into quiescent G0 state (Pedruzzi et al. 2000; Pedruzzi et al. 2003).

Evidence has been accumulated that the TOR (target of rapamycin) pathway, through its TORC1 complex (complex made of Tor1p or Tor2p with three other partners, Kog1p, Lst8p, Tco89p; see (De Virgilio and Loewith 2006 for a review), also affects storage carbohydrates as shown by rapamycin-induced glycogen and trehalose accumulation in yeast cells growing on glucose (Barbet et al. 1996). This accumulation is accompanied by upregulation of the glycogen and trehalose genes (Zurita-Martinez and Cardenas 2005). This upregulation can be explained by the effect of rapamycin to induce nuclear localization of Msn2/4p via inhibition of TORC1 (Santhanam et al. 2004). Therefore, TORC1 may negatively control storage metabolism through a signaling pathway involving phosphorylation of Tap42p, which in turn inhibits Ser/Thr protein phosphatase 2A by direct binding. Consequently, this latter protein is no longer able to dephosphorylate Msn2p, which therefore remains sequestered in the cytoplasm (Zaman et al. 2008). This mechanism can therefore account for previous reports showing effects of this type 2 phosphatase on glycogen levels (Clotet et al. 1995). Additionally, TORC1 has been shown to negatively control Rim15-Gis1p cascade through the protein kinase Sch9 (De Virgilio and Loewith2006). Altogether, these data bring into light a converging effect of the two main nutrient-sensing pathways, PKA and TOR, on Msn2/4p to regulate expression of glycogen and trehalose genes. Hence, any changes in the balance of the activities of PP2A and PKA may directly impact the expression levels of these genes and eventually on the attendant metabolism. This model is in fact more complex since the PKA is able to phosphorylate the Msn2 protein on both the nuclear localization signal (NLS) and the nuclear export signal (NES) of this protein, whereas PP2A only dephosphorylates NES (Gorner et al. 2002; Santhanam et al. 2004), indicating a prominent effect of the PKA in controlling the localization of Msn2/4p and consequently on Msn2-dependent gene expression. Nonetheless, the mechanism by which nitrogen starvation, heat, or osmotic shock promote activation

of glycogen and trehalose-related genes is mainly due to the PP2A-dependent dephosphorylation as there is no evidence that the PKA activity is modified under these conditions (Zaman et al. 2008) (Fig. 4). However, this model cannot account for the independency between the TOR and the PKA in glycogen accumulation as shown by the fact that yeast cells bearing unbridled PKA activity (i.e. *bcy1* mutant) and treated with rapamycin are still able to accumulate glycogen (Barbet et al. 1996; Zurita-Martinez and Cardenas2005). A possible explanation for this effect is to propose that the apparent glycogen accumulation is actually a consequence of the sequestration of this glucose polymer into the vacuole by the rapamycin-dependent induction of the autophagy process (Noda and Ohsumi 1998; Dubouloz et al. 2005), implying inhibition of the TORC1 but being independent to Msn2/4p and Rim15p (Budovskaya et al. 2004; Yorimitsu et al. 2007) (Fig. 4).

Two additional nutrient-sensor kinases, namely the cyclin-dependent Pho85 kinase and the Snf1 kinase, have been reported to control glycogen and trehalose. However, contrary to the PKA and the TORC1 pathways, which merely exert control at the transcriptional level (see Fig. 4), the regulation by Snf1p and Pho85p takes place at both transcriptional and posttranslational levels. A two- to threefold upregulation or downregulation of glycogen and trehalose metabolism-related genes has been reported in *pho85* and *snf1* mutants, respectively (Timblin and Bergman 1997; Parrou et al. 1999b), but the mechanisms of this control are not yet determined. The presence of binding sites for the transcriptional factors Adr1p or Mig1p in most of glycogen and trehalose-related genes could be the mechanism through which Snf1 kinase exerts its positive transcriptional effect (Fig. 4), whereas effects of Pho85 kinase on expression of these genes are still unclear (Enjalbert et al. 2004). At the posttranslational level, the positive control on glycogen accumulation by Snf1p appears to involve two distinct pathways. On the one hand, the Snf1 kinase has been shown to antagonize the Pho85-dependent phosphorylation of Gsy2p (Huang et al. 1996; Wilson et al. 1999), but how this antagonism takes place is not yet understood. On the other hand, Snf1p can indirectly affect glycogen store through its positive control of the autophagy process involving *APG1* and *APG13* (Wang et al. 2001), as this latter mechanism causes part of glycogen particles to be stored into the vacuole. This process being defective in a *snf1* mutant would account in part for the lack of glycogen in this mutant. Finally, the recovery of glycogen in an *snf1pho85* mutant is explained by a concomitant hyperactivation of glycogen synthase and an apparent recovery of the autophagy process, indicating that Pho85 also controls in an antagonistic manner to Snf1 the autophagy process (Wang et al. 2001).

In summary, several nutrient-sensing pathways impinge on glycogen and trehalose metabolic systems at the transcriptional and posttranslational levels. The PKA pathway is clearly the major transcriptional mechanism of control, whereas Glc6P is the major metabolic effector as it is a direct substrate for trehalose synthesis, a potent activator of glycogen synthase and an inhibitor of glycogen phosphorylase, and last but not least, its binding to these two enzymes favors the dephosphorylation and inhibits the phosphorylation processes (Fig. 4) (Francois and Parrou 2001). Under efficient growth related to available nutrients, such as during growth on glucose rich medium, glycogen and trehalose accumulation is prevented because

the PKA and the TORC1 are fully operative, whereas under growth imbalance related with some nutrients shortage, accumulation of these two glucose stores may be favored. However, the nature of the growth-limiting nutrient is critical for effective accumulation of glycogen and trehalose. In excess of glucose in a nitrogen-depleted medium, the high Glc6P prevailing in this condition favors synthesis of glycogen and trehalose (Francois et al. 1988; Parrou et al. 1999b; Hazelwood et al. 2009). On the other hand, limitation or depletion of sulfate, phosphate, or zinc is not accompanied by the rise of glycogen or trehalose because under this growth-limiting condition, the PKA and the TOR pathways are activated, as indicated by low transcript levels of glycogen and trehalose-related genes (Hazelwood et al. 2009). Finally, it is noteworthy that the coexpression of genes in the biosynthetic and the biodegradation pathways mainly due to the presence of STRE in their promoter may lead to a recycling of trehalose and glycogen (Blomberg 2000; Voit 2003). Whether this recycling, which has been genetically demonstrated to exist under heat shock, saline stresses, and during growth on glucose (Parrou et al. 1997; Parrou et al. 1999b; Pedreno et al. 2002; Mahmud et al. 2009), has any physiological meaning or is a fortuitous consequence of the coexpression of these genes remains to be addressed.

4 Biological Function of Storage Carbohydrates in Yeast

It is well established that glycogen and trehalose are two energy stores for the yeast cells. This section discusses more precisely how and when yeast cells are playing with these two glucose stores thanks to the use of more sophisticated bioprocess conditions combined with the use of dedicated mutants. Besides, the trehalose synthesis pathways are endowed with a peculiar function that is likely needed in the regulation of the energy and carbon metabolism in yeast.

4.1 Function as Energy and Carbon Stores

Glycogen agrees with the concept of an energy store since it is found to accumulate when glucose is still present in the medium, and is only mobilized when all exogenous carbon sources have been exhausted (Parrou et al. 1999b; Wang et al. 2001). Trehalose does not exactly fit with this concept since it accumulates only after glucose has been consumed (Francois et al. 1991). Nevertheless, several biological situations indicate that both glucose stores have an energetic function in yeast cells. A relevant example is found with respiratory-deficient mutant cells, which accumulate larger amount of glycogen during the growth phase on glucose and then readily mobilize it at the onset of glucose depletion because these cells are respiratory deficient and hence cannot resume on the accumulated ethanol or amino acids present in the growth medium. This rapid mobilization coincides with a drop of Glc6P, accompanied by an increase of glycogen phosphorylase and decrease of

glycogen synthase activity (Enjalbert et al. 2000) by a mechanism that may implicate Pho85p kinase as well as other uncharacterized partners (Wilson et al. 2002a). It is worth noting that respiratory mutants are also unable to accumulate trehalose (J. François, unpublished data). A seemingly direct function of trehalose in carbon and energy metabolism has been recently underscored from studies aiming at characterizing whether Ath1 can hydrolyze endogenous trehalose. In this work, an *nth1nth2* mutant defective in cytosolic trehalases was grown on trehalose and then subjected to carbon starvation. This extreme situation resulted in a rapid mobilization of trehalose by a mechanism involving first its export out of the cell by a yet uncharacterized exporter, the hydrolysis of the exported trehalose at the cell surface by Ath1p, and the subsequent uptake of the released glucose (Jules et al. 2008).

It is well known that accumulation of reserve carbohydrates is favored at lower growth rates under carbon- or nitrogen-limited conditions. In fact, this accumulation is proportional to the duration of the G1 phase of the growth cycle (Sillje et al. 1999; Paalman et al. 2003) and correlates with the transcriptional activation of glycogen and trehalose-related genes (Brauer et al. 2008; Hazelwood et al. 2009). In contrast, it is reduced by overexpression of the G1 cyclin Cln3, the translation rate of which is positively regulated by the TORC1 kinase (Barbet et al. 1996). These data are in accordance with the recent proposition that TORC1 is the major controller of growth rate in response to nutrient availability (Castrillo et al. 2007). The stored carbohydrates can be readily mobilized upon raising the growth rate, and this rapid mobilization is likely to supply ATP surplus required for budding process since a good correlation has been obtained between the increase in the budding index and the extension of reserve carbohydrates mobilization, when the growth rate was suddenly increased from 0.05 to 0.15 h^{-1} (Guillou et al. 2004). This experimental approach is strongly reminiscent to the energy-metabolism oscillations (EMO) that arise spontaneously under glucose- or nitrogen-limited continuous cultures at low dilution (growth) rate, showing periodicity of approximately 300 min of waves of accumulation and mobilization of reserve carbohydrates, as first reported almost 40 years ago (Kuenzi and Fiechter 1972). Such oscillatory behavior has been also observed in batch culture of yeast on trehalose (Jules et al. 2005). This EMO has been recently investigated in a system-level approach, showing that it is composed of two distinct phases termed respiro-fermentative and respiratory period, respectively. The transition between these two periods is basically characterized by a periodic change in the $NADH/NAD^+$ ratio, where the ratio is high during the respiro-fermentative period and low during the respiratory period (Xu and Tsurugi 2006). The importance of trehalose and glycogen in controlling EMO has been illustrated using mutants defective in the synthesis of trehalose (*tps1* mutant) that exhibit destabilized EMO (Xu and Tsurugi 2007), while mutants defective in glycogen (*gsy2* or *gsy2gsy1p* mutants) show very weak oscillatory waves (Xu and Tsurugi 2006; J. Francois, unpublished results). At a global transcriptomic level, these spontaneous oscillations, which were also termed yeast metabolic cycle (YMC), revealed that over half of yeast genes exhibited periodic expression patterns, with a common period of transcript oscillation of ~300 min (Tu et al. 2005). Using an

unbiased k-means cluster analysis, these authors identified three superclusters defining a temporal compartmentalization of the oscillations in three major phases, namely the Ox (oxidative), the R/B (reductive-building), and R/C (reductive-charging) phase, respectively. The Ox clusters mainly comprises genes involved in ribosome and protein synthesis, the R/B cluster was enriched of genes encoding proteins required for DNA replication and genes encoding mitochondrial proteins, whereas the R/C supercluster contained proteins involved in protein degradation, peroxisomes, fatty acid oxidation as well as genes of the glycogen and trehalose metabolism. Taking into account the metabolic events identified in the EMO, the respiratory phase would correspond to the last part of R/B and R/C, as it is the period during which storage carbohydrate accumulates and the respiratory quotient (RQ) is close to 1.0. On the other hand, the respiro-fermentative phase corresponds to Ox and to the beginning of R/B during which stored carbohydrates are liquidated and the RQ $>$ 1.0, corresponding to a reductive, highly glycolytic metabolism. Recently, an interesting model was proposed that the temporal compartmentalization of respiration and the restriction of DNA replication to the reductive phase of the metabolic cycle are to protect cells for genomic integrity (Chen et al. 2007). However, another model assigns the sudden mobilization of reserve carbohydrates to specific metabolic requirements to pass the START at the G1/S transition of the cell cycle, as proposed by Futcher (Futcher 2006). This author proposed the "finishing kick" hypothesis which states that at low growth rate, the cell organizes its metabolism to store sufficient carbohydrates during the G1 phase then suddenly burns it to provide an additional burst of ATP for biosynthesis processes in late G1, resulting in increased budding rate. This finishing kick hypothesis also suggests that the critical size that has to be reached to pass through the Start could be correlated to the stored carbohydrates. However, the function of reserve carbohydrate as cell sizer remains to be verified. Moreover, the hypothesis of a finishing kick is only valid for slow growing cells since rapidly growing cells do not store glycogen or trehalose but show normal cell cycle progression.

While the mechanism that governs the synthesis of glycogen and trehalose during G1 may be dependent on a reduction of the TORC1 activity, the rapid mobilization of the stored carbohydrates in late G1 coincided with a transient burst of cAMP (Xu and Tsurugi 2006; Muller et al. 2003). This suggests that mobilization of the stored carbohydrate is mediated by the PKA pathway. In favor of this model, trehalase and glycogen phosphorylase activity was found to increase at this period (Muller et al. 2003; J. François, unpublished data).

4.2 Specific Function of Trehalose as a Stress Protectant

A number of reports have shown that the trehalose molecule is endowed with the unique property to act as a replacement of water molecule to stabilize proteins and membranes from dessication. However, recent results indicate that trehalose is neither necessary nor sufficient for dessication tolerance in yeast (Ratnakumar and

Tunnacliffe 2006). Thermotolerance has also been reported to be a synergistic effect due to the accumulation of trehalose acting as chemical chaperones and molecular chaperones (Singer and Lindquist 1998; Lee and Goldberg 1998). However, Thevelein's group recently showed that additional unidentified factors may participate in this resistance since a mutant strain defective in adenylate cyclase (*fil1* mutant) that was rendered unable to accumulate trehalose and lacking also Hsp104 protein still exhibited elevated thermotolerance (Versele et al. 2004). Besides, the role of trehalose in the acquisition of thermotolerance may be dependent on the property of this disaccharide to activate Hsf1 (Bulman and Nelson 2005; Conlin and Nelson 2007), which is an essential transcriptional regulator of heat shock response in eukaryote (Amoros and Estruch 2001). On the other hand, recovery of viability of cells from heat shock or saline stress required that the accumulated trehalose is mobilized, to allow proper recovery to normal conditions (Wera et al. 1999; Garre and Matallana 2009). The proposed explanation is that the disaccharide can interfere with the refolding of denatured proteins by HSPs that takes place upon return from heat shock or salt stress (Singer and Lindquist 1998). The adaptation of yeast cells to near-freezing temperatures seems also to be linked to the presence of trehalose. Shifting temperature from 25 to $< 10°C$ is accompanied by a dramatic rise in trehalose and by a Msn2/Msn4p-dependent induction of genes related to its synthesis as well as genes encoding some HSP proteins (Panadero et al. 2006; Schade et al. 2004). It has been observed that an *msn2*/*msn4* mutant dies quickly when maintained at temperature below 10°C (Kandror et al. 2004), but it has not been shown whether this rapid death was due to the lack of trehalose. Finally, trehalose protects cells from damage induced by oxygen radicals as well as from ethanol toxicity (Benaroudj et al. 2001; van Voorst et al. 2006). These protective effects are likely due to the property of this disaccharide to prevent proteins to be damaged under these harsher conditions.

5 The Role of Tps1/ Trehalose-6-Phosphate in Carbon and Energy Metabolism

An unexpected link between the trehalose and the glycolytic pathway is that mutations in *TPS1* prevent growth on rapidly fermentable carbon sources (reviewed in (Gancedo and Flores 2004). The metabolic phenotype that characterizes this mutant is a massive accumulation of sugar phosphates and precipitous depletion of ATP immediately after glucose addition. These effects are likely responsible for the inability of a *tps1* mutant to growth on glucose. The *tps1* mutant can grow on less rapidly fermented sugars such as galactose or raffinose that also depend on the function of the glycolytic pathway causing, however, lower flux over this pathway. In addition, catabolism of these sugars differs from the one of glucose in the sensing and the uptake mechanisms, respectively (Gancedo 2008). This led to the hypothesis that the lack of growth of the *tps1* mutant could be caused by deregulation of the transport step or of the glucose phosphorylating activity.

Three models have been put forward trying to explain the involvement of Tps1 in the control of the sugar influx and, by extension, in the regulation of glycolysis. The first model is based on the finding that the main hexokinase in yeast encoded by *HXK2* is inhibited by Tre6P, the product of the Tps1p reaction (Blazquez et al. 1993). Though quite attractive at first sight, the Tre6P inhibition model of hexokinase is probably incomplete since yeast cells growing exponentially on glucose (and thus in a highly glycolytic state) or overexpressing *TPS2* that encodes the trehalose 6-P phosphatase have barely detectable Tre6P levels while growth on glucose is not impaired (Hohmann et al. 1996). Also, growth and fermentative capacity of yeast are not altered after replacement of a Tre6P-sensitive hexokinase by an enzyme insensitive to this metabolite (Bonini et al. 2003). More importantly, it was recently found that *TPS1* from *Y. lypolitica* fully complemented growth of an *S. cerevisiae tps1Δ* mutant on fructose, even though Tre6P was barely detected in this mutant (C. Gancedo and J. François, unpublished results). Such a result brings us to the second hypothesis which proposes that besides its catalytic function, Tps1p may have a regulatory role, as for instance by restricting sugar influx through a yet unidentified protein interaction. Evidence in support of this hypothesis came from work on the pathogenic fungus *Magnaporthe grisea*, in which the introduction of a noncatalytic form of Tps1p into *tps1* mutants from this fungus recovered its capacity to invade rice leaves which was lost upon deletion of the protein. Another interesting observation was made in the model plant *A. thaliana* where a single point mutation in the *AtTPS6* gene resulted in many phenotypes, although the mutated variant protein kept its catalytic function (Chary et al. 2008). In yeast, there is so far no direct evidence supporting the hypothesis of a regulatory role exerted by the Tps1 protein independently from its reaction product, Tre6P. However, the fact that *tps1* mutants are also unable to undergo sporulation, a process that occurs in the absence of fermentable carbon sources and thus independently from Tre6P, supports the idea that the Tps1 protein has functions other than the simple formation of the Tre6P from UDP-Glc and Glc6P (Silva-Udawatta and Cannon 2001). In addition, it was shown that Tps1p may be present as a free protein (i.e., not bound to the TPS protein complex) (Bell et al. 1998), and recent global interactomics studies indicate that Tps1p may belong to a large interactomic network, whose partners mainly fall into the MIPS categories of energy and metabolism (27%), cell rescue and defense (12%), and cell cycle and DNA processing (10%) (Krogan et al. 2006; Gavin et al. 2006). The third hypothesis proposes that the trehalose biosynthetic pathway can serve an additional function, i.e., in the recovery of inorganic phosphate that is required for the functioning of glycolysis at the level of glyceraldehyde 3-P dehydrogenase. The importance of Pi replenishment in rescuing growth of *tps1* on highly fermentative sugars has been illustrated by hyperactivity of the Gpd1p and/or of the glycerol facilitator encoded by *FPS1* (Van Aelst et al. 1991) that both result in excess glycerol formation at the expense of triose intermediates DHAP. The rapid drop Pi in a *tps1* mutant is likely not collateral effect of the lack of sugar influx but may be a direct consequence of the lack of activation of H^+-ATPase in a *tps1* mutant (Th Walther and J François, unpublished results).

Recent genome-wide analyses on pairwise genetic interactions have provided new insights on how Tps1p may impinge on cellular growth. These results indicated that *TPS1* negatively interacts with more than 200 genes, whose functions mainly fall into the MIPS functional categories vesicle formation and vesicular transport, phosphate metabolism, budding/cell polarity, cell wall, and general stress response. It suggests that the levels of Tre6P or the Tps1 protein itself may be critical in regulating some targeted cellular functions by coordinating sugar metabolism with cell growth, budding, and cell wall synthesis according to carbon availability. A similar hypothesis has been raised for the role of Tre6P in plant in coordinating sugar metabolism with development, particularly with the cell wall synthesis that depends on the supply of Glc6P and UDP-Glc (Paul et al. 2008). Also and quite intriguingly, a considerable number of genes that negatively interact with *TPS1* were found to positively interact with *TPS2* and *vice versa* (Fiedler et al. 2009; Costanzo et al. 2010). These genes provide candidate cellular functions that are controlled by Tre6P since this metabolite is absent in *tps1* mutants and exhibits hyperaccumulation in *tps2* strains. Genes that show negative interaction with *TPS1* and positive interaction with *TPS2* include (among others) *ANP1, RIM20, CHS5, PSD1, WHI2, COG7, RSP5, DFG16, ADO1, ATX1,* and *VSP9* , whereas the opposite situation is found for *HXK2, UBR1, PAP1, PMA1, YHC1*, and *CAK1*. These findings support a potential *direct* implication of Tre6P in the regulation of vesicle formation, phospholipid metabolism, and Pi/ATP homeostasis. In short, converging data strongly support an essential function of Tps1 and its metabolite Tre6P in the regulation of carbon and energy metabolism in yeast, for which the precise mechanism of the action and relevant cellular targets remain to be identified.

6 Conclusion and Perspectives

The yeast *S. cerevisiae* accumulates two storage carbohydrates, glycogen and trehalose, that fulfill and share, in some cases, specific functions. The control of the metabolism of these two glucose stores is extremely sophisticated and is likely meant to satisfy rapidly changing energetic needs during cell cycle and upon changes in nutrient availability. Among numerous questions regarding mechanisms by which the nutrient-sensing pathways impact on storage carbohydrate metabolism, two major problems need to be solved. The first one is to identify the alternative priming system that allows glycogen to be synthesized without glycogenin that also includes the elucidation of the stochastic nature of this alternative mechanism. Answering this question can be expected to have a strong impact on human glycogen and its related metabolic disorders. The second question is to unravel the complete mechanism by which the TPS complex and/or Tre6P regulate glycolysis and energy metabolism. It is essential to address this question because it is indispensable for our general understanding of fermentative growth. It is also a necessary step toward rational engineering of the glycolytic pathway, being it dedicated to improve fermentation of

natural substrates or to enable fermentation of nonnaturally consumed carbon sources like xylose.

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References

Alonso MD, Lagzdins EJ, Lomako J, Lomako WM, Whelan WJ (1995a) New and specific nucleoside diphosphate glucose substrates for glycogenin. FEBS Lett 359:110–112

Alonso MD, Lomako J, Lomako WM, Whelan WJ (1995b) A new look at the biogenesis of glycogen. FASEB J 9:1126–1137

Amoros M, Estruch F (2001) Hsf1p and Msn2/4p cooperate in the expression of Saccharomyces cerevisiae genes HSP26 and HSP104 in a gene- and stress type-dependent manner. Mol Microbiol 39:1523–1532

App H, Holzer H (1989) Purification and characterization of neutral trehalase from the yeast ABYS1 mutant. J Biol Chem 264:17583–17588

Avonce N, Mendoza-Vargas A, Morett E, Iturriaga G (2006) Insights on the evolution of trehalose biosynthesis. BMC Evol Biol 6:109

Avonce N, Wuyts J, Verschooten K, Vandesteene L, Van Dijck P (2010) The Cytophaga hutchinsonii ChTPSP: first characterized bifunctional TPS-TPP protein as putative ancestor of all eukaryotic trehalose biosynthesis proteins. Mol Biol Evol 27:359–369

Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN (1996) TOR controls translation initiation and early G1 progression in yeast. Mol Biol Cell 7:25–42

Baskaran S, Roach PJ, DePaoli-Roach AA, Hurley TD (2010) Structural basis for glucose-6-phosphate activation of glycogen synthase. Proc Natl Acad Sci USA 107:17563–17568

Bell W, Sun W, Hohmann S, Wera S, Reinders A, De Virgilio C, Wiemken A, Thevelein JM (1998) Composition and functional analysis of the Saccharomyces cerevisiae trehalose synthase complex. J Biol Chem 273:33311–33319

Benaroudj N, Lee DH, Goldberg AL (2001) Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. J Biol Chem 276:24261–24267

Blazquez MA, Lagunas R, Gancedo C, Gancedo JM (1993) Trehalose-6-phosphate, a new regulator of yeast glycolysis that inhibits hexokinases. FEBS Lett 329:51–54

Blomberg A (2000) Metabolic surprises in *Saccharomyces cerevisiae* during adaptation to saline conditions: questions, some answers and a model. FEMS Microbiol Lett 182:1–8

Bonini BM, Van Dijck P, Thevelein JM (2003) Uncoupling of the glucose growth defect and the deregulation of glycolysis in *Saccharomyces cerevisiae* Tps1 mutants expressing trehalose-6-phosphate-insensitive hexokinase from *Schizosaccharomyces pombe*. Biochim Biophys Acta 1606:83–93

Brauer MJ, Huttenhower C, Airoldi EM, Rosenstein R, Matese JC, Gresham D, Boer VM, Troyanskaya OG, Botstein D (2008) Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast. Mol Biol Cell 19:352–367

Budovskaya YV, Stephan JS, Reggiori F, Klionsky DJ, Herman PK (2004) The Ras/cAMP-dependent protein kinase signaling pathway regulates an early step of the autophagy process in *Saccharomyces cerevisiae*. J Biol Chem 279:20663–20671

Bulman AL, Nelson HC (2005) Role of trehalose and heat in the structure of the C-terminal activation domain of the heat shock transcription factor. Proteins 58:826–835

Castrillo JI, Zeef LA, Hoyle DC, Zhang N, Hayes A, Gardner DC, Cornell MJ, Petty J, Hakes L, Wardleworth L, Rash B, Brown M, Dunn WB, Broadhurst D, O'Donoghue K, Hester SS, Dunkley TP, Hart SR, Swainston N, Li P, Gaskell SJ, Paton NW, Lilley KS, Kell DB, Oliver SG (2007) Growth control of the eukaryote cell: a systems biology study in yeast. J Biol 6:4

Chary SN, Hicks GR, Choi YG, Carter D, Raikhel NV (2008) Trehalose-6-phosphate synthase/phosphatase regulates cell shape and plant architecture in Arabidopsis. Plant Physiol 146:97–107

Chen Z, Odstrcil EA, Tu BP, McKnight SL (2007) Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity. Science 316:1916–1919

Cheng C, Mu J, Farkas I, Huang D, Goebl MG, Roach PJ (1995) Requirement of the self-glucosylating initiator proteins Glg1p and Glg2p for glycogen accumulation in *Saccharomyces cerevisiae*. Mol Cell Biol 15:6632–6640

Cheng C, Huang D, Roach PJ (1997) Yeast *PIG* genes: *PIG1* encodes a putative type 1 phosphatase subunit that interacts with the yeast glycogen synthase Gsy2p. Yeast 13:1–8

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. Science 282:699–705

Clancy MJ, Smith LM, Magee PT (1982) Developmental regulation of a sporulation-specific enzyme activity in *Saccharomyces cerevisiae*. Mol Cell Biol 2:171–178

Clotet J, Posas F, Hu GZ, Ronne H, Arino J (1995) Role of protein phosphatase 2A in the control of glycogen metabolism in yeast. Eur J Biochem 229:207–214

Colonna WJ, Magee PT (1978) Glycogenolytic enzymes in sporulating yeast. J Bacteriol 134:844–853

Conlin LK, Nelson HC (2007) The natural osmolyte trehalose is a positive regulator of the heat-induced activity of yeast heat shock transcription factor. Mol Cell Biol 27:1505–1515

Corominas J, Clotet J, Fernandez BI, Boles E, Zimmermann FK, Guinovart JJ, Arino J (1992) Glycogen metabolism in a *Saccharomyces cerevisiae* phosphoglucose isomerase (*pgil*) disruption mutant. FEBS Lett 310:182–186

Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, Prinz J, St Onge RP, VanderSluis B, Makhnevych T, Vizeacoumar FJ, Alizadeh S, Bahr S, Brost RL, Chen Y, Cokol M, Deshpande R, Li Z, Lin ZY, Liang W, Marback M, Paw J, San Luis BJ, Shuteriqi E, Tong AH, van Dyk N, Wallace IM, Whitney JA, Weirauch MT, Zhong G, Zhu H, Houry WA, Brudno M, Ragibizadeh S, Papp B, Pal C, Roth FP, Giaever G, Nislow C, Troyanskaya OG, Bussey H, Bader GD, Gingras AC, Morris QD, Kim PM, Kaiser CA, Myers CL, Andrews BJ, Boone C (2010) The genetic landscape of a cell. Science 327:425–431

Daran JM, Dallies N, Thines-Sempoux D, Paquet V, Francois J (1995) Genetic and biochemical characterization of the *UGP1* gene encoding the UDP-glucose pyrophosphorylase from *Saccharomyces cerevisiae*. Eur J Biochem 233:520–530

Daran JM, Bell W, Francois J (1997) Physiological and morphological effects of genetic alterations leading to a reduced synthesis of UDP-glucose in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 153:89–96

De Virgilio C, Loewith R (2006) The TOR signalling network from yeast to man. Int J Biochem Cell Biol 38:1476–1481

Dubouloz F, Deloche O, Wanke V, Cameroni E, De Virgilio C (2005) The TOR and EGO protein complexes orchestrate microautophagy in yeast. Mol Cell 19:15–26

Elliott B, Haltiwanger RS, Futcher B (1996) Synergy between trehalose and Hsp104 for thermotolerance in *Saccharomyces cerevisiae*. Genetics 144:923–933

Enjalbert B, Parrou JL, Vincent O, Francois J (2000) Mitochondrial respiratory mutants of *Saccharomyces cerevisiae* accumulate glycogen and readily mobilize it in a glucose-depleted medium. Microbiology 146(Pt 10):2685–2694

Enjalbert B, Parrou JL, Teste MA, Francois J (2004) Combinatorial control by the protein kinases PKA, PHO85 and SNF1 of transcriptional induction of the *Saccharomyces cerevisiae* GSY2 gene at the diauxic shift. Mol Genet Genomics 271:697–708

Farkas I, Hardy TA, DePaoli-Roach AA, Roach PJ (1990) Isolation of the *GSY1* gene encoding yeast glycogen synthase and evidence for the existence of a second gene. J Biol Chem 265:20879–20886

Farkas I, Hardy TA, Goebl MG, Roach PJ (1991) Two glycogen synthase isoforms in *Saccharomyces cerevisiae* are coded by distinct genes that are differentially controlled. J Biol Chem 266:15602–15607

Ficarro SB, McCleland ML, Stukenberg PT, Burke DJ, Ross MM, Shabanowitz J, Hunt DF, White FM (2002) Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae*. Nat Biotechnol 20:301–305

Fiedler D, Braberg H, Mehta M, Chechik G, Cagney G, Mukherjee P, Silva AC, Shales M, Collins SR, van Wageningen S, Kemmeren P, Holstege FC, Weissman JS, Keogh MC, Koller D, Shokat KM, Krogan NJ (2009) Functional organization of the *S. cerevisiae* phosphorylation network. Cell 136:952–963

Fletterick RJ, Burke JA, Hwang PK, Nakano K, Newgard CB (1986) Structural relationships in glycogen phosphorylases. Ann N Y Acad Sci 478:220–232

Francois J, Hers HG (1988) The control of glycogen metabolism in yeast. 2. A kinetic study of the two forms of glycogen synthase and of glycogen phosphorylase and an investigation of their interconversion in a cell-free extract. Eur J Biochem 174:561–567

Francois J, Parrou JL (2001) Reserve carbohydrates metabolism in the yeast *Saccharomyces cerevisiae*. FEMS Microbiol Rev 25:125–145

Francois J, Villanueva ME, Hers HG (1988) The control of glycogen metabolism in yeast. 1. Interconversion in vivo of glycogen synthase and glycogen phosphorylase induced by glucose, a nitrogen source or uncouplers. Eur J Biochem 174:551–559

Francois J, Neves MJ, Hers HG (1991) The control of trehalose biosynthesis in Saccharomyces cerevisiae: evidence for a catabolite inactivation and repression of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase. Yeast 7:575–587

Francois JM, Thompson-Jaeger S, Skroch J, Zellenka U, Spevak W, Tatchell K (1992) GAC1 may encode a regulatory subunit for protein phosphatase type 1 in *Saccharomyces cerevisiae*. EMBO J 11:87–96

Fukuhara H (2003) The Kluyver effect revisited. FEMS Yeast Res 3:327–331

Futcher B (2006) Metabolic cycle, cell cycle, and the finishing kick to start. Genome Biol 7:107

Gancedo JM (2008) The early steps of glucose signalling in yeast. FEMS Microbiol Rev 32:673–704

Gancedo C, Flores CL (2004) The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. FEMS Yeast Res 4:351–359

Garre E, Matallana E (2009) The three trehalases Nth1p, Nth2p and Ath1p participate in the mobilization of intracellular trehalose required for recovery from saline stress in *Saccharomyces cerevisiae*. Microbiology 155:3092–3099

Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, Marzioch M, Rau C, Jensen LJ, Bastuck S, Dumpelfeld B, Edelmann A, Heurtier MA, Hoffman V, Hoefert C, Klein K, Hudak M, Michon AM, Schelder M, Schirle M, Remor M, Rudi T, Hooper S, Bauer A, Bouwmeester T, Casari G, Drewes G, Neubauer G, Rick JM, Kuster B, Bork P, Russell RB, Superti-Furga G (2006) Proteome survey reveals modularity of the yeast cell machinery. Nature 440:631–636

Gorner W, Durchschlag E, Wolf J, Brown EL, Ammerer G, Ruis H, Schuller C (2002) Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. EMBO J 21:135–144

Guillou V, Plourde-Owobi L, Parrou JL, Goma G, Francois J (2004) Role of reserve carbohydrates in the growth dynamics of *Saccharomyces cerevisiae*. FEMS Yeast Res 4:773–787

Han EK, Cotty F, Sottas C, Jiang H, Michels CA (1995) Characterization of *AGT1* encoding a general alpha-glucoside transporter from *Saccharomyces*. Mol Microbiol 17:1093–1107

Hardy TA, Roach PJ (1993) Control of yeast glycogen synthase-2 by COOH-terminal phosphorylation. J Biol Chem 268:23799–23805

Hazelwood LA, Walsh MC, Luttik MA, Daran-Lapujade P, Pronk JT, Daran JM (2009) Identity of the growth-limiting nutrient strongly affects storage carbohydrate accumulation in anaerobic chemostat cultures of Saccharomyces cerevisiae. Appl Environ Microbiol 75:6876–6885

He S, Bystricky K, Leon S, Francois JM, Parrou JL (2009) The Saccharomyces cerevisiae vacuolar acid trehalase is targeted at the cell surface for its physiological function. FEBS J 276:5432–5446

Hohmann S, Bell W, Neves MJ, Valckx D, Thevelein JM (1996) Evidence for trehalose-6-phosphate-dependent and -independent mechanisms in the control of sugar influx into yeast glycolysis. Mol Microbiol 20:981–991

Huang D, Farkas I, Roach PJ (1996) Pho85p, a cyclin-dependent protein kinase, and the Snf1p protein kinase act antagonistically to control glycogen accumulation in *Saccharomyces cerevisiae*. Mol Cell Biol 16:4357–4365

Huang D, Wilson WA, Roach PJ (1997) Glucose-6-P control of glycogen synthase phosphorylation in yeast. J Biol Chem 272:22495–22501

Huang D, Moffat J, Wilson WA, Moore L, Cheng C, Roach PJ, Andrews B (1998) Cyclin partners determine Pho85 protein kinase substrate specificity in vitro and in vivo: control of glycogen biosynthesis by Pcl8 and Pcl10. Mol Cell Biol 18:3289–3299

Huang J, Reggiori F, Klionsky DJ (2007) The transmembrane domain of acid trehalase mediates ubiquitin-independent multivesicular body pathway sorting. Mol Biol Cell 18:2511–2524

Hwang PK, Tugendreich S, Fletterick RJ (1989) Molecular analysis of *GPH1*, the gene encoding glycogen phosphorylase in *Saccharomyces cerevisiae*. Mol Cell Biol 9:1659–1666

Jules M, Guillou V, Francois J, Parrou JL (2004) Two distinct pathways for trehalose assimilation in the yeast *Saccharomyces cerevisiae*. Appl Environ Microbiol 70:2771–2778

Jules M, Francois J, Parrou JL (2005) Autonomous oscillations in *Saccharomyces cerevisiae* during batch cultures on trehalose. FEBS J 272:1490–1500

Jules M, Beltran G, Francois J, Parrou JL (2008) New insights into trehalose metabolism by *Saccharomyces cerevisiae*: NTH2 encodes a functional cytosolic trehalase, and deletion of TPS1 reveals Ath1p-dependent trehalose mobilization. Appl Environ Microbiol 74:605–614

Kandror O, Bretschneider N, Kreydin E, Cavalieri D, Goldberg AL (2004) Yeast adapt to near-freezing temperatures by STRE/Msn2,4-dependent induction of trehalose synthesis and certain molecular chaperones. Mol Cell 13:771–781

Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP, Punna T, Peregrin-Alvarez JM, Shales M, Zhang X, Davey M, Robinson MD, Paccanaro A, Bray JE, Sheung A, Beattie B, Richards DP, Canadien V, Lalev A, Mena F, Wong P, Starostine A, Canete MM, Vlasblom J, Wu S, Orsi C, Collins SR, Chandran S, Haw R, Rilstone JJ, Gandi K, Thompson NJ, Musso G, St Onge P, Ghanny S, Lam MH, Butland G, Altaf-Ul AM, Kanaya S, Shilatifard A, O'Shea E, Weissman JS, Ingles CJ, Hughes TR, Parkinson J, Gerstein M, Wodak SJ, Emili A, Greenblatt JF (2006) Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. Nature 440:637–643

Kuenzi MT, Fiechter A (1972) Regulation of carbohydrate composition of *Saccharomyces cerevisiae* under growth limitation. Arch Microbiol 84:254–265

Kwon HB, Yeo ET, Hahn SE, Bae SC, Kim DY, Byun MO (2003) Cloning and characterization of genes encoding trehalose-6-phosphate synthase (TPS1) and trehalose-6-phosphate phosphatase (TPS2) from Zygosaccharomyces rouxii. FEMS Yeast Res 3:433–440

Lee DH, Goldberg AL (1998) Proteasome inhibitors cause induction of heat shock proteins and trehalose, which together confer thermotolerance in *Saccharomyces cerevisiae*. Mol Cell Biol 18:30–38

Lin K, Hwang PK, Fletterick RJ (1995) Mechanism of regulation in yeast glycogen phosphorylase. J Biol Chem 270:26833–26839

Lin K, Rath VL, Dai SC, Fletterick RJ, Hwang PK (1996) A protein phosphorylation switch at the conserved allosteric site in GP. Science 273:1539–1542

Lomako J, Lomako WM, Whelan WJ (2004) Glycogenin: the primer for mammalian and yeast glycogen synthesis. Biochim Biophys Acta 1673:45–55

Londesborough J, Varimo K (1984) Characterization of two trehalases in baker's yeast. Biochem J 219:511–518

Londesborough J, Vuorio OE (1993) Purification of trehalose synthase from baker's yeast. Its temperature-dependent activation by fructose 6-phosphate and inhibition by phosphate. Eur J Biochem 216:841–848

Mahmud SA, Nagahisa K, Hirasawa T, Yoshikawa K, Ashitani K, Shimizu H (2009) Effect of trehalose accumulation on response to saline stress in *Saccharomyces cerevisiae*. Yeast 26:17–30

Melendez R, Melendez-Hevia E, Canela EI (1999) The fractal structure of glycogen: a clever solution to optimize cell metabolism. Biophys J 77:1327–1332

Muller D, Exler S, Aguilera-Vazquez L, Guerrero-Martin E, Reuss M (2003) Cyclic AMP mediates the cell cycle dynamics of energy metabolism in *Saccharomyces cerevisiae*. Yeast 20:351–367

Neves MJ, Jorge JA, Francois JM, Terenzi HF (1991) Effects of heat shock on the level of trehalose and glycogen, and on the induction of thermotolerance in *Neurospora crassa*. FEBS Lett 283:19–22

Ni HT, LaPorte DC (1995) Response of a yeast glycogen synthase gene to stress. Mol Microbiol 16:1197–1205

Noda T, Ohsumi Y (1998) Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J Biol Chem 273:3963–3966

Nwaka S, Mechler B, Destruelle M, Holzer H (1995) Phenotypic features of trehalase mutants in *Saccharomyces cerevisiae*. FEBS Lett 360:286–290

Nwaka S, Mechler B, Holzer H (1996) Deletion of the ATH1 gene in *Saccharomyces cerevisiae* prevents growth on trehalose. FEBS Lett 386:235–238

Paalman JW, Verwaal R, Slofstra SH, Verkleij AJ, Boonstra J, Verrips CT (2003) Trehalose and glycogen accumulation is related to the duration of the G1 phase of *Saccharomyces cerevisiae*. FEMS Yeast Res 3:261–268

Panadero J, Pallotti C, Rodriguez-Vargas S, Randez-Gil F, Prieto JA (2006) A downshift in temperature activates the high osmolarity glycerol (HOG) pathway, which determines freeze tolerance in *Saccharomyces cerevisiae*. J Biol Chem 281:4638–4645

Panni S, Landgraf C, Volkmer-Engert R, Cesareni G, Castagnoli L (2008) Role of 14-3-3 proteins in the regulation of neutral trehalase in the yeast *Saccharomyces cerevisiae*. FEMS Yeast Res 8:53–63

Parrou JL, Teste MA, François J (1997) Effects of various types of stress on the metabolism of reserve carbohydrates in *Saccharomyces cerevisiae*: genetic evidence for a stress-induced recycling of glycogen and trehalose. Microbiology 143:1891–1900

Parrou JL, Enjalbert B, Francois J (1999a) STRE- and cAMP-independent transcriptional induction of *Saccharomyces cerevisiae GSY2* encoding glycogen synthase during diauxic growth on glucose. Yeast 15:1471–1484

Parrou JL, Enjalbert B, Plourde L, Bauche A, Gonzalez B, Francois J (1999b) Dynamic responses of reserve carbohydrate metabolism under carbon and nitrogen limitations in *Saccharomyces cerevisiae*. Yeast 15:191–203

Parrou JL, Jules M, Beltran G, Francois J (2005) Acid trehalase in yeasts and filamentous fungi: localization, regulation and physiological function. FEMS Yeast Res 5:503–511

Paul MJ, Primavesi LF, Jhurreea D, Zhang Y (2008) Trehalose metabolism and signaling. Annu Rev Plant Biol 59:417–441

Pederson BA, Cheng C, Wilson WA, Roach PJ (2000) Regulation of glycogen synthase. Identification of residues involved in regulation by the allosteric ligand glucose-6-P and by phosphorylation. J Biol Chem 275:27753–27761

Pederson BA, Wilson WA, Roach PJ (2004) Glycogen synthase sensitivity to glucose-6-P is important for controlling glycogen accumulation in *Saccharomyces cerevisiae*. J Biol Chem 279:13764–13768

Pedreno Y, Gimeno-Alcaniz JV, Matallana E, Arguelles JC (2002) Response to oxidative stress caused by H(2)O(2) in *Saccharomyces cerevisiae* mutants deficient in trehalase genes. Arch Microbiol 177:494–499

Pedruzzi I, Burckert N, Egger P, De Virgilio C (2000) Saccharomyces cerevisiae Ras/cAMP pathway controls post-diauxic shift element-dependent transcription through the zinc finger protein Gis1. EMBO J 19:2569–2579

Pedruzzi I, Dubouloz F, Cameroni E, Wanke V, Roosen J, Winderickx J, De Virgilio C (2003) TOR and PKA signaling pathways converge on the protein kinase Rim15 to control entry into G0. Mol Cell 12:1607–1613

Peng ZY, Trumbly RJ, Reimann EM (1990) Purification and characterization of glycogen synthase from a glycogen- deficient strain of *Saccharomyces cerevisiae*. J Biol Chem 265:13871–13877

Plourde-Owobi L, Durner S, Parrou JL, Wieczorke R, Goma G, Francois J (1999) *AGT1*, encoding an alpha-glucoside transporter involved in uptake and intracellular accumulation of trehalose in *Saccharomyces cerevisiae*. J Bacteriol 181:3830–3832

Posas F, Clotet J, Muns MT, Corominas J, Casamayor A, Arino J (1993) The gene PPG encodes a novel yeast protein phosphatase involved in glycogen accumulation. J Biol Chem 268:1349–1354

Ratnakumar S, Tunnacliffe A (2006) Intracellular trehalose is neither necessary nor sufficient for desiccation tolerance in yeast. FEMS Yeast Res 6:902–913

Reinders A, Burckert N, Hohmann S, Thevelein JM, Boller T, Wiemken A, De Virgilio C (1997) Structural analysis of the subunits of the trehalose-6-phosphate synthase/phosphatase complex in *Saccharomyces cerevisiae* and their function during heat shock. Mol Microbiol 24:687–695

Rutter J, Probst BL, McKnight SL (2002) Coordinate regulation of sugar flux and translation by PAS kinase. Cell 111:17–28

Santhanam A, Hartley A, Duvel K, Broach JR, Garrett S (2004) PP2A phosphatase activity is required for stress and Tor kinase regulation of yeast stress response factor Msn2p. Eukaryot Cell 3:1261–1271

Schade B, Jansen G, Whiteway M, Entian KD, Thomas DY (2004) Cold adaptation in budding yeast. Mol Biol Cell 15:5492–5502

Sillje HHW, Paalman JWG, ter Schure EG, Olsthoorn SQB, Verkleij AJ, Boonstra J, Verrips CT (1999) Function of trehalose and glycogen in cell cycle progression and cell viability in *Saccharomyces cerevisiae*. J Bacteriol 181:396–400

Silva-Udawatta MN, Cannon JF (2001) Roles of trehalose phosphate synthase in yeast glycogen metabolism and sporulation. Mol Microbiol 40:1345–1356

Singer MA, Lindquist S (1998) Multiple effects of trehalose on protein folding in vitro and in vivo. Mol Cell 1:639–648

Skroch Stuart J, Frederick DL, Varner CM, Tatchell K (1994) The mutant type 1 protein phosphatase encoded by glc7-1 from *Saccharomyces cerevisiae* fails to interact productively with the GAC1- encoded regulatory subunit. Mol Cell Biol 14:896–905

Slattery MG, Liko D, Heideman W (2008) Protein kinase A, TOR, and glucose transport control the response to nutrient repletion in *Saccharomyces cerevisiae*. Eukaryot Cell 7:358–367

Smith TL, Rutter J (2007) Regulation of glucose partitioning by PAS kinase and Ugp1 phosphorylation. Mol Cell 26:491–499

Sunnarborg SW, Miller SP, Unnikrishnan I, LaPorte DC (2001) Expression of the yeast glycogen phosphorylase gene is regulated by stress-response elements and by the HOG MAP kinase pathway. Yeast 18:1505–1514

Teste MA, Enjalbert B, Parrou JL, Francois JM (2000) The Saccharomyces cerevisiae YPR184w gene encodes the glycogen debranching enzyme. FEMS Microbiol Lett 193:105–110

Teste MA, Duquenne M, Francois JM, Parrou JL (2009) Validation of reference genes for quantitative expression analysis by real-time RT-PCR in *Saccharomyces cerevisiae*. BMC Mol Biol 10:99

Thon VJ, Vigneron LC, Marianne PT, Montreuil J, Decq A, Rachez C, Ball SG, Cannon JF (1992) Coordinate regulation of glycogen metabolism in the yeast *Saccharomyces cerevisiae*. Induction of glycogen branching enzyme. J Biol Chem 267:15224–15228

Timblin BK, Bergman LW (1997) Elevated expression of stress response genes resulting from deletion of the *PHO85* gene. Mol Microbiol 26:981–990

Timblin BK, Tatchell K, Bergman LW (1996) Deletion of the gene encoding the cyclin-dependent protein kinase Pho85 alters glycogen metabolism in *Saccharomyces cerevisiae*. Genetics 143:57–66

Torija MJ, Novo M, Lemassu A, Wilson W, Roach PJ, Francois J, Parrou JL (2005) Glycogen synthesis in the absence of glycogenin in the yeast *Saccharomyces cerevisiae*. FEBS Lett 579:3999–4004

Tu BP, Kudlicki A, Rowicka M, McKnight SL (2005) Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science 310:1152–1158

Ugalde JE, Parodi AJ, Ugalde RA (2003) De novo synthesis of bacterial glycogen: agrobacterium tumefaciens glycogen synthase is involved in glucan initiation and elongation. Proc Natl Acad Sci USA 100:10659–10663

Van Aelst L, Hohmann S, Zimmermann FK, Jans AW, Thevelein JM (1991) A yeast homologue of the bovine lens fibre MIP gene family complements the growth defect of a *Saccharomyces cerevisiae* mutant on fermentable sugars but not its defect in glucose-induced RAS-mediated cAMP signalling. EMBO J 10:2095–2104

van Voorst F, Houghton-Larsen J, Jonson L, Kielland-Brandt MC, Brandt A (2006) Genome-wide identification of genes required for growth of *Saccharomyces cerevisiae* under ethanol stress. Yeast 23:351–359

Vandercammen A, Francois J, Hers HG (1989) Characterization of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase of *Saccharomyces cerevisiae*. Eur J Biochem 182:613–620

Versele M, Thevelein JM, Van Dijck P (2004) The high general stress resistance of the *Saccharomyces cerevisiae* fil1 adenylate cyclase mutant (Cyr1Lys1682) is only partially dependent on trehalose, Hsp104 and overexpression of Msn2/4-regulated genes. Yeast 21:75–86

Voit EO (2003) Biochemical and genomic regulation of the trehalose cycle in yeast: review of observations and canonical model analysis. J Theor Biol 223:55–78

Walther T, Novo M, Rossger K, Letisse F, Loret MO, Portais JC, Francois JM (2010) Control of ATP homeostasis during the respiro-fermentative transition in yeast. Mol Syst Biol 6:344

Wang Z, Wilson WA, Fujino MA, Roach PJ (2001) Antagonistic controls of autophagy and glycogen accumulation by Snf1p, the yeast homolog of AMP-activated protein kinase, and the cyclin-dependent kinase Pho85p. Mol Cell Biol 21:5742–5752

Ward MP, Gimeno CJ, Fink GR, Garrett S (1995) SOK2 may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. Mol Cell Biol 15:6854–6863

Wera S, De Schrijver E, Geyskens I, Nwaka S, Thevelein JM (1999) Opposite roles of trehalase activity in heat-shock recovery and heat-shock survival in *Saccharomyces cerevisiae*. Biochem J 343:621–626

Wilson WA, Mahrenholz AM, Roach PJ (1999) Substrate targeting of the yeast cyclin-dependent kinase pho85p by the cyclin pcl10p. Mol Cell Biol 19:7020–7030

Wilson WA, Wang Z, Roach PJ (2002a) Analysis of respiratory mutants reveals new aspects of the control of glycogen accumulation by the cyclin-dependent protein kinase Pho85p. FEBS Lett 515:104–108

Wilson WA, Wang Z, Roach PJ (2002b) Systematic identification of the genes affecting glycogen storage in the yeast *Saccharomyces cerevisiae:* implication of the vacuole as a determinant of glycogen level. Mol Cell Proteomics 1:232–242

Wilson WA, Hughes WE, Tomamichel W, Roach PJ (2004) Increased glycogen storage in yeast results in less branched glycogen. Biochem Biophys Res Commun 320:416–423

Wilson WA, Wang Z, Roach PJ (2005) Regulation of yeast glycogen phosphorylase by the cyclin-dependent protein kinase Pho85p. Biochem Biophys Res Commun 329:161–167

Winderickx J, de WJ, Crauwels M, Hino A, Hohmann S, Van DP, Thevelein JM (1996) Regulation of genes encoding subunits of the trehalose synthase complex in *Saccharomyces cerevisiae*: novel variations of STRE-mediated transcription control. Mol Gen Genet 252:470–482

Wu X, Hart H, Cheng C, Roach PJ, Tatchell K (2001) Characterization of Gac1p, a regulatory subunit of protein phosphatase type I involved in glycogen accumulation in *Saccharomyces cerevisiae*. Mol Genet Genomics 265:622–635

Xu Z, Tsurugi K (2006) A potential mechanism of energy-metabolism oscillation in an aerobic chemostat culture of the yeast *Saccharomyces cerevisiae*. FEBS J 273:1696–1709

Xu Z, Tsurugi K (2007) Destabilization of energy-metabolism oscillation in the absence of trehalose synthesis in the chemostat culture of yeast. Arch Biochem Biophys 464:350–358

Yorimitsu T, Zaman S, Broach JR, Klionsky DJ (2007) Protein kinase A and Sch9 cooperatively regulate induction of autophagy in *Saccharomyces cerevisiae*. Mol Biol Cell 18:4180–4189

Zahringer H, Thevelein JM, Nwaka S (2000) Induction of neutral trehalase Nth1 by heat and osmotic stress is controlled by STRE elements and Msn2/Msn4 transcription factors: variations of PKA effect during stress and growth. Mol Microbiol 35:397–406

Zaman S, Lippman SI, Zhao X, Broach JR (2008) How *Saccharomyces* responds to nutrients. Annu Rev Genet 42:27–81

Zurita-Martinez SA, Cardenas ME (2005) Tor and cyclic AMP-protein kinase A: two parallel pathways regulating expression of genes required for cell growth. Eukaryot Cell 4:63–71

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