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Jean Marie François, Jean-Luc Parrou

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Jean François *, Jean Luc Parrou

*Centre de Bioingenierie Gilbert Durand, UMR-CNRS 5504, UMR-INRA 792, Département de Génie Biochimique et Alimentaire, Institut National des Sciences Appliquées, 135 Avenue de Rangeuil, 31077 Toulouse Cedex 04, France*

Received 26 May 2000; accepted 30 September 2000

**Abstract**

Glycogen and trehalose are the two glucose stores of yeast cells. The large variations in the cell content of these two compounds in response to different environmental changes indicate that their metabolism is controlled by complex regulatory systems. In this review we present information on the regulation of the activity of the enzymes implicated in the pathways of synthesis and degradation of glycogen and trehalose as well as on the transcriptional control of the genes encoding them. cAMP and the protein kinases Snf1 and Pho85 appear as major actors in this regulation. From a metabolic point of view, glucose-6-phosphate seems the major effector in the net synthesis of glycogen and trehalose. We discuss also the implication of the recently elucidated TOR-dependent nutrient signalling pathway in the control of the yeast glucose stores and its integration in growth and cell division. The unexpected roles of glycogen and trehalose found in the control of glycolytic flux, stress responses and energy stores for the budding process, demonstrate that their presence confers survival and reproductive advantages to the cell. The findings discussed provide for the first time a teleonomic value for the presence of two different glucose stores in the yeast cell.

*Keywords:* Glycogen; Trehalose; Glycolysis; Signal transduction; Stress; Growth; Viability

**Contents**



* Corresponding author. Tel.: +33 (5) 61-559492; Fax: +33 (5) 61-559400; E-mail: fran_jm@insa-tlse.fr

*Abbreviations:* Glc6*P*, glucose-6-phosphate; Tre6*P*, trehalose-6-phosphate; UDP-Glc, UDP-glucose

## 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. Trehalose is a non-reducing disaccharide composed of two α (1,1)-linked glucose molecules. Several reports have illustrated important variations in the levels of these two glucose stores during incubation of cells to prolonged starvation conditions [1], resumption of vegetative growth on a fresh growth medium [2,3] and sporulation or germination of spores [2,4,5]. The enormous investment devoted to the biochemical and molecular characterization of glycogen and trehalose metabolism over the past 10 years provided important new insights into the function of these glucose stores in yeast. Our purpose is to give a comprehensive and integrated overview on the control and the role of glycogen and trehalose metabolism in the life cycle of the yeast *S. cerevisiae*. The reader may also refer to recent reviews focused on specific enzymatic systems participating in glycogen or trehalose metabolism [6–8].

## 2. The glycogen metabolic pathway: genes, proteins and regulation

### 2.1. Glycogen biosynthesis

#### 2.1.1. Nucleation by glycogenin (Glg1p/Glg2p)

As in the case of other high molecular mass biopolymers, the synthesis of glycogen involves initiation, elongation, and ramification steps. The initiation step is carried out by a protein designated 'glycogenin' which bears a self-autoglucosylating activity and produces from UDP-glucose (UDP-Glc) a short α (1,4)-glucosyl chain that is covalently attached to a tyrosine residue (Fig. 1). This protein was originally identified as a 38-kDa 'contaminant' of a purified preparation of glycogen synthase in skeletal muscle [9,10]. Roach and colleagues took advantage of this interesting property to isolate, by a two-hybrid screen using glycogen synthase encoded by *GSY2* as the bait, a yeast homologue of the mammalian glycogenin. This gene, designated *GLG2*, is located on chromosome X and is highly homologous to another open reading frame identified on chromosome XI and named *GLG1*. Loss of either *GLG* genes does not result in apparent glycogen defect, whereas the lack of glycogen in *glg1glg2* cells would indicate an absolute requirement for a self-glucosylating initiator protein in the biosynthesis of glycogen [11]. However, preliminary results suggest that a *glg1glg2* mutant strain recovers about 30% of the wild-type glycogen levels when it is additionally disrupted for *TPS1* encoding the Tre6*P* synthase catalytic subunit (unpublished).

Glg1p differs from Glg2p in being higher in molecular mass (67 kDa versus 43 kDa). They exhibit 55% sequence identity over a 260 residues $NH_2$-terminal segment and 33% identity with the mammalian glycogenin in this region. All eukaryotic glycogenins display a high affinity ($K_m$ around 5–10 μM) for UDP-Glc and exist in vivo as oligomers of unknown stoichiometry [6]. Unlike mammalian glycogenins, yeast Glg proteins possess multiple Tyr residues that are needed for sustained glycogen accumulation and harbor a COOH-terminal domain that interacts with the yeast glycogen synthase. The truncation of this domain reduces glycogen synthase activity and severely impairs glycogen accumulation [12]. The observation that a *glg1* mutant strain which additionally carries a Tyr-230/Phe and Tyr-232/Phe substitution in Glg2p still accumulates 10% of the wild-type level of glycogen suggested that these substitutions had forced another tyrosine residue (e.g. Tyr-237) at the C-terminus to be a glucosyl acceptor. Alternatively glycogen synthase may elongate short primers (maltose, maltotriose) produced by the intrinsic transglucosidase activity of Glg proteins. In either case, disruption of the interaction with glycogen synthase by removal of the C-terminal domain of the mutant Glg proteins abolished glycogen synthesis [12]. Surprisingly, glycogen levels are not increased in yeast cells overexpressing *GLG* genes, suggesting that either yeast glycogenins are in molar excess over glycogen synthase, or that these proteins could be reiteratively used. However, this reiteration would require the existence of an additional enzyme to release the glycogenin from the growing glucosyl chains, but such an enzyme has not been found yet.

#### 2.1.2. Elongation by glycogen synthases (Gsy1p/Gsy2p)

Glycogen synthase catalyzes the formation of α (1,4)-glucosidic bonds from UDP-Glc to the non-reducing end of linear α (1,4)-chains of glycogen (Fig. 1). *S. cerevisiae* contains two genes, *GSY1* and *GSY2*, encoding 80 501 and 79 963 Da polypeptides that correspond respectively to glycogen synthase isoform I and II [13]. The two proteins are 80% identical and share 50% similarity with mammalian muscle and rat liver glycogen synthase. Deletion analysis indicated that *GSY2* encodes the predominant glycogen synthase since loss of its function resulted in a 90% reduction in both enzyme activity and glycogen levels.

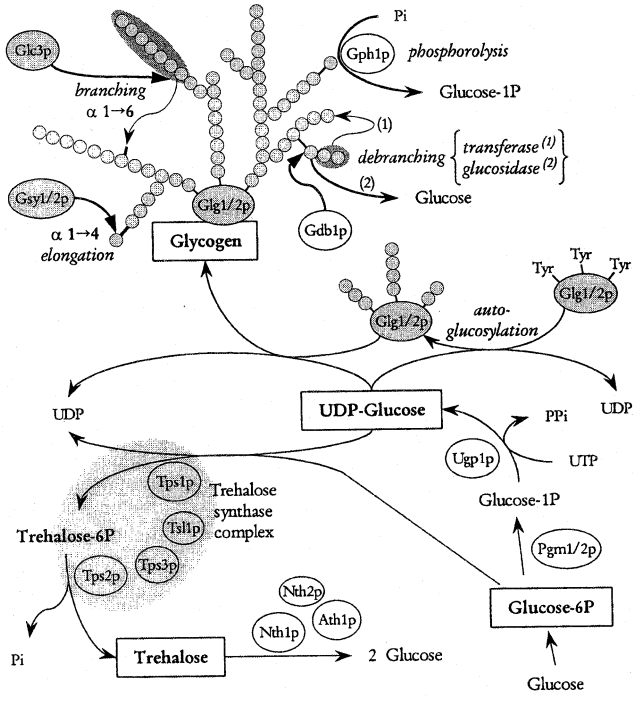


Fig. 1. Glycogen and trehalose metabolic pathways in the yeast *S. cerevisiae*. A two-step reaction catalyzed by phosphoglucomutase (two isoforms, Pgm1p and Pgm2p) and UDP-Glc pyrophosphorylase (Ugp1p) leads to the synthesis of UDP-Glc. Glycogen synthesis is initiated by glycogenin (two isoforms, Glg1p and Glg2p), that produces a short α (1,4)-glucosyl chain that is elongated by glycogen synthase (two isoforms, Gsy1p and Gsy2p). The chains are ramified by the branching enzyme (Glc3p) which transfers a block of 6–8 residues from the end of a linear chain to an internal glucosyl unit and creates an α (1,6)-linkage. Glycogen degradation occurs by the combined action of glycogen phosphorylase (Gph1p) which releases glucose-1-P, and a debranching enzyme (Gdb1p) which transfers a maltosyl unit to the end of an adjacent linear α (1,4)-chain and releases glucose by cleaving the remaining α (1,6)-linkage. Trehalose biosynthesis is catalyzed by the trehalose synthase complex composed of four subunits. The trehalose-6-phosphate (Tre6*P*) synthase subunit (Tps1p) produces Tre6*P* from UDP-Glc and glucose-6-phosphate (Glc6*P*), which is dephosphorylated in trehalose by the Tre6*P* phosphatase subunit (Tps2p). Tps3p and Tsl1p are two regulatory subunits that stabilize the complex. Trehalose is degraded by the neutral (Nth1p) or the acid (Ath1p) trehalase. The role of Nth2p in this degradation process is not yet clarified.

Deletion of both genes produced cells with no other apparent phenotype than glycogen synthesis deficiency [13].

The original work of Cabib and colleagues [14–16], extended by others [13,17–20], established that yeast glycogen synthase shares many properties with its mammalian counterpart, including allosteric stimulation by Glc6*P* and reversible covalent phosphorylation. As the active – non-phosphorylated – form of glycogen synthase is almost insensitive to the sugar phosphate, the ratio of the activities assayed in the absence and in the presence of Glc6*P* (−/+ Glc6*P* activity ratio) can provide a good estimate of the phosphorylation state of the glycogen synthase [15,17,19]. Partial proteolysis of a purified preparation of glycogen synthase (both isoforms) resulted in its Glc6*P* insensitivity and provided the first evidence that the phosphorylation occurred at the COOH-terminus [15,16]. A cluster of three putative sites for cAMP-dependent phosphorylation was identified at the C-terminus of the two isoforms [13,21], in agreement with the finding of a maximum of 3 mol phosphate per mol subunit in the purified inactive glycogen synthase [18]. Moreover, it was demonstrated that the in vivo phosphorylated sites are Ser-650, Ser-654 and Thr-667. While a change of one of the three amino acid residues to Ala only caused a 35% increase in the activity ratio, full activation of Gsy2p can be obtained either by mutation of the three amino acids, or by removal of a 60-amino acids fragment of the C-terminus where the amino acids were located [19].

The next question was to identify the protein kinases implicated in glycogen synthase phosphorylation. The cAMP-dependent protein kinase (PKA) is likely involved in the control of the glycogen synthase phosphorylation state as indicated by an inverse relationship between the −/+ Glc6*P* activity ratio of this enzyme and the PKA activity in yeast strains altered in the cAMP-PKA pathway ([19,22,23], unpublished results). These in vivo results contrast with the fact that none of the three sites on Gsy2p could be phosphorylated in vitro by PKA [19]. This indicates that this kinase can act, most probably indirectly, in the control of glycogen synthase phosphorylation (Fig. 2). A partial purification of a yeast extract on phenyl-Sepharose column revealed two distinct peaks, termed Gpk1p and Gpk2p, that phosphorylated a recombinant Gsy2p. Gpk1p, whose corresponding gene is not yet known, phosphorylates Gsy2p in a cAMP-, $Ca^{2+}$- and calmodulin-independent way, but the phosphorylated amino acid residues have not been identified [24]. Gpk2p is identical to the protein kinase Pho85, a member of the cyclin-dependent protein kinase family [25]. The implication of this kinase in glycogen synthesis has been shown by two other independent approaches. Roach and coworkers [24] isolated *PHO85* as a second site suppressor of the glycogen storage defect of *snf1* cells, whereas Bergman's group [26] found that disruption of *PHO85* resulted in pleiotropic phenotypes, including a hyperaccumulation of glycogen. In addition, Huang et al. [27] identified the cyclin-like Pcl8p and Pcl10p as targeting subunits of Pho85p to phosphorylate Gsy2p at Ser-654 and Thr-667 (Fig. 2). It remains to determine whether the Gpk1p may phosphorylate Ser-650. The protein kinase responsible for the inactivation of glycogen synthase in response to the treatment of MAT**a** haploid cells with the pheromone α-factor [28] has not been identified yet. Since the pheromone-induced MAP kinase Fus3p cannot directly phosphorylate Gsy2p (P. Roach, personal communication), this inactivation may result from a Fus3p-dependent stimulation of either Pho85p or Gpk1p. Alternatively, the mating pheromone may inhibit specific glycogen synthase phosphatases.

Protein phosphatases play critical roles in cellular activities [29,30] and in glycogen metabolism in particular

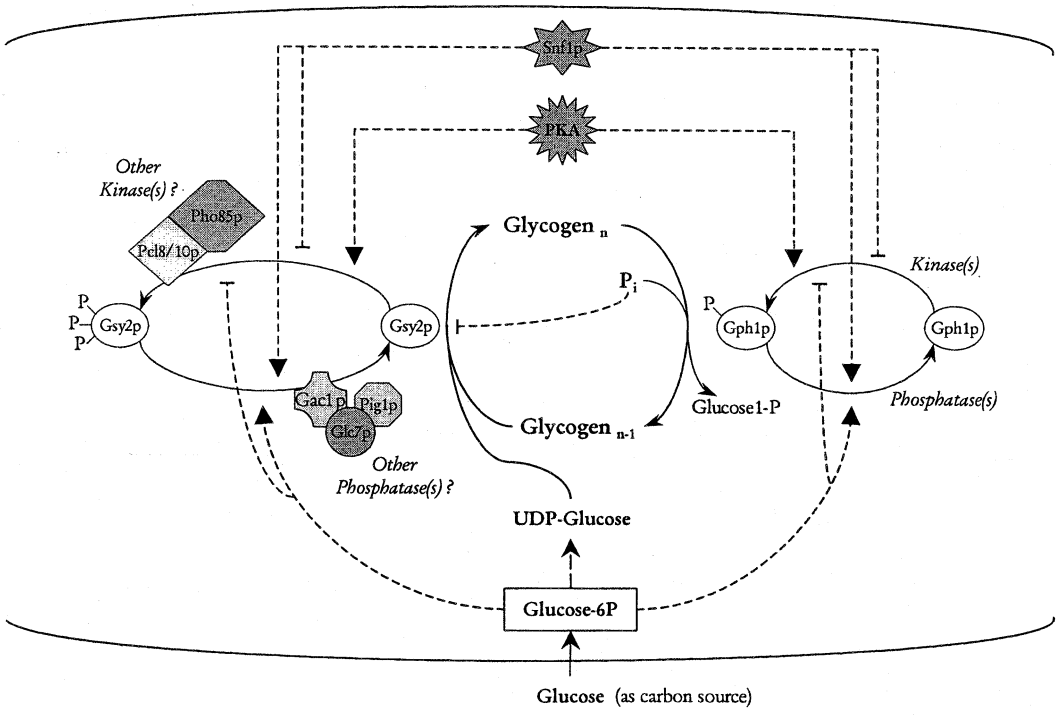


Fig. 2. Metabolic control of glycogen metabolism in *S. cerevisiae*. PKA and Snf1p antagonistically control the phosphorylation state of Gsy2p and Gph1p. Pho85p, in association with Pcl8p and Pcl10p, phosphorylates and inactivates Gsy2p. The reverse reaction is catalyzed by the phosphatase Glc7p and its targetting subunits Gac1p and Pig1p. The existence of other kinases and phosphatases is still hypothetic. Kinase(s) and phosphatase(s) that control the phosphorylation state of Gph1p have not been identified yet. The main effector of this process is Glc6*P* which acts as a potent stimulator of the dephosphorylation and inhibitor of the phosphorylation processes. Pi is also another important effector in glycogen control, being the substrate of Gph1p and an inhibitor of glycogen synthase. (Arrows) positive interaction; (bars) negative interaction; (dashed lines) direct interaction not yet determined.

[31,32]. Yeast possesses the four different classes of Ser/Thr protein phosphatases catalytic subunit designated PP1, 2A, 2B and 2C, and a large number of targeting subunits that direct the catalytic subunits to particular locations within the cell and selectively enhance their activity towards specific substrates [29,33]. The first targeting subunit identified in yeast was the product of *GAC1* which was isolated as a dosage-dependent suppressor of the glycogen storage defect of a mutant with high adenylate cyclase activity. This gene encodes a 88-kDa polypeptide 30% identical to the mammalian regulatory subunit $R_{GI}$ of the PP1 [33,34]. Using the two-hybrid system, Skroch-Stuart et al. [35] demonstrated that Gac1p physically interacts with the catalytic subunit of PP1 encoded by *GLC7* and that this interaction is defective in the glycogen-deficient *glc7-1* mutant cells. These authors also suggested that the productive Glc7p–Gac1p interaction could be negatively affected by the phosphorylation of Gac1p, which is reminiscent of the negative cAMP-dependent control of $R_{GI}$–PP1 association [36]. All *glc7* alleles isolated conferred glycogen deficiency or glycogen hyperaccumulation, likely due to an alteration of Glc7p–Gac1p interaction [37,38]. Two additional genes, *PIG1* and *PIG2*, were isolated using a two-hybrid screen with Gsy2p as bait [39]. *PIG1* encodes a protein 38% identical over a 230-amino acids segment to Gac1p, whereas *PIG2* has 30% identity with *GIP2* which was isolated using Glc7p as bait [40]. Sequence analysis indicated that Gac1p, Pig1p, Pig2p and the product of YER054c (unknown function) are the only four proteins of the yeast genome sharing a common 25-amino acid stretch designated 'GVNK'. This stretch is also identifiable in the mammalian $R_{GI}$ and may be required for interaction with either glycogen particles, PP1 catalytic subunits and/or Gsy2p. Although the Gac1p–Glc7p complex appears to be the most potent glycogen synthase phosphatase in yeast as the loss of *GAC1* function strongly reduces glycogen accumulation and leaves glycogen synthase in an inactive form [34] (Fig. 2), *PIG1* may participate in concert with *GAC1* in the control of glycogen synthesis since a *gac1pig1* double mutant shows more severe glycogen defect than a *gac1* single mutant [39]. However, the *pig1* single mutant had no phenotype and the role of *PIG2* in glycogen metabolism is still unknown. In a screen for suppressors of the glycogen accumulation defect in *glc7-1* cells, Huang et al. [41] uncovered a mutant form of the *REG1* which encodes another PP1 targeting subunit implicated in glucose repression [42]. The mechanism by which *REG1* influences glycogen synthesis is unclear, and it is independent on its role in glucose repression [41]. Besides the major role of PP1 in

controlling glycogen synthase, type 2A protein phosphatase also appears to exert a control, albeit minor, on this enzyme, as illustrated by two experimental results. On the one hand, Reimann and colleagues [43] purified a yeast glycogen synthase phosphatase which exhibits typical enzymological properties of PP2A, and which was able to increase in vitro the −/+ Glc6*P* activity ratio of glycogen synthase from 12 to 30%. On the other hand, the progressive depletion of major PP2A activities, which are encoded by *PPH21* and *PPH22*, resulted in a concomitant 30% decrease of glycogen synthase activity [44]. Moreover, the yeast genome encodes other Ser/Thr protein phosphatases 2A closely related to, but different from, the catalytic subunits of PP1 and PP2A previously mentioned. The loss of one of them encoded by *SIT4* [45] caused a 25% reduction in the activity ratio of glycogen synthase [46], whereas disruption of another PP2A homologue encoded by *PPG* led to a 30% decrease in the amount of total glycogen synthase with no change in the activity ratio [47]. In both cases, a slight 30% reduction in glycogen levels was measured, indicating that these two PP2A-related protein phosphatases exert a minor influence on the control of glycogen metabolism. The action of these type 2A phosphatases on glycogen metabolism is likely due to their role in the regulation of cell growth [48].

To summarize, the activation state of glycogen synthase is ultimately dependent on the relative activity of protein kinases and protein phosphatases (Fig. 2). Biochemical data indicate that this phosphorylation–dephosphorylation 'equilibrium' is antagonistically controlled by the levels of Glc6*P* and cAMP, which are themselves affected by external stimuli. While the mechanism by which high levels of cAMP favor glycogen synthase phosphorylation is not yet clarified, several data indicate that the major role of Glc6*P* is to act as a stimulator of the dephosphorylation and as an inhibitor of the phosphorylation processes [17] (Fig. 2). Such a role of Glc6*P*, which has been recognized for years in mammals [31,32], has now been highlighted by a genetic approach in yeast. Looking for second site suppressor mutations of glycogen deficiency in *snf1* cells, Huang et al. [20] identified a mutation in *PFK2* which encodes the β-subunit of 6-phosphofructo-1-kinase. This mutation leads to the hyperaccumulation of glycogen associated with a 10-fold higher intracellular pool of Glc6*P*. Similar phenotypic traits have previously been observed in phosphoglucose isomerase (*PGI*) defective strains [49]. In addition, the glycogen synthase kinase present in a cell-free extract obtained from a *pfk2* or a *pgi1* mutant was less efficient than that of a wild-type to phosphorylate a recombinant Gsy2p. However, a gel filtration on Sephadex G-25 of extracts from these mutant strains, which removed small metabolites including Glc6*P*, resulted in the recovery of a 'wild-type' glycogen synthase kinase activity [20]. Hence, it remains to determine whether Glc6*P* mediates these effects through its binding to glycogen synthase or to some modifying enzymes.

#### *2.1.3. Branching by amylo (1,4) → (1,6)-transglucosidase (Glc3p)*

After initiation by glycogenin and elongation by glycogen synthase, the linear α (1,4)-glucosyl chains are ramified by the action of amylo (1,4) → (1,6)-transglucosidase (branching enzyme) which transfers a block of 6–8 residues from the end of a linear chain to create an α (1,6)-linkage to an internal glucosyl unit on an adjacent chain [50] (Fig. 1). It is estimated that yeast glycogen particles contain 7–10% of α (1,6)-glucosidic linkages, which is 2–3 times higher than the proportion of such linkages found in the amylopectin structure of starch [51,52]. The highly branched structure of glycogen is responsible for the brown staining of yeast cells upon exposure to iodine crystals vapor, whereas a lower branching gives rise to a green–purple color of the cells. *GLC3* was cloned by complementation of iodine staining-deficient mutants. This gene is located on chromosome V and encodes a protein 42% and 67% similar to prokaryotic and human glycogen branching enzyme, respectively. Disruption of *GLC3* showed that the branching activity is essential for efficient glycogen accumulation since a *glc3* null mutant accumulated only ca. 10% of the wild-type glycogen [53,54].

### *2.2. Glycogen degradation*

In most eukaryotic cells including yeast, the degradation of glycogen can occur either by amylolysis catalyzed by α-glucosidases that produce glucose, or by sequential reactions involving phosphorolysis and debranching activities which produce glucose-1-P and glucose (Fig. 1). In yeast, no peculiar phenotype associated with the lack of glycogen degradation has been noticed so far, in deep contrast with metabolic disorders in mammals resulting from defects in enzymes or regulatory mechanisms involved in this process [55].

#### *2.2.1. Phosphorolysis by glycogen phosphorylase and debranching by (1,4)-glucanotransferase and (1,6)-glucosidase (Gph1p and Gdb1p)*

Yeast glycogen phosphorylase releases glucose-1-P from the linear α (1,4)-glucosidic bonds, shortening the glycogen molecule to limit dextrin, and the debranching enzyme transfers a maltosyl or maltotriosyl unit to an adjacent linear α (1,4)-chain and releases glucose by cleaving the remaining α (1,6)-linkage, allowing glycogen phosphorylase to pursue its action (Fig. 1). These two enzymes are encoded respectively by *GPH1* [56] and *YPR184w* (renamed *GDB1*). Disruption of either gene results in a failure to degrade glycogen, indicating that the breakdown of this polymer requires the combined action of both enzymes, and it only leads to a minor glycogen hyperaccumulation ([57], unpublished). Yeast glycogen phosphorylase exhibits kinetic and regulatory properties distinct from its mammalian counterpart despite having an identical metabolic function. Yeast and rabbit muscle phosphor-

ylases are 45% similar, but the poor conservation of the residues involved in the binding of AMP may explain that yeast phosphorylase is not activated by this effector. Conversely, residues involved in the binding of glucose are well conserved [56], but the yeast phosphorylase is insensitive to glucose and non-competitively inhibited by Glc6*P* ($K_i$ = 2–5 mM) ([58,59], unpublished). A common feature in yeast and mammalian glycogen phosphorylases is their interconversion between a phosphorylated – apparently dimeric – (active) and a dephosphorylated – apparently tetrameric – (inactive) form [58,60,61]. The protein kinases and protein phosphatases implicated in this process in yeast have not yet been genetically identified. A 35-kDa molecular mass cAMP-independent protein kinase and a glycogen phosphorylase phosphatase harboring some properties of type 2A phosphatase have been purified from yeast ([62,63], A. Schwartz, personal communication). In addition, it was shown that PKA could phosphorylate and activate the yeast glycogen phosphorylase in vitro [64]. The structural basis of the phosphorylation-initiated activation of yeast glycogen phosphorylase has been worked out by Fletterick and coworkers [56, 59,65,66]. These authors showed that the phosphorylation site on yeast phosphorylase is a threonine located within a 39-amino acid $NH_2$-terminal extension relative to the residue $NH_2$-terminus of the mammalian enzyme. The phosphorylation initiates a mechanism of local refolding in the N-terminus, causing activation of the protein. This refolding also results in the protection of the phosphorylated group from protein phosphatases. Crystal structure analysis of phosphorylated and non-phosphorylated enzyme complexes with Glc6*P* revealed that this metabolite serves as a dephosphorylation facilitator by modifying the accessibility of the phosphorylation site to protein phosphatases [66]. Another important effector in controlling the activated state of glycogen phosphorylase is glycogen which serves as a facilitator of phosphorylation by shifting the enzyme from a tetrameric to a dimeric state. Hence, as for the glycogen synthase interconversion, Glc6*P* appears to be the most important regulator controlling the activation state of glycogen phosphorylase (Fig. 2).

*GDB1* encodes a 1536-amino acids sequence which is 39% identical to the eukaryote glycogen debranching enzyme and which carries both the α (1,4)-glucanotransferase and the α (1,6)-glucosidase activities as found in the eukaryote counterpart (Fig. 1). Based on biochemical studies and on sequence comparison with the α-amylase superfamily [67], it was suggested that the transferase activity may encompass the N-terminal part of the protein, leaving the α (1,6)-glucosidase activity for the C-terminus [68–70]. Preliminary experiments with the yeast enzyme do not agree with this claim, and rather suggest that the two enzymatic activities are localized within the N-terminal half of the protein, whereas the C-terminus would be involved in glycogen binding. In addition, the yeast Gdb1p preferentially transfers a maltosyl unit from the branch to an adjacent α (1,4)-glucosyl chain ([71,72], unpublished).

#### *2.2.2. Amylolysis by amylo (1,4-1,6)-glucosidase (Sga1p)*

Reserve carbohydrates deposition accounts for about 65% of the increase in cell dry mass of sporulating and non-sporulating diploid cells when they are transferred to a nitrogen-free acetate-containing medium [73,74]. Only in sporulating diploid cells, the huge accumulation of glycogen is followed by a period of extensive breakdown which culminates with the production of mature ascospores [5]. It was demonstrated that the degradation is achieved by a glucoamylase bearing α (1,4)- and α (1,6)-glucosidase activities that releases glucose as the final product [4,75]. This activity is absent in vegetative cells, and is induced coincidentally with the initiation of glycogen breakdown to reach a maximum at the spores maturity. The gene *SGA1* encoding this sporulation-specific glucoamylase has been cloned using a DNA probe from the *STA1* gene that encodes an extracellular glucoamylase from the distant yeast *S. diastaticus* [76]. A recent genome wide analysis of the sporulation process in budding yeast classified *SGA1* as a middle to mid-late induced gene that increases by about 35 times at the end of the sporulation period, and which is under the control of transcriptional activator encoded by *NDT80* [77]. In contrast to expectation, this glucoamylase activity is not required for spore formation since more than 90% of the *sga1*/*sga1* homozygous cells still undergo meiosis and form four viable spores [76]. The non-requirement of glycogen in this process has also been confirmed using mutants unable to synthesize this polysaccharide ([13,76], unpublished).

## 3. The trehalose metabolic pathway: genes, proteins and regulation

### *3.1. Biosynthesis by the trehalose synthase complex (Tps1p, Tps2p, Tsl1p, Tps3p)*

Leloir and Cabib originally demonstrated that a 20-fold purified yeast enzymatic preparation catalyzes, from UDP-Glc and Glc6*P*, the formation of Tre6*P* which is subsequently dephosphorylated into trehalose and Pi [78] (Fig. 1). This two-step process is carried out by a protein complex [79,80] that is composed of four different subunits encoded by *TPS1*, *TPS2*, *TSL1* and *TPS3* [81,82]. The smallest 56-kDa subunit of the purified protein complex corresponds to the Tre6*P* synthase catalytic subunit. Antibodies raised against this polypeptide have been used to clone *TPS1* from a yeast cDNA library [83,84]. It was also independently cloned by complementation of *cif1* [85] and *fdp1* mutants [86] which are unable to grow on fermentable carbon sources [87,88], and from a *glc6-1* mutant that accumulates lower glycogen than wild-type. This latter phenotype has not been elucidated yet and it contrasts

with the fact that a null mutation of *GLC6* hyperaccumulates glycogen due to very high levels of Glc6*P* and due to the presence of a more active form of glycogen synthase ([89], unpublished data). These interesting phenotypes revealed for the first time a regulatory role of trehalose metabolism in the control of glucose metabolism (see Section 5). Peptide sequencing of the 100-kDa band of the purified trehalose synthase complex was used to clone *TPS2* which encodes the Tre6*P* phosphatase catalytic subunit [90]. Loss of *TPS2* function results in a temperature sensitive growth phenotype accompanied by a hyperaccumulation of Tre6*P* [90,91]. This gene was also isolated in a screen for mutants unable to acquire heat shock resistance in stationary phase [92], mutants able to neutralize the pleiotropic drug resistance phenotype caused by overexpression of *YAP1* [93], and by complementation of a thermosensitive glucose-negative mutant harboring very low 6-phosphofructo-1-kinase activity [94]. The isolation of *TPS2* by these apparent well disparate genetic screens is likely linked to the critical function of trehalose in stress and of Tre6*P* in glycolysis (see Section 5). The larger 123-kDa polypeptide found in purified preparations of trehalose synthase complex is encoded by *TSL1* [84]. A homologue of this gene (about 55% at the protein sequence) designated *TPS3* has been identified by systematic genome sequencing. Using the two-hybrid method, Reinders et al. showed that Tsl1p and Tps3p do not interact with each other while they interact with Tps1p and Tps2p, and that the latter two proteins also interact with each other [81]. Interestingly, the four polypeptides share about 33% identity over a stretch of 500 amino acids, but it is not known whether these interactions occur through this common sequence. Construction of isogenic strains carrying deletions of *TPS1*, *TPS2*, *TSL1* and *TPS3* has shown that the catalytic activity of *TPS1* was ca. 30–50% reduced by deletion of *TPS2* and reciprocally, that *TPS3* and *TSL1* play an interchangeable regulatory function in trehalose synthase since the *tps3tsl1* double mutant was impaired in trehalose synthase activity. This latter result supports the idea that the function of Tps3p and Tsl1p is to stabilize the trehalose synthase complex [81,82]. Moreover, the large amounts of Tre6*P* measured upon heat shock and trehalose in stationary phase *tps2tps3tsl1* mutant cells indicate that Tps1p can function independently of the complex [82] and that Tre6*P* can be dephosphorylated by other (unspecific) phosphatases. The free form of Tps1p may also participate in the sharp increase of Tre6*P* observed upon glucose addition to the cells and to the control of growth on glucose or fructose ([82,83,95], see Section 5).

The Tre6*P* synthase activity of the complex displays affinities for Glc6*P* ($K_m \cong 5$–$20$ mM) and UDP-Glc ($K_m \cong 1.5$ mM) which are 3–10 times higher than the bulk concentration in the cells. This enzyme is also strongly non-competitively inhibited by Pi ($K_i = 2$ mM) while fructose-6-P acts as a potent activator [79,96]. In contrast to other enzymatic systems involved in reserve carbohydrate metabolism, the trehalose synthase complex is not subject to reversible phosphorylation [79,97]. A peculiar property of this protein complex is its strong temperature activation, with an optimum at 42–45°C in the presence of physiological concentrations of substrates and effectors [3,80,98,99]. Accordingly, it is anticipated that the rate of trehalose synthesis can be strongly influenced by changes in substrates concentration (Glc6*P* and UDP-Glc), temperature and the steady-state levels of the protein.

### 3.2. Hydrolysis by neutral (Nth1p, Nth2p) and acid (Ath1p) trehalases

Most yeast and fungi species exhibit two types of trehalose hydrolyzing activities, one termed acid trehalase which is optimally active at pH 4.5–5.0, and the other one, designated neutral trehalase with an optimum of activity at pH 6.8–7.0 [2,100–102]. It is also considered that the acidic trehalase is vacuolar, while the neutral enzyme is cytosolic [2,101,103,104]. *NTH1* was cloned by complementation of a neutral trehalase-deficient yeast mutant that was isolated by a sophisticated enzymatic overlay assay [105]. The gene is localized on chromosome IV and encodes a 80-kDa protein which is 77% identical with the product of *YBR0106* (*NTH2*), another gene identified by systematic sequencing of the yeast chromosome II [106]. Amino acid sequence comparison with the trehalases from *S. cerevisiae*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, insect, rabbit small intestine and *Escherichia coli* reveals that regions of homology (about 25–28% identity) are restricted to two domains corresponding to the central (likely catalytic) core and C-terminal parts, and that only neutral trehalases from yeasts exhibit a N-terminal extension that contains the phosphorylation regulatory domain [8]. Deletion of *NTH1* leads to inability of yeast cells to mobilize endogenous trehalose and to an apparent complete loss of measurable trehalase activity. In contrast, no change in trehalose and trehalase activity was observed in a *nth2* mutant. The only phenotypic feature found for *nth2* mutant is its lower thermotolerance also found for *nth1* mutants ([107], Section 5). The purified neutral trehalase is a homodimer of two 80–86-kDa subunits which displays a $K_m$ for trehalose between 5–35 mM [100, 102,108]. The great discrepancy in the $K_m$ might be related to the existence of neutral trehalase into two forms interconvertible by reversible phosphorylation. However, the kinetic properties of each form have not been established [102,108]. PKA is the only protein kinase known to directly phosphorylate and activate Nth1p [100,102, 108,109]. The Ser-20, -21 and -83 in the N-terminal part of the protein are the phosphorylatable sites and their absence leads to a complete inactivation of Nth1p [110]. However, nothing is known about the protein phosphatases that dephosphorylate the phosphorylated form of Nth1p.

In order to clone *ATH1* which encodes the acidic trehalase, it was made use of the property that vacuolar proteins, when they are overproduced, mislocalize at the cell surface [111]. Therefore, colonies that expressed higher acidic trehalase activity after transformation with a multicopy yeast DNA library were screened on plates for their capacity to develop a dark green color after permeabilization and incubation with glucose oxidase reagents [112]. A surprising phenotype associated with the loss of acid trehalase function is the apparent inability of *ath1* mutants to growth on trehalose, since the vacuolar localization of Ath1p is in contrast with this property [113]. In addition, the transport of Ath1p to vacuoles via the secretory pathway as proposed from previous works [101,104] is doubtful since the amino acid sequence of this protein does not present any signal sequence and consensus cleavage sites characteristic of proteins following this pathway [8]. It is therefore suggested that the acid trehalase can be secreted to the periplasm similarly to the externalization of some permeases and $H^+$-ATPase [114,115]. The acid trehalase and extracellular trehalose will then be internalized from the surface and delivered to the vacuoles by endocytosis. The fact that mutants in the endocytosis pathway (*end1* mutants) cannot grow on trehalose like *ath1* mutant is consistent with this scenario [8]. The ability of acid trehalase to sustain growth on low trehalose concentration also fits with its high affinity for this substrate ($K_m$ 1.5–4.5 mM) [100,101].

### 3.3. Trehalose transport

Growth of certain yeast species is possible on trehalose. In *S. cerevisiae* it is preceded by a long lag phase due to the 'induction' of the trehalose uptake. Prior incubation of yeast cells with α-methylglucoside or maltose favors the subsequent uptake and growth on trehalose whereas glucose, galactose or ethanol are strongly inhibitory [116]. Kinetic studies showed the existence of at least two different trehalose transporter activities: a high affinity $H^+$-trehalose symporter ($K_m = 2$–$4$ mM) and a low affinity – non-concentrative – trehalose transporter ($K_m > 50$ mM). The high affinity transporter is repressed in glucose and highly expressed in maltose-containing medium, whereas the low affinity transporter appears insensitive to the carbon source [117,118]. Michels' group isolated *AGT1* as encoding a permease that restores growth on maltose of a *mal1* yeast strain [119]. The protein sequence is 57% identical to the maltose permease encoded by *MAL61*, and exhibits structural homology with members of the 12 transmembrane domain superfamily of sugar transporters [120]. Analysis of the *AGT1* promoter revealed the presence of a 489-bp region identical to that of the maltose-inducible *MAL61* gene, and the presence of the $UAS_{mal}$, which could explain the tight control of *AGT1* expression by maltose [121]. Unlike Mal61p which is specific for maltose uptake, Agt1p can also actively transport other α-glucosides including trehalose, turanose, isomaltose, α-methylglucoside and maltotriose [119]. The proposed function of Agt1p to actively transport trehalose from the medium [118] was genetically confirmed by showing that a maltose-positive yeast strain defective in trehalose synthase subunit (*tps1* mutant) no longer accumulates trehalose upon deletion of *AGT1* in the presence of a low concentration of the disaccharide ($< 4$ mg $ml^{-1}$) in the medium [122]. However, measurable intracellular trehalose in a *agt1Δtps1Δ* double mutant cultivated with a high exogenous concentration of the disaccharide ($> 10$ mg $ml^{-1}$) supports the existence of a low affinity trehalose uptake. The gene encoding this transporter has not been characterized yet. The accumulation of trehalose from the medium may provide a molecular explanation to the results of Panek's group who suggested the existence of a maltose-dependent, *TPS1*-independent trehalose synthesis pathway in yeast [123,124].

## 4. Integrated mechanisms of control

Cells remarkably adapt to their immediate environment by complex regulatory pathways which make the connections between external stimuli and growth (reviewed in [125–127]). Glycogen and trehalose are typical hall-marks of rapid adaptations of yeast cells to environmental changing conditions. The purpose of this section is to discuss the mechanisms by which nutrients availability and stress affect the levels of these two glucose stores and to integrate the regulation of reserve carbohydrates metabolism in the context of cell growth and cell division.

### 4.1. Genetic and metabolic control in response to stress

Cells subjected to stresses develop within minutes genetic and metabolic responses that eventually lead to the acquisition of a 'stress resistance' state [128,129]. Trehalose and glycogen likely belong to the early metabolic response as exposure of exponentially growing cells to high temperature, sodium chloride, hydrogen peroxide, copper sulfate, high ethanol concentration ($> 7\%$), or weak organic acids (sorbate, benzoate) causes, albeit to various extents, a rapid increase of one or both of the two carbohydrates [98,99,130–137]. A general genetic trait of stress conditions is the induction of a wide class of genes that relies on the presence of at least one *cis*-element in their promoter, the '*st*ress *r*esponsive *e*lement' (STRE, core consensus CCCCT, reviewed in [128,129,138]), which is under the positive control of the transactivator Msn2p/Msn4p [139,140]. However, the mere presence of one to several STREs in the promoter of genes involved in glycogen and trehalose pathways as well as in *PGM2* and *UGP1*, two genes required for the production of UDP-Glc [141,142], does not explain in most cases the increase in trehalose and glycogen in response to these stresses. In fact, increase

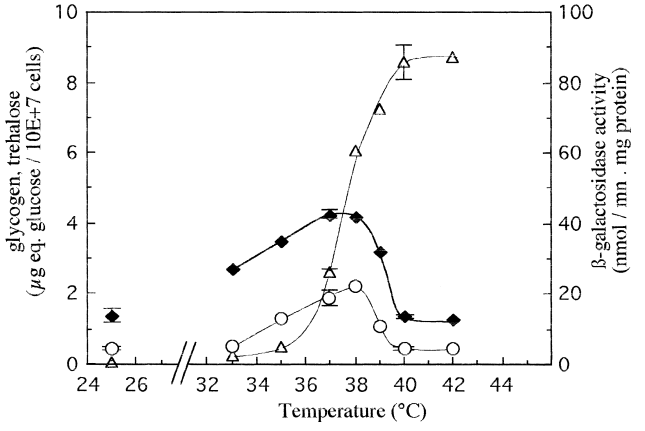


Fig. 3. Temperature effects on *TPS1* expression and on the levels of reserve carbohydrates. Exponentially growing yeast culture at 25°C was divided into portions and transferred to different water baths set at the indicated temperatures. During a 2-h period, samples were taken to measure β-galactosidase activity to obtain a *TPS1-lacZ* construct (integrated at the *URA3* locus of yeast strain) and to assay for glycogen and trehalose accumulation. Symbols are: β-galactosidase (♦), glycogen (○) and trehalose (△). Adapted from Parrou et al. [99] with the permission of Microbiology.

in the amount of enzymes, change in enzymatic activities by allosteric effectors or by covalent modification, increase in the levels of substrates or a combination of these parameters must be invoked to account for the accumulation of these two glucose stores in response to a specific stress condition. For instance, exposure of yeast cells to high ethanol concentration results in a modest trehalose accumulation mainly due to increased concentration of the Tre6*P* synthase substrates (UDP-Glc and Glc6*P*) while *TPS1* expression is not affected [137]. When yeast cells are challenged with sodium chloride (0.3–0.5 M) or $H_2O_2$ (0.4–1.0 mM), very low or even no accumulation of glycogen and trehalose is found in spite of transcriptional activation of the genes involved in their metabolism [99,143]. A net deposition of reserve carbohydrates is nevertheless observed after disabling the degradation pathways by deletion of *GPH1* and *NTH1*. This indicates that a major outcome of this coordinated stress-induced gene activation is to stimulate a recycling of glycogen and trehalose molecules [99,144].

The effect of temperature upshift perfectly illustrates the interplay between the transcriptional and post-translational mechanisms that promote trehalose and glycogen accumulation, as illustrated in Fig. 3. During a temperature upshift from 25°C to 33–38°C, the transcriptional activation of *TPS1* and *GSY2* mainly accounts for the net accumulation of glycogen and trehalose, since this deposition is prevented in a mutant strain defective in the STRE binding transcriptional activators Msn2/Msn4p. The coordinated transcriptional activation of the genes involved in the degradation reactions nevertheless leads to a relatively low deposition of these two glucose stores due to their potent turnover [131]. This is further indicated by higher accumulation in mutants defective in glycogen or trehalose degradation pathway [99]. Above 40°C, the induction of STRE-containing genes is totally abolished, glycogen does not accumulate while trehalose deposition reaches very high levels. The great efficacy to accumulate trehalose and the absence of glycogen is the consequence of a direct stimulation of the Tre6*P* synthase complex activity, and inhibition of trehalase and glycogen synthase by high temperature [96,98,99,145]. The high temperature dependency of Tre6*P* synthase complex could also explain why the accumulation of trehalose is largely independent of de novo protein synthesis [98,134,135,146,147]. In addition, greater substrate availability (Glc6*P* and UDP-Glc) should favor trehalose accumulation, but there is a controversy whether a temperature upshift causes an increase [135,136] or a decrease in Glc6*P* [98,137]. A weak trehalose recycling still persisted at this elevated temperature since an even higher trehalose content was measured in a *nth1* null mutant [99,148]. During a temperature upshift to 38–40°C, the intracellular concentration of substrates might be decisive to account for the mild trehalose accumulation, since in this temperature window the positive effect of temperature on Tre6*P* synthase complex hardly compensates for the sharp reduction of *TPS1* transcriptional activation [99]. The importance of the availability of the substrates for trehalose and glycogen accumulation in response to stress has been strengthened by strains with deleted or overexpressed *PGM2* and *UGP1* [141,149]. Finally, it should be emphasized that the capacity of cells to accumulate trehalose and glycogen in response to stress is linked to the amount of enzymes they contain before exposition to stress, as revealed by *msn2/msn4* and *hsp104* mutants which accumulate less glucose stores after heat shock because of a lower basal *TPS1* and *GSY2* expression [99,146,150,151].

### 4.2. *Genetic and metabolic control in response to nutrients availability*

Two extreme nutritional situations currently investigated in *S. cerevisiae* are (i) the lack of one essential nutrient (i.e. nutrient starvation) which causes growth arrest in $G_0/G_1$ of the cell cycle and which is generally accompanied by a high glycogen and trehalose content and (ii) the abundance of all essential nutrients (i.e. nutrient sufficiency) which permits cells to grow and divide at maximal rate with a low content of storage carbohydrates. Nutrient starvation is obtained by transfer of exponentially growing cells to a medium lacking a nitrogen, carbon, phosphorus or sulfur source. The genetic response of genes involved in reserve carbohydrate metabolism is strictly dependent upon the presence of the 'STRE' *cis*-elements or the transactivator Msn2p/Msn4p [151–155]. In response to nitrogen starvation, the accumulation of reserve carbohydrates is explained by a moderate 2–3-fold transcriptional activation of the genes involved in these metabolic pathways

and by a greater availability of the substrates (Glc6*P* and UDP-Glc). Glycogen deposition is further favored by the Glc6*P*-promoted activation of glycogen synthase and inactivation of glycogen phosphorylase (see Section 2 and Fig. 2). With respect to carbon starvation, the immediate and sustained gene activation contrasts with a very weak deposition of reserve carbohydrates (unpublished). This is in part due to the fact that the complete absence of sugar in the medium strongly impairs RNAs translation [156,157] and also due to very low levels of Glc6*P* and UDP-Glc (unpublished).

Conversely, resuspension of starved cells or stationary phase cells in a growth medium with a fermentable carbon source elicits a mobilization of glycogen and trehalose which is associated with a sustained activation of neutral trehalase and glycogen phosphorylase, and an inactivation of glycogen synthase [2,3,22,158,159]. These changes are likely brought about by the transient glucose-induced cAMP-dependent activation of PKA, followed by a sustained nutrient stimulation of PKA activity to high level by a still poorly characterized cAMP-independent pathway (referred as the fermentable growth medium or FGM pathway, reviewed in [160]). In addition, the high glycolytic flux prevailing in this condition likely maintains the intracellular pool of Glc6*P* relatively low and reduces its effect in the activation of protein phosphatases and in the inhibition of protein kinases involved in the reversible phosphorylation of glycogen synthase and glycogen phosphorylase (see Fig. 2).

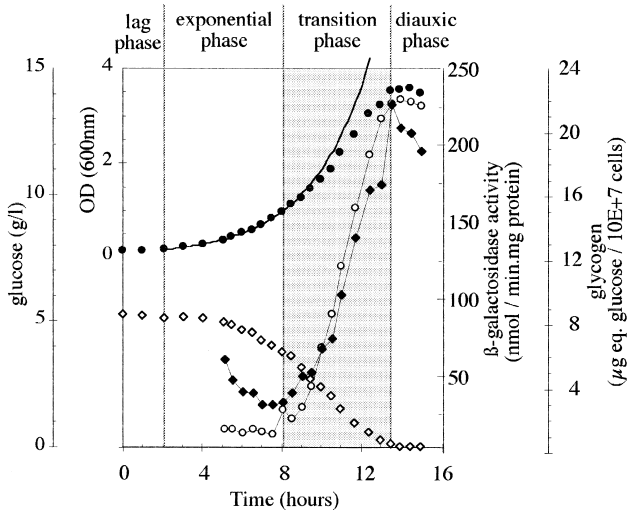


Fig. 4. glycogen accumulation and expression of *GSY2* during the diauxic growth of *S. cerevisiae* on glucose. Accurate monitoring of yeast culture on glucose allows to divide the growth in four specific phases: (i) lag to accelerating growth phase, (ii) a pure (and actually quite short) exponential phase during which the growth rate ($\mu$) is maximum, (iii) a transition phase for which the growth rate is decelerating, and (iv) the diauxic shift which starts at the time when glucose is exhausted from the medium. The exponential fitting curve that provides maximal growth rate ($\mu_{max}$) was calculated using the experimental points in the 'exponential phase' of the growth. Glycogen levels and expression of *GSY2* were assessed from β-galactosidase levels of a *GSY2-lacz* (integrated at the *URA3* locus of the yeast strain) and were determined during the diauxic growth until glucose exhaustion. Symbols are: glucose (○); cell density (●); β-galactosidase (♦); glycogen (◇). Modified from Parrou et al. [162] with the permission of Yeast.

### 4.3. Genetic and metabolic control during the diauxic growth on glucose

#### 4.3.1. Phenomenological aspects

In between the two conditions reported above, yeast cells may face in their natural habitat a more common situation which is the progressive change in the concentration of an essential nutrient during growth. Such a situation leads to a permanent adjustment of the genetic and metabolic machinery of the cell. Experimentally, this condition can be simulated in a continuous culture in the presence of a limiting nutrient (e.g. carbon source) or in a discontinuous culture providing that only one nutrient is progressively depleted and disappears while all other nutrients remained in large excess in the growth medium. A typical situation is the diauxic growth of yeast on a fermentable carbon source, which exhibits unique genetic and metabolic changes, including an early accumulation of glycogen and a reduction of glycolysis [1,161] before cells enter the diauxic phase of growth. As illustrated in Fig. 4, an accurate monitoring of cell growth shows that glycogen accumulation occurs during a transition period of growth that follows the pure exponential phase and precedes the diauxic phase [162]. Such a phase of growth is expected in cell cultures limited for a nutrient, but in this case a limitation for glucose is nevertheless puzzling since it occurs when approximately half of the extracellular sugar has been consumed, independently of its initial concentration in the medium [1,162]. The induction of glycogen at the beginning of this linear phase of growth perfectly coincided with the transcriptional activation of all the genes involved in glycogen metabolism [13,23,34, 53,54,57,152,162]. The transcriptional activation is accompanied by the accumulation of the respective proteins and by the activation of glycogen synthase and the inactivation of glycogen phosphorylase by dephosphorylation [14,18, 22,34,162,163]. These covalent modifications are essential to achieve normal glycogen accumulation since this latter is missing in *glc7-1* or *gac1* mutants defective in the activity of the major glycogen synthase phosphatase [34,89,164]. Unlike glycogen, trehalose accumulation does not occur in the 'linear' phase of growth, despite a sharp transcriptional activation of the genes involved in this metabolic pathway and the accumulation of Tps1p [83,84,148,151,153,162]. The accumulation of this disaccharide is delayed to the diauxic phase of growth since *NTH1* is co-expressed with *TPS1*, leading to high degradation activity and to the recycling of trehalose [161]. Consistent with this is the simultaneous accumulation of trehalose and glycogen in a *nth1* mutant [162]. There is however a paradoxical situation with respect to Nth1p, since its expression is low while its activity is high in glu-

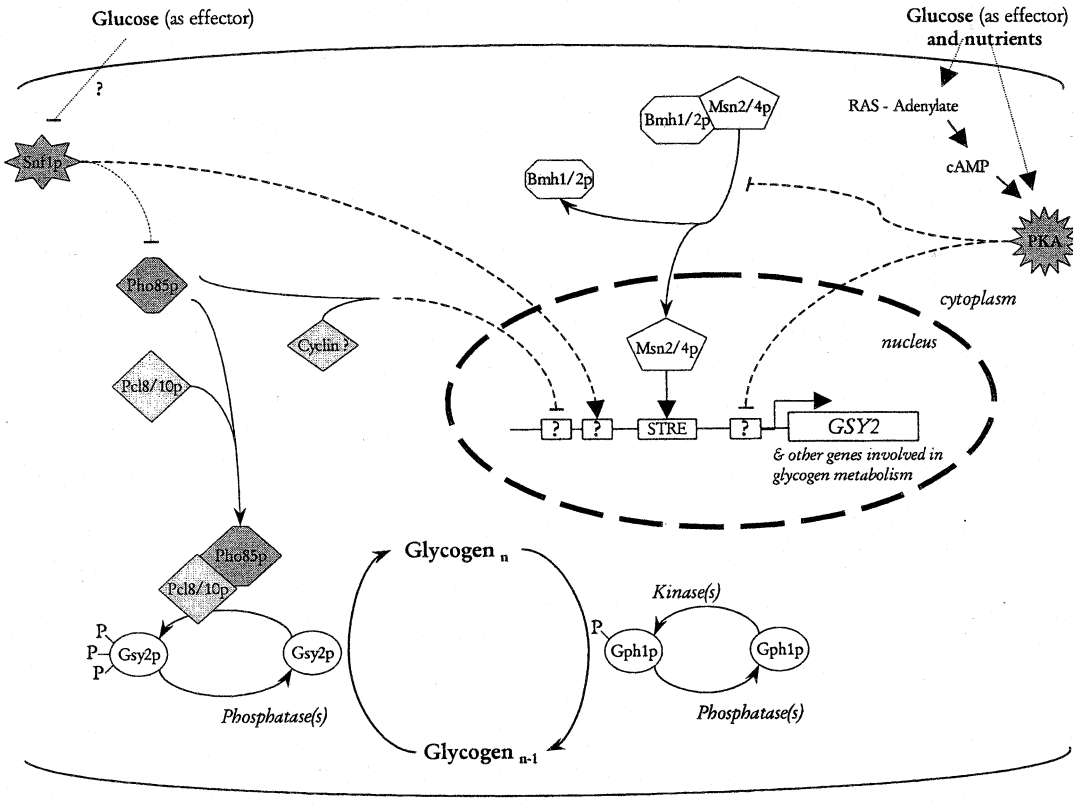


Fig. 5. Genetic and metabolic control of glycogen metabolism in *S. cerevisiae*. PKA negatively controls gene expression by inhibiting nuclear localization of the transcriptional activator Msn2p, and probably Msn4p, through the anchor protein Bmh1/2p. The STRE- and/or Msn2/4p-independent control of transcription by the PKA favors the existence of an independent still uncharacterized pathway. In contrast to PKA, the protein kinase Snf1p positively controls gene expression. It may act as an upstream negative effector of the cyclin-dependent protein kinase Pho85p. Similarly to its role in phosphate metabolism, Pho85p may associate with a cyclin to regulate the nuclear localization of a putative transcription factor that controls *GSY2* expression in a STRE-independent way. In addition, PKA and Snf1p antagonistically control the phosphorylation state of Gsy2p and Gph1p, which is not depicted for clarity of the cartoon (see Fig. 2 for additional details with respect to this post-translational control). Arrows, positive interaction; bars, negative interaction; dashed lines, direct interaction not yet determined.

cose-growing cells, whereas the reciprocal is observed in the post-diauxic phase [102,151,161,162,165].

*4.3.2. The genomic target(s)*

A common denominator for most of the genes showing an early transcriptional induction, including those involved in glycogen and trehalose metabolism, is the presence of one to several STREs in their promoter [162,166,167]. Although an absolute requirement has been claimed for these *cis*-elements for transcriptional activation of *GSY2* at the end of growth on glucose [152], the co-induction of these genes can occur in the absence of STREs [151,153,162]. This conclusion was further illustrated by the co-expression of *GLG1* encoding glycogenin 1 which does not possess any STRE in its promoter [162]. Moreover, removal of the two STREs in *GSY2* promoter does not alter the growth-related transcriptional induction of this gene [154]. Detailed *GSY2* promoter analysis also reveals the existence of PDS and HAP2/3/4/5 consensus, but none of them is implicated in the growth-related transcriptional induction of *GSY2* [152,154]. According to the function of Msn2/4p to bind STREs and to activate transcription [139,140], the absence of these two redundant factors results in a significant reduction of glycogen and trehalose accumulation during growth, likely due to an overall reduction in the expression of genes involved in the metabolism of these two glucose stores ([151,155], unpublished).

*4.3.3. The transduction pathways*

Compelling evidences indicate that the RAS-cAMP cascade exerts a potent negative effect on the reprogramming of gene expression at the diauxic transition of growth [168–170]. This negative control on the expression of genes involved in reserve carbohydrates implicates STREs and additional *cis*-elements since a substantial cAMP-dependent repressive effect persisted on a *ΔSTRE-gsy2-lacZ* construct integrated in the yeast genome [154]. It also requires more than Msn2p/4p transcription factors (Fig. 5) since a 3-fold repression of *GSY2* can be observed in *pde2cyr1msn2msn4* cells grown with high exogenous cAMP as compared to growth without cAMP (unpublished). Our recent data showed that the transcriptional induction of *GSY2* is almost totally abolished in *ira1ira2*

and *bcy1* null mutants which support the idea that the reduction of the PKA activity may be part, if not all, of the triggering pathway that promotes this growth-related transcriptional induction. It is worth noting that the previous claim of a PKA-independent control of cellular responses during the diauxic growth [154,171,172] is likely misleading due to the use of mutant strains (*tpk*$^{w}$*bcy1* and *sra1-14* mutant) in which the *bcy1* allele does not probably yield to a constitutive and non-regulatable PKA activity. Reduction of the cAMP-PKA activity occurs at least at the transcriptional level as reported by a decrease in *CDC35*/*CYR1* and *CDC25* mRNAs and an increased expression of the negative regulators Ira1/2p and bcy1p [168,173]. In addition, Cdc25p stabilization by positive interaction with chaperone proteins of the Hsp70 family [174] could give an explanation for a reduction of Cdc25p activity due to the recruitment of these chaperones by unfolded proteins that are accumulated as cells progress towards the diauxic transition. One of the most challenging questions is therefore to identify the trigger of this growth-related change of the activity of the cAMP-PKA pathway.

In addition to its major role in the derepression of glucose-repressed genes (review in [175]), the protein kinase Snf1p positively controls reserve carbohydrates metabolism [176] (Fig. 5). This positive control takes place at the transcriptional level since *snf1* mutant cells exhibit a 2–4-fold reduction in the expression of genes involved in glycogen and trehalose metabolism ([23], unpublished data). The best characterized effect of Snf1p is however to favor glycogen synthase activation (Fig. 2) as shown by the suppression of the glycogen deficiency of *snf1* mutants by a mutation in *GLC7* (*glc7-1*), by deletion of *GAC1* or by truncation of the carboxy-terminus of Gsy2p [19,23]. Interestingly, the cyclin-dependent glycogen synthase kinase Pho85p has been identified in a screen for second site suppressors of the glycogen storage defect of *snf1* cells, indicating an antagonistic action between the two protein kinases on glycogen metabolism. Epistasis studies indicate that Snf1p may act in a parallel pathway or upstream Pho85p as a negative regulator of this kinase. The parallel pathway could involve the inhibition of other glycogen synthase kinases or activation of protein phosphatases such as Glc7p–Gac1p. However, the recovery of glycogen synthase activity in a *snf1* mutant deleted for *PCL8* and *PCL10*, which encode Pho85p targeting subunits to phosphorylate Gsy2p [177], favors a direct control of Pho85p by Snf1p. In addition to this post-translational control, Pho85p negatively controls the expression of genes involved in glycogen metabolism in a STRE-independent way [26,178] (Fig. 3). There are no available genetic data to conclude whether Snf1p and Pho85p act independently or not, but the STRE-independent control of gene expression by Snf1p (unpublished data) and the similar but opposite effect of these two kinases on gene expression [26,178] support the model of upstream control of Pho85p by Snf1p (Fig. 5). Moreover, the lack of glycogen accumulation in a *pcl8pcl10snf1* mutant is in agreement with the idea that Pho85p could associate with a yet unknown cyclin [27], to repress genes expression through the phosphorylation-dependent nuclear localization of a transcriptional factor, similarly to its role in phosphate metabolism [179].

Fig. 5 illustrates an integrated working model on the genetic and metabolic control of glycogen metabolism during growth and in response to different nutritional and stress conditions. As long as the growth is optimal (nutrient sufficient medium), the high PKA activity represses glycogen and trehalose accumulation synergistically with Pho85p, both at the genetic and the enzymatic levels. When an essential nutrient is progressively consumed from the growth medium, the activity of the cAMP-PKA pathway is reduced and releases gene repression (or promotes gene induction), whereas Snf1p possibly inhibits Pho85p (and/or other kinases) and possibly activates the Gac1p–Glc7p phosphatase complex (or other phosphatases) (see also Fig. 2 for further details on the post-translational control). This coordinated control results in a sharp rise of glycogen, probably concomitant with other metabolic and genetic changes as it has been elegantly illustrated by a genome wide scale analysis of gene expression during the diauxic growth [180], and which characterizes the switch from respiro–fermentative to respiratory metabolism in yeast [171,180,181]. However, neither Snf1p nor Pho85p are necessary to trigger this growth-related gene induction, as it is not prevented by the loss of function of either kinase (unpublished). In contrast, the cAMP-PKA pathway is indispensable as indicated by specific mutations (e.g. *ira1ira2* or *bcy1* null mutant) that abolish this growth-related event. There are still numerous partners or intermediates missing in this model. In particular the mechanism of Snf1p regulation during growth is unknown. By shifting yeast cultures from high (10%) to low (0.1%) glucose medium, Wilson et al. [182] showed a good correlation between the increase in Snf1p activity and in AMP/ATP ratio, suggesting that the change in this ratio might be part of the triggering signal for Snf1p activation. Therefore, it should be interesting to determine whether this energetic ratio may vary along the diauxic growth to trigger this singular induction.

### *4.4. Integration in the growth control through the TOR pathway*

Recent data revealed a central role of the TOR pathway to control cell growth and cell division in response to nutrients [127,183]. *TOR1/2* (*t*arget *o*f *r*apamycin) encode two related phosphatidylinositol kinase homologues (Tor1p and Tor2p) which are part of a signal pathway that stimulates protein synthesis by the control of translation initiation [184] and of ribosome biogenesis by transcriptional activation [185,186] in response to nutrient suf-

ficiency. These positive actions lead to optimal growth rate which can be explained in part by a fast $G_1$/S progression through the stimulation of Cln3p translational initiation [184]. Inhibition of TOR activity by rapamycin treatment of exponential growing cells resulted in genes expression patterns similar to those observed during the diauxic shift [180,181,187]. This result might suggest that the growth-related events associated with the entrance of yeast cells in the diauxic shift are in part controlled by the TOR pathway. Therefore, the striking similarity between TOR and cAMP-PKA pathways in the control of these events, including a negative control of Msn2/4p nuclear localization through the anchor protein Bmh1/2p which mediates nuclear exclusion [188,189], raises the question of how these two signalling pathways are interconnected in the control of growth in general and in storage carbohydrates in particular. Preliminary results suggest that they act independently since glycogen accumulation in rapamycin-treated cells is not abrogated by hyperactivation of the cAMP-PKA pathway [184].

Results from our laboratory support the idea that the initiation of the reserve carbohydrate accumulation is always associated with a drop in the growth rate, and this independently of the maximal growth rate the culture may have had during the truly exponential phase of growth ([162], unpublished). Since it is unlikely that such a drop of growth rate triggers these metabolic events, we favor the idea that a reduction of TOR- and cAMP-PKA pathway, in an independent way or synergistically, promotes reserve carbohydrates deposition by increasing gene expression, enzymatic activation and increasing the length of the $G_1$ phase, as the longer this phase is the more time for glycogen and trehalose synthesis is available [190]. This model also agrees with the observation that the $G_1$ duration of asynchronously growing cells increased as glucose is being depleted [191] and with the fact that storage carbohydrates deposition is inversely proportional to the growth rate ([1,192,193], unpublished). As stated above, the sensing process that initiates these events and triggers the reduction of TOR and PKA activity is unknown.

## 5. Function of reserve carbohydrates in yeast

### 5.1. Carbon and energy reserves

Glycogen accumulation agrees with the concept that a reserve carbohydrate accumulates when nutrients are still abundant since it is synthesized during the diauxic growth of yeast when glucose remains in the medium, and mobilized during the stationary phase of growth when yeast cells are deprived of nutrients [1,3,22]. Trehalose does not exactly fit with this concept since it is produced during the second growth phase on ethanol and it is consumed much later under non-stressed conditions [1,194]. A rather convincing evidence that the major function of glycogen is to provide carbon and energy for maintenance of cellular activities when nutrients are scarce is provided by mutant strains defective in respiration. It has been claimed for a long time that respiratory mutants are defective in glycogen synthesis based on a lack of brownish coloration upon exposure to iodine vapor [89,176,195,196]. However, in deep contrast with this claim, respiration-deficient cells accumulate even more glycogen during the fermentative growth phase on glucose, but they mobilize it much faster than wild-type cells at the onset of glucose depletion [197]. This metabolic trait which is specific to respiratory defects is associated with an inactivation of glycogen synthase and activation of glycogen phosphorylase, likely through reversible phosphorylation [197,198]. Taking into account the key role of Glc6*P* in the control of phosphorylation of these two enzymes (Fig. 2), we propose that the sudden drop in this metabolite observed in respiratory mutants at the onset of glucose depletion might be the trigger that promotes this fast glycogen degradation, as respiratory defective cells are unable to reuse by-products of glucose fermentation [197].

Experiments performed with partially synchronized carbon-limited continuous cultures of yeast showed that glycogen and trehalose mainly accumulate in $G_1$ phase of the cell cycle and are subsequently mobilized shortly before bud emergence, coincidentally with a rise in the activity of trehalase and glycogen phosphorylase ([190,191,199–202], unpublished). Moreover, a good correlation was obtained between the increase in the budding index and the extend of reserve carbohydrates mobilization, when the glucose feed in a chemostat was suddenly increased to rise the dilution rate (i.e. growth rate) of the continuous culture of yeast from 0.05 to 0.15 $h^{-1}$ (unpublished). These data suggest that these glucose stores are essential to generate a surplus of carbon and/or energy for the progression through the cell cycle at low growth rate. This idea was nevertheless challenged by the observation that a mutant unable to synthesize glycogen and trehalose still divided and grew at low growth rate [200]. However, the ATP production rate in this mutant had been increased by 1.6 times over the wild-type, and may therefore compensate for the energy production arising from reserve carbohydrates mobilization in the wild-type cells [200]. Finally, the reason why glycogen and trehalose accumulate so much during formation of spores and are rapidly degraded during spores maturation and germination [5,203–205] is totally unclear as these latter two processes can be performed without reserve carbohydrates synthesis or degradation [13,76,107,148,206].

### 5.2. Interaction with glycolysis

An unexpected link between trehalose and glycolytic pathways is that mutations in *TPS1* prevent growth on rapidly fermentable carbon sources (reviewed in [207]).

The metabolic features of *tps1* mutants are a hyperaccumulation of glycolytic intermediates up to fructose-1,6-bisphosphate, a reduction of intracellular Pi and a depletion of ATP within a few seconds after sugar addition [85,86]. Such an immediate ATP drop reveals an imbalance between the rate of the upper – ATP consuming – part of the glycolysis and the lower – ATP regenerating – part of this pathway. This suggests that an initial step of glycolysis is relieved from an inhibitory effect associated with the loss of *TPS1* function, thereby enhancing the sugar influx into glycolysis. Accordingly, the glucose sensitivity of *tps1* mutants can be suppressed by mutations which reduce the glucose transport [208,209] or lower the sugar phosphorylation rate [208,210]. The inhibition of the major hexokinase PII isoenzyme activity by sub-millimolar concentrations of Tre6*P* is still today the sole direct evidence for such a control at the gate of the glycolytic pathway [211]. The restriction of the glucose influx by Tre6*P* inhibition of hexokinase is probably incomplete as indicated by the following experiments. A 50-fold overexpression of hexokinase PII does not lead to a *tps1* null phenotype [212] and deletion of *TPS2* only causes growth inhibition above 35°C [90,91]. However, the inhibition of hexokinase by Tre6*P* is competitive with glucose, and therefore any correct conclusion requires precise determination of intracellular glucose and Tre6*P*. Using a novel methodology for exhaustive metabolites determination, we found that intracellular Tre6*P* transiently rose up ca. 7 mM in less than 5 min after glucose addition to resting cells [213]. This concentration is large enough to transiently reduce by 90% the activity of hexokinase (considering an intracellular glucose of 1.5 mM and $K_i$ for Tre6*P* of 0.04 mM [211,214]). Since the Tre6*P* inhibition of hexokinase does not apparently satisfy all the criteria of *tps1* phenotype, two additional models were proposed [205]. One of them, the phosphate recovery model, was suggested from the finding that intracellular phosphate, which is critical for the glyceraldehyde 3-phosphate (glyceraldehyde 3-P) dehydrogenase reaction, dropped to much lower levels in *tps1* than in the wild-type after glucose addition [86,95]. Thus, it was stressed that the trehalose synthase could recycle inorganic phosphate to avoid a blockage at the glyceraldehyde 3-P step, and the consequently ATP drop. This model actually could not hold for two reasons. First, an allele mutant of *TPS1* (the *byp1-3* mutant) displaying a very low Tre6*P* synthase activity had improved growth on glucose upon additional deletion of *TPS2* [95]. Second, the glycolysis flux is at least 100-fold faster than the trehalose formation, a result that makes inconceivable for this pathway to recycle the Pi needed to sustain the glyceraldehyde-3-P dehydrogenase reaction. The other model suggests that Tps1p itself could have a direct inhibitory action on the sugar influx, for instance by a direct interaction with glucose transporters and/or with hexokinase [82,83,95]. Even though Tps1p may exist as a free subunit in the cell [83], this model remains a speculative one.

Teusink et al. [215] have discussed the thermodynamical reasons for yeast cells to control the gate of the glycolysis, in comparing it with the 'turbo design' of a motor. Lack of control of the 'turbo design' nature of glycolysis would result in a 'substrate-accelerated death', with the prediction of a steady rise of the sugar phosphates until ATP and Pi are depleted. Interestingly, yeast cells are faced with a similar 'substrate-accelerated death' problem when challenged with stress conditions. Because these conditions cause growth retardation, yeast cells have to reduce ATP demand due to a decreased anabolic activity. This correction can be performed by reducing the sugar transport, the sugar kinase activity, or by increasing storage of carbohydrates. Alternatively, the induction of ATP futile cycles might be a fine tuning system to counteract the stress-induced ATP imbalance. The recycling of trehalose and glycogen molecules which is observed upon exposure of cells to heat, salt and oxidative stresses [99,131,144] may fulfil this function. In agreement with Blomberg [216], we suggest that glycogen and trehalose turnover may function as 'glycolytic safety valves' to avoid 'substrate-accelerated death' under stress.

### 5.3. *Specific function of trehalose as a stress protectant*

A high content of trehalose protects cells from autolysis and increases leavening capacity in dough [217–219]. In addition, it is well recognized that the capacity of yeast cells to withstand harmful environments is correlated with the intracellular content of trehalose [218,220–224]. Slight improvement of tolerance to freezing and dehydration have been obtained by disabling the trehalose hydrolysis pathway in laboratory and industrial strains [225,226]. Although other players including molecular chaperones participate in synergy with trehalose in stress resistance [218,227], trehalose fulfils two unique properties that make this molecule a stress protectant. A first one, recognized for a long time in anhydrobiotic organisms, is the capacity of trehalose to protect membranes from desiccation. This action known as 'the water-replacement hypothesis' [228] received support from in vivo $^1$H nuclear magnetic resonance experiments on freeze-dried yeast and from thermogravimetry and differential thermal analysis of rehydrated yeast cells [229]. These studies revealed that only 2–3% of dry mass of intracellular trehalose greatly improves viability of dried yeast. Varying the intracellular content of trehalose in yeast by means of its active transport, a similar threshold value was found for survival of cells during nutrient starvation and after a temperature shock at 50°C [230]. Preserving the biological structure of membranes is likely essential for its cellular function. Recent work on inhibition of maltose permease endocytosis by ethanol unraveled an important role of trehalose in releasing this inhibition, possibly because trehalose can displace ethanol in yeast membranes, and in doing so, maintaining the membrane integrity (R. Lagunas, personal

communication). Furthermore, the intrinsic property to substitute for water molecules and to stabilize membrane structure may account for the fact that trehalose greatly improves the survival of fibroblast cells during cryopreservation when the disaccharide has been introduced by microinjection or produced in low amount by genetic engineering of *E. coli* trehalose synthesis genes in mammalian cells [231,232]. The other and complementary function of this disaccharide is its ability to exclude water from the protein surface and hence to protect proteins from denaturation in hydrated cells. It was recently shown that both trehalose and the Hsp104 chaperone are required for refolding proteins in the cytosol [233] and also for conformational repair of heat-damaged glycoprotein in the endoplasmic reticulum lumen [234]. A high level of trehalose can protect native proteins from denaturation and also suppresses the aggregation of denatured proteins, which prevents their subsequent refolding by molecular chaperones [233]. Collectively, the proposed model is that the synergy between trehalose and some Hsps in stress tolerance is effective only if the accumulation of the disaccharide in response to stress is followed by its rapid mobilization as soon as the cells return to normal conditions [227]. This model reconciles contradictory results showing that mutants unable to accumulate or to degrade trehalose have a diminished resistance to heat stress [92,148,235, 236]. In addition, Tps1p itself seems to positively modulate the expression of genes encoding Hsps by a mechanism that is still not understood [237,238].

## 6. Concluding remarks

The yeast *S. cerevisiae* accumulates two storage carbohydrates, glycogen and trehalose, that fulfil either specific and in some cases shared functions. Their synthesis and degradation are controlled by complex transcriptional and post-translational mechanisms, which is not surprising since the survival of yeast cells is ultimately dependent on a positive carbon and energetic balance whatever the environmental challenging conditions. Many questions regarding control of reserve carbohydrates in yeast remain to be clarified, especially the identification of the different protein kinases or the protein phosphatases involved in interconversion of glycogen synthase, phosphorylase and trehalase, the identification of the *cis*- and *trans*-acting elements implicated in gene expression in response to nutrient, and more importantly, the sensing process that triggers the early glycogen induction when cells switch from fermentation to respiration mode of growth. The great challenge in the near future is to elucidate the mechanism by which the different nutrient signalling pathways (i.e. cAMP-PKA, SNF1, PHO85 and TOR protein kinase pathways) interplay each other to integrate the control of reserve carbohydrates metabolism in cell growth and cell division. From an applied point of view, the improvement of viability and leavening activity by genetic manipulation of baker's and brewer's yeast strains would require a better understanding of the regulation of reserve carbohydrate metabolism under industrial conditions. The emergence of novel technologies in genomics and proteomics will help to address these issues, as it has recently been carried out to explore the global response of the yeast *S. cerevisiae* to saline conditions [239,240].

## Acknowledgements

We are grateful to many colleagues for stimulating discussion and for providing unpublished data. We thank in particular Dr. C. Gancedo for many valuable suggestions that have improved the manuscript. Part of the work has been supported by Grants from Region Midi-Pyrenees (no. 97002787), CNRS (project PIR-GP-BIO12) and European Commission (BIO95-CT132-0192).

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