Post-translational modifications on yeast carbon metabolism: regulatory mechanisms beyond transcriptional control

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Abstract

Background Yeast cells have developed a variety of mechanisms to regulate the activity of metabolic enzymes in order to adjust their metabolism in response to genetic and environmental perturbations. This can be achieved by a massive reprogramming of gene expression. However, the transcriptional response cannot explain the complexity of metabolic regulation, and mRNA stability regulation, non-covalent binding of allosteric effectors and post-translational modifications of enzymes (such as phosphorylation, acetylation and ubiquitination) are also involved, especially as short term responses, all converging in modulating enzyme activity.

Scope of Review The functional significance of post-translational modifications (PTMs) to the regulation of the central carbon metabolism is the subject of this review.

Major Conclusions A genome wide analysis of PTMs indicates that several metabolic enzymes are subjected to multiple PTMs, suggesting that yeast cells can use different modifications and/or combinations of them to specifically respond to environmental changes. Glycolysis and fermentation are the pathways where phosphorylation, acetylation and ubiquitination are most frequent, while enzymes of storage carbohydrate metabolism are especially phosphorylated. Interestingly, some enzymes, such as the 6-phosphofructo-2-kinase Pfk26, the phosphofructokinases Pfk1 and 2 and the pyruvate kinase Cdc19, are hubs of PTMs, thus representing central key regulation nodes. For the functionally better characterized enzymes, the role of phosphorylations and lysine modifications is discussed.

General Significance This review focuses on the regulatory mechanisms of yeast carbon metabolism, highlighting the requirement of quantitative, systematical studies to better understand PTMs contribution to metabolic regulation.

1 Introduction

The budding yeast *Saccharomyces cerevisiae* preferentially uses glucose and fructose over other carbon sources as they can directly enter the glycolytic pathway [1]. Glucose is converted to pyruvate through the glycolysis and then fermented to give ethanol even in the presence of oxygen (Crabtree effect), leading to the production of ATP, metabolic intermediates and NADH to be used for other biosynthetic pathways (Fig. 1). However, when glucose is unavailable, a wide variety of alternative and non-fermentable carbon sources (such as galactose, sucrose, maltose, ethanol, glycerol, acetate and others) can be used for the production of metabolic energy and cellular biomass. Indeed, yeast cells can rapidly switch between respiratory and fermentative metabolism in response to variations in the availability of oxygen and fermentable sugars. This response is achieved by changes in the pattern of gene expression and protein regulation, allowing optimal adaptation to the most convenient substrate available in a certain situation and ensuring that enzymes needed for a specific pathway are produced only when required [1,2]. Yet, although glycolysis and gluconeogenesis are two opposite pathways for glucose metabolism, a number of enzymes are common to both pathways while only a few enzymes are specific for gluconeogenesis (see Fig. 1).

The tricarboxylic acid (TCA) cycle occurs in the mitochondrial matrix and plays a pivotal role in utilizing non-fermentable carbon sources via generation of NADH, driving aerobic respiration to yield ATP (Fig. 1). However, the TCA cycle is important also under fermentative conditions, since it is a source of biosynthetic building blocks, such as α-ketoglutarate, succinyl-CoA and oxaloacetate required for the synthesis of amino acids, glucose and of the prosthetic group heme. When cells are grown on two-carbon compounds, such as acetate, the TCA cycle by itself cannot supply adequate amounts of biosynthetic precursors unless alternative reactions are available. In this case, yeast cells use the glyoxylate cycle, which converts two-carbon units into four-carbon dicarboxylic acids bypassing oxidative decarboxylation. The glyoxylate cycle shares three of the five reactions with the TCA cycle, that are catalyzed by malate dehydrogenase, aconitase and citrate

synthase. Instead, the first two enzymes, isocitrate lyase and malate synthase, are unique to the glyoxylate cycle and are encoded by *ICL1* [3,4] and *MLS1* [5,6], respectively (see Fig. 1).

There are other biosynthetic pathways that branch from glycolysis. The pentose phosphate pathway starts from glucose-6-phosphate through the cytoplasmic glucose-6-phosphate dehydrogenase Zwf1, which catalyzes the first irreversible and rate-limiting step of this pathway (Fig. 1). It is required for generating NADPH, which is a source of reducing energy and of sugar molecules that are needed for the biosynthesis of nucleic acids and amino acids. It is also important for protecting yeast cells from oxidative stress, since NADPH is an essential cofactor for glutathione- and thioredoxin-dependent enzymes, that defend cells against oxidative damage [7,8].

Glucose-6-phosphate is also the debranching point for the synthesis of the storage carbohydrate glycogen, a high molecular mass branched polysaccharide and of the stress protectant trehalose, a non-reducing disaccharide (Fig. 1). Their concentration is high during nutrient limitations and in resting 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 [9]. Moreover, a short branch of glycolysis consisting of NAD-dependent glycerol-3-phosphate dehydrogenase (Gpd1, Gpd2) and glycerol-1-phosphatase (Rhr2, Hor2), produces glycerol from dihydroxyacetone phosphate (Fig. 1). Glycerol synthesis is not only required under osmotic stress, but plays an important role in lipid synthesis and it is necessary in anaerobiosis, since conversion of NADH excess to yield NAD^+ is essential for balancing the redox potential [10,11].

2 Regulation of metabolism

Yeast cells have to adjust their metabolism in response to genetic and environmental perturbations and this can be achieved in many different ways. The most intensively studied is the transcriptional mechanism, which implies a massive reprogramming of gene expression and involves many different factors, widely studied and described in several reviews (see for instance [1,2]). However,

there is a time delay between changes in mRNA levels and the corresponding changes of protein concentrations and enzyme activities, suggesting that transcriptional rearrangement cannot explain fast and rapid changes in cellular metabolism in response to internal or external stimuli. In addition, the transcriptional response cannot account for the complexity of metabolic regulation, since metabolic fluxes are the result of a complex interplay of gene expression, protein concentrations, post-translational modifications, enzymatic kinetics, allosteric regulation and metabolite concentrations [12]. Therefore, beyond transcriptomics, proteomics analysis as well as metabolomics and fluxomics technologies are gaining more and more relevance to provide a deeper understanding of cellular metabolic properties, since they allow to achieve quantitative and dynamic information on cellular metabolites, whose changes can be really fast. Thus, the quantitative measurement of fluxes through each metabolic reaction reflects the integrated response from all levels of cellular regulation.

In the following paragraphs, the functional relevance of post-transcriptional modifications to carbon metabolism regulation will be discussed, with particular regard to mRNA stability regulation, non-covalent binding of allosteric effectors and post-translational modifications, which are crucial short term mechanisms to modulate enzyme activity.

2.1 Modulation of mRNA stability

When glucose is available, genes involved in mitochondrial biogenesis and oxidative phosphorylation, as well as genes involved in metabolism of non-fermentable carbon sources, are not expressed, while genes involved in glycolysis and fermentation are actively transcribed. Among the different mechanisms of glucose repression, regulation of mRNA turnover plays a critical, although often poorly considered, role. In fact, the degradation of a number of yeast mRNAs is accelerated in response to high glucose signals (>1% glucose). These include the *SDH1* and *2* mRNAs, which encode the flavoprotein subunit (Fp) and the iron-protein subunit (Ip) of succinate dehydrogenase (complex II), respectively [13,14], the *SUC2* mRNA encoding invertase [15] and the

FBP1 and *PCK1* mRNAs, encoding the gluconeogenic specific enzymes fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase [16].

One of the best characterized is the turnover of *SDH2* mRNA, which is regulated by its 5' UTR and requires the 5'→3' exonuclease Xrn1 [14,17]. The stability of *SDH2* mRNA is determined by a competition, affected by glucose concentration, between formation of the translation initiation complex and the degradation machinery at the 5'cap [14,17].

The rapid degradation of mRNAs for gluconeogenic enzymes (such as *FBP1* and *PCK1*) is also sensitive to low glucose signals (0.01% glucose). The high sensitivity of gluconeogenic mRNAs to glucose might reflect the need for tight repression of gluconeogenesis when glycolysis is active. In contrast, the TCA cycle is required to provide important metabolic precursors during both gluconeogenic and glycolytic growth. It was shown that degradation of gluconeogenic transcripts requires sugar (glucose or fructose) phosphorylation, but further glycolytic metabolism is dispensable [18].

Moreover, Young and colleagues recently showed that a rapid and specific decay of mRNAs of several glucose-repressible genes (such as *PCK1* and *FBP1*) is induced by the inhibition of the activity of protein kinase Snf1/AMPK [19]. Snf1 is a kinase implicated in various processes, including metabolism of non-fermentable substrates, regulation of phospholipid and fatty acid biosynthesis, response to environmental stresses, cell cycle, invasive growth, meiosis and sporulation [20–23]. The mechanism through which Snf1 regulates mRNA stability was recently suggested to involve Snf1-dependent phosphorylation of proteins involved in mRNA metabolism, such as the major cytoplasmic deadenylase Ccr4, the RNA helicase Dhh1 and the exonuclease Xrn1 [24].

2.2 Allosteric regulations

A relevant mechanism to regulate enzymatic activity (especially studied for the glycolytic pathway) is the non-covalent binding of allosteric effectors, which allows the adjustment of metabolic fluxes

in response to changes in metabolite concentrations. One of the more relevant allosterically regulated steps is the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate, catalyzed by phosphofructokinases Pfk1 and Pfk2, which is a major checkpoint in the control of glycolysis. Phosphofructokinase activity is subject to two different control mechanisms: (*i*) it is inhibited by ATP and activated by AMP; (*ii*) it is stimulated by fructose-2,6-bisphosphate, produced by 6-phosphofructo-2-kinase (Pfk26, 27, Fig. 1).

The second control point of glycolysis is the final conversion of phosphoenolpyruvate to pyruvate; this reaction is essentially irreversible and is catalyzed by pyruvate kinase. Yeast cells express two isozymes of pyruvate kinase, Cdc19 and Pyk2, being Cdc19 the principal isoform, essential for fermentative growth [25,26] and allosterically activated by fructose-1,6-bisphosphate [25,27,28].

Finally, another well characterized allosteric regulator of the glycolytic flux is trehalose 6-phosphate, which acts as a feedback inhibitor of hexokinase [29], thereby preventing the hyper-accumulation of sugar phosphates (Fig. 1).

2.3 Post-translational modifications (PTM)

Post-translational modifications (PTM) are particularly relevant events because they allow the cell to modulate protein activity in a fast, dynamical and reversible way, but more steadily than the allosteric regulation, to adapt to intracellular and extracellular stimuli. The interest of PTMs is given by the fact that a number of different enzymes are modified at the same time in a coordinate way in response to a signaling stimulation that activates or inactivates a given regulatory network (for instance a specific kinase phosphorylating a set of metabolic enzymes). In addition, several metabolic enzymes are subjected to multiple post-translational modifications, suggesting that yeast cells can use different PTMs and/or combinations of them to specifically respond to environmental changes. Phosphorylation and lysine modifications (ubiquitination and acetylation) will be discussed in details in the following paragraphs.

2.3.1 Phosphorylation

Phosphorylation is the best studied and more frequent reversible post-translational modification in yeast. The advent of high throughput techniques to study phosphorylation is producing more and more information about the phosphorylation status of proteins, although specific data on the functional relevance of these phosphorylations are still far from being achieved. Recently, an integrated proteomic approach was described, to identify *in vivo* functionality of enzyme phosphorylation under different growth conditions, opening the route for quantitative studies to relate phosphorylation status with physiological enzyme activity [30]. In addition, the connection with signaling pathways leading to specific phosphorylation events will be of great interest, since it will greatly improve our understanding on the regulation of metabolic networks. According to phospho-proteomic data, a large number of metabolic enzymes is phosphorylated, on one or more sites [31]. Figure 2A represents the phosphorylation network of the central carbon metabolism, showing the known substrate-kinase pairs, with red bordered nodes indicating kinases and filled nodes indicating substrates. As can be noticed, some enzymes seem to be phosphorylation hubs, being target of many different kinases (node size represents the degree of connection), thus representing central key regulatory nodes. The most connected enzymes are reported in Fig. 2B. Interestingly, the main target of phosphorylation is Pfk26, one of the two isoforms of 6-phosphofructo-2-kinase, responsible for the synthesis of the allosteric regulator fructose-2,6-bisphosphate. Also Pfk1 and 2 and Cdc19, catalyzing the two irreversible steps of the glycolytic pathway, appear to be phosphorylation hubs, thus indicating that these steps of glycolysis are the most regulated by phosphorylation. In addition, also three enzymes involved in glycogen and trehalose metabolism, the glycogen phosphorylase Gph1, the glycogen synthase Gsy2 and the neutral trehalase Nth1 present a high degree of connections (Fig. 2B), indicating that phosphorylation is a key regulatory process of carbohydrate storage. As concerns the kinases of the network (Fig. 2C), the cyclin-dependent kinase Pho85 is the main responsible for these phosphorylations. Pho85 is a multifunctional kinase, which regulates cell-cycle progression, cell

polarity and the actin cytoskeleton, gene expression, metabolism and the signaling of environmental changes [32]. Pho85 activity depends on the association with cyclin regulatory subunits, which confer substrate specificity and targets the enzyme to different cellular functions [32]. The role of Pho85-dependent phosphorylation on some of these enzymes has been functionally characterized and will be discussed below. Also two catalytic subunits of protein kinase A (PKA), Tpk1 and Tpk2, present many connections in this analysis (Fig. 2C). The cAMP/PKA pathway regulates many aspects of cellular physiology, such as growth, response to glucose and mass accumulation during cell-cycle progression [33–35]. Finally, two other interesting kinase clusters emerge from the substrate-kinase pair analysis: the putative serine/threonine protein kinase Ptk2 [36], whose role is still poorly understood, and the interesting DNA-damage cluster (Fig. 2C), comprising the three kinases Mec1, Tel1 and Rad53, activated upon DNA damage [37].

2.3.2 Lysine modification: ubiquitination and acetylation

Another system to regulate metabolic pathways under specific growth conditions is by modulating enzyme stability, which is mainly achieved through the ubiquitin/proteasome pathway. The covalent binding of ubiquitin (Ub) is mediated by a hierarchical cascade of E1-E2-E3 enzymes [38]. By multiple runs of reactions, ubiquitin is attached to the ε-amino group of an internal lysine residue of the target protein, to form K48-linked poly-ubiquitinated conjugates that are rapidly recognized and degraded by the 26S proteasome [39].

Lysine residues can also be modified by acetylation, another dynamic and reversible post-translational modification. Acetylation of lysine residues on their ε-amino groups is catalysed by lysine acetyl-transferases (KAT), also known as histone acetyltransferases (HATs) and reversed by lysine deacetylases (KDAC), also known as histone deacetylases (HDACs) [40]. Acetylation depends on the concentration of acetyl-CoA, a central metabolite that is produced by at least three major pathways in yeast (Fig. 3A): (*i*) the mitochondrial pyruvate dehydrogenase complex, which converts pyruvate into acetyl-CoA; (*ii*) the fatty acid β-oxidation which occurs in yeast peroxisomes

[41]; (*iii*) acetyl-CoA synthetases Acs1 and Acs2, which catalyse the ligation of acetate and CoA and hydrolyze ATP into AMP + pyrophosphate. Notably, acetylation may occur non-enzymatically by exposure to acetyl-CoA, particularly in mitochondria where a protein acetyltransferase activity has not been identified yet [42,43]. The best characterized acetylation events are related to transcription and several studies have shown that transcription factors can recruit acetyl-transferase-containing coactivator complexes to DNA to catalyse histone acetylation, which consequently facilitates gene activation [44,45]. More recently, the role of cell regulation by lysine acetylation has been extended to that of other major PTMs such as phosphorylation and ubiquitination, although functional relevance of lysine acetylation has been specifically examined only for few metabolic enzymes in yeast, and a lot of work still needs to be done in order to elucidate the importance of acetylation on metabolism. In Fig. 3B we mapped ubiquitinated (blue circles) and acetylated (red circles) enzymes in the central carbon metabolism, identified querying the CPLM database [46,47]. The two pathways with the highest concentration of modified lysines are glycolysis (Pgi1, Pfk26, Pfk1, Pfk2, Fba1, Tpi1, Pgk1, Gpm1, Eno1, Eno2 and Cdc19) and fermentation (Pdc1, Adh1, Ald4 and Ald6). Strikingly, many of the modified enzymes catalyze irreversible reactions, which need to be finely regulated under different metabolic and environmental conditions. It is also interesting to note that most of these enzymes were found to be either ubiquitinated or acetylated, suggesting that acetylation and ubiquitination can be mutually exclusive post-translational modifications.

3 Regulation of carbon metabolism by PTMs

Post-translational modifications, and especially phosphorylation -which seems to be the predominant mechanism for protein modification-, can regulate metabolism in different ways, such as through modification of the enzyme structure and consequent alteration of its functioning or through regulation of the total amount of available enzyme, which results from the balance between its synthesis and degradation. In order to understand the impact of PTMs on metabolism, the

identification of PTM sites and their functional relevance is not enough, since pools of modified and non-modified protein coexist in the cell, and what changes is often the ratio between these pools under different conditions. At the moment, essentially qualitative data on PTMs on enzymes involved in carbon metabolism are available, but quantitative, systematical studies are required to better understand their contribution to metabolic regulation, as well as kinetic analysis of their changes, to investigate their persistence over time and the time-scale in which they contribute to metabolic rearrangements.

3.1 Glycolysis

One of the more studied positive regulators of glycolysis is protein kinase A (PKA) [33–35]. It phosphorylates proteins catalyzing key enzymatic steps, such as both isoforms of pyruvate kinase, Cdc19 and Pyk2 [48–50]. PKA phosphorylation on Ser22 was shown to stimulate Cdc19 activity, although cells expressing Cdc19-S22A are still able to grow in glucose [50]. PKA also phosphorylates the 6-phosphofructo-2-kinase Pfk26 on Ser644, increasing its enzymatic activity [51–53], as well as on some other residues, whose physiological relevance have not been fully understood [53].

As highlighted above (see Fig. 2B), Pfk26 is phosphorylated by several other kinases. For instance, it was reported as a substrate of protein kinase C (Pkc1), a Ser/Thr protein kinase involved in the regulation of cell wall remodeling, through the activation of a mitogen-activated protein (MAP) kinase cascade [54]. Under hypotonic stress conditions, Pkc1 is activated to respond to the environmental change [55] and phosphorylates the 6-phosphofructo-2-kinase Pfk26 on Ser8 and Ser652, causing its inactivation [56]. This inactivation could be part of the cellular response to the osmotic stress, since the reduction of the glycolysis could lead to an accumulation of glucose-6-phosphate for the synthesis of glucane (the main constituents of the yeast cell wall [57]), as well as to a reduction of the synthesis of the strong osmolyte glycerol [56]. Moreover, hypotonic stress stimulates Pfk26 acetylation on Lys3, which affects its N-terminal phosphorylation under hypo-

osmotic stress and slightly inhibits its activity [58]. On the contrary, hyper-osmotic stress stimulates the HOG-MAPK pathway [59] and leads to multiple phosphorylations on Pfk26 within the peptide 67–101, causing its activation and possibly rerouting glycolytic flux towards lower glycolysis [60,61].

Pfk27, the second isoform of 6-phosphofructo-2-kinase, was shown to be phosphorylated by protein kinase Snf1/AMPK [62]. Upon glucose removal, Snf1 phosphorylates Pfk27 in its N-terminal domain, leading to the SCF^{Grr1}-dependent degradation of Pfk27 [62]. In particular, Snf1-dependent phosphorylation is required to promote Pfk27 association with the F-box protein Grr1 [62], thus leading to Pfk27 turnover and consequently to a reduction of fructose-2,6-bisphosphate. The importance of Pfk27 turnover is highlighted by the fact that expression of a non-phosphorylatable and non-degradable Pfk27 protein inhibits growth on glycerol [62].

3.2 Gluconeogenesis

Remarkably, PKA also modulates gluconeogenesis. It phosphorylates the fructose-1,6-bisphosphatase Fbp1 on Ser11 in a glucose-induced manner [63–65], as well as on other non-mapped residues [66], leading to its inactivation [67] through a proteolytic mechanism [63–66,68,69]. The ubiquitin ligase responsible for poly-ubiquitination of Fbp1 is the Gid complex. This complex consists of seven subunits of which Gid2/Rmd5 subunit contains a RING finger domain providing E3 ligase activity [70,71]. After glucose addition, Gid4 activates the Gid E3 ligase. The Gid2 and Gid9 dimer together with the E2 ubiquitin-conjugating enzyme Gid3/Ubc8, poly-ubiquitinate the substrate proteins by triggering their degradation by the proteasome [71]. It was shown that when cells are starved for long periods of time in glucose-limited medium the degradation of Fbp1 progressively shifts from a non-vacuolar proteolytic pathway to a vacuolar one [66]. Glucose phosphorylation by hexokinase Hxk1,2 and glucokinase Glk1 is required for Fbp1 degradation in the proteasome and the vacuole; instead, the cAMP-dependent signaling pathway is exclusively involved in the vacuolar degradation of Fbp1 [66].

A similar degradation mechanism has been proposed for the phosphoenolpyruvate carboxykinase Pck1, another gluconeogenic enzyme [71]. Interestingly, Pck1 is also regulated through acetylation. Pck1 is acetylated and deacetylated by the nucleosome acetyltransferase of H4 complex and Sir2 deacetylase, respectively [72]. Acetylation occurs on Lys19 and Lys514 and the acetylation on Lys514 was shown to be crucial for its enzymatic activity and for the ability of yeast cells to grow on non-fermentable carbon sources [72].

3.3 Storage carbohydrates metabolism

A part from its positive role in stimulating glycolysis, PKA is also involved in glucose storage mobilization and inhibition of their synthesis. In fact, PKA inhibits glycogen synthase activity through phosphorylation of the C-terminal tail of Gsy2 [73], the most important glycogen synthase involved in the accumulation of glycogen upon entry into stationary phase [74]. Gsy2 is also inhibited by two other protein kinases: PAS kinases, which phosphorylate Gsy2 on Ser654, [75] and Pho85/Pcl8,10 complexes, which phosphorylate Gsy2 on Ser654 and Thr667, preventing hyper-accumulation of glycogen during exponential phase, when its function is not required [76–78].

In addition, PKA stimulates glucose storage mobilization through phosphorylation of the glycogen phosphorylase Gph1, on Thr31 and Ser333 [79,80], as well as through phosphorylation of several residues of the *NTH1*-encoded neutral trehalase, which is responsible for trehalose breakdown [81].

Finally, the metabolism of storage carbohydrates is regulated by the nutrient sensing PAS kinases [82,83]. They phosphorylate Ugp1, the UDP-glucose pyrophosphorylase, which produces UDP-glucose, a substrate for both glucan and glycogen synthesis [84]. Ugp1 phosphorylation on Ser11 by PAS kinase is required for growth under cell wall stress conditions [75,85,86] and leads to a conformational change that targets Ugp1 to the cell periphery, where its product UDP-glucose is generated in proximity to the site of glucan synthesis [86].

3.4 Other pathways

Little information is so far available on other metabolic pathways, but some data indicate that PTMs can regulate key steps of glycerol synthesis, glyoxylate cycle and pentose phosphate cycle. Glycerol synthesis is inhibited during the diauxic shift by Snf1 phosphorylation on Gpd2, the glycerol-3-phosphate dehydrogenase required for anaerobic growth. In fact, it was recently reported that Snf1 phosphorylates Gpd2 on Ser72 priming Gpd2 for subsequent phosphorylation on Ser75, probably by Yck1 [87]. Instead Gpd1, the other isoform of the glycerol-3-phosphate dehydrogenase, is a target of TORC2-dependent kinases Ypk1 and Ypk2. Hyperosmotic stress prevents Ypk1-dependent inhibitory phosphorylation of Gpd1, causing its dephosphorylation and activation to enhance glycerol production [30,87].

Two key enzymes of the glyoxylate cycle, malate dehydrogenase Mdh2 and isocitrate lyase Icl1, are subjected to proteolysis after glucose addition. Mdh2 is degraded through the proteosomal pathway when cells are starved for short time and is degraded in the vacuole when cells are starved for longer [66,88]. Also Icl1 is subjected to catabolite repression, since its activity is dispensable when fermentable carbon sources are available [89]. It was shown that phosphorylation of Thr53 by PKA is involved in a regulatory mechanism necessary for short-term inactivation of Icl1, but other unidentified mechanisms mediate the irreversible long-term inactivation of Icl1 [90].

Finally, glucose 6-phosphate dehydrogenase (G6PD, Zwf1), which catalyses the first step of the pentose phosphate pathway, is acetylated at Lys191, inhibiting its enzymatic activity [91].

4 Concluding remarks

The analysis of the enzymes involved in carbon metabolism (shown in Fig. 1), as targets of phosphorylation, acetylation and ubiquitination, highlights that 54% of them are subjected to more than one PTM, showing the importance of PTMs in the regulation of the enzyme function at the network level. Interestingly, 21% of metabolic enzymes are targets of all three PTMs and they are mainly enzymes of glycolysis and fermentation. For instance, the phosphofructokinases Pfk1 and 2

are phosphorylated by several kinases (Fig. 2B) and are also extensively acetylated and ubiquitinated (Fig. 3B). The same is true for the pyruvate kinase Cdc19, which is a known target of different kinases (Fig. 2B) and is acetylated on 19 lysines and ubiquitinated on 23 lysines (Fig. 3B). Therefore, these enzymes, which catalyze irreversible steps of glycolysis and are known to be allosterically modulated, also represent regulation hubs on which different kinds of stimuli converge, maximizing the flexibility of the cellular metabolism in adapting to internal and environmental changes. Strikingly, a comparison between these data and those reported by Ande and co-workers on human enzymes [92] demonstrates that, besides the well-known conservation of the metabolic pathways, there is also a strong evolutionary conservation of post-translational modifications as regulatory mechanisms of enzymatic activities. Thus, considering that changes in cellular metabolism are associated with a variety of diseases, studies, tools and new innovative methodologies conducted in *S. cerevisiae*, one of the most studied eukaryotic model organisms [93], are going to be of applicative relevance also in medical biotechnology. Therefore, systematic genetic analysis and experiments using steady-state chemostat cultures, which are the most suitable to collect quantitative and reproducible data, are required, in order to get to a complete understanding of metabolic fluxes and to develop mathematical models of metabolism.

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Figure legends

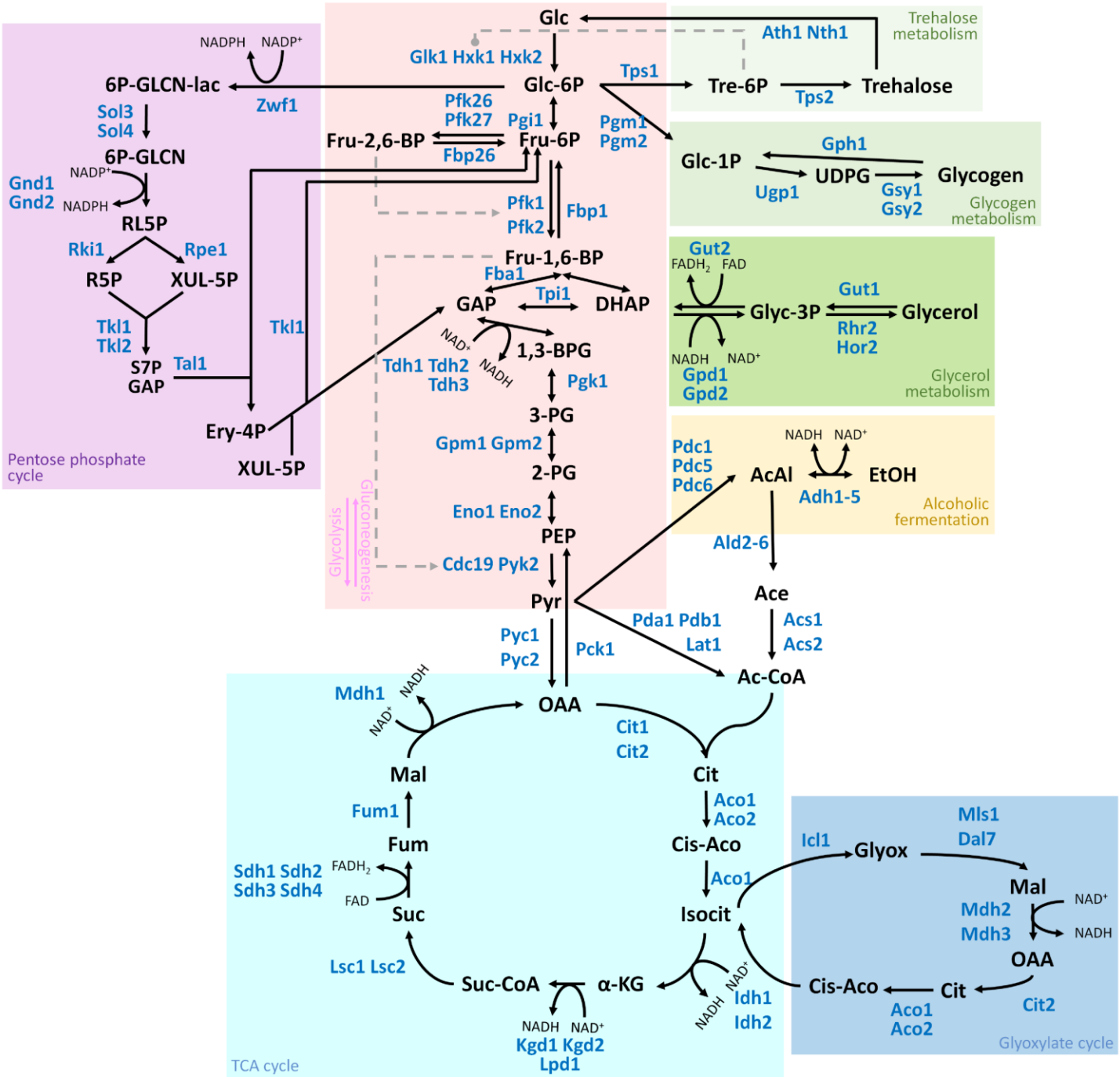
Figure 1 Metabolic pathways involved in central carbon metabolism. Metabolic pathways for carbons utilization are schematically shown (glycolysis, gluconeogenesis, alcoholic fermentation, TCA cycle, glyoxylate cycle, pentose phosphate cycle, trehalose metabolism, glycogen metabolism, glycerol metabolism) as well as key enzymes (in blue) involved in these processes. Compartmentalization information was omitted for graphical reasons. Black arrows indicate enzymatic reactions while dashed grey arrows correspond to regulatory steps. Colored boxes group metabolic reactions and enzymes of the same pathway.

Figure 2 (A) Phosphorylation network of the central carbon metabolism enzymes. The network reports the known target-kinase associations. Red bordered nodes indicate a kinase, filled nodes indicate a substrate. Red nodes indicate glycolytic enzymes, different shades of green indicate

enzymes of glycolysis-branching pathways (trehalose, glycogen and glycerol biosynthesis), pink nodes indicate pentose phosphate enzymes, orange nodes indicate fermentation enzymes and blue nodes indicate TCA/glyoxylate cycle enzymes. Node size represents the degree of connection. The network was generated querying the Yeast Kinase Interaction Database (KID, http://www.moseslab.csb.utoronto.ca/KID/; [31]; updated 2014-01-10) with the full list of enzymes of the central carbon metabolism pathways. Data visualization and analysis was performed with Cytoscape [94]. (B-C) Sub-networks of the most connected substrates (B) and kinases (C), extracted from the network in (A).

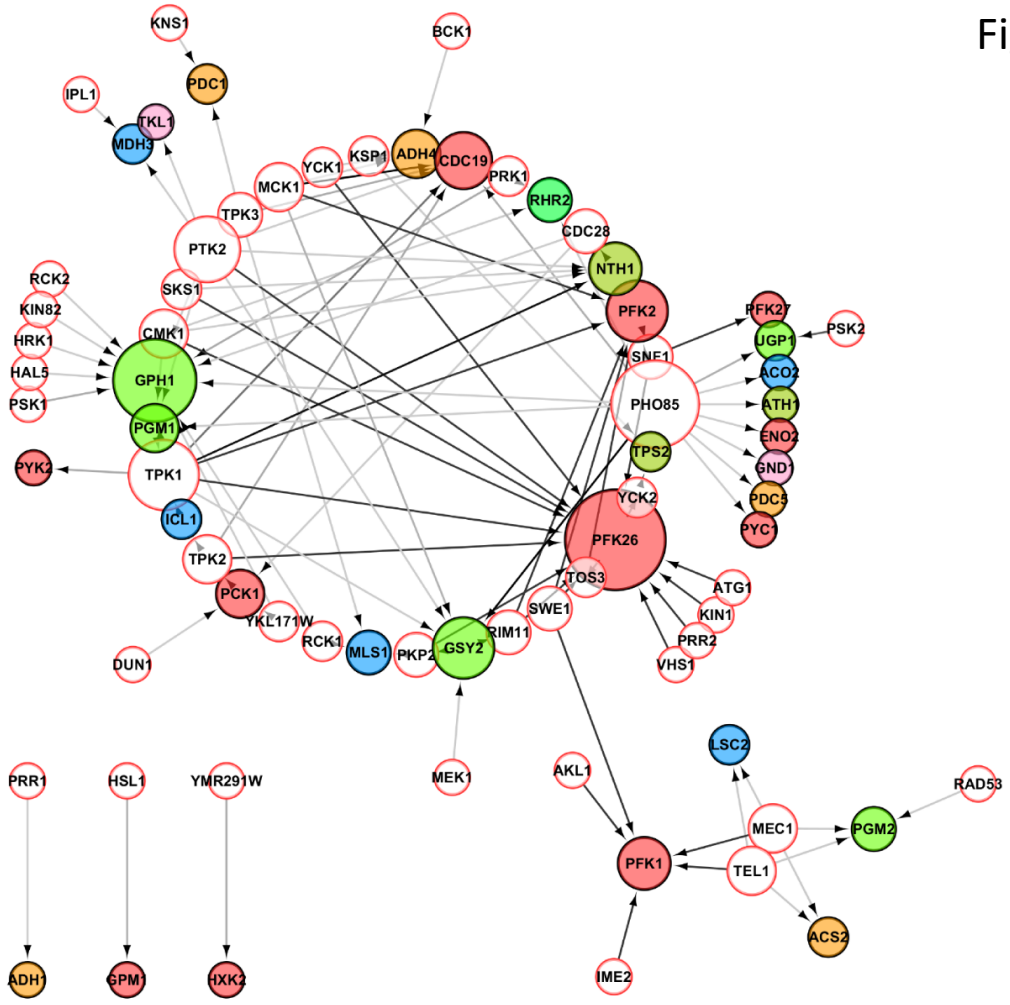
Figure 3 (A) Schematic representation of acetyl-CoA metabolism. Black arrows indicate direct enzymatic reactions while dashed arrows correspond to multi-step pathways. (B) Acetylated (red circles) and ubiquitinated (blue circles) enzymes mapped on the central carbon metabolism scheme. Circle size represents the number of modified sites. Only enzymes modified on more than 5 lysines are marked. The map was generated querying CPLM database [46,47].

Fig. 1

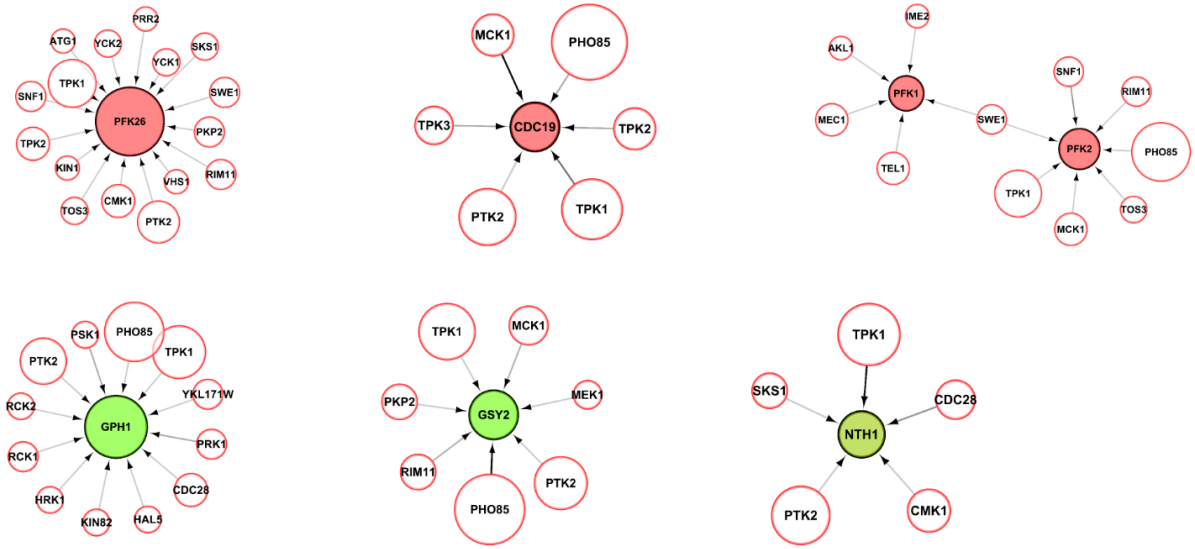

Glc
Trehalose metabolism
Ath1 Nth1
Glk1 Hxk1 Hxk2
Tps1
Glc-6P
Tre-6P
Tps2
Trehalose
NADPH NADP+
6P-GLCN-lac
Zwf1
Pfk26
Pfk27
Pgi1
Pgm1
Pgm2
Fru-2,6-BP
Fru-6P
Fbp26
Glc-1P
Gph1
UDPG
Ugp1
Gsy1
Gsy2
Glycogen
Glycogen metabolism
Sol3
Sol4
6P-GLCN
NADP+
NADPH
Gnd1
Gnd2
RL5P
Rki1
Rpe1
R5P
XUL-5P
Pfk1
Pfk2
Fbp1
Fru-1,6-BP
Gut2
FADH2 FAD
Fba1
Tpi1
GAP
DHAP
Gut1
Glyc-3P
Glycerol
Rhr2
Hor2
NADH NAD+
Gpd1
Gpd2
Glycerol metabolism
Tkl1
Tkl2
Tkl1
S7P
GAP
Tal1
NAD+
NADH
Tdh1 Tdh2
Tdh3
1,3-BPG
Pgk1
3-PG
Ery-4P
XUL-5P
Pentose phosphate cycle
Gpm1 Gpm2
2-PG
Pdc1
Pdc5
Pdc6
AcAl
NADH NAD+
EtOH
Adh1-5
Alcoholic fermentation
Eno1 Eno2
PEP
Glycolysis
Gluconeogenesis
Ald2-6
Cdc19 Pyk2
Pyr
Ace
Pda1 Pdb1
Lat1
Acs1
Acs2
Pyc1
Pyc2
Pck1
Ac-CoA
Mdh1
NADH
NAD+
OAA
Cit1
Cit2
Mal
Cit
Fum1
Aco1
Aco2
Fum
Cis-Aco
Mls1
Dal7
Icl1
Glyox
Sdh1 Sdh2
Sdh3 Sdh4
FADH2
FAD
Aco1
Mal
Suc
Isocit
NAD+
Mdh2
Mdh3
NADH
OAA
Lsc1 Lsc2
Suc-CoA
α-KG
NAD+
NADH
Idh1
Idh2
Cis-Aco
Cit
Cit2
NADH NAD+
Kgd1 Kgd2
Lpd1
Aco1
Aco2
TCA cycle
Glyoxylate cycle

(A)

Fig. 2



(B) Hub substrates



(C) Hub kinases

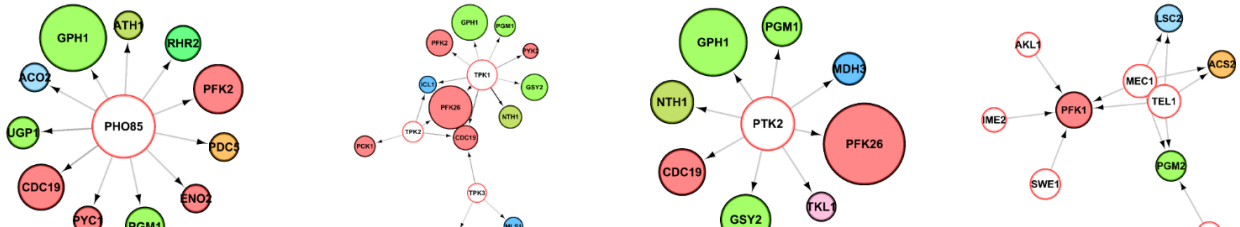
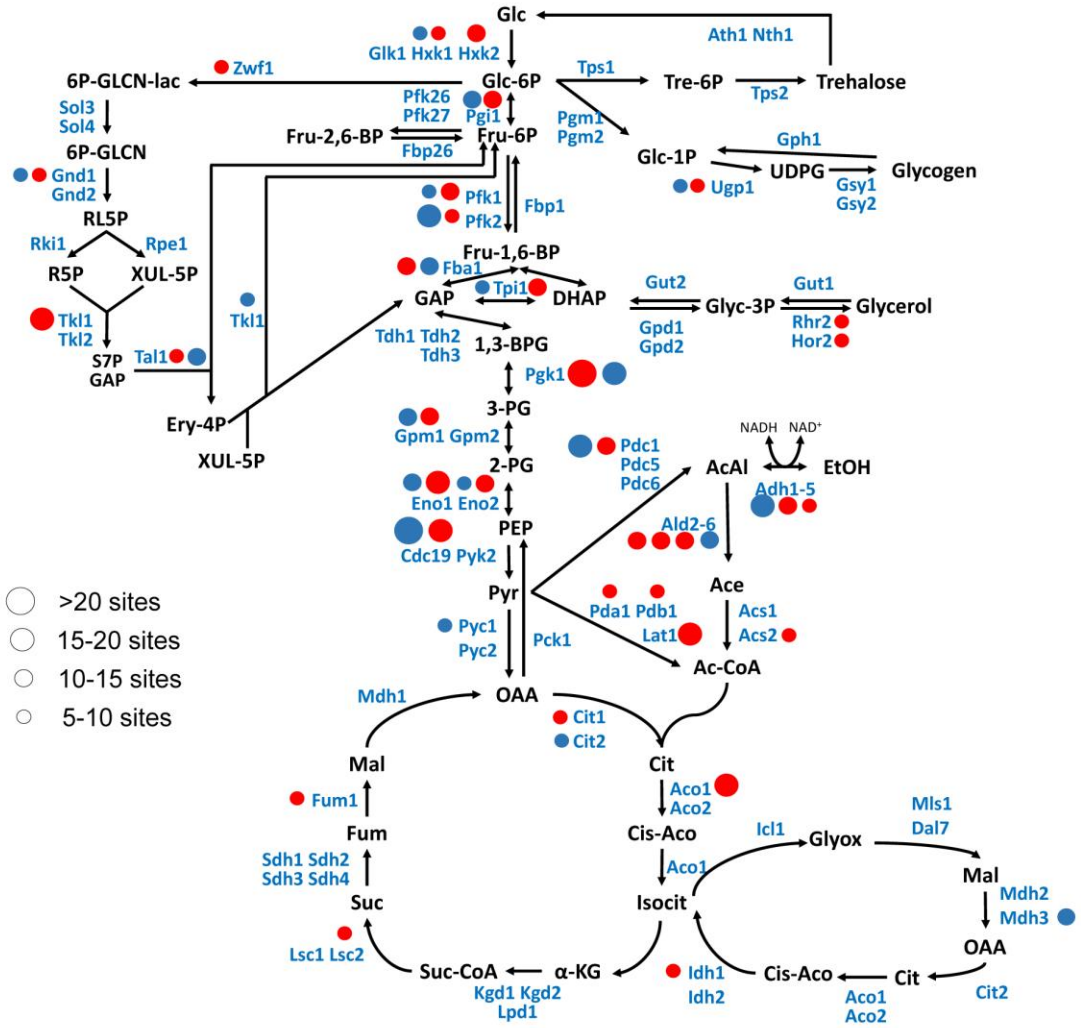
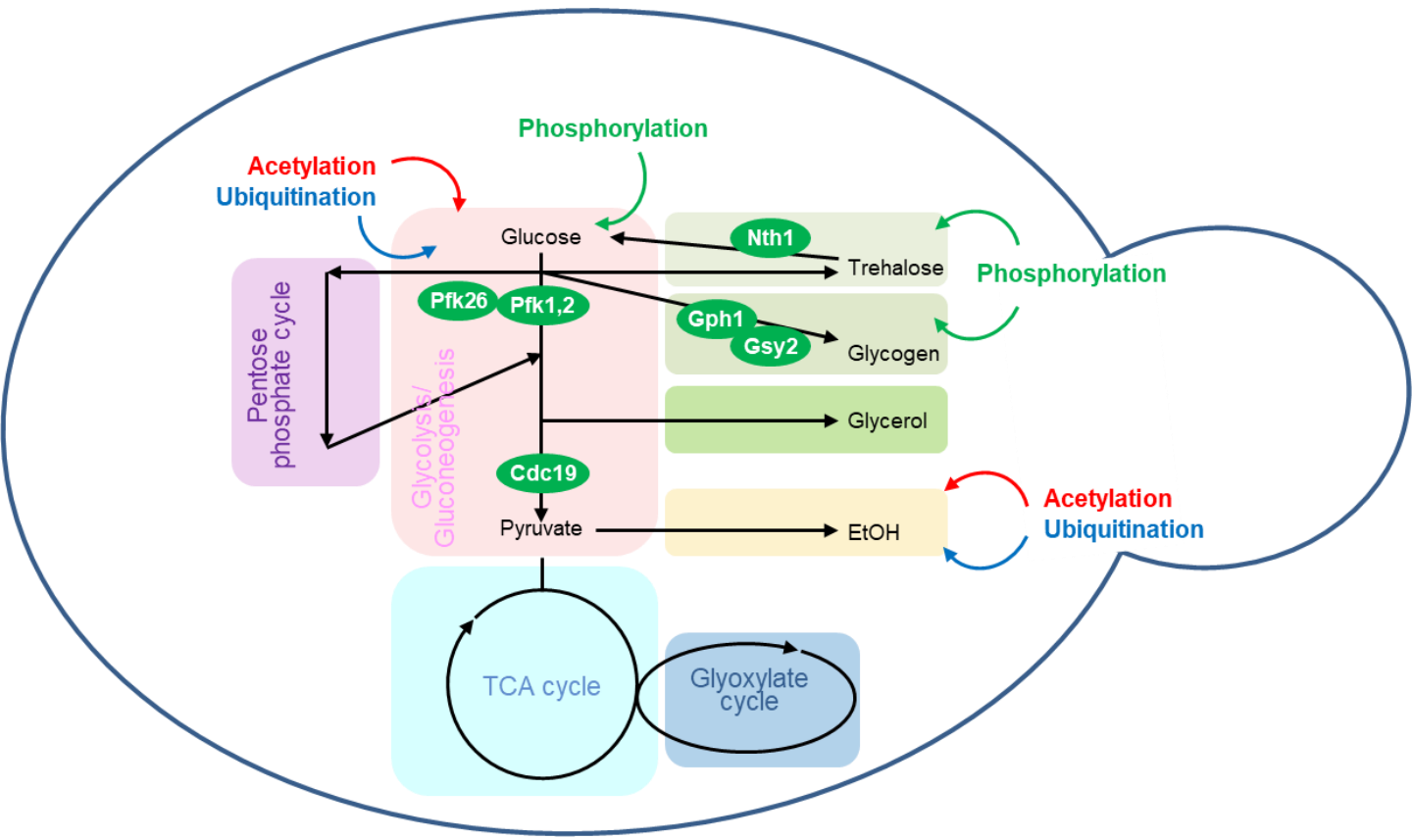


Fig. 3


Glc
Glk1 Hxk1 Hxk2
Ath1 Nth1
6P-GLCN-lac
Zwf1
Glc-6P
Tps1
Tre-6P
Tps2
Trehalose
Sol3
Sol4
Pfk26
Pfk27
Pgi1
Pgm1
Pgm2
Fru-2,6-BP
Fbp26
Fru-6P
Glc-1P
Gph1
UDPG
Glycogen
Gsy1
Gsy2
Ugp1
6P-GLCN
Gnd1
Gnd2
Pfk1
Pfk2
Fbp1
RL5P
Rki1
Rpe1
R5P
XUL-5P
Fru-1,6-BP
Fba1
GAP
Tpi1
DHAP
Gut2
Glyc-3P
Gut1
Glycerol
Rhr2
Hor2
Gpd1
Gpd2
Tkl1
Tkl2
S7P
GAP
Tal1
Tdh1 Tdh2
Tdh3
1,3-BPG
Pgk1
3-PG
Ery-4P
XUL-5P
Gpm1 Gpm2
2-PG
Pdc1
Pdc5
Pdc6
AcAl
NADH
NAD+
EtOH
Adh1-5
Eno1 Eno2
PEP
Ald2-6
Cdc19 Pyk2
Pyr
Ace
Pda1 Pdb1
Lat1
Acs1
Acs2
Pyc1
Pyc2
Pck1
Ac-CoA
Mdh1
OAA
Cit1
Cit2
Mal
Cit
Fum1
Aco1
Aco2
Fum
Cis-Aco
Icl1
Glyox
Mls1
Dal7
Sdh1 Sdh2
Sdh3 Sdh4
Aco1
Mal
Suc
Isocit
Mdh2
Mdh3
OAA
Lsc1 Lsc2
Suc-CoA
α-KG
Idh1
Idh2
Cis-Aco
Cit
Kgd1 Kgd2
Lpd1
Aco1
Aco2
Cit2
>20 sites
15-20 sites
10-15 sites
5-10 sites

Graphical Abstract



Highlights

- Yeast cells adjust metabolism in response to genetic and environmental changes.
- The transcriptional response cannot explain the complexity of metabolic regulation.
- Many metabolic enzymes are subjected to multiple post-translational modifications.
- These metabolic hubs, target of many PTMs, represent key regulatory nodes.
- We discuss the role of PTMs for the functionally better characterized enzymes.