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Review

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 Pfk2 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 PTM 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 and acetate) 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

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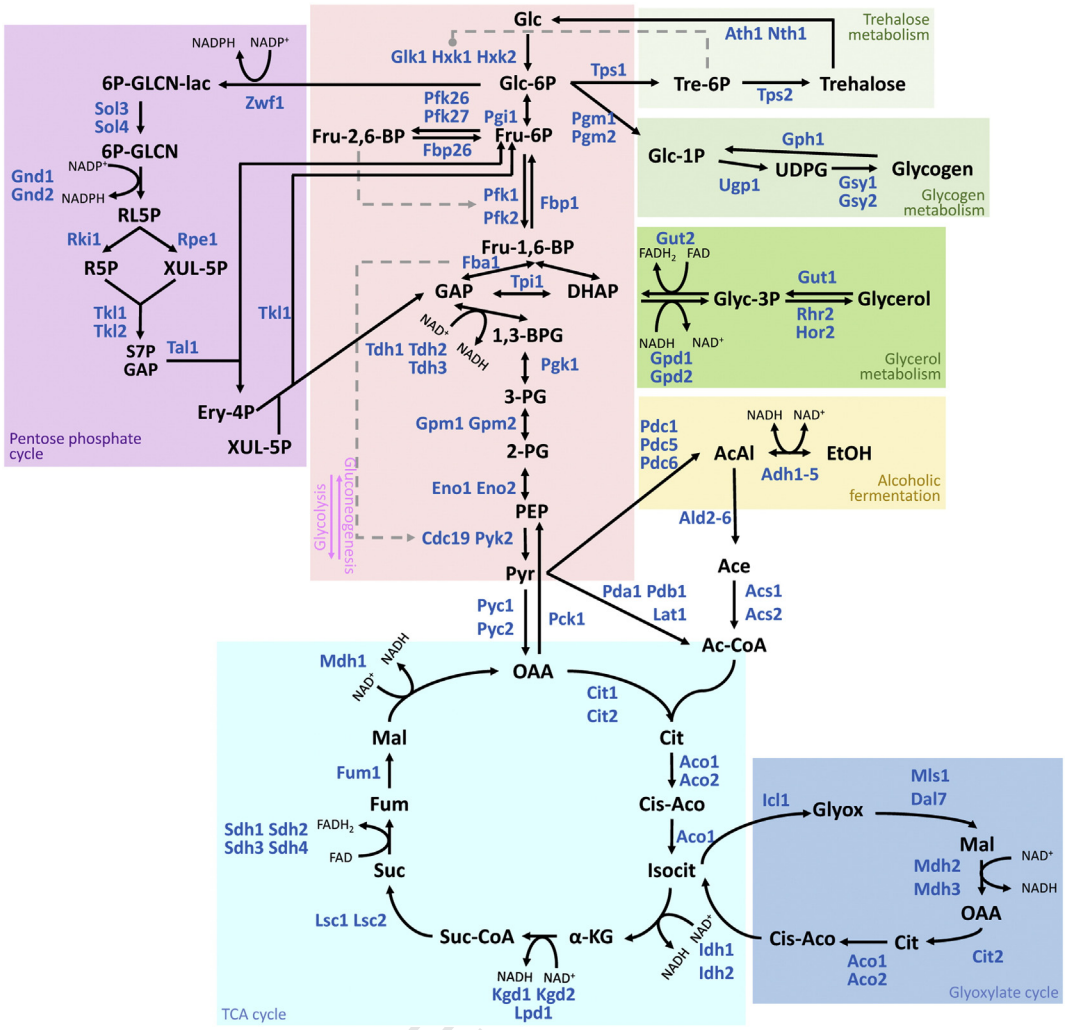

Fig. 1. Metabolic pathways involved in central carbon metabolism. Metabolic pathways for carbons utilization are schematically shown (glycolysis, gluconeogenesis, alcoholic fermentation, TCA cycle, glyoxylate cycle, pentose phosphate cycle, trehalose metabolism, glycogen metabolism, glycerol metabolism) 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 gray arrows correspond to regulatory steps. Colored boxes group metabolic reactions and enzymes of the same pathway.

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 also 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

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