Hagen Hofmann is in the Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel.
e-mail: hagen.hofmann@weizmann.ac.il

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METABOLISM

A new layer of glycolysis

Glucose metabolism has long been thought to operate with exquisite specificity and near-optimal efficiency. New findings show, however, that two glycolytic enzymes produce minor products that inhibit other enzymes involved in central carbon metabolism unless they are further metabolized by a novel enzyme.

Maria V Liberti & Jason W Locasale

The breakdown of carbohydrates through a series of enzymatic reactions is tuned to meet both catabolic and anabolic demands and to communicate information about nutritional status to other elements of cellular physiology[1]. Glucose metabolism operates with remarkable efficiency: each enzymatic reaction is able to satisfy both the requirements of phenotypic function and exact physicochemical principles[2,3]. In this issue of *Nature Chemical Biology*, Collard *et al.* now show that not only do certain enzymes in glycolysis catalyze alternative reactions, but these resulting byproducts can inhibit other enzymes in the pathway unless they are further metabolized by phosphoglycolate phosphatase (PGP, or PHO13 in yeast)[4].

Glycolytic enzymes are highly abundant, accounting for as much as 10% of the entire protein mass in a cell, and the rate of glycolysis can be very high (around 100 mM/h) in certain settings such as the Warburg effect[5,6]. Thus a glycolytic enzyme can generate a product concentration of over 1 M during the course of a cell cycle. If any of these enzymes catalyzes an alternative reaction with even 10^{-6} of the efficiency of the main reaction, this could result in the accumulation of a byproduct at micromolar concentrations unless that product is also metabolized. There is evidence for the presence of such metabolic side products during glucose metabolism. Recent work has demonstrated that 2-hydroxyglutarate (2HG) (a metabolite produced by cancer-associated mutations of IDH1 and/or IDH2) can be a minor product of the enzymes lactate dehydrogenase A (LDH-A) and phosphoglycerate dehydrogenase (PHGDH, an enzyme that commits glycolysis to serine synthesis), with each enzyme producing different enantiomeric forms[7,8]. 2HG generated from LDH-A can accumulate during hypoxia at concentrations that could approach those needed to inhibit certain dioxygenase enzymes.

It is likely that there are other instances of alternative reactions that can produce side products that have remained uncharacterized. Collard *et al.*[4] found that both glyceraldehyde phosphate dehydrogenase (GAPDH), the enzyme that catalyzes the sixth step in glycolysis, and pyruvate kinase (PK), the enzyme that catalyzes the ninth and final step in glycolysis, have minor alternative activities (**Fig. 1a**). GAPDH carries out the oxidative phosphorylation of glyceraldehyde-3-phosphate (GA3P) to generate 1,3-bisphosphoglycerate (1,3BPG), but can also catalyze the oxidative phosphorylation of erythrose-4-phosphate to produce 1,4-bisphosphoerythronate (1,4BPE), which is dephosphorylated to produce 4-phosphoerythronate (4PE). PK, which phosphorylates ADP using the substrate phosphoenolpyruvate (PEP) to generate ATP and pyruvate, can also use lactate as a substrate to generate 2-phospholactate (2PL). In both cases, the alternative substrate is a structural analog of the more specific substrate, and the enzyme has a far weaker activity (~10^{-6} that of its conventional substrate) for the alternative reaction.

Given that the accumulation of these products should lead to physiological consequences, the authors presumed that there must exist an enzyme to metabolize these side products. They identified phosphoglycolate phosphatase (PGP; PHO13 in yeast) as an enzyme that metabolizes 4PE and 2PL. Genetic ablation of this phosphatase using CRISPR–Cas9 led to the accumulation of both 4PE and 2PL. As hypothesized, the increased levels of these metabolites appeared to be able to reach concentrations that are sufficient to inhibit other metabolic enzymes (**Fig. 1b**). The authors showed that 4PE could inhibit glucose-6-phosphate dehydrogenase (6PGDH), an enzyme in the oxidative branch of the pentose phosphate pathway (PPP), and 2PL could inhibit phosphofructokinase 2 (PFK2), an enzyme involved in mediating the activity of glycolysis through the generation of fructose 2,6-bisphosphate, which is an allosteric effector of the glycolytic enzyme phosphofructokinase 1 (PFK1)[9]. Consistently with this, a recent study has shown that *PHO13* deletion strains upregulate PPP-related genes, presumably to compensate for decreased activity due to accumulation of 4PE[10]. For both metabolites, the product inhibits an enzyme involved in glucose metabolism and reduces the output of glycolysis, thus adding new layers of feedback interaction in the already complex reaction network of glucose metabolism.

Further structural elucidation of the binding properties of 4PE and 2PL to their targets (by, for example, defining mutations that can abrogate binding) and systems-level analysis of the fluxes and concentrations in the absence of PGP should provide further insights into the regulatory principles that define these feedback loops. Future work should also identify the physiological contexts in which regulation of the phosphatase may be important. For example,

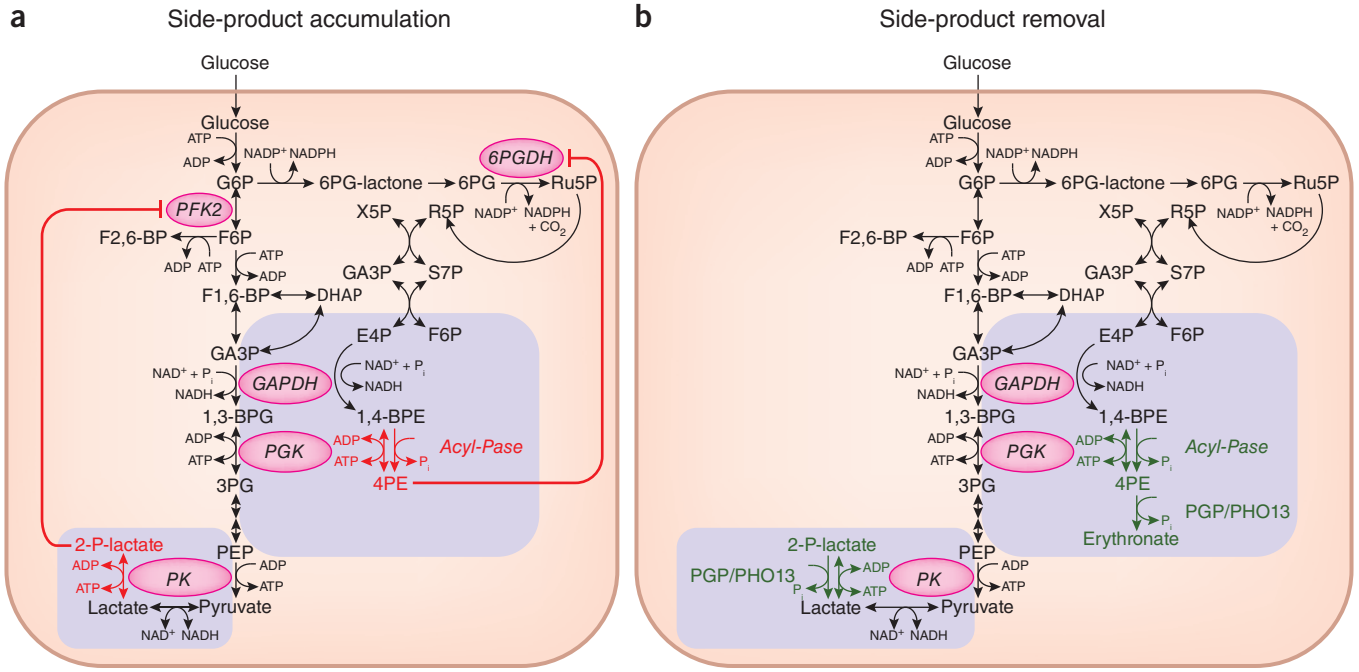

Figure 1 | Glycolysis, side-product formation, and repair by *PGP/PHO13*. (**a**) Inhibitory mechanisms of alternative product accumulation in the absence of *PGP/PHO13* (shown in red). 2-Phospholactate (2-P-lactate) causes inhibition of *PFK2* and 4-phosphoerythronate (4PE) causes inhibition of *6PGDH* in the pentose phosphate pathway. Circled, named enzymes are those involved in the formation of side products and their interacting enzymes. Purple shaded areas of the pathway denote reactions with alternative substrates. (**b**) Metabolic repair activities (shown in green) involving the enzyme *PGP/PHO13*. This enzyme prevents the accumulation of metabolites that inhibit the activity of enzymes in the pathway. 2-P-lactate is converted back to lactate, and 4-phosphoerythronate (4PE) is converted to erythronate. *6PGDH*, 6-phosphogluconate dehydrogenase; *PFK2*, pyruvate fructose kinase 2; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *PGK*, phosphoglycerate kinase; *PK*, pyruvate kinase; *G6P*, glucose-6-phosphate; F6P, fructose-6-phosphate; F1,6-BP, fructose 1,6-bisphosphate; F2,6-BP, fructose 2,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GA3P, glyceraldehyde-3-phosphate; E4P, erythrose 4-phosphate; 1,3-BPG, 1,3-bisphosphoglycerate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; 6PG-lactone, 6-phosphogluconolactone; Ru5P, ribulose-5-phosphate; X5P, xylulose-5-phosphate; R5P, ribose-5-phosphate; S7P, sedoheptulose-7-phosphate; 1,4BPE, 1,4-bisphospho-erythronate.

during conditions of fasting, the liver has multiple mechanisms that act to temper the pace of glycolysis, and downregulating this phosphatase could also serve this function. Conversely, during increased glycolysis associated with conditions such as hypoxia or growth factor signaling, one might expect that the phosphatase could be coordinately upregulated. The enzyme may also provide some protective function against diseases such as cancer and aging. Beyond aiding the understanding of this basic physiology, disrupting the overall balance of glycolysis is a therapeutic strategy for targeting aberrant glucose metabolism in cancer, but it has been challenging to define the specificity towards cancer cells. Addressing the allosteric mechanisms involved in this study brings another opportunity in this area. Nevertheless, the work by Collard *et al.*[4] provides an important forward step in understanding the generality of this intriguing mechanism. ■

Maria V. Liberti and Jason W. Locasale are at the Department of Pharmacology and Cancer Biology, Duke University, Durham, North Carolina, USA.
e-mail: jason.locasale@duke.edu

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