

New concepts in feedback regulation of glucose metabolism

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Abstract

Glycolysis, the breakdown of glucose, is one of the most conserved and extensively studied biochemical pathways. Designing principles from chemistry and thermodynamics allow for energy production, biosynthesis and cellular communication. However, the kinetics or metabolic flux through the pathway also determines its function. Recently, there have been numerous developments that establish new allosteric interactions of glycolytic enzymes with small molecule metabolites and other mechanisms that may cooperate to allow for additional complex regulation of glycolysis. This review surveys these newfound sources of glycolysis regulation and discusses their possible roles in establishing kinetic design principles of glycolysis.

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Kinetics, Glucose metabolism, Network design principles, Feedback regulation, Feedforward regulation.

Glycolysis: structure and function

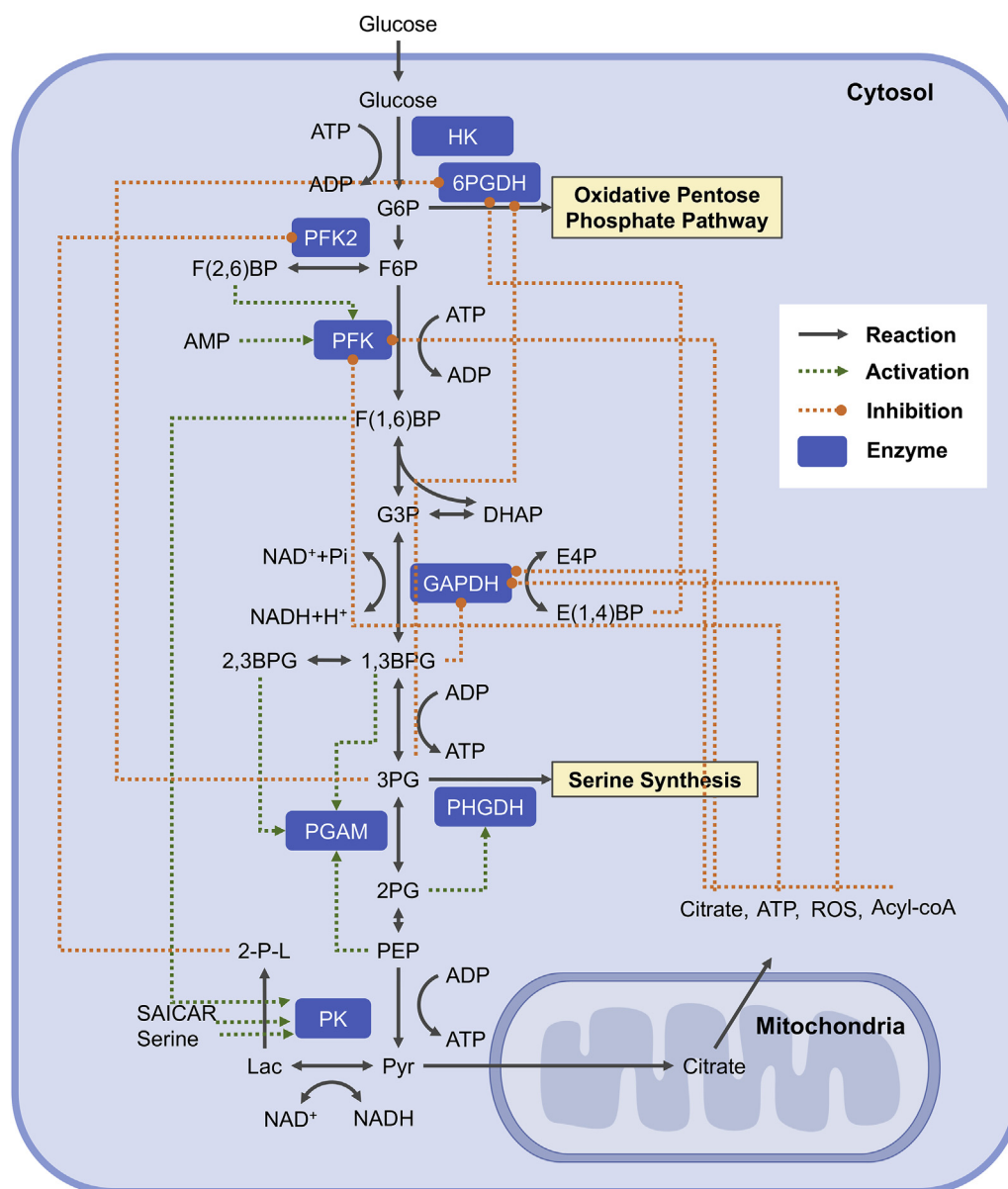
Glycolysis, the breakdown of glucose, is a pathway that exemplifies the core principles of how metabolic biochemistry can achieve biological function [1,2] (Figure 1). It is the oldest studied biological pathway with much of its discovery dating back to the 1950s. Thus, it is remarkable and speaks to the complexity of biology that almost 70 years later, there is still much to learn about both its biochemical makeup and biological function. Recently, several studies have discovered new elements of biochemistry within the pathway that may function to create feedback and feedforward forms of interaction throughout the pathway. How these

newfound interactions confer regulatory principles for glucose metabolism is largely unknown. This review will provide a brief overview of glycolysis and mention, non-exhaustively, some classic forms of glycolysis regulation. It will next discuss recent developments in defining new aspects of the structure of the pathway, through allosteric interactions and other means, and speculate on the possible roles for these additional layers of wiring. These mechanisms likely confer numerous additional properties to glycolysis regulation but our knowledge is still in its infancy. I ultimately hope to give the reader a sense of the new possibilities for glycolysis regulation that may be present in certain contexts.

Glycolysis involves the uptake and metabolism of glucose through a set of chemical reactions that together constitute one of the oldest and most broadly studied biochemical pathways. As a result, energy in the form of ATP is generated and electrons are passed from glucose to the cofactor NADH. The intermediates in the pathway can undergo additional chemistry to generate biomass in the form of nucleic acids, proteins, and lipids. The product of glycolysis, pyruvate, can be further oxidized in the mitochondria or reduced and thus fermented to produce lactic acid or lactate. The lactate produced rapidly exchanges with pyruvate to enter the mitochondria as well [3,4]. Furthermore, the process of metabolizing glucose also signals to other aspects of the cellular milieu such as the chromatin state by affecting histone acetylation or the activation of receptor tyrosine kinase pathways by changing the redox state that leads to oxidation of the catalytic cysteine and thus catalytic inactivation of protein phosphatases [5–7]. Altogether each of these fates of glucose confers numerous essential functions to cells many of which are evolutionarily conserved from the most primitive of organisms to the most complex of multicellular species.

The biochemical setup of glycolysis contains several design principles that allows for each step in the pathway to achieve a specific outcome. This topic has been reviewed and analyzed extensively [2,8,9]. These goals either achieve thermodynamic favorability, satisfy physiochemical constraints such as membrane permeability, or allow for the creation of intermediates that can initiate biosynthesis from enzymatically feasible chemistry. As a result, much and in some cases, all demand for biomass and energy can be met from the breakdown and chemical conversion of sugar alone.

Figure 1



New concepts of regulation in glycolysis. Overview of glycolysis and new mechanisms of feedforward and feedback regulation. The pathway of glycolysis begins with the uptake of glucose and ends with the production of lactate or CO_2 in the mitochondria. Classic and newly identified feedback and feedforward interactions in the pathway are highlighted. Abbreviations: G6P - Glucose-6-phosphate, F6P - Fructose-6-phosphate, F(1,6)BP - Fructose-1,6-bisphosphate, DHAP - Dihydroxyacetone phosphate, G3P - Glyceraldehyde-3-phosphate, 1,3BPG - 1,3-bisphosphoglycerate, 2,3BPG - 2,3-bisphosphoglycerate, 3PG - 3-phosphoglycerate, 2PG - 2-phosphoglycerate, PEP - phosphoenolpyruvate, Pyr - pyruvate, Lac - lactate, SAICAR - 2-[5-Amino-1-(5-phospho-D-ribose)]imidazole-4-carboxamide succinate, NAD^+ - Nicotinamide adenine dinucleotide (oxidized), NADH - Nicotinamide adenine dinucleotide (reduced), ATP - Adenosine triphosphate, ADP - Adenosine diphosphate, AMP - Adenosine monophosphate, ROS - Reactive oxygen species, Acyl-coA - Acyl coenzyme A, 2-P-L 2-phospholactate, E4P - 4-phosphoerythronate, E(1,4)BP - 1,4-bisphosphoerythronate, HK - hexokinase, PFK - phosphofructokinase, PFK2 - phosphofructokinase 2, 6PGDH - 6-glucose-6-phosphate dehydrogenase, GAPDH - glyceraldehyde phosphate dehydrogenase, PHGDH - phosphoglycerate dehydrogenase, PGAM - phosphoglycerate mutase, PK - pyruvate kinase.

While the structure (i.e. the chemical reactions and thermodynamic properties) of glycolysis enables numerous cellular functions, metabolism is never in thermodynamic equilibrium and the kinetic properties of metabolism determine the function of the pathway.

For example, during normal tumor development, cells exhibit larger rates of glycolysis and this leads to overflow metabolism such as fermentation of lactate (i.e. the Warburg Effect). As a result, cells carrying out this Warburg Effect have dramatically different phenotypes

because of this differential in the kinetics of glucose metabolism [5,10]. The mechanism for how the rate of glycolysis can influence biological outcomes is complex. For instance, glycolysis is coupled to the activity of nearly all other metabolic pathways. The flux through glycolysis is also coupled to the concentrations of glycolytic intermediates that are sensed by other cellular processes. Thus, cells have evolved intricate mechanisms to regulate the kinetics of glycolysis through both positive and negative feedback and feedforward mechanisms.

Regulation of glycolysis

Classic examples

Typically metazoan cells when supplied with an abundance of glucose maintain low rates of glycolysis due to the saturation of enzymes in glycolysis [11]. Thus mechanisms in addition to increasing nutrient availability are needed to increase the rate of glycolysis. This has been extensively studied during insulin signaling and in cancer cell biology where the Warburg Effect is induced by oncogenic signaling pathways and transcription factors which decrease the K_m and increase the V^{\max} of glycolytic enzymes through posttranslational modifications and increased expression. For example master transcription factors such as MYC and HIF1 can increase the V^{\max} by several fold for multiple glycolytic enzymes at once [12].

Allosteric binding of small-molecules to glycolytic enzymes is another pervasive theme in positive glycolysis regulation. Examples include pyruvate kinase, the enzyme that catalyzes the final step in glycolysis, is binds to and is activated by fructose-1,6-bisphosphate (F1,6BP) an early intermediate in the pathway [13]. This allows for the creation of a feedforward loop. When glycolysis is backed up and FBP accumulates, its binding to pyruvate kinase (PK) facilitates an increase in glycolytic rate and relief from accumulation of glycolytic intermediates. Also, nucleotides and amino acids derived from glycolytic intermediates including SAICAR and serine bind to and activate PK creating additional feedforward loops [14–16]. Another feedforward loop occurs through the generation of fructose-2,6-bisphosphate (F2,6BP) from fructose-6-phosphate (F6P) by phosphofructokinase 2 (PFK2) [17]. F2,6BP allosterically activates phosphofructokinase 1 (which also is activated by AMP) to allow for additional layers of rate control at the phosphofructokinase (PFK) step.

In addition to these ways of increasing the rate of glycolysis, there are also numerous modes of negative regulation. Both citrate and ATP bind to and inhibit PFK and thus rising ATP or citrate levels that might occur from enhanced mitochondria TCA cycle activity provide negative feedback to PFK and glycolysis. Other negative feedback loops via other glycolytic intermediates exist as well.

New elements of glycolysis regulation

While many aspects of glycolysis regulation have been known for several decades, the surge of interest in metabolism has allowed for deeper investigations into glycolysis and as a result has uncovered many new elements of glycolysis regulation. Historically in mammals, much of the positive regulation has focused on the front end of glycolysis such as the trafficking of the glucose transporter from endomembranes to the cell surface during insulin stimulation [18] and the phosphorylation of glycolysis enzymes such as hexokinase by AKT [19]. More recently it is now appreciated that other steps in glycolysis are subjected to substantial positive regulation. Several of these mechanisms only recently discovered are highlighted below.

Glyceraldehyde phosphate dehydrogenase (GAPDH) catalyzes the fifth step in glycolysis that converts glyceraldehyde-3-phosphate (G3P) to 1,3-bisphosphoglycerate (1,3BPG). The reaction occurs through an oxidative phosphorylation mechanism using a catalytic cysteine that is coupled to the oxidation of G3P. It has long been considered an enzymatic step in glycolysis that lacks regulation. This conclusion has largely been based on reported estimates of the free energy of this reaction in red blood cells being near zero and thus the reaction is in equilibrium [9,20]. It is also generally thought that reactions farther away from equilibrium tend to be under more regulation, although recent analyses have questioned this assertion [21]. As can be seen in both the chemical and kinetic equations, the multiple cofactors and the transient nature of 1,3BPG due to its reactive acyl-phosphate groups, large variations in concentrations can alter the kinetics and also shift the free energy far from equilibrium. For example, the $NAD^+/NADH$ ratio can vary over 100-fold when cells undergo differing rates of glycolysis [22,23]. Inorganic phosphate and intracellular pH also are thought to vary over several orders of magnitude and regulate GAPDH activity [24]. As a result, regulated changes in these variables exert both positive and negative influences on the pace of glycolysis. Metabolic control analysis has shown that under certain conditions GAPDH exerts a surprising amount of control over glycolytic pathway flux [10,23–25]. For example, during the Warburg Effect, some recent calculations have placed GAPDH as having as much control of glycolysis as the enzymes placed in more canonical rate-determining steps such as PFK and pyruvate kinase [10,23,24].

A recent study using mathematical modeling, flux measurements, and single cell analysis reported in a subset of yeast cells that during adaptation to increases in glucose availability, an imbalance in glycolysis can be created that marked by differences in the flux through upper and lower glycolysis leading to an accumulation of FBP and depletion of ATP that leads to cytotoxicity [24]. Phosphate release from the vacuole and changes in

pH affect the extent of this imbalance and can restore the overall rate of glycolysis. A form of control analysis showed that these results were largely connected to the activity of GAPDH that was mediated by nutrient availability.

Numerous other elements of negative regulation are now appreciated to also occur at the GAPDH step. 1,3BPG has recently been shown to covalently modify a lysine near the active site of GAPDH [26]. Thus, if this acylation reaction can occur with appreciable kinetics in cells, it establishes a negative feedback loop. Other products of metabolism downstream of glycolysis such as acyl-coenzyme A species derived from mitochondrial metabolism also react with GAPDH to covalently modify and inhibit its activity [27–29] although whether and how these metabolites generated in the mitochondria may react with GAPDH in the cytosol or nucleus is unclear.

Another aspect of negative regulation of GAPDH activity occurs through reactive oxygen species generation via hydrogen peroxide mediated oxidation and inactivation of the catalytic cysteine [30]. Thus oxidative stress and the generation of hydrogen peroxide that comes for example from uncoupling of the TCA cycle from or excessive activity in the electron transport chain in the mitochondria [31]. For example, an interesting and provocative concept for a cancer therapy is to overload cancer cells that can differentially uptake Vitamin C which at high concentrations acts as an oxidant and among other toxic effects to cells, inhibits GAPDH by inducing oxidative stress [30]. Given its potential effects on rate-control in glycolysis, oxidative inactivation or other covalent modifications to GAPDH even in small amounts could exert large reductions to the rate of glycolysis. Additionally, positive regulation of GAPDH has recently been discovered to occur via protein–protein interactions by glutathione S-transferase Pi 1 (GSTP1) as well [32].

Another enzyme that has historically been considered to have less regulatory capacity in glycolysis due to the thermodynamics of the reaction is phosphoglycerate mutase (PGAM) [20]. PGAM, the 7th step in glycolysis catalyzes the conversion of 3-phosphoglycerate (3PG) to 2-phosphoglycerate (2PG). 3PG binds to and inhibits, phosphogluconate dehydrogenase (6PGDH), an enzyme in the pentose phosphate pathway which branches from glycolysis [33]. Its product 2PG can bind to and activate phosphoglycerate dehydrogenase (PHGDH). Each of these interactions confer feedback and possibly feedforward regulation to the pathway. Inhibition of the PPP may increase the overall rate of glycolysis conferring either positive feedback or negative feedback (depending on the kinetics of the interaction) to glycolysis. Further, activation of serine synthesis

which occurs through PHGDH possibly provides negative feedback to the pathway [33,34]. For its reaction mechanism, PGAM is first primed using a phosphorylated histidine on the enzyme to mediate the transfer of the phosphate. Thus the acceptance of a phosphate on the histidine which is required for its complete catalytic activity can result in positive regulation and possibly feedback if that phosphate is derived from an intermediate in glycolysis. Indeed, it has been shown that phosphoenolpyruvate (PEP) can prime this reaction which creates a positive feedback loop [35]. Additionally, a recent study has now demonstrated both 1,3BPG and another acylphosphate, 2,3-bisphosphoglycerate (2,3BPG), can prime PGAM [36]. Both thus provide feedforward regulation and therefore PGAM and related metabolites provide numerous aspects of positive and regulation to glycolysis.

Minor products in glycolysis as allosteric regulators

While enzymes are in principle thought to operate with unique specificity for a single substrate and product, their properties do allow for catalysis of alternative reactions from the same or alternative substrates yielding additional products. The relative extent of the generation of these products depends on the kinetic and thermodynamic properties associated with the reactions. In the case of glycolysis, the overall rate can be very high relative to other metabolic reactions in cells. Glycolysis enzymes are in high abundance in cells (up to 50% of all metabolic enzymes and 10% of the entire proteome in mammalian cells) and thus have high V^{Max} values [37]. Thus, if side products are created at even very small extents, they can have biological functions. For example, lactate dehydrogenase (LDH), which predominantly reduces pyruvate to generate lactate, has recently been shown to produce S-2-hydroxyglutarate (2HG) as a minor product in quantities sufficient to affect biology [38]. Recently, a study also reported that two enzymes in glycolysis produce minor product that interact with other enzymes in glycolysis. LDH produces 2-phospholactate (2-P-L), and GAPDH 1,4-bisphosphoerythronate (E(1,4)BP) from 4-phosphoerythronate (4EP) [39]. 2-P-L inhibits PFK2 and 1,4PE was shown to bind to and inhibit 6PGDH in the Pentose Phosphate Pathway. These products were shown to be catalytically degraded by a newly identified enzyme phosphoglycolate phosphatase (PGP). Since these products are coupled to the rate of glycolysis, they provide interesting and unexplored possibilities for avenues of feedback regulation of glycolysis. Binding of 2-P-L to PFK2 creates a negative feedback loop whereas binding of 4PE creates a positive feedback. These possibilities are in addition to transcriptional regulation of PGP or epigenetic alterations by 2HG which have been extensively reported [40].

Outlook: from cataloguing interactions to mechanistic systems biology

This review has reported multiple newly characterized aspects feedback and feedforward interaction in glycolysis. Undoubtedly there are many discoveries left as the biochemistry thus far reported is by no means exhaustive. Nevertheless, with these many catalogued interactions, the challenge is to synthesize them collectively to understand what biological functions they might confer. For example, in the context of both cancer and development, high rates of glycolysis maintain numerous biological functions such as maintenance of chromatin structure and can have kinetic advantages over acquiring ATP from oxidative phosphorylation when competing for limited glucose [41–44]. Lowering the rate of glycolysis may also be advantageous when glucose is environmentally limited and thus negative regulation enhances the fitness of a multicellular community by providing more glucose to other cells [41–43]. Higher rates may also in some contexts have toxicities associated with increased ATP or ROS production or general mitochondrial overload [45]. Whether and how these mechanisms may be involved in any of these process remains to be determined.

In addition to increasing or decreasing the rate of glycolysis through feedback regulation, the kinetics of glucose metabolism is highly nonlinear. Nonlinear feedback results in more complex behavior. Positive feedback can result in bi- or multi-stability where cells can stably over long periods of time operate in multiple steady states and exhibit hysteresis or biochemical memory such that when one state (i.e. a set of fluxes and concentrations related to glycolysis) is reached the transitioning to another stable state is irreversibly slow. Modeling results of glycolysis have pointed to this bi- or multi-stability as having a regulatory function [46,47]. Thus, a high glycolytic rate that was established by for example the presence of insulin or a growth factor could be maintained long after removal of the stimuli that initiated the high rate of glycolysis. Such concepts of irreversibility due to bistability could be established through any of the feedback loops mentioned [48].

Negative feedback loops also can give rise to complex behavior. They can give rise to oscillations by establishing limit cycles [49]. Such circadian behavior could confer numerous biological properties to glycolysis. While the period of such oscillations would be commensurate with the kinetics of glycolysis thus rendering them unlikely to account for circadian rhythms which are slower, the accumulation of minor products that negatively feedback for example could occur over hours giving glycolysis a periodicity that would occur over similar timings as the mammalian circadian transcriptional networks [50]. These would allow for central carbon metabolism to be coupled to

transcriptional processes in a multitude of ways. Finally, feedforward loops can also result in other complex kinetics including their ability to establish robustness or consistency of rates of glycolysis in the presence of changes in nutrient availability in the environment [51].

In summary, the consequences of these feedback loops remain largely unexplored and further research is needed to define their biological functions. Advances in technology such as flux analysis [52] and quantitative metabolomics [53] and single cell analysis [54,55] will enable further understanding of these iterations. Nevertheless, with the wealth of available data, the development of quantitative models that can understand the biological consequences of these phenomenon are likely imminent.

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