Strength in numbers: Phosphofructokinase polymerization prevails in the liver

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Accessibility
Numerous metabolic enzymes assemble into filamentous structures, which are thought to serve additional regulatory functions. In this issue, Webb et al. (2017. J. Cell Biol. https://doi.org/10.1083/jcb.201701084) show that the liver-specific isoform of phosphofructokinase-1 forms filaments in vitro and localizes as puncta in cells along the plasma membrane. This suggests spatial organization of glycolysis in higher organisms.

Glycolysis is the core of central carbon metabolism; its intermediates provide precursors important for generating ATP through glucose oxidation, serine for one carbon metabolism, sugars for protein glycosylation, and building blocks for nucleotide synthesis through the pentose phosphate shunt (Fig. 1 A). Although glucose metabolism is highly studied, spatiotemporal aspects of glycolysis remain largely unexplored. Polymerization of metabolic enzymes is one means of spatially regulating cellular processes, and it has been observed for numerous enzymes including acetyl-CoA carboxylase (ACC), cytidine triphosphate (CTP) synthase, glutamate dehydrogenase, and β-glucosidase (O’Connell et al., 2012). A recent study has shown that PFK1, the rate-determining step of glycolysis that converts fructose 6-phosphate (F6P) to fructose 1,6-bisphosphate (F1,6BP) polymerizes in yeast (Shen et al., 2016). However, it is unclear whether PFK1 can polymerize in higher organisms, how PFK1 polymerization is regulated, and the structural and functional features of these fibers. Webb et al. set out to address these uncertainties.

Using both transmission electron microscopy and 90° light scattering, Webb et al. (2017) demonstrated that a recombinant liver-specific isoform of PFK1 (liver PFK1; PFKL) forms filaments in vitro. However, filament assembly was not observed in platelet PFK1 (PFKP)- or muscle PFK1–specific isoforms. Remarkably, the PFKL filaments were an average of 65.4 nm in length, approximately six tetramers long. Binding of F6P, which is a substrate of PFK1, strongly induced PFKL polymerization, and mutagenesis of the F6P binding pocket revealed that F6P binding was crucial for filament formation.

In addition to identifying PFKL polymerization, Webb et al. (2017) examined the filament structure with negative-stain EM and iterative helical real-space reconstruction. They found that PFKL filaments are distinct from other filamentous enzymes. PFKL filaments formed through interaction of the C-terminal regulatory domains of tetramers. In the filament structure, each tetramer is composed of two structurally distinct dimers, dimers A and B. Each dimer has two interfaces, in which interface 1 of dimer A interacts with interface 1 of dimer A on the adjacent tetramer, and interface 2 of dimer B is solvent exposed. Furthermore, longitudinal contacts alternate, with interface 2 of dimer A interacting with interface 1 of dimer B on the adjacent tetramer. This creates an unusual helical symmetry such that the last subunit can either (a) bind another subunit in a linear manner or (b) bind a subunit and introduce a kink into the filament. The kinks within the PFKL filaments appeared abundant and random.

Because the C-terminal regulatory domain enables tetramers to interact within PFKL filaments, Webb et al. (2017) assessed whether this domain is sufficient for assembly. Using a chimera of the PFKL C-terminal regulatory domain and PFKP catalytic region, they identified that the C-terminal regulatory domain of PFKL is sufficient to form filaments. This result underscores the importance of isoform specificity in filament formation.

The liver is a critical anabolic organ that supports systemic metabolism (Rui, 2014). In particular, the liver senses the levels of glucose in an organism and uses the processes of glycogenolysis and gluconeogenesis to generate glucose, which is subsequently delivered to other tissues in the body. The process of glycolysis (glucose breakdown) and gluconeogenesis (glucose synthesis) are opposing. Therefore, most of the enzymes in glucose metabolism are bidirectional to support both anabolic and catabolic glucose metabolism. The catalytic activity of most enzymes involved in glucose metabolism is allosterically regulated by metabolites. PFK1 is distinct, however, because it is not reversible, and it is the rate-determining step of oxidative glucose metabolism (Fig. 1 A).

PFK1 has two states of quaternary structure: the inhibitory T state and the activated R state (Fig. 1 B; Webb et al., 2015). ATP, citrate, and phosphoenolpyruvate bind PFK1, stabilizing the T state and therefore inhibiting catalytic activity. These metabolites are elevated in a cell when there is sufficient energy production through TCA cycle flux and glycolysis, respectively. Alternatively, metabolites such as AMP and F2,6BP stabilize the PFK1 R state, activating it to increase glycolytic flux in low-energy conditions. In addition to allosteric modifications, PFK1 is regulated by posttranslational modifications such as glycosylation. Glycosylation inhibits PFK1 activity and rewires glucose metabolism to the pentose phosphate pathway (Yi et al.,
Metabolic enzymes have evolved to rapidly respond to ever-changing cellular conditions. Therefore, a high order of regulation is required to fine-tune their activity. Advances in metabolomics have allowed us to assess global changes in cellular metabolic profiles, and yet the spatiotemporal aspects of metabolism are still largely unexplored and represent an exciting area for future studies. The existence of metabolic enzyme filaments implies the importance of localized metabolism and suggests an additional layer of complexity and regulation of glycolysis.

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References


