A humble hexose monophosphate pathway metabolite regulates short- and long-term control of lipogenesis

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Most modern biologists and contemporary textbooks of biochemistry present the glycolytic pathway and the synthesis of fats as history where all of the relevant facts are known. An article in a recent issue of PNAS by Kosaku Uyeda and his group (1) combines new insights into the regulation of lipogenesis with heroic protein chemistry and an elegant combination of enzymology and molecular biology. This paper describes how a small and ignored metabolite of the hexose monophosphate pathway, xylulose 5-phosphate (Xu-5-P), activates protein phosphatase 2A to mediate the acute effects of carbohydrate feeding on the glycolytic pathway, as well as the coordinate long-term control of the enzymes required for fatty acid and triglyceride synthesis.

It has long been known that feeding of cholesterol suppresses cholesterol synthesis. In the early 1990s a transcription factor designated as SREBP, a sterol response element binding protein, was identified that regulated the transcription of the genes encoding a number of the key enzymes of cholesterol biosynthesis, including hydroxymethylglutaryl (HMG)-CoA synthase (EC 4.1.3.5), HMG-CoA reductase (EC 1.1.1.88), farnesyl-diphosphate synthase (EC 2.5.1.10), and the low-density lipoprotein receptor protein (for review, see ref. 2). SREBP also regulates the transcription of the gene encoding glucokinase (EC 2.7.1.12), an enzyme responsible for catalysis of the first step of hepatic glycolysis. The processing of SREBP is regulated by proteolysis by mechanisms analogous to those involved in the processing of amyloid precursor protein. Cleavage of SREBP requires an activating protein designated SCAP and is responsible for the feedback inhibition of cholesterol on sterol synthesis. It was also found that, in addition to affecting sterol synthesis, SREBP also modulates the expression of some of the genes encoding enzymes necessary for fatty acid biosynthesis. These findings led to the conclusion that SREBP coordinates the synthesis of the two major building blocks of membranes, fatty acids and cholesterol. Soon, however, more data were to emerge.

Feeding of a high-carbohydrate diet has long been known to stimulate the synthesis of fatty acids and to cause the coordinate induction not only of the enzymes of the fatty acid synthesis pathway but also of glycolytic enzymes required for the supply of pyruvate, the precursor of acetyl-CoA, and the enzymes required for the synthesis of NADPH, the essential cofactor for all lipid biosynthesis. Further studies of the signaling involved in lipogenesis in isolated hepatocytes showed that two distinct transcription factors were involved. One, SREBP-1c, was stimulated by insulin. However, another DNA-binding site promoting the transcription of lipogenic enzymes after stimulation by high glucose in the absence of insulin was identified and designated the carbohydrate-response element, ChoRE (3). In a heroic feat of protein isolation, Uyeda and his group (4) managed to purify 100 μg of ChREBP, the protein that bound to the ChoRE of the promoter region of the gene encoding liver pyruvate kinase (LPK; EC 2.7.1.40) from the livers of 800 rats that had been fasted and then refed a high-carbohydrate diet. The protein isolated was a 100-kDa polypeptide of the basic helix–loop–helix leucine zipper (bHLH-ZIP) family of transcription factors which bind to E-box motifs, CACGTG, within their target promoters. DNA-binding activity assays, so called gel shifts, disclosed its presence in kidney and small intestine in addition to liver. In another paper (5) Uyeda's group reported that ChREBP contained three consensus sequences for protein kinase A phosphorylation sites and that phosphorylation of Ser-196 inhibited nuclear entry of ChREBP, whereas phosphorylation of Thr-666 inhibited its binding to the LPK promoter site. This inhibition by ChREBP of nuclear entry and DNA binding gave a further mechanism to the well known observation that glucagon, a stimulator of cAMP production, inhibits fatty acid synthesis.

Biochemistry textbooks present glycolysis and fat synthesis as history, with all of the facts known.

Just as feeding a high-cholesterol diet inhibits cholesterol synthesis, Uyeda's group next analyzed the mechanisms whereby feeding of fat inhibits carbohydrate metabolism (6). Activation of fatty acids produces AMP in a reaction of the type fatty acid + ATP + CoA $\rightarrow$ acyl-CoA + AMP + PP_i. Using hepatocytes transfected with ChREBP, they showed that administration of the fatty acids acetate, octanoate, or palmitate resulted in a 30-fold increase in the concentration of free cytosolic AMP and a 2-fold increase in the activity of the AMP-stimulated protein kinase. AMP kinase phosphorylated ChREBP at a specific site on the molecule and inhibited its binding to DNA.

The unification of the long-term control of fat synthesis by the transcription factor ChREBP and the short-term control on glycolysis and NADPH generation at the phosphofructokinase (PFK; EC 2.7.1.11) was explained by changes in the concentration of the hexose monophosphate pathway metabolite Xu-5-P (1). During carbohydrate feeding, pyruvate produced in glycolysis enters the mitochondria, where pyruvate dehydrogenase multienzyme complex converts it to acetyl-CoA. The acetyl-CoA produced is condensed with oxaloacetate by citrate synthase (EC 4.1.3.7) to form citrate, the first step in the tricarboxylic acid (TCA) cycle. Both fatty acids and cholesterol are synthesized from citrate produced in the mitochondrial TCA cycle and exported to the cytosol on the tricarboxylate carrier. There citrate is converted to cytosolic acetyl-CoA by citrate-cleavage enzyme (EC 4.1.3.8). HCO_3^- is added to acetyl-CoA by the biotin enzyme acetyl-CoA carboxylase (EC 6.4.1.2) to form malonyl-CoA, the first committed step of fatty acid synthesis. The synthesis of fatty ac-

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ids requires both malonyl-CoA, produced largely from pyruvate, and reducing power. That reducing power is supplied from the reactions of the malic enzyme (EC 1.1.1.39) and the first two enzymes of the hexose monophosphate pathway, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.43). The malic enzyme and 6-phosphogluconate dehydrogenase reactions are both in near equilibrium with the cytosolic free $[NADP^+]/[NADPH]$ system and as such are sensitive to changes in the concentrations of their products and reactants. The reactions catalyzed by these dehydrogenases create the low-potential cytosolic free $NADP^+$ system, whose redox potential is −0.41 V. In comparison, the free cytosolic NAD^+ system, formed by the glycolytic dehydrogenases, glycerol-3-phosphate dehydrogenase (EC 1.2.1.12), whose redox potential is around −0.19 V, is unable to drive the reduction of the β-oxoacyl-CoA to β-hydroxyacyl-CoA, whose half-reduction potential is −0.24 V, during fatty acid synthesis (7). The concentration of malonyl-CoA and the V_{max} of fatty acid synthase complex (EC 2.3.1.85) are major rate controlling steps in the process of fatty acid synthesis (8). Malonyl-CoA molecules are repeatedly added to the lengthening fatty acid chain until the complete fatty acid is released from the surface of the multienzyme complex.

How the disparate pathways of glycolysis, fatty acid synthesis, and gene transcription are integrated has been described in several recent papers by the Uyeda group. The enzyme PFK sits astride the intersection of the glycolytic pathway and the termination of the hexose monophosphate pathway at the metabolites fructose 6-phosphate and glyceraldehyde 3-phosphate (see Fig. 1). PFK activity is controlled in liver by the concentration of Fru-2,6-P_2, which is produced and destroyed by a bifunctional enzyme called 6-phosphofructo-2-kinase/Fru-2,6-P_2 phosphatase (EC 2.7.1.105/3.1.3.46). The kinase activity is inhibited, and the phosphatase is activated (9), by phosphorylation by cAMP-dependent protein kinase. Approximately 10 years later it was shown that dephosphorylation of the bifunctional enzyme, and hence activation of 6-phosphofructo-2-kinase, was stimulated, and the Fru-2,6-P_2 phosphatase was inhibited, in response to high-carbohydrate feeding by a specific protein phosphatase that was activated by Xu-5-P (10). The Xu-5-P-stimulated protein phosphatase responsible for the activation of the enzyme was identified as an isozyme of the protein phosphatase 2A class, PP2A

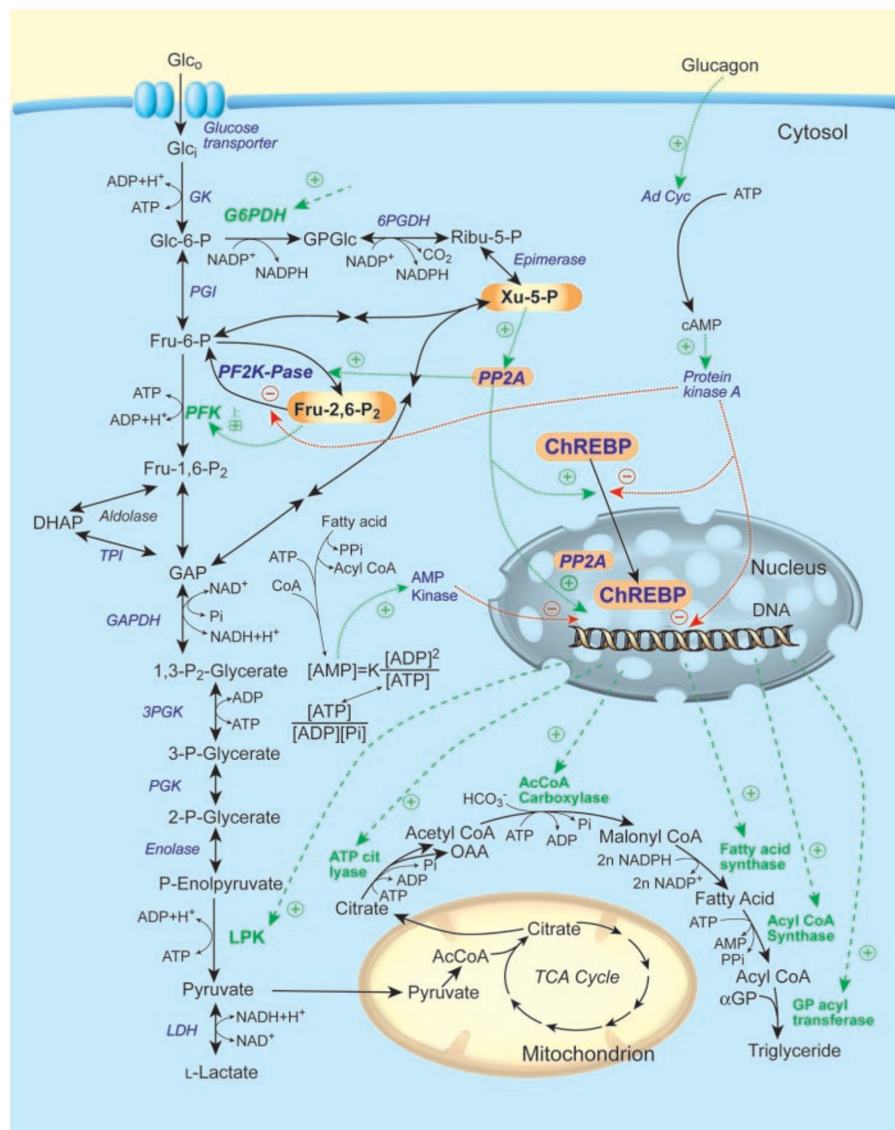

Fig. 1. Xu-5-P is the signal for the coordinated control of lipogenesis. Feeding carbohydrate causes levels of liver glucose, Glc-6-P, and Fru-6-P to rise. Elevation of [Fru-6-P] leads to elevation of [Xu-5-P] in reactions catalyzed by the near-equilibrium isomerases of the nonoxidative portion of the hexose monophosphate pathway. The elevation of [Xu-5-P] is the coordinating signal that both acutely activates PFK in glycolysis and promotes the action of the transcription factor ChREBP to increase transcription of the genes for the enzymes of lipogenesis, the hexose monophosphate shunt, and glycolysis, all of which are required for the *de novo* synthesis of fat. The figure depicts the increase in enzyme transcription caused by the carbohydrate response element binding protein, ChREBP, in green dashed lines. Stimulation of the Fru-2,6-kinase reaction by protein phosphatase 2A (PP2A) and its stimulation by Xu-5-P are by indicated green dotted lines. Metabolic reactions are indicated by solid black lines. Those reactions that are reversible *in vivo* are indicated with double arrows, and those catalyzing unidirectional reactions have only a single arrowhead. $[ATP]/[ADP][P_i]$ represents the free cytosolic phosphorylation potential catalyzed by the combined glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 3-phosphoglycerate kinase reactions, and [AMP] represents the free cytosolic value catalyzed by the myokinase reaction. The names and EC numbers of the enzymes in green are given in the text. Inhibitions by AMP-stimulated protein kinase and cAMP-stimulated protein kinase are indicted by red dotted lines.

(11). The K_a for the activation of the PP2A by Xu-5-P was shown both in cellular preparations and in the isolated enzyme, to be ≈9 μM (12), in the midrange for concentrations of Xu-5-P in liver *in vivo* (13).

In their recent paper (1), the Uyeda group completes the circle. They show that the transport of ChREBP into the nucleus and DNA binding are inhibited by phosphorylation by cAMP-dependent protein kinase, and that activation of nuclear transport and binding is stimulated in response to high-carbohydrate feeding by a xylulose-stimulated PP2A of the ABδC class. Transcription of the

genes of the enzymes of glycolysis producing malonyl-CoA, the enzymes of NADPH production, and the enzymes of the fatty acid synthesis pathway are activated by PP2A in response to changes in the cellular levels of Xu-5-P. Similar acute effects catalyzed by the same Xu-5-P stimulated PP2A by increasing the activity of the 6-phosphofructo-2-kinase (EC 2.7.1.105).

Why should an obscure metabolite such as Xu-5-P be such an important signal? The products of the four isomerases of the nonoxidative portion of the hexose monophosphate pathway, ribulose 5-phosphate (Ru5P) epimerase (EC 5.1.3.1), ribose 5-phosphate (Rib5P) isomerase (EC 5.3.1.6), transaldolase (EC 2.2.1.2), and transketolase (EC 2.2.1.1) produce Fru-6-P and glyceraldehyde-3-P, with their substrates and products being in approximate near equilibrium in liver *in vivo* (13). One may therefore write

$$[\text{Xu-5-P}] = \frac{(K_{\text{Ru5P epimerase}})^{1/3}(K_{\text{transketolase-E4P}})^{1/3}}{(K_{\text{Rib5P isomerase}})^{1/3}(K_{\text{transaldolase}})^{1/3} \times (K_{\text{transketolase-S7P}})^{1/3}} \times [\text{glyceraldehyde-3-P}]^{1/3}[\text{Fru-6-P}]^{2/3},$$

in which E4P represent erythrose 4-phosphate and S7P represents sedoheptulose 7-phosphate, representing two of the reactions catalyzed by transketolase (13). What this equation says is that, as [Fru-6-P] increases during carbohydrate feeding, the concentration of Xu-5-P must also increase, with the resultant effects of the stimulation of PP2A cited above. Furthermore, the product of the PFK reaction, Fru-1,6-P_2, is in near equilibrium through aldolase (EC 4.1.2.13) with the near-equilibrium system catalyzed by glyceraldehyde-3-P dehydrogenase and 3-phosphoglycerate kinase (EC 2.7.2.3) (14). Hence changes in [glyceraldehyde-3-P] must conform to changes in both the free cytoplasmic $[NAD^+]/[NADH]$ ratio and the free cytoplasmic $[ATP]/[ADP][P_i]$ ratio. Additionally, the product of the 6-phosphogluconate reaction, Ru5P, is in near equilibrium with 6-phosphogluconate and the free cytosolic $[NADP^+]/[NADPH]$ ratio (15). Ru5P, a component of the nonoxidative portion of the hexose monophosphate pathway, is thus sensitive to changes in the concentrations of the combined PFK and aldolase reactions in liver. The substrates of this system are thus responsive to changes in all of the great nucleotide systems of the cell, the free $[NAD^+]/[NADH]$, the $[NADP^+]/[NADPH]$, and the $[ATP]/[ADP][P_i]$ systems.

It is widely stated in textbooks that PFK is "the rate-controlling step" in glycolysis. More systematic analysis of the control of fluxes in glycolysis suggests that there are instead multiple rate-controlling steps, which vary depending upon hormonal and substrate concentration (16). The principle of distributive control is well illustrated by Uyeda's findings on the enzymes increased by the action of ChREBP. In the glycolytic pathway there are increases in PFK, but also in pyruvate kinase. Were PFK the only "limiting" step in the pathway, such increases would be hard to explain. Coordinate control of transcription is also illustrated by the finding that both glucose-6-phosphate dehydrogenase and the malic enzyme, producers of the NADPH needed for fat synthesis, are increased by ChREBP. Finally, all of the enzymes required for fat synthesis, starting with ATP citrate lyase, are increased. The coordinate changes in the disparate pathways required to achieve fat synthesis represent an elegant example of distributive control. It is remarkable that such complex control mechanisms are all sensitive to the simple hexose monophosphate shunt metabolite Xu-5-P. I know of no other way that one could come to such unexpected, insightful conclusions other than through the application of protein chemical, biochemical, and molecular biological techniques informed by a global knowledge of metabolic systems and their enormous plasticity made possible by distributed, context-dependent control.

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