**2011 Cold Spring Harbor Symposium on Quantitative Biology**

**BACK TO THE FUTURE: MOLECULAR BIOLOGY MEETS METABOLISM**

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Excerpts

The 76th Annual Cold Spring Harbor Symposium on Quantitative Biology brought together molecular biologists and physiologists. This event was confirmation that the webs we are seeking to connect and understand will be composed equally of chemicals (metabolites) and polymers (proteins, RNAs and genes).

Historically, the field of physiology-as prosecuted with focus on nutrient metabolism-formed a corner­stone for the foundation of modem genetics and the successful reductionism of molecular biology.

The field of molecular biology began its dominance some four decades ago. So powerful were the techniques, however, that molecular biologists were no longer tied to the metabolic pathways. Indeed, one needed to know essentially nothing about metabolism to clone any gene one wanted. Pull-down and yeast two-hybrid experiments can treat metabolism as if it were no more important than tris-(hydroxymethyl)-aminomethane (Tris) buffer. Gene knockouts, in situ hybridizations, immuno­ histochemical stainings, DNA microarrays, CHIP:Seq, RNA:Seq, and chromosomome capture assays-none of these approaches need pay any mind to the fact that the regulatory state of a cell, organ, or organism must be integrally intertwined with metabolic state.

ENZYMES AND SUBSTRATES

A useful starting point is an illumination of the contrasting way we think of the relationship of adenosine triphosphate (ATP) as the substrate for protein kinase enzymes as compared with the prototypical relationship between enzymes and substrates. Although classical metabolic enzymes can be regulated in many ways, including rate of synthesis, turnover, and posttranscriptional modification, the major factor controlling the activity of a metabolic enzyme is its access to substrate and product. If substrate levels are high, and products low, the enzyme converts substrate to product. Because many enzymes are reversible, the opposing reaction can happen when substrate levels are low and product high. Contrast this with the operative manner in which most protein kinase enzymes work. With the exception of the adenosine monophosphate (AMP)-activated protein kinase (AMPK), studied so beautifully by Graham Hardie and colleagues (Hardie 2012), the vast majority of protein kinase enzymes could care less about the concentration of their substrate (ATP). These enzymes have evolved to shield themselves from regulation via substrate abundance so that they can instead be regulated by alternative strategies. If a cell had too little ATP to feed its hundreds of protein kinase enzymes, it would be dead. Simply put, protein kinase enzymes are not substrate regulated with respect to ATP.

Although the distinction between protein kinases and classically studied metabolic enzymes is both simple and obvious, it helps us consider the question of how newly discovered enzymes are regulated.

PIVOT POINTS CONTROLLING SUGAR METABOLISM

The 10 enzymes responsible for the catabolic conversion of glucose to lactate were discovered by German and American scientists between 1933 and 1947 (Lohmann 1933; Meyerhof and Lohmann 1934; Kendal and Sticklanld 1937; Meyerhof and Schultz 1938; Warburg and Christian 1939, 1941; Colowick and Kalckar 1941; Kubowitz and Ott 1943, 1944; Bucher 1947). With one exception, the icons of biochemistry-including Kalckar, Lohmann, Meyerhoff, Warburg, Kubowitz, and Ott-fully solved the enzymatic pathway used by cells to catabolize glucose.

The single shortcoming of these remarkable experimental accomplishments was at the very hub of the glycolytic pathway. The rate limiting enzyme of the pathway, phosphofructokinase (PFK), could only recognize its substrate-fructose-6-phosphate (F-6-P)--at a Km 1000-fold the ambient, physiological level of the enzyme substrate. This enigma stood for nearly 40 years until the discoveries by Belgian and American scientists revealing an allosteric "activating factor" for PFK and the "bifunctional enzyme" that makes and destroys the activating factor (Furuya and Uyeda 1980, 1981; Van Schaftingen et al. 1980, 1981; EI-Maghrabi et al. 1981; Uyeda et al. 1981). The activating factor turned out to be fructose-2,6- bisphosphate (F-2,6-P), which binds to PFK and increases its affinity for F-6-P 1000-fold. The enzyme that makes and destroys the activating factor, 6-phosphofructo-2-ki­ nase/fructose-2,6-bisphophatase (PFKFB), most aptly termed "bifunctional enzyme," contains both a kinase domain that adds a phosphate onto F-6-P (generating F- 2,6-P) and a phosphatase domain that regenerates F-6-P. Fittingly, Kosaku Uyeda reported these spectacular discoveries at the 1980 Cold Spring Harbor meeting on Protein Phosphorylation organized by Ed Krebs 30 years ago (Furuya et al. 1981).

Aside slotting in the final piece to complete the puzzle of glycolysis, the discovery of the bifunctional enzyme led to an understanding of the principal manner in which cells regulate glycolysis. Under fasting conditions, glucagon activates cyclic-AMP which, in turn, activates protein kinase A (PKA). Activated PKA phosphorylates an amino-terminal regulatory domain of the bifunctional enzyme, thereby preventing its kinase domain from making the F-2,6-P activator of glycolysis and blocking glucose catabolism at the central, rate-limiting pivot point (Furuya and Uyeda 1982). How is this reversed When cells or tissues are availed of an ample source of glucose? Because glycolysis is initially blocked via glucagon-mediated phosphorylation of the bifunctional enzyme, new­ found glucose is backed up and driven into the pentose phosphate pathway-thereby leading to enhanced levels of 5-carbon sugars. Here comes the beauty of biology:

Uyeda and colleagues found that xylulose-5-phosphate, a product of the pentose phosphate pathway, activates protein phosphatase 2A (PP2A) via one of its regulatory subunits, leading to the dephosphorylation and activation of the bifunctional enzyme (Ishikawa et al. 1990). Uyeda went on to clone the genes encoding four different isozymes of the bifunctional enzyme (Algaier and Uyeda 1988), and solved the X-ray crystal structure of one of the isozymes (Hasemann et al. 1996).

Why am I ending this meeting summary chapter on a topic that was hardly even mentioned at the Symposium? I do so because of my belief that regulation of glycolysis via the pathways discovered principally by Uyeda will be of vital importance to cancer. As an example, the various isoforms of the bifunctional enzyme come in flavors that differentially balance phosphatase and kinase activity. Uyeda's placental isoform of the PFKFB enzyme has virile kinase activity, balanced by little or no phosphatase activity-it is hard-wired to produce the F-2,6-P activating factor. This isoform, also called the inducible variant of PFKFB3, is frequently overexpressed in human tumors (Chesney et al. 1999; Atsumi et al. 2002; Bando et al. 2005; Kessler et al. 2008). What better way for a cancer cell to step on the gas and accelerate glycolysis!

Digging deeper, what about the opposite? Karen Vousden offered a masterful symposium presentation of her discovery of a gene product that she calls T1GAR (TP53- induced glycolysis and apoptosis regulator). TIGAR turns out to represent an entirely new isoform of Uyeda 's bifunctional enzyme having no kinase activity at all, only the phosphatase activity that destroys the F-2,6-P activating factor (Mor et al. 2012). The interpretation could not be more simple: p53 activates T1GAR as a means of controlling glycolytic flux-perhaps backing up intermediates upstream of F-6-P to feed the pentose phosphate pathway so that damaged cells can be supplied with a replete abundance of NADP(H). 1n the absence of p53, TIGAR is no longer inducible and-irrespective of the form of damage-the brakes cannot be applied to glycolysis.

Kosaku Uyeda did not stop after solving the final riddle of how cells adapt to short-term variations in the abundance of glucose. He and others knew that prolonged exposure of mammalian cells to an abundant supply of glucose led to the transcriptional activation of genes encoding lipogenic enzymes. These enzymes, including the cytosolic ATP citrate lyase enzyme discovered by Uyeda's long-time colleague, Paul Srere (1959), facilitate the conversion of catabolic products of glycolysis into long-chain fatty acids for the purpose of energy storage. Uyeda was first to purify the long-sought glucose-inducible transcription factor, designated carbohydrate response element binding protein (ChREBP), clone its encoding gene, and prepare and study ChREBP-deficient

laboratory mice (Yamashita et al. 2001; lizuka ct al. 2004; Uyeda and Repa 2006).

Most satisfyingly, Uyeda discovered that the exact same regulatory logic used to control the short-term regulation of sugar consumption (glycolysis) is also used to control long-term adaptation to glucose abundance (lipogenesis). ln the starved state, glucagon activates PKA, leading to phosphorylation of an amino-terminal regulatory domain of ChREBP. This phosphorylation facilitates binding of the transcription factor to 14-3-3 proteins, keeping ChREBP in a latent, cytoplasmic state. Upon exposure to a new-found abundance of glucose, the pentose phosphate shunt yields xylulosc-5-phosphate, which activates protein phosphatase 2A. In turn, activated PP2A de-phosphorylates ChREBP, allowing it to move from cytoplasm to nucleus, where it activates the expression of genes encoding lipogenic enzymes (Kabashima et al. 2003).

That the precise same regulatory logic is used to control both the short- and long-term adaptation of cells to glucose abundance is satisfying. It is remarkable that the same scientist-Kosaku Uyeda-solved both of these puzzles. His discoveries revealing the intimate details and common logic of short- and long-term adaptation of cells and tissues to glucose abundance bookend a 30 year era wherein the fields of molecular biology and metabolism have been largely separated. It is hoped that the 76th Cold Spring Harbor Symposium on Quantitative Biology will be looked back upon as the celebratory event putting an end to that extended period of separation.