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The Logic Linking Protein Acetylation and Metabolism

Essay

The Logic Linking Protein Acetylation and Metabolism

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Protein acetylation now rivals phosphorylation in frequency of occurrence but is incompletely understood. A picture is presented in which protein acetylation is linked to available energy via the NAD-dependent deacetylases. This model suggests that protein acetylation regulates metabolic strategy and also helps store energy in cells.

It is now apparent that a large number of cellular proteins are acetylated. For example, recent studies have uncovered acetylation of a high fraction of mitochondrial proteins (Kim et al., 2006), as well as most metabolic enzymes in the cytosolic/nuclear pool (Wang et al., 2010; Zhao et al., 2010). Acetylation in this latter cellular compartment is carried out by histone acetyl transferases (HATs) and deacetylation by class I and II histone deacetylases (HDACs), as well as the class III deacetylases termed sirtuins. Among the sirtuins, SIRT1 appears to have the broadest range of substrates and affect the greatest number of physiological pathways (Finkel et al., 2009; Imai and Guarente, 2010; Haigis and Sinclair, 2010). In the mitochondria, the source of protein acetylation is not known, but the most prominent deacetylase appears to be the sirtuin SIRT3 (Lombard et al., 2007).

Despite our growing understanding of the scope of protein acetylation, many questions regarding this posttranslational modification remain unanswered. How is protein acetylation globally regulated? And is there an underlying logic that links diet, metabolism, and protein acetylation? In this Essay, I attempt to integrate recent findings from numerous labs to suggest how the diet may dictate the level of protein acetylation, which in turn molds the metabolic strategy of cells.

Cells have two basic ways to produce ATP: glycolysis, which takes place in the cytosolic/nuclear location, and oxidative phosphorylation, which occurs in the mitochondria. When energy is abundant in the diet, growth factors will drive glucose uptake leading to activation of glycolysis, generation of ATP, and conversion of NAD to NADH in the cytosolic/nuclear pool (Figure 1A). Because

the sirtuins are NAD-dependent deacetylases, a high flux through glycolysis is expected to lower the activity of SIRT1, as well as the other sirtuins in the nucleus and cytoplasm (SIRT2, SIRT6, and SIRT7). Consistent with this idea, many studies show that SIRT1 activity is low in growth conditions of glucose excess and high during energy limitation (Finkel et al., 2009; Imai and Guarente, 2010; Haigis and Sinclair, 2010). Low SIRT1 activity should increase the acetylation status of many metabolic enzymes in the cytosolic/nuclear compartment, although any physiological effects on the activities of HATs and HDACs may also be important.

Indeed in *Salmonella*, the acetylation level of many glycolytic enzymes was shown to be higher in glucose-grown cells than in cells grown on a nonfermentable carbon source (Wang et al., 2010). This difference was at least partly mediated by the bacterial sirtuin cobB, since acetylation of these enzymes appeared to be higher in cobB mutants under all growth conditions. One enzyme regulated in this way is glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate in glycolysis, and catalyzes the reverse reaction in gluconeogenesis. Remarkably, acetylated GAPDH drives the forward reaction used in glycolysis, while deacetylated GAPDH is more effective at the reverse reaction used in gluconeogenesis.

These simple findings suggest a feedforward mechanism, in which diets rich in carbohydrate energy drive glycolysis, convert NAD to NADH, inactivate sirtuins, and increase acetylation and activity of glycolytic enzymes (Figure 1A). Intriguingly, mammalian GAPDH is also acetylated, and it will be important to determine

whether it—as well as other acetylated metabolic enzymes in mammalian cells—is a SIRT1 substrate, and whether acetylation will generally be activating for glycolytic enzymes.

The increase in protein acetylation in times of plenty is analogous to the increased phosphorylation of proteins by CDKs to drive the cell cycle under these conditions. More generally, phosphorylation-based signaling pathways may impinge on sirtuins to exert an additional layer of control over protein acetylation.

How will the flow of carbon provide acetyl-CoA for acetylation of cytosolic/nuclear proteins under glycolytic conditions? The pyruvate produced by glycolysis has two possible fates (Figure 1A). First, it can be reduced to fermentation products (bacteria), ethanol (yeast), or lactate (mammals), thereby converting some of the NADH produced in glycolysis back to NAD. But second, it can be used to synthesize fatty acids as a primary mechanism of cell growth and energy storage. In this case, the pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase (PDH) in mitochondria, and then rederived back into cytoplasmic acetyl-CoA (Figure 1A). This latter step occurs by transport of citrate from mitochondria to the cytosol, where ATP-citrate lyase (ACL) converts it to acetyl-CoA. ACL was recently shown to be required for normal histone acetylation in mammalian cells (Wellen et al., 2009), suggesting the primary pathway of storing energy as fat may also provide the carbon for protein acetylation in the cytosolic/nuclear pool. By this view, the high degree of protein acetylation in the cytosolic/nuclear pool during energy excess can be seen as another mechanism, along with fat synthesis, of storing carbon energy.
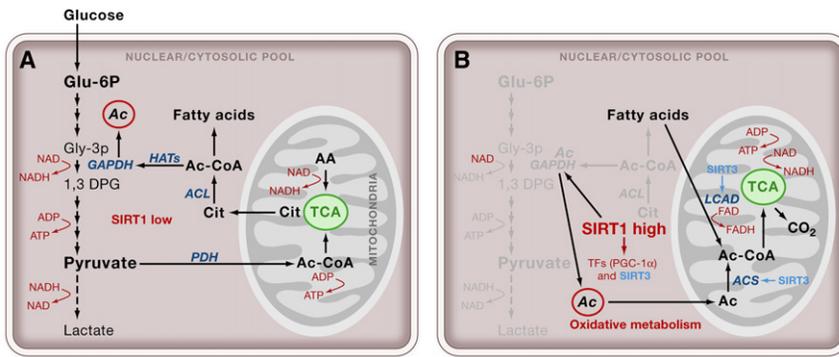
Figure 1. SIRT1 Links Protein Acetylation and Metabolic Strategy of Cells
(A) Glycolytic strategy for ATP production and flow of carbon (black text and arrows) in energy storage tissues when energy is in excess. Mitochondria (Mito) and the TCA cycle are indicated and the remainder of the cell represents the cytosolic and nuclear compartments combined. Enzymes in blue italics are glyceraldehyde-3-phosphate dehydrogenase (GAPDH), histone acetyl transferases (HATs), pyruvate dehydrogenase (PDH), and ATP-citrate lyase (ACL). Note a feed-forward loop of high glycolysis and NADH production, low SIRT1 activity, and high acetylation and activity of glycolytic enzymes (e.g., GAPDH). Carbon flows as follows. Glucose 6-phosphate (Glu-6P) is converted to pyruvate, which can be reduced to lactate or converted to acetyl-CoA (Ac-CoA) by PDH. In this case, citrate from the TCA cycle is transported to the cytosol and converted to Ac-CoA for fatty-acid synthesis by ACL. Depletion of citrate from the TCA cycle necessitates anapleurotic conversion of amino acids (AA) to α-ketogluterate to replenish the cycle.
(B) Oxidative strategy for ATP production and flow of carbon (black text and arrows) in energy producing tissues upon energy deprivation. Glycolytic feed-forward loop in (A) is reversed, thereby activating SIRT1 and deacetylating and inhibiting glycolytic enzymes. SIRT1 generates O-acetyl ADP ribose and thus acetate (Ac) by deacetylating glycolytic proteins—and induces mitochondrial biogenesis and β-oxidation of fatty acids by deacetylating transcription factors (TFs) like PGC-1α. SIRT3 is also induced under energy limitation. Note that deacetylation of acetyl-CoA synthetase (ACS) and long-chain acyl dehydrogenase (LCAD) by SIRT3 in the mitochondria is known to activate those enzymes and generate acetyl-CoA.

Conversely, diets poor in energy would limit glycolysis, activate SIRT1, and trigger protein deacetylation in the cytosolic/nuclear pool (Figure 1B). These conditions would fit energetically with one of the main outputs of SIRT1 activity, the deacetylation of PGC-1α to promote mitochondrial biogenesis and oxidative metabolism (Rodgers et al., 2005). Oxidative phosphorylation produces much more ATP per input glucose than fermentation, because the fuel is oxidized all the way to CO_2. Since fatty acids are oxidized in mitochondria under these conditions, acetyl-CoA (and FADH) will be generated in this compartment. In contrast, the generation of acetyl-CoA in the cytoplasm by ATP-citrate lyase will be limited. Both the high SIRT1 activity and low cytosolic acetyl-CoA levels would favor maintenance of cytosolic/nuclear proteins in the deacetylated state during energy-poor diets.

Changes in the acetylation of mitochondrial proteins with diet appear to vary from tissue to tissue and may be conceptually distinct from the above framework for proteins in the nuclear/cytoplasmic pool (Schwer et al., 2009). In fact, there appear to be competing processes affecting protein acetylation in this organelle. On the one hand, oxidative metabolism in mitochondria, for example fatty acid oxidation, may be expected to increase acetyl-CoA levels in the organelle and thereby favor protein acetylation (Figure 1B). On the other, one of the mitochondrial proteins activated under these conditions is the SIRT3 deacetylase. What does seem clear is that deacetylation by SIRT3 activates mitochondrial metabolic enzymes for oxidative metabolism and detoxification of reactive oxygen species (Verdin et al., 2010).

Evolution has evidently selected for protein acetylation as a regulatory mechanism, as well as an energy-storage mechanism, when energy is in excess. The regulatory aspects minimally include histone acetylation to regulate gene transcription, acetylation of metabolic enzymes to favor glycolysis for ATP production, and acetylation of many transcription factors, such as p53, FOXO, PGC-1α, nuclear receptors, etc. to adjust expession levels of pathways for oxidative versus glycolytic metabolism (Finkel et al., 2009; Imai and Guarente, 2010; Haigis and Sinclair, 2010). In the case of transcription factors, the acetylation of SIRT1-sensitive lysines can either be activating

(e.g., p53) or repressing (e.g., PGC-1α), and this would allow flexibility in regulating the many affected pathways.

The storage aspect of acetylation may be important in transitioning from energy excess to energy limitation (Figure 1B). Under these conditions acetate generated by SIRT1 deacetylation of many proteins would be a substrate for acetyl-CoA synthetase (ACS). Along with the oxidation of fatty acids, this would drive the TCA cycle and oxidative phosphorylation to yield ATP and CO_2. Reinforcing this model, SIRT3, which is upregulated during this energy transition, deacetylates and activates the mitochondrial ACS and long-chain acyl dehydrogenase (LCAD) in the fatty acid oxidation pathway, both of which generate acetyl-CoA (Verdin et al., 2010).

It is intriguing that SIRT1 cleaves NAD to nicotinamide and O-acetyl ADP-ribose each reaction cycle, even though protein deacetylation is energetically favorable. NAD must subsequently be regenerated by salvage pathways (Haigis and Sinclair, 2010; Imai and Guarente, 2010). How the cleavage and recycling of NAD make sense biologically is an important topic for future studies.

In the above framework, protein acetylation governs how cells choose glycolytic versus oxidative metabolism as a function of available energy and helps determine the storage or utilization of carbon energy. Because the substrates for protein acetylation are NAD and acetyl-CoA, it would make sense that SIRT1, an NAD-dependent deacetylase, be a central metabolic sensor and mediator of this process. The protein acetylation cycle depicted in the figure may interact with other biological processes to determine the metabolic strategy of cells. For example PARP-1 inhibition drives SIRT1 activity and oxidative metabolism by raising NAD levels (Bai et al., 2011). Finally, cancer cells often display a preference for glycolytic/fermentative metabolism over oxidative metabolism, termed the Warburg effect. It will be important to determine whether manipulation of NAD or sirtuin levels is a general strategy to tip the balance of cancer cells toward oxidative metabolism and inhibit their potential for tumorigenesis.

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