the new analysis provides insight into how that happens, revealing that upon LTC_4 binding both TM bundles move inward (particularly around the substrate-binding site), bringing the separation of the two NBDs to a distance that is believed to prime the protein for ATP hydrolysis, similar to what is seen for bacterial maltose transporter (Oldham and Chen, 2011).

The recent structures of ABCC transporters have provided new insights into the functional diversity of these biomedically important membrane proteins. Using the complementary tools of X-ray crystallography and high-resolution single-particle cryo-EM, the next challenges will be to determine structures of these proteins in nucleotide-bound and outward-open conformations in order to fully understand the structural changes underlying the transport cycle. For these highly disease-relevant membrane proteins, such knowledge will have major implications for drug discovery.

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Energy in Ancient Metabolism

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http://dx.doi.org/10.1016/j.cell.2017.02.032

Modern metabolism would not work without ATP and phosphate, but in primordial biochemical networks, energy currencies might have been simpler. Goldford et al. report a novel systems approach to reconstructing energetics in ancient metabolism, with very interesting results.

Life is a chemical reaction. Metabolism and life move forward only if energy is released in the overall reaction, as stipulated by the second law of thermodynamics, which permits no exceptions. Because many individual metabolic reactions are energetically uphill (endergonic), the biochemical pathways of a cell are all linked to a central energy supply. At the core of every cell's biochemistry there is thus a main energy releasing (exergonic) reaction that generates a diffusable energetic currency, usually adenosine triphosphate (ATP). Hydrolysis of ATP to ADP and phosphate (P_i) releases energy, such that coupling of ATP hydrolysis to uphill steps can energetically pull the reaction forward. ATP, phosphate, and coupling are universal to life today, but at the evolutionary onset of metabolism 4 billion years ago, the chemistry had to be simpler. What came before ATP? In innovative computer work in this issue of *Cell*, Goldford et al. (2017) remove all of the reactions from metabolism that are ATP-dependent or that even involve phosphate-containing cofactors to see if anything remains and whether what remains might hold clues about ancient metabolism. They find a connected reaction network of small molecular weight carbon compounds, a prevalence of thioesters, and an enrichment of FeS-dependent enzymes. Their findings shed light on the nature of chemical energy currencies in early evolution.

Of life's energy currencies, ATP is the most familiar and the most widely used, but that does not mean that it is also the most ancient. Where does ATP come from in metabolism? At the most basic level, there are only two ways in which cells synthesize ATP, both require sources of environmental energy.

The evolutionarily more advanced mechanism of ATP synthesis is electron transfer phosphorylation, or chemiosmotic coupling. In chemiosmotic coupling, exergonic reactions at the plasma membrane are coupled to the pumping of ions from the inside of the cell to the outside, generating electrochemical ion gradients that can be harnessed by highly complex multisubunit proteins, rotor stator type

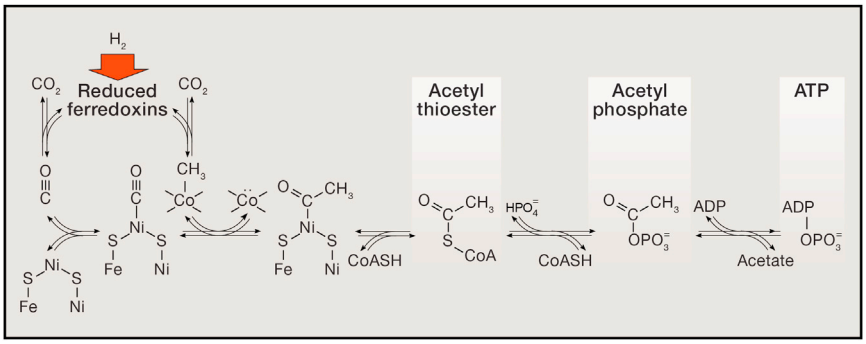

Figure 1. Energy Conservation from Ferredoxin to ATP in the Acetyl CoA Pathway
The pathway is drawn from Fuchs (2011) and references therein. The large red arrow denotes that the synthesis of reduced ferredoxins from H_2 is not a trivial step, it involves a process called electron bifurcation (Buckel and Thauer, 2013) in which redox reactions of differing midpoint potentials are coupled. In methanogenic archaea none of the coenzymes or prosthetic groups participating in CO_2 reduction with H_2 to acetyl-CoA or methane directly involve phosphate in catalysis.

ATPases, to transfer P_i to the β-phosphate of ADP. The evolutionary success of chemiosmotic coupling lies in its flexibility: by natural variation in membrane proteins a myriad of different energy releasing reactions can be converted into a universal currency of ion gradients and ATP. Once life had invented chemiosmotic coupling, virtually any source of environmental energy harboring enough free energy to pump an ion could be tapped. The invention of proteins that could harness gradients posed, however, a great hurdle in early evolution. Rotor stator type ATPases are as universal as the ribosome, but they are highly sophisticated proteins.

The chemically simpler, and probably older, biochemical mechanism to generate ATP is substrate-level phosphorylation (SLP). In SLP, exergonic chemical reactions of carbon compounds generate intermediates with high energy phosphate bonds that can phosphorylate ADP in a subsequent energy releasing reaction. The enzymes involved are typically soluble with a simple subunit composition. There are about a dozen compounds in metabolism that directly phosphorylate ADP, they were known 50 years ago (Decker et al., 1970) and the list has not grown since. In SLP, phosphate enters metabolism via acyl phosphate or phosphoenolate bonds through energy releasing reactions of carbon compounds. Although there are many reactions in nature that harbor enough energy to support SLP, the reactions that are used by metabolism for the purpose of SLP involve changes in the oxidation state of carbon. In SLP, the energy to phosphorylate ADP resides in carbon chemistry.

Phosphate bonds are used as an energy currency because they have a high free energy of hydrolysis. That, in turn, is because phosphorus, in contrast to carbon, nitrogen and oxygen, possesses a third, more open, shell with *d* orbitals available for further combination, which can readily accept lone pairs of electrons (Wald 1964). Sulfur also has *d* orbitals and is also involved in biological energy conservation. Sulfur functions as a biological energy currency in thioesters. Thioesters also have a high free energy of hydrolysis, making them chemically reactive. Goldford et al. (2017) found a prevalence of thioester-linked reactions in their phosphate-free network, suggesting an ancient role for thioesters in metabolism.

In line with that, Semenov et al. (2016) recently showed that thioesters can spontaneously generate autocatalytic chemical reaction networks with oscillating properties. Both studies point to a role for thioesters as simple carriers of chemical energy in the assembly of early metabolic networks. Can metabolic energy get even simpler than thioesters? Yes, but that brings us all the way back to FeS clusters and H_2, which in terms of chemical simplicity are hard to underbid.

H_2 is a rich source of chemical energy for microbes. Under anaerobic conditions, the reaction of CO_2 with H_2, releases energy—a reason why biologists have always thought that anaerobic autotrophs are ancient (Decker et al., 1970; Fuchs 2011). Both H_2 and CO_2 were abundant on the early Earth (Sleep et al., 2011). Their exergonic reaction to form a thioester is the basis of the most ancient CO_2 fixation pathway, the acetyl-CoA pathway (Fuchs 2011).

Among the six CO_2 fixation pathways known, the acetyl-CoA pathway (Figure 1) stands out from an energetic standpoint. It provides carbon backbones while releasing energy that cells harness to make ATP. The other five pathways fix CO_2 at ATP expense (Fuchs 2011), a reason why Goldford et al. (2017) included acetate among their starting compounds. The electrons that flow to CO_2 in the acetyl-CoA pathway flow through FeS clusters of ferredoxins, which are ancient electron carriers and, like H_2, an energy currency in their own right (Herrmann et al., 2008).

In some groups of anaerobic autotrophs, the acetyl-CoA pathway provides CO_2 fixation, chemiosmotic coupling, and ATP synthesis via SLP all at the same time (Basen and Müller, 2017). That it affords SLP while reducing CO_2 is noteworthy because most pathways of SLP involve the oxidation of biologically derived reduced carbon compounds (Decker et al., 1970), the reduction of glycine via the Stickland reaction being an interesting exception (Andreesen, 1994). A look at the main reactions of the acetyl-CoA pathway (Figure 1), reveals many ancient forms of chemical energy—the H_2/CO_2 couple, reduced ferredoxins (FeS), thioesters, acyl phosphates and ATP—in their biologically, and thermodynamically, ordered sequence of energy conserving reactions. Such a sequence might even trace the emergence of biochemical energy currencies at the evolutionary onset of metabolic networks.

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