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Electron bifurcation

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Electron bifurcation is the recently recognized third mechanism of biological energy conservation. It simultaneously couples exergonic and endergonic oxidation–reduction reactions to circumvent thermodynamic barriers and minimize free energy loss. Little is known about the details of how electron bifurcating enzymes function, but specifics are beginning to emerge for several bifurcating enzymes. To date, those characterized contain a collection of redox cofactors including flavins and iron–sulfur clusters. Here we discuss the current understanding of bifurcating enzymes and the mechanistic features required to reversibly partition multiple electrons from a single redox site into exergonic and endergonic electron transfer paths.

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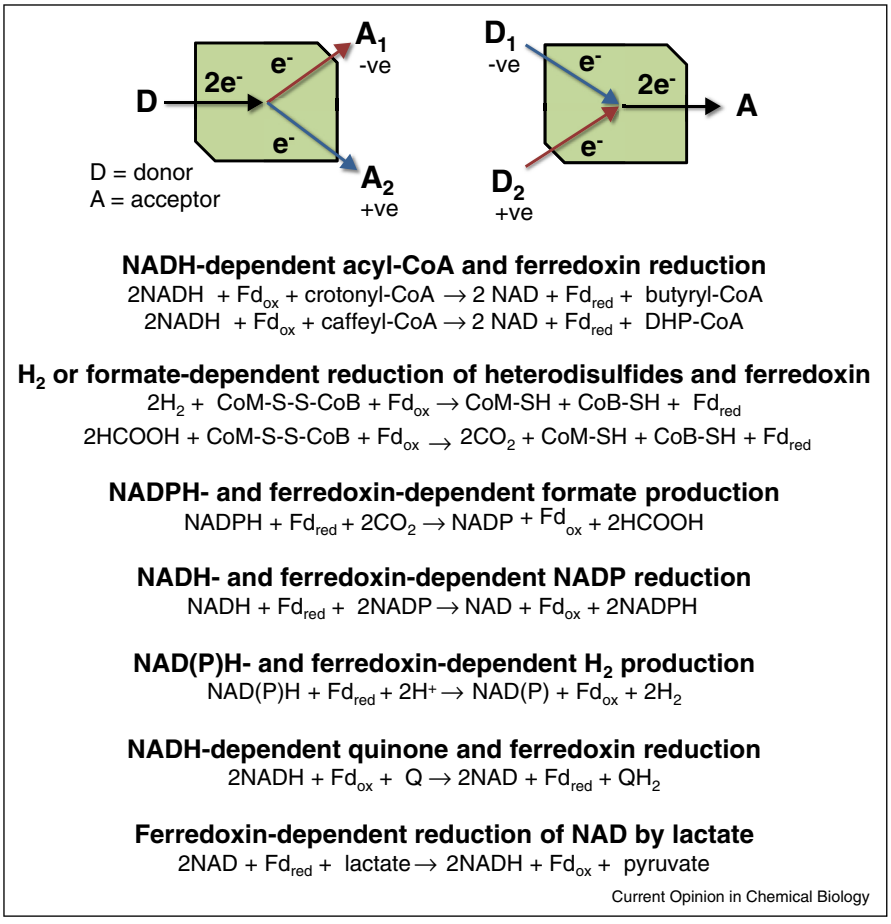
The unifying feature of biological energy conservation mechanisms is the conversion of electrochemical potential to chemical bond energy. During substrate-level phosphorylation, metabolism of organic compounds is accompanied by formation of phosphorylated high-energy intermediates that can undergo phosphoryl transfer reactions with ADP to form ATP. Similarly, both organic and inorganic electron donors serve as substrates for electron transport chain complexes in electron transport-linked phosphorylation. This process couples exergonic electron transfer from a more negative to a more positive electrochemical potential, to ion pumping and formation of a chemiosmotic potential. This in turn drives production of ATP by the ATPase. For decades it was generally accepted that substrate level phosphorylation and electron transport-linked phosphorylation were the two fundamental biological energy conservation mechanisms. However, a third mode of energy conservation, referred to as electron bifurcation, has been recently recognized.

A conceptual framework

Electron bifurcation involves coupling exergonic and endergonic electron transfer reactions to limit energy loss. A redox reaction $A \rightarrow B$ with a significant positive free energy change at the concentrations of A and B in the cell does not occur spontaneously. In contrast, the reaction $A \rightarrow C$ may have a significantly negative free energy change and occur spontaneously at cellular concentrations of A and C. In biology, significant negative free energy represents energy wasted as heat unless it is harnessed through energy-conserving mechanisms. Electron bifurcation in its simplest terms is a mechanism through which the reactions $A \rightarrow B$ and $A \rightarrow C$ are coupled ($2A \rightarrow B + C$) to generate a net exergonic reaction with minimal negative free energy change and maximal energy conservation.

The thermodynamics of electron transfer is dictated by the relative reduction potentials of the redox partners. Electron transfers that occur from a more negative to a more positive potential are favorable. Reduction potentials are sensitive to concentrations of reactants and products according to the Nernst equation. In complex electron transfer pathways having multiple stepwise electron transfer events, individual intermediate electron transfer reactions from more positive to more negative potentials can occur readily if the overall sequence of reactions is exergonic and thermodynamically favorable [1]. Alternately by coupling reactions via electron bifurcation, reactions that at face value appear to be thermodynamically unfavorable can be made to occur spontaneously. In short, a redox reaction from a more positive to a more negative reduction potential can occur spontaneously as long as the net change in electrochemical potential of the coupled reactions is still from more negative to more positive (Figure 1). Note that some types of bifurcating enzymes actually catalyze a 'confurcating' reaction, where the oxidation of low and high potential donors are coupled to simultaneously reduce

Figure 1



Bifurcating enzymes. The schemes depict electron transfer in the two types of bifurcating enzyme. *Left*: a two-electron donor (D) of intermediate reduction potential simultaneously provides electrons to electron acceptors with more negative (A_1) and more positive (A_2) potentials. *Right*: a two-electron acceptor (A) of intermediate reduction potential simultaneously accepts electrons from electron donors with more negative (D_1) and more positive (D_2) potentials. Seven types of reaction currently known to be catalyzed by bifurcating flavoenzymes are listed. Details on the enzymes can found in [6,11,12].

an acceptor of intermediate potential, although they are still referred to as bifurcating enzymes (Figure 1).

Electron bifurcation in biology was first proposed based on quinones some 40 years ago by Peter Mitchell to explain the so-called Q-cycle catalyzed by Complex III of the aerobic respiratory chain [2]. In this cycle, reducing equivalents produced by oxidation of quinol ($E_0' \sim +100$ mV) are fed into two distinct redox chains directed to opposite sides of the membrane. The first electron reduces a Rieske FeS cluster ($E_0' \sim +300$ mV) at the starting-point of a thermodynamically favorable, higher potential chain. The second electron reduces a low potential b-type heme ($E_0' \sim -100$ mV) [3]. In comparison, flavin-based electron bifurcation occurs at much more negative potentials, typically in the −300 to −500 mV range, than does quinone-based bifurcation. Flavin-based bifurcation can therefore be exploited by anaerobic microorganisms to generate low potential, high energy compounds such as hydrogen gas and reduced ferredoxin. Hence, as pointed out by Thauer [4•], flavin-based bifurcation is probably as important for energy coupling in anaerobic metabolism as the quinone-based system is in aerobic metabolism. Indeed, it has been speculated that electron bifurcation may have played a key role in origin of life [5•].

The discovery of flavin-based bifurcating enzymes

Flavin-based electron bifurcation was discovered as a result of observations by Wolfgang Buckel and Rolf Thauer in 2008. They showed that metabolism in some anaerobic microorganisms couples oxidation of NAD(P)H, with a standard reduction potential of −320 mV (at pH 7, E_0'), to reduction of the low potential electron carrier ferredoxin, a small iron and sulfur-containing protein with E_0' typically near −500 mV. This seemingly thermodynamically unfavorable reaction