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Commentary

Redox loops and proton pumps

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In a *Review–Hypothesis*, Mitchell [(1987) FEBS Lett. 222, 235–245] has recently suggested possible molecular mechanisms for proton translocation by cytochrome oxidase. In describing these mechanisms, he extended his own concept of a redox loop in a manner expected to lead to confusion. He also stated that the term redox-linked proton pump implies an indirect coupling between electron transfer and proton translocation, and that this type of coupling is very difficult to test experimentally. Here it is argued that the original meaning of a redox loop should be maintained, and proper definitions of the terms redox-linked proton pump and direct or indirect coupling are formulated. In addition, it is reasoned that both types of coupling are amenable to experimental tests.

Cytochrome oxidase; Cytochrome *a*; Proton translocation; Proton pump; Redox loop; Redox-linked

Mitchell, in his famous 'gray book' of 1966 [1], described a redox (or o/r) loop as a system which "translocates hydrogen groups one way and electrons the other way", and he pointed out that "it thus gives a net translocation of protons". Mitchell further suggested that cytochrome oxidase transfers 4 e^- from cytochrome *c* on the cytosol side to the matrix side of the inner mitochondrial membrane, where they combine with 4 H^+ and O_2 to yield 2 H_2O. In this way the oxidase would provide the electron-translocating arm of a redox loop.

Obviously the H^+/e^- stoichiometry of this kind of redox loop (type II in the original terminology of Mitchell [1]) can only be 1 (a type I loop has a stoichiometry of 1/2). It was therefore a surprise when Wikström [2] reported in 1977 that the oxidation of cytochrome *c* results in the net release of about 1 H^+/e^- into the extramitochondrial phase, thus providing evidence that cytochrome oxidase functions as an H^+ pump. It was not until August 1985 that Mitchell accepted the proton-pump concept for cytochrome oxidase, when he and collaborators published a *Review–Hypothesis* [3], in which they described hypothetical O loop and O cycle mechanisms for the pump. In this communication, Mitchell still retained the original redox-loop concept with e^- and H^+ moving in different directions across the membrane. In October of last year, however, he formulated a new redox loop formality [4], in which he extends the use of the term to include any mechanism having a direct involvement of a redox center, for example Cu_A, in the H^+ translocation, even if the H^+/e^- stoichiometry is >1.

I believe Mitchell's extension of the concept of redox loops to be most unfortunate. The original meaning is so well established among biochemists that it is described in modern textbooks. Thus, to use the term in any other sense can lead to nothing but confusion. In addition, we do need a term to

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designate systems which move H and e^- in opposite directions, and have an intrinsic stoichiometry of 1, as opposed to proton pumps in a more general sense. So why force us to introduce a new term, when an established one exists? I would like to appeal to investigators in the field to restrict their use of the term redox loop to the original loop concept also in the future.

In his recent writings [3,4], Mitchell interprets the term redox-linked pump as describing an indirectly coupled pump, and this usage of terms is also most confusing. A redox-linked pump, which is synonymous with an electron-transport-driven pump [5], is, of course, a pump in which a redox (or electron-transfer) reaction provides the free energy necessary for ion translocation, whether the coupling be direct or indirect. Direct coupling means that the H^+ (or OH^-)-binding group is associated with a redox center, i.e. an e^--binding group, as cytochrome *a* according to a hypothesis of Wikström and Krab [6] or Cu_A in one of Mitchell's mechanisms [4]. Indirect coupling, on the other hand, means that the H^+- and e^--binding groups are topologically remote, like the substrate- and effector-binding sites in an allosteric protein.

In an indirectly coupled pump the interaction between H^+ and e^- must be mediated by conformational changes, and for this reason such pumps have been said to operate by a conformational mechanism [6]. In my view a better term would be allosteric mechanism, as directly coupled pumps must also involve at least two conformational states to provide an alternating access for the ion to be translocated to the two sides of the membrane.

Mitchell [3,4] maintains that indirectly coupled pumps, in contrast to directly coupled ones, are not easily approached experimentally, but the extremely detailed experimental information available for allosteric systems, such as hemoglobin and aspartate transcarbamylase, certainly belies this attitude. My own recent work has been guided by an indirect model [5], which predicts a specific form of pH dependence of cytochrome oxidase kinetics, and this can be tested experimentally [7]. On the basis of the effect of temperature on the rate of the internal electron transfer in cytochrome oxidase, Brzezinski and I [8] have also found it possible to formulate a molecular mechanism for electron gating, a fundamental aspect of any electron-transport-driven pump [9].

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