PERSPECTIVES: STRUCTURAL BIOLOGY

PMF Through the Redox Loop

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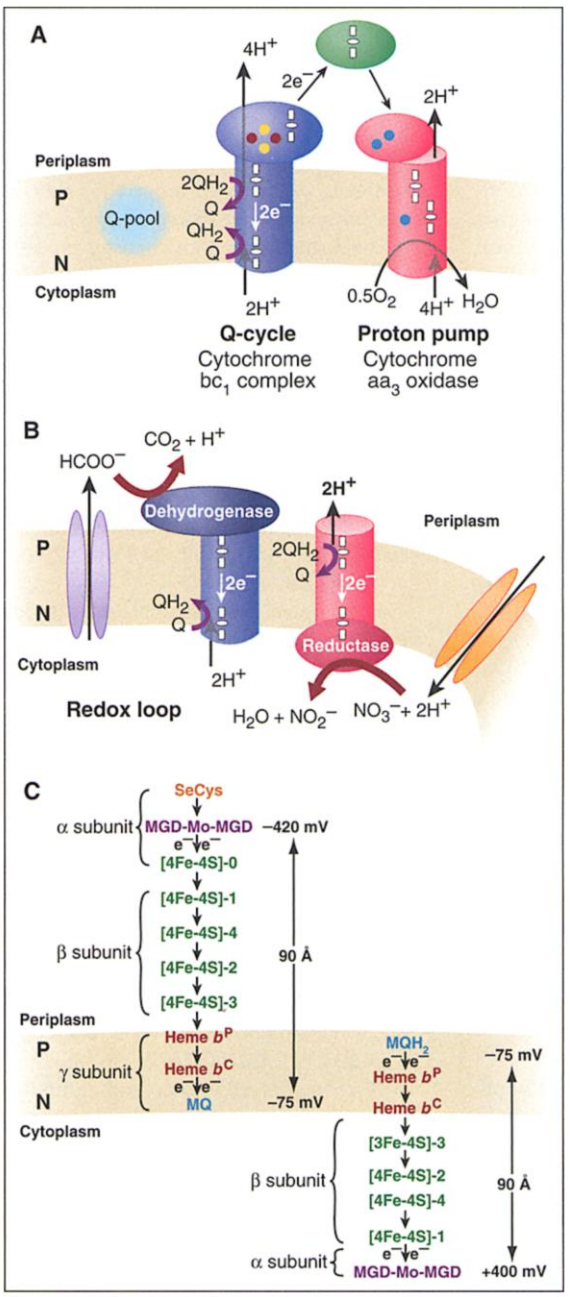
More than 30 years ago, Peter Mitchell proposed his chemiosmotic model of energy coupling (*1*). In this model, he postulated that "energy-consuming" integral membrane proteins, such as ATP synthase and secondary transporters, are driven by a transmembrane proton electrochemical gradient called the proton motive force (pmf) (*1*). A pmf has been detected across many energy-conserving biological membranes, with proton translocation being driven by membrane-associated proteins that couple electron flow from low–redox potential electron donors to higher-potential electron acceptors. A well-known example of this process is the respiration of oxygen by mitochondria, during which NADH or succinate serves as an electron donor. Two key integral membrane enzymes involved in respiration, cytochrome aa_3 oxidase and the cytochrome bc_1 complex, use different methods (proton pumping and the Q-cycle, respectively) for coupling electron transfer to pmf generation (see the figure, A) (*2*–*4*). However, in early formulations of the chemiosmotic theory, Mitchell envisaged that proton translocation was driven by a "redox loop." He proposed that in this loop, two electrons are transferred from the positive (P) side of the membrane to the negative (N) side where, in combination with two protons, they reduce a quinone to quinol. The quinol then diffuses back across the membrane lipid bilayer and is reoxidized at the P face, releasing protons (see the figure, B). In contrast to the conformational proton-pump and Q-cycle, the full proton-motive redox loop requires the participation of two catalytically distinct enzyme complexes. The redox loop does not actually operate in mitochondria but has been discovered in the inner membrane of bacteria (*5*). On page 1863 of this issue, Jormakka *et al.* (*6*) provide a molecular description of one of the electron-carrying enzyme complexes in a redox loop of *Escherichia coli*: the nitrate-induced formate dehydrogenase (Fdh-N).

In contrast to most eukaryotes, many bacterial species have inducible respiratory chains that enable the organism to respire using alternative terminal electron acceptors, such as nitrate, nitrite, fumarate, and dimethylsulfoxide, when oxygen is unavailable (*7*). Reduction of these respiratory substrates can be coupled to a wide range of electron donors, including formate and hydrogen. The Fdh-N–nitrate reductase (NR) respiratory chain of *E. coli* is a paradigm for a proton-motive redox loop (see the figure, B). It has been the subject of intense study for 60 years, since the recognition that formate is an important source of reductant for anaerobically growing bacteria (*8*). *E. coli* synthesizes three Fdh isozymes (*9*), all seleno-molybdoenzymes. They catalyze the oxidation of formate, which is produced by *E. coli* under anaerobic conditions by the enzyme pyruvate formate–lyase. Fdh-N, together with its close homolog Fdh-O, are involved in respiration, whereas Fdh-H (which produces hydrogen) is not thought to be energy conserving.

Resolution of the Fdh-N structure at 1.6 Å by Jormakka *et al.* sheds light on a number of enzymes that contribute to the respiratory flexibility of bacteria. The generic framework



Three mechanisms for proton translocation. (**A**) The Q-cycle and proton pump operate together in mitochondria and in the aerobic respiratory chains of many bacteria. In the Q-cycle of the cytochrome bc_1 complex, a single protein complex oxidizes two molecules of quinol at the periplasmic P (positive) face of the membrane and four protons are released. Two electrons move across the membrane to the cytoplasmic N (negative) face and combine with the two protons to reduce quinone, resulting in recycling of a quinol molecule. Thus, there is net consumption of one quinol molecule by the cytochrome bc_1 complex as two electrons also flow from this complex via cytochrome c to the proton-pumping cytochrome aa_3 oxidase, which directly couples oxygen reduction to proton translocation through the protein milieu. (**B**) In the proton-motive redox loop composed of the formate dehydrogenase (Fdh-N) and nitrate reductase (NR) enzymes of *E. coli*, quinone reduction and quinol oxidation take place at different faces of the membrane and on different protein complexes. (**C**) In both enzyme complexes, two electrons flow from the P face to the N face and then loop back with two protons (carried by a diffusible quinol) from the N face to the P face. In Fdh-N, electrons move via a 90 Å "ladder" of redox cofactors, with the energy difference between the donating and accepting redox couples being sufficient to drive electron transfer against a negative-inside membrane potential.

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consists of an integral membrane quinol dehydrogenase, a peripheral membrane ferredoxin with four associated iron-sulfur clusters, and a peripheral membrane subunit with an active-site bis-MGD (molybdopterin-guanine-dinucleotide). Other enzymes that have this framework include the respiratory reductases for nitrate (NarGHI), dimethylsulfoxide, and tetrathionate (*7*). In Fdh-N the catalytic site is in the periplasm (between the outer and inner bacterial membranes), and the electrons generated pass down a 90 Å "ladder" of redox centers that connect a molybdenum-bis-guanine dinucleotide cofactor (Mo-bis-MGD), located in the periplasm, to a menaquinone reductase site at the cytoplasmic (N) face of the inner membrane (see the figure, C). The ladder comprises five iron sulfur clusters and two hemes, each within 12 Å of its nearest neighbor to ensure rapid electron transfer. The large ~340-mV potential "drop" (–420 to –75 mV at the N face) allows efficient electron transfer against the membrane potential. The NR electron-carrying arm of the Fdh-N–NR proton-motive redox loop has a cofactor composition similar to that of Fdh-N (see the figure, C), making it likely that a similar ladder arrangement, though with opposite orientation across the membrane, exists for this enzyme. Consequently, the full Fdh-N–NR redox loop may span an electron-transfer distance of some 150 Å (see the figure, C). The structure of Fdh-N reveals the arrangement of two pairs of iron-sulfur centers in the β subunit, which was correctly predicted from detailed spectroscopic studies on *E. coli* NarGH (*10*). In this case, the low redox potentials of two of the centers raised the possibility that they are not directly involved in electron transfer between quinol and nitrate. These low–redox potential centers correspond to FeS-2 and FeS-4 in the Fdh-N structure (see the figure, C). The Fdh-N structure leaves no doubt that these centers are directly involved in electron transfer between formate and menaquinone. The structure of Fdh-N, and predicted organization of NR, adds to other recent structural definitions of extended electron-transfer chains in bacteria (*11*, *12*).

In both the Fdh-N and NR complexes, much of the redox ladder is outside the membrane (see the figure, C), with the two hemes in the membrane being critical for providing charge separation. In Fdh-N, these hemes bind within a four-helical bundle where three helices provide the four histidine ligands required for iron binding. Here, there is likely to be a difference in the NR where the four histidines are predicted to be provided by only two of five transmembrane helices (*5*). Notably, in Fdh-N, the N-face heme interacts with the bound menasemiquinone analog HQNO. Many integral membrane electron transfer proteins for which structures are available are quinone-binding proteins, yet despite some basic principles governing a Q-binding site, structural variation can sometimes make identification of this site difficult (*13*). In the case of Fdh-N, the bound napthoquinone accepts a hydrogen bond from one of the histidine ligands of the N-face heme. This is the first time that a heme ligand has been shown to be directly involved in quinone binding, and it may be that other examples will emerge, such as the γ subunit of NR (*10*).

The structure of the α subunit of Fdh-N confirms that formate oxidation is carried out at a selenocysteine-coordinated derivative of the Mo-bis-MGD cofactor. In this respect Fdh-N is similar to its water-soluble counterpart Fdh-H, except that the latter is located in the cytoplasm (*14*). A molecular mechanism for electron and proton extraction from formate, derived from structure-informed spectroscopy, has been proposed for the Fdh-H subunit of the hydrogen-producing formate-hydrogen lyase (*15*). This mechanism involves a conserved active-site histidine serving as a proton-accepting base, and is supported by the structure of the respiratory Fdh-N α subunit. This suggests that the biochemistry of formate oxidation is the same regardless of enzyme function. When the whole electron transfer ladder from the Se-Mo-bis MGD to the menaquinone is considered, intermediary electron carriers are found to be one-electron transfer centers. However, formate oxidation and menaquinone reduction are two-electron reactions. Thus, the Se-Mo-MGD and Q-reductase site at either end of the electron transfer ladder are crucial for coupling the two-electron to one-electron oxido-reductions.

A fundamental criterion of the proton-motive redox loop is that the two enzyme complexes involved are oriented with the sites of substrate oxidation and substrate reduction on opposite faces of the membrane at P and N locations, respectively (see the figure, B). Compelling evidence from bioenergetic studies that electron transport from formate to nitrate is energy conserving (*16*) is now substantiated by the structure of Fdh-N, which shows unambiguously that the α and β subunits are located at the periplasmic (positive) face of the membrane. Although this location is essential for the proton-motive redox loop, the finding of the active site at the P face raises a number of important questions. First, how does the organism assemble such a large protein complex with multiple redox centers in the periplasm? The nascent Fdh-N α subunit has a signal peptide (removed from the mature enzyme) that includes a "twin arginine translocase" (Tat) motif. Seminal studies by Berks *et al.* (*17*) demonstrate that this translocase transports prefolded proteins with assembled redox cofactors. The Fdh-N β subunit does not have a signal peptide and must therefore be translocated as a passenger with the α subunit. It is now important to determine whether the fully assembled $\alpha_3\beta_3\gamma_3$ complex is translocated or whether it is assembled from αβ pairs on a γ_3 membrane scaffold. Transport of αβ pairs requires a maximum diameter for the pore of the Tat apparatus of around 70 Å, but if the $\alpha_3\beta_3\gamma_3$ is transported, the pore would have to be around 130 Å. Clearly, the resolution of the translocase structure will provide insights into the "gating" mechanism that maintains an "ionic seal" upon transport of such large substrates.

The second question posed by the orientation of the Fdh-N–NR proton-motive redox loop is how the two substrates for the loop, formate and nitrate, are delivered to their respective active sites. The nitrate anion is encountered in the external environment of the bacterium and so must be transported into the cell (against the "negative-inside" membrane potential) to serve as the substrate for NarGH. This could, in principle, be a pmf-consuming process that would then affect the net energetics of the Fdh-N–NR electron transfer system (see the figure, B). In contrast, formate is generated inside the cell and must be exported to the periplasm. In both cases the molecular mechanisms of anion transport are not yet fully understood, although candidate transport proteins have emerged (*18*, *19*). The Fdh-N structure instructs us that a full evaluation of a proton-motive redox loop requires an understanding of the molecular mechanism underlying enzyme catalysis and electron transfer. Further, it demands an appreciation of the relationship that the loop enzymes have, both at a molecular and gene-regulation level, with other electron transport enzymes and transport proteins in the energy-conserving membrane.

References

1. P. Mitchell, *Science* **206**, 1148 (1979).
2. S. Iwata *et al.*, *Nature* **376**, 660 (1995).
3. T. Tsukihara *et al.*, *Science* **269**, 1069 (1995).
4. D. Xia *et al.*, *Science* **277**, 60 (1997).
5. B. C. Berks *et al.*, *Mol. Microbiol.* **15**, 319 (1985).
6. M. Jormakka, S. Törnroth, B. Byrne, S. Iwata, *Science* **295**, 1863 (2002).
7. D. J. Richardson, *Microbiology* **146**, 551 (2000).
8. E. J. Ordal, H. O. Halvorson, *J. Bacteriol.* **38**, 199 (1939).
9. G. Sawers, *Antonie Leeuwenhoek* **66**, 57 (1994).
10. F. Blasco *et al.*, *Cell. Mol. Life Sci.* **58**, 179 (2001).
11. A. Volbeda *et al.*, *Nature* **373**, 580 (1995).
12. C. R. Lancaster *et al.*, *Nature* **402**, 377 (1999).
13. N. Fisher, P. R. Rich, *J. Mol. Biol.* **296**, 1153 (2000).
14. J. C. Boyington *et al.*, *Science* **275**, 1305 (1997).
15. S. V. Kangulov *et al.*, *Biochemistry* **37**, 3518 (1998).
16. R. W. Jones, *FEMS Microbiol. Lett.* **8**, 167 (1980).
17. B. C. Berks *et al.*, *Mol. Microbiol.* **35**, 260 (2000).
18. B. Suppmann, G. Sawers, *Mol. Microbiol.* **11**, 965 (1994).
19. J. W. B. Moir, N. J. Wood, *Cell. Mol. Life Sci.* **58**, 215 (2001).