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Invited review

Targeting bacterial energetics to produce new antimicrobials

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ABSTRACT

From the war on drug resistance, through cancer biology, even to agricultural and environmental protection: there is a huge demand for rapid and effective solutions to control infections and diseases. The development of small molecule inhibitors was once an accepted "one-size fits all" approach to these varied problems, but persistence and resistance threaten to return society to a pre-antibiotic era. Only five essential cellular targets in bacteria have been developed for the majority of our clinically-relevant antibiotics. These include: cell wall synthesis, cell membrane function, protein and nucleic acid biosynthesis, and antimetabolites. Many of these targets are now compromised through rapidly spreading antimicrobial resistance and the need to target non-replicating cells (persisters). Recently, an unprecedented medical breakthrough was achieved by the FDA approval of the drug bedaquiline (BDQ, trade name Sirturo) for the treatment of multidrug-resistant tuberculosis disease. BDQ targets the membrane-bound F_1F_o-ATP synthase, validating cellular energy generating machinery as a new target space for drug discovery. Recently, BDQ and several other FDA-approved drugs have been demonstrated to be respiratory "uncouplers" disrupting transmembrane electrochemical gradients, in addition to binding to enzyme targets. In this review, we summarize the role of bioenergetic systems in mycobacterial persistence and discuss the multi-targeting nature of uncouplers and the place these molecules may have in future drug development.

1. Bioenergetic systems in persistent bacteria

1.1. Overview of the respiratory circuit

Although the term "bioenergetics" could be broadly applied to any biological reaction involving an energy change, it colloquially applies to the mechanisms that organisms use to store and utilize different energy sources (Nicholls and Ferguson, 2013a). All life has evolved to harness the energy inherent in disequilibria, whether it be in chemical mass:action ratios, abiotic gradients (*i.e.* salt, pH) or electrical potential differences (Schoepp-Cothenet et al., 2013). The main feature of bioenergetic systems are biological membranes (Nicholls and Ferguson, 2013a): they act as a barrier to allow the establishment of electrochemical gradients that are efficiently transduced into chemical energy, according to cellular demand. The study of bioenergetics is focused on understanding the membrane-bound enzyme complexes that effect these transductions.

All organisms require an electrochemical gradient, in the form of either a proton motive force (PMF) or sodium motive force (SMF), to survive and grow (Cook et al., 2014a). The energy in these gradients is consumed for a variety of processes, such as the synthesis of ATP and active transport of solutes from the environment. ATP and strong metabolic reductants, like NADH, can in fact be generated by soluble cytoplasmic enzymes, which is the primary mechanism used by many fermentative organisms. However, the PMF or SMF is still required and these organisms will use alternative mechanisms, such as ATP hydrolysis or solute export, to drive their generation (Cook et al., 2014a). Furthermore, the generation of the PMF or SMF by membrane-bound respiratory complexes are evolutionarily conserved and existed in the lowest universal common ancestor of Eubacteria and Archaea (Schoepp-Cothenet et al., 2013). Due to their prevalence and conservation, this review will consider only those organisms that encode the intact respiratory chains that defines the backbone of bioenergetics: henceforth "*respiratory organisms*".

Respiratory organisms require three components for the generation and maintenance of an electrochemical gradient (Fig. 1A): a physical barrier, enzymes to generate the gradient and enzymes to consume the gradient. The electrochemical gradient itself links these components and can be likened to a simple electrical circuit (Fig. 1B). This analogy holds even when discussing complex systems and most bioenergetic parameters are derived from electrical theory (Nicholls and Ferguson, 2013b). For example, the electrochemical gradient can be expressed in

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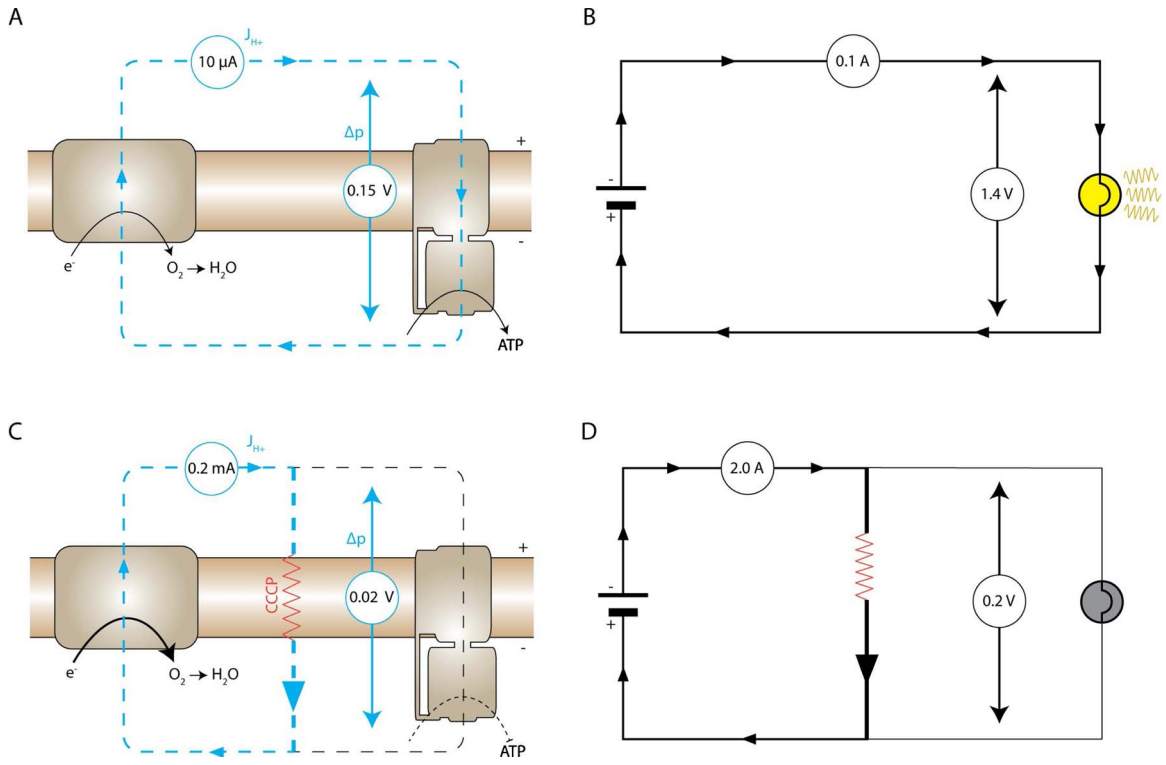

Fig. 1. Electric circuit analogy for the respiratory chain.
Biological membranes (A) form a circuit of protons that have a potential difference (ΔpH, usually measured in mV) across the membrane. The respiratory chain and ATP synthase are analogous to the battery and lightbulb of a simple electrical circuit (B). The proton permeability of the membrane and activity of proton translocating enzymes (*e.g.* ATP synthase) determine the resistance and hence proton current. Introducing an uncoupler (C), like CCCP, provides a low resistance pathway; causing current and respiratory activity to increase substantially. This is analogous to introducing a wire (D) across the circuit described in (B). Current and voltage values as described in (Nicholls and Ferguson, 2013c).

millivolts and proton (or sodium ion) flux is measured in amps. Most organisms tend to generate an electrochemical gradient of ~150 mV (Schoepp-Cothenet et al., 2013), which is comprised by varying amounts of both a charge (Δy) and proton/sodium (ΔpH, ΔpNa^+) gradients. It is important that consumption and generation are precisely balanced: overly high magnitudes are susceptible to proton-leaks (Brand et al., 1994) and/or increase of the total system's resistance (respiratory backpressure), while an overly high proton current decreases the total voltage due to internal resistance in the battery-like components (Nicholls and Ferguson, 2013b). In both cases, the mass:action ratio of reducing equivalents (like the NADH/NAD^+ couple) will be disturbed, having a feedback effect on all intracellular reactions.

An important proof of this theory was the observation that bioenergetically-active membranes could be short-circuited (Nicholls and Ferguson, 2013b). Chemicals that can disrupt the permeability of membranes, by forming physical pores or specifically binding protons or ions, are equivalent to introducing a small wire across an electrical circuit (Fig. 1C & D). The cellular response is to increase the activity of PMF/SMF-generating complexes to be commensurate with the reduced resistance (Nicholls and Ferguson, 2013c). This is ultimately futile due to internal resistance and the lack of useful work performed in this low resistance circuit. Chemicals achieving this are called "uncouplers" as they uncouple the generation of electrochemical gradients from ATP synthesis by F-type ATP synthases. A summary of canonical uncouplers is provided in Fig. 2, where molecules can either dissipate both components of the electrochemical gradient (*e.g.* CCCP) or only one component (*e.g.* valinomycin, nigericin, gramicidin). It is therefore important that the functions of individual components of the respiratory circuit are considered in light of the electrochemical gradients, which link the entire system.

1.2. Electron transfer and components of bacterial respiratory chains

Most reactions involved in the generation of electrochemical gradients are redox driven. High-energy precursors, such as NADH, are oxidized to release high-energy electrons, depending on the reduction potential of the particular redox couple (Table 1). This is transferred through several enzyme complexes and lipid-soluble electron carriers, with increasing reduction potentials, and the released energy is harnessed to generate electrochemical gradients by vectorial proton pumping or charge separation/redox loop mechanisms (Fig. 3). Electrons generally terminate on the reduction of oxygen, due to its high reduction potential, but alternatives such as fumarate and nitrate are also commonly used (Cook et al., 2014a). The total system is referred to as the electron transport chain or respiratory chain.

Bacterial respiratory chains are far more diverse than their eukaryotic counterparts (Cook et al., 2014a), but follow the same general structure: oxidative electron liberating complexes are connected to reductive electron terminating complexes *via* electron carriers (quinones, cytochrome *c*) and intermediary complexes in certain cases. For electron donating complexes, bacteria most frequently encode from two types of NADH:quinone oxidoreductase (Ndh1 c.f. complex I, Ndh2), succinate dehydrogenases (c.f. complex II), formate dehydrogenases and hydrogenases. While for electron accepting complexes, bacteria most frequently encode two types of terminal cytochrome oxidase (heme-copper oxidases, c.f. complex IV and cytochrome *bd*), nitrate reductases, nitrite reductases, fumarate reductases, tetrathionate reductases and hydrogenases (Cook et al., 2014a). Menaquinone derivatives are the most frequently utilized electron carriers, as they are the ancestral quinone (Schoepp-Cothenet et al., 2013), while higher potential ubiquinones can be utilized in some Alpha-, Beta- and Gamma-proteobacteria. Given that complex IV frequently exists in functional association with quinol:cytochrome *c* oxidases (c.f. complex III) (Melo and Teixeira, 2016), it is unlikely that cytochrome *c* exists as an