The Rnf Complex of Clostridium ljungdahlii Is a Proton-Translocating Ferredoxin: NAD+ oxidoreductase essential for autotrophic growth

It has been predicted that the Rnf complex of Clostridium ljungdahlii is a proton-translocating ferredoxin: NAD(+) oxidoreductase which contributes to ATP synthesis by an H+-translocating ATPase under both autotrophic and heterotrophic growth conditions. The recent development of methods for genetic manipulation of C. ljungdahlii made it possible to evaluate the possible role of the Rnf complex in energy conservation. Disruption of the C. ljungdahlii rnf operon inhibited autotrophic growth. ATP synthesis, proton gradient, membrane potential, and proton motive force collapsed in the Rnf-deficient mutant with H-2 as the electron source and CO2 as the electron acceptor. Heterotrophic growth was hindered in the absence of a functional Rnf complex, as ATP synthesis, proton gradient, and proton motive force were significantly reduced with fructose as the electron donor. Growth of the Rnf-deficient mutant was also inhibited when no source of fixed nitrogen was provided. These results demonstrate that the Rnf complex of C. ljungdahlii is responsible for translocation of protons across the membrane to elicit energy conservation during acetogenesis and is a multifunctional device also implicated in nitrogen fixation. IMPORTANCE Mechanisms for energy conservation in the acetogen Clostridium ljungdahlii are of interest because of its potential value as a chassis for the production of biocommodities with novel electron donors such as carbon monoxide, syngas, and electrons derived from electrodes. Characterizing the components implicated in the chemiosmotic ATP synthesis during acetogenesis by C. ljungdahlii is a prerequisite for the development of highly productive strains. The Rnf complex has been considered the prime candidate to be the pump responsible for the formation of an ion gradient coupled with ATP synthesis in multiple acetogens. However, experimental evidence for a proton-pumping Rnf complex has been lacking. This study establishes the C. ljungdahlii Rnf complex as a proton-translocating ferredoxin: NAD(+) oxidoreductase and demonstrates that C. ljungdahlii has the potential of becoming a model organism to study proton translocation, electron transport, and other functions of the Rnf complex in energy conservation or other processes.