Biochimica et Biophysica Acta 1817 (2012) S1–S7

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Plenary

P1

Lactose permease: A beautiful chemiosmotic machine

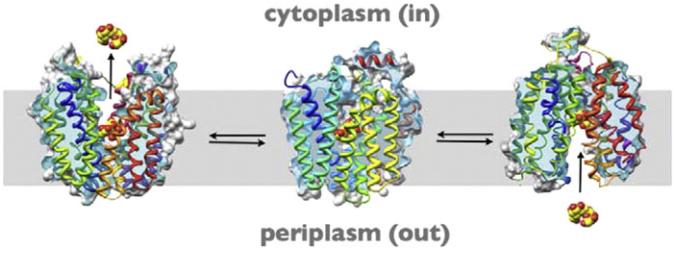
H. Ronald Kaback

Department of Physiology, Molecular Biology Institute, University of California Los Angeles, USA

Department of Microbiology, Immunology & Molecular Genetics, Molecular Biology Institute, University of California Los Angeles, USA

E-mail address: RKaback@mednet.ucla.edu

The lactose permease (LacY), a paradigm for membrane transport proteins and a member of the Major Facilitator Superfamily, catalyzes the coupled, stoichiometric translocation of a galactoside and an H^+ across the cytoplasmic membrane of *Escherichia coli*. Thus, sugar accumulation against a concentration gradient is driven by the free energy released from the downhill movement of H^+ with the electrochemical H^+ gradient ($\Delta\tilde{\mu}_H+$; interior negative and/or alkaline). Moreover, since transport is obligatorily coupled, downhill sugar translocation drives uphill H^+ translocation with the generation of $\Delta\tilde{\mu}_H+$, the polarity of which depends on the direction of the sugar concentration gradient. X-ray crystal structures reveal an inward-facing conformation and confirm many conclusions from biochemical and biophysical experiments. LacY contains N- and C-terminal domains, each with 6 largely irregular transmembrane helices positioned pseudo-symmetrically and surrounding a deep water-filled cavity open to the cytoplasm only. Sugar- and H^+-binding sites are located primarily in the N- and C-terminal helix bundles, respectively, at the apex of the cavity in the approximate middle of the molecule, and the periplasmic side is tightly sealed. Every residue in LacY has been mutagenized, and those involved in sugar and H^+ binding have been identified. Surprisingly, those involved in H^+ binding and translocation are aligned parallel to the membrane at the same level as the sugar-binding site. Both sites are exposed reciprocally to water-filled cavities in the inward- or outward-facing conformation, thereby allowing sugar and H^+ release from either side of LacY via an alternating access mechanism. These features likely explain how LacY catalyzes lactose/H^+ symport in both directions across the membrane utilizing the same residues. Site-directed alkylation, single molecule FRET, double electron–electron resonance, thiol cross-linking and Trp quenching/unquenching studies provide converging evidence for an alternating access mechanism. The primary driving force for alternating access is sugar binding and dissociation, and $\Delta\tilde{\mu}_H+$ changes the rate-limiting step. Evidence for an occluded intermediate will also be discussed.



doi:10.1016/j.bbabio.2012.06.012

P2

The ATP synthase: The understood, the uncertain and the unknown

John E. Walker

Medical Research Council Mitochondrial Biology Unit, Cambridge, UK

E-mail address: walker@mrc-mbu.cam.ac.uk

The F-ATPases, or F_1F_o-ATPases, are multisubunit enzyme complexes found in energy transducing membranes in eubacteria, mitochondria and chloroplasts. Their role is to synthesize ATP from ADP and phosphate under aerobic conditions using the proton-motive force generated by respiration or photosynthesis as a source of energy. The ATP hydrolase activities of the enzymes from mitochondria, chloroplasts and some eubacteria are inhibited, and they can only synthesize ATP. However, other eubacterial enzymes hydrolyze ATP, made by glycolysis under anaerobic conditions, to generate the proton motive force required for essential cellular functions, such as chemotaxis and transmembrane transport processes. The lecture will describe the common features and differences between the F-ATPases from these various sources. It has been assumed widely that information from one F-ATPase would apply to all or many other F-ATPases, but it is increasingly evident that while there are common principles in the operation of F-ATPases from diverse sources, there are also significant differences. This is most evident in the variety of the symmetries of the membrane bound c-rings in the rotors of the enzyme from various species, which has profound consequences for the bioenergetic cost of making ATP.

doi:10.1016/j.bbabio.2012.06.013

P3

Structure, function and regulation of ion pumps

Poul Nissen

Danish National Research Foundation, Center for Membrane Pumps in Cells and Disease — PUMPKIN, Aarhus University, Department of Molecular Biology and Genetics, DK — 8000 Aarhus C, Denmark

E-mail address: pn@mb.au.dk

0005-2728/$ – see front matter.