Biochimica et Biophysica Acta 1818 (2012) 1097–1107

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Molecular genetic and biochemical approaches for defining lipid-dependent membrane protein folding☆

William Dowhan *, Mikhail Bogdanov **

Department of Biochemistry and Molecular Biology, University of Texas Medical School-Houston, Houston, TX 77030, USA

ARTICLE INFO

Article history:
Received 26 June 2011
Received in revised form 30 August 2011
Accepted 12 September 2011
Available online 17 September 2011

Keywords:
Phosphatidylethanolamine
Lactose permease
Protein topology
Lipochaperone
Positive-inside rule

ABSTRACT

We provide an overview of lipid-dependent polytopic membrane protein folding and topogenesis. Lipid dependence of this process was determined by employing *Escherichia coli* cells in which specific lipids can be eliminated, substituted, tightly titrated or controlled temporally during membrane protein synthesis and assembly. The secondary transport protein lactose permease (LacY) was used to establish general principles underlying the molecular basis of lipid-dependent effects on protein domain folding, protein transmembrane domain (TM) orientation, and function. These principles were then extended to several other secondary transport proteins of *E. coli*. The methods used to follow proper conformational organization of protein domains and the topological organization of protein TMs in whole cells and membranes are described. The proper folding of an extramembrane domain of LacY that is crucial for energy dependent uphill transport function depends on specific lipids acting as non-protein molecular chaperones. Correct TM topogenesis is dependent on charge interactions between the cytoplasmic surface of membrane proteins and a proper balance of the membrane surface net charge defined by the lipid head groups. Short-range interactions between the nascent protein chain and the translocon are necessary but not sufficient for establishment of final topology. After release from the translocon short-range interactions between lipid head groups and the nascent protein chain, partitioning of protein hydrophobic domains into the membrane bilayer, and long-range interactions within the protein thermodynamically drive final membrane protein organization. Given the diversity of membrane lipid compositions throughout nature, it is tempting to speculate that during the course of evolution the physical and chemical properties of proteins and lipids have co-evolved in the context of the lipid environment of membrane systems in which both are mutually dependent on each other for functional organization of proteins. This article is part of a Special Issue entitled: Protein Folding in Membranes

Contents

Abbreviations: TM, transmembrane domain; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PA, phosphatidic acid; PS, phosphatidylserine; lys, lysyl; PC, phosphatidylcholine; GlcDAG, monoglucosyl diacylglycerol; GlcGlcDAG, diglucosyl diacylglycerol; PI, phosphatidylinositol; LacY, lactose permease; CscB, sucrose permease; PheP, phenylalanine permease; GabP, γ-aminobutyrate permease; mAb, monoclonal antibody; SCAM™, substituted cysteine accessibility method as applied to TMs

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* Corresponding author at: W. Dowhan, Department of Biochemistry and Molecular Biology, University of Texas Medical School-Houston, 6431 Fannin St., Suite 6.200, Houston, TX 77030, USA. Tel.: +1 713 500 6051; fax: +1 713 500 0652.
** Corresponding author. Tel.: +1 713 500 6125; fax: +1 713 500 0652.
E-mail addresses: William.Dowhan@uth.tmc.edu (W. Dowhan), Mikhail.V.Bogdanov@uth.tmc.edu (M. Bogdanov).

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doi:10.1016/j.bbamem.2011.09.013

1. Introduction

The Singer–Nicolson [1] fluid mosaic concept of biological membranes envisioned individual protein units moving through a sea of lipids that form a bilayer composed of a hydrophobic core bounded on each side by the hydrophilic domains of polar lipids. The main role of lipids was to provide a hydrophobic sink for membrane barrier function and residency for the hydrophobic portion of protein domains that are inserted into or traverse the membrane. Membrane proteins are now well established to be composed of multiple transmembrane domains (TMs) alternately oriented in opposite directions with respect to the plane of the bilayer and connected by hydrophilic domains on alternating sides of the membrane. Since many membrane proteins can be purified in a functional and structurally compact form using detergents in place of natural lipids, less attention has been paid to the native lipid environment in defining the structure and function of membrane proteins. Taking into account the different hydrophobic and hydrophilic domains of natural lipids, the diversity within the lipidome probably exceeds that of the proteome [2]. When lipids are added back to purified membrane proteins, they are usually single or simple mixtures of synthetic lipids that do not reflect the diversity of lipids found in biological membranes. However, increasing evidence [3] indicates that individual native lipids and lipid composition of biological membranes play a more specific role in membrane structure and function than originally envisioned by Singer and Nicolson. Specific lipids have also been found associated with the surface and even integrated into the structure of purified membrane proteins [4]. Addressing this problem only through purification using detergents followed by reconstitution with even native lipids still requires in vivo evidence for a specific function of lipids, which has not been done extensively.

There are major obstacles to defining specific roles for lipids in vivo. Lipids have no catalytic activity so their effects are generally determined secondary to effects on biological processes usually reconstituted in vitro. In vivo importance of proteins has generally been established through gene mutation. Genes do not encode lipids so that changes in lipid composition must be done by mutations in the enzymes that define their biosynthesis. Mutations early in the pathway eliminate minor lipids resulting in loss of the major endpoint lipids, and mutations late the pathway result in accumulation of minor intermediates. Changes in membrane lipid composition often affect multiple processes, especially in eukaryotic cells containing several membranes containing the same lipids. Large changes in lipid composition can result in compromising membrane barrier function resulting in cell death before affecting a specific biological process. In spite of these limitations it has been possible to define specific roles for lipids by establishing complimentary effects of lipids in vivo by genetic manipulation and in vitro through reconstitution.

This review will summarize the use of a set of mutants in *Escherichia coli* in which membrane lipid composition can be systematically altered while maintaining cell viability. Examples will be reviewed in which changes in lipid environment affects membrane protein structure and function in vivo with in vitro verification of a specific effect.

2. Systematic alteration in membrane lipid composition

2.1. Genetic manipulation of E. coli phospholipid metabolism

The cell envelope of *E. coli* is composed of an outer membrane that, due to the presence of porins, is a barrier to molecules>600 Da [5]. The outer membrane is made up of an inner leaflet of glycerol-based

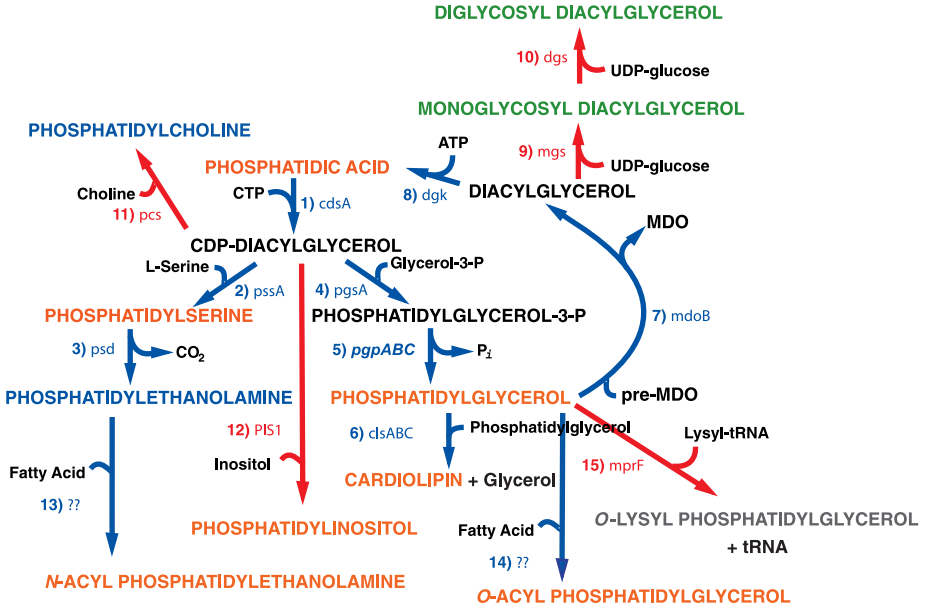

Fig. 1. Native and foreign lipid biosynthesis in *E. coli*. Pathways native to *E. coli* are noted with *blue* arrows, and pathways resulting from foreign genes introduced into *E. coli* are noted with *red* arrows. Lipids emphasized in the text are color coded as zwitterionic (*blue*), neutral (*green*), anionic (*orange*) or cationic (*gray*). The genes encoding the following enzymes and associated with each biosynthetic step are listed next to the arrows: (1) CDP-diacylglycerol synthase; (2) PS synthase; (3) PS decarboxylase; (4) PG-P synthase; (5) PG-P phosphatases [8]; (6) CL synthases; (7) PG:MDO (membrane derived oligosaccharide) *sn*-glycerol-1-P transferase; (8) diacylglycerol kinase; (9) GlcDAG synthase (*Acholeplasma laidlawii*); (10) GlcGlcDAG synthase (*A. laidlawii*); (11) PC synthase (*Legionella pneumophila*); (12) PI synthase (*Saccharomyces cerevisiae*); (13) *N*-acyl PE synthase; (14) *O*-acyl PG synthase and (15) *O*-lys PG synthase (*Staphylococcus aureus*) [9, 10].