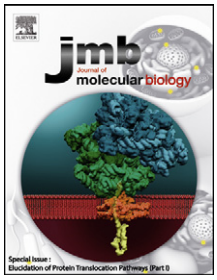

Mechanisms of Integral Membrane Protein Insertion and Folding

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Abstract

The biogenesis, folding, and structure of α-helical membrane proteins (MPs) are important to understand because they underlie virtually all physiological processes in cells including key metabolic pathways, such as the respiratory chain and the photosystems, as well as the transport of solutes and signals across membranes. Nearly all MPs require translocons—often referred to as protein-conducting channels—for proper insertion into their target membrane. Remarkable progress toward understanding the structure and functioning of translocons has been made during the past decade. Here, we review and assess this progress critically. All available evidence indicates that MPs are equilibrium structures that achieve their final structural states by folding along thermodynamically controlled pathways. The main challenge for cells is the targeting and membrane insertion of highly hydrophobic amino acid sequences. Targeting and insertion are managed in cells principally by interactions between ribosomes and membrane-embedded translocons. Our review examines the biophysical and biological boundaries of MP insertion and the folding of polytopic MPs *in vivo*. A theme of the review is the under-appreciated role of basic thermodynamic principles in MP folding and assembly. Thermodynamics not only dictates the final folded structure but also is the driving force for the evolution of the ribosome–translocon system of assembly. We conclude the review with a perspective suggesting a new view of translocon-guided MP insertion.

Introduction

Membrane proteins (MPs) occur in all living cells and form part of key metabolic pathways, such as the respiratory chain and the photosystems. MPs also mediate the transport of solutes and the transduction of signals across membranes. For proper insertion into their target membrane, nearly all MPs require the aid of translocons, so-called protein-conducting channel proteins. In the past decade, structures of key translocon complexes that mediate the translocation or insertion of MPs have been solved and biochemical studies have yielded important insights into key aspects of MP insertion and folding. The cartoon in Fig. 1 illustrates a general scheme for the assembly of multi-span MPs. We will add many details to this scheme in the course of this review, but many questions will still remain about the membrane insertion of proteins, how they fold into their final tertiary structure, and how they assemble into homo- and hetero-oligomeric complexes.

Our aim in this review is not to provide a comprehensive overview of the literature but rather to appraise critically current models of MP insertion and folding *in vivo* and to identify the most important gaps in our understanding of these processes. We start with a discussion of the basic biophysics of protein–lipid interactions and then turn our attention to how the biophysical principles are played out in the *in vivo* context. We suggest in the concluding section some outstanding questions that the field needs to address. We close with some general thoughts on how translocons may facilitate insertion

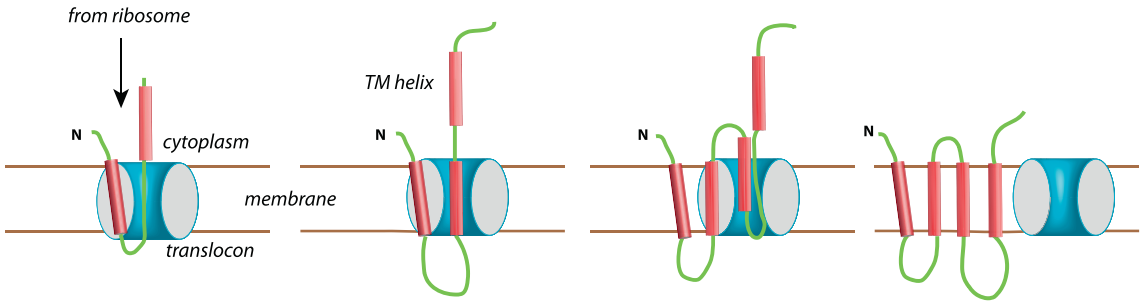

Fig. 1. This schematic cartoon represents in broad terms current thinking about the insertion of multi-span proteins into membranes. Two ideas are captured in the cartoon. First, TM segments (red) emerge from the ribosome and pass into the translocon (blue). Second, the nascent segments partition into the membrane from the translocon. As a starting point for discussion, we present alternative views of the MP insertion pathway in Fig. 15.

and folding. Through out this review, our focus is on helix-bundle integral MPs and SecYEG/Sec61-type translocons [found in the inner membrane of bacteria and the endoplasmic reticulum (ER) membrane of eukaryotic cells]; β-barrel and peripheral MPs will not be discussed.

Biophysical Boundaries of MP Insertion and Folding

MPs and equilibrium

Molecular dynamics (MD) simulations of MPs in fluid lipid bilayers reveal graphically the complex environment of MPs. The environment of the SecYEG translocon is especially complex as a result of its large hour-glass-shaped interior filled with about 250 water molecules (Fig. 2). The structural stability of SecYEG and other MPs depends upon numerous physicochemical interactions, summarized in a simplistic way in Fig. 3. Despite the complexity, the equilibrium structures of MPs are in principle calculable given a complete quantitative description of the interactions. These interactions are understood in broad terms [1–3], but many essential details are lacking. Even though the physical chemistry of soluble proteins is also complex, we at least have a simple measure of their stability defined by their free energies of folding, ΔG_{fold}. These free energies, usually obtained using calorimetric or chemical denaturation methods [4], are typically -5 to -10 kcal mol^{-1} regardless of the number of amino acid residues [5].

Even defining what "unfolding" of an α-helical MP means is problematic [6]. For example, bacteriorhodopsin (bR), a protein with seven transmembrane helices (TMHs), can be unfolded by heating, but unfolding is irreversible [7]. Other MPs can be unfolded reversibly with detergents, but the structure of the unfolded detergent–protein complex is unknown [8]. All we know for sure in either case is that the denatured proteins remain about 50% helical, which means that α-helices are extremely stable in lipids and detergents; this is expected from simple thermodynamic considerations (below). Despite the unsolved challenges of MP folding, understanding the thermodynamic stability of MPs is important because it defines the energetic framework within which the translocon apparatus must operate.

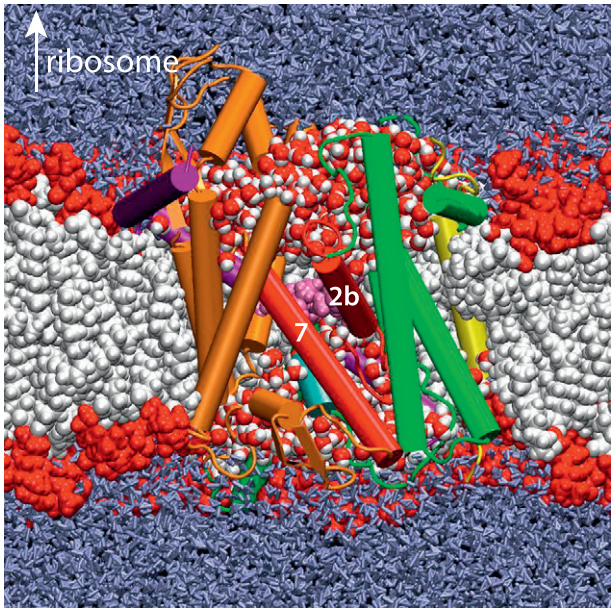

Fig. 2. α-Helical MPs exist in their native state in highly thermally disordered lipid bilayers, as illustrated here for the SecYEG translocon [78] from *Methanococcus jannaschii*. The image is from an MD simulation of the translocon (PDB code 1RHZ) executed in a phospholipid bilayer. In this view, parallel with the membrane plane, the so-called gate helices 2b and 7 (red cylinders) were exposed by cutting away the lipid bilayer. Water molecules within the translocon are shown as van der Waals spheres in red (oxygen) and white (hydrogen). Waters surrounding the bilayer are shown as H-O-H bonds in blue-gray. Lipid acyl chains are white and the phospholipid headgroups are red. The image is provided courtesy of J. Alfredo Freites and Stephen H. White.