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Minireview

Membrane protein folding: beyond the two stage model

Donald M. Engelman[a,*], Yang Chen[a], Chen-Ni Chin[a], A. Rachael Curran[a], Ann M. Dixon[a], Allison D. Dupuy[a], Albert S. Lee[a], Ursula Lehnert[a], Erin E. Matthews[a], Yana K. Reshetnyak[a], Alessandro Senes[a], Jean-Luc Popot[b]

[a] *Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 208114, New Haven, CT 06520-8114, USA*
[b] *CNRS/Université Paris-7 UMR 7099, Institut de Biologie Physico-Chimique, 13 rue Pierre-et-Marie-Curie, F-75005 Paris, France*

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Abstract The folding of α-helical membrane proteins has previously been described using the two stage model, in which the membrane insertion of independently stable α-helices is followed by their mutual interactions within the membrane to give higher order folding and oligomerization. Given recent advances in our understanding of membrane protein structure it has become apparent that in some cases the model may not fully represent the folding process. Here we present a three stage model which gives considerations to ligand binding, folding of extramembranous loops, insertion of peripheral domains and the formation of quaternary structure.

Key words: Membrane protein; Folding; Two stage model

1. Introduction

The two stage model, proposed more than a decade ago, postulates that membrane protein folding includes a stage in which independently stable helices are formed across the membrane lipid bilayer, followed by a stage in which the helices interact with each other to form higher order structures (see [1] for review, see Fig. 1). But, what happens next? Several kinds of events follow, including the binding of prosthetic groups, the folding of loops into ordered structures, the entry of other regions of polypeptide into the transbilayer region, and the oligomerization of separate polypeptide chains. Here we present a discussion of some of these subsequent steps, selecting key illustrative experimental evidence without attempting to be exhaustive.

A number of arguments support the idea of independent helices in stage I. Perhaps the simplest is that transmembrane helices in the known crystal structures are well predicted by algorithms based on the idea that they are each immersed in lipid [2], yet most are in fact in contact with other helices much more than with lipid. That helices are well predicted on the basis of lipid contact suggests that they may have passed through such an environment during synthesis and folding. This pathway is in accord with the concept of progressive emergence from the translocon, where hydrophobic helices are progressively moved into the lipid as the nascent polypeptide is synthesized [3]. Recent studies reinforce this concept [4].

That helix association could drive folding became apparent in the first split protein experiments on bacteriorhodopsin [5]. Fig. 2 shows the association of two fragments of the protein, observed by incorporation of each into a population of lipid vesicles, followed by fusion of the vesicles to place the fragments in the same bilayer. Kinetic analysis of retinal binding showed that the retinal binds after the fragments associate via helix interactions, effectively in a 'third stage' of the folding process [5]. Subsequent experiments showed that the bacteriorhodopsin molecule can be cut in several loops and re-assembled [6,7], and related experiments on other proteins have demonstrated that co-expression of fragments cut in loop regions results in functional proteins inserted into membranes (see table in [1]). These experiments have been taken as strong support for the idea of the two stage model. But, the subsequent stage of retinal binding by associated bacteriorhodopsin fragments suggests a further paradigm for folding: helix association can create an environment for further folding events.

By inserting into the bilayer and interacting, helices necessarily create a local environment that is less hydrophobic than the surrounding bulk lipid. This effect arises from: (A) the membrane insertion of partially polar helix backbones, in which the main chain hydrogen bonding does not fully satisfy the carbonyl group hydrogen bond acceptor potential, (B) the partitioning of space away from the lipid by the array of helices, and (C) the creation of surfaces that can be used for specific binding events, such as those involved in prosthetic group interactions. We will now consider each of these in turn.

2. Polar backbone effects

For stable helix insertion into a bilayer, the hydrophilicity of backbone and side chain groups must be overcome by the hydrophobicity of other side chain groups. Polar side chains must be included for function since the chemistry of purely aliphatic side chains is very limited. The polarity of the helix backbone has been recognized as a factor in helix–bilayer interactions for some time, and the energy associated with sequestering the backbone from water has been recently mea-

*Corresponding author. Fax: (1)-203-432 6381.
E-mail address: donald.engelman@yale.edu (D.M. Engelman).

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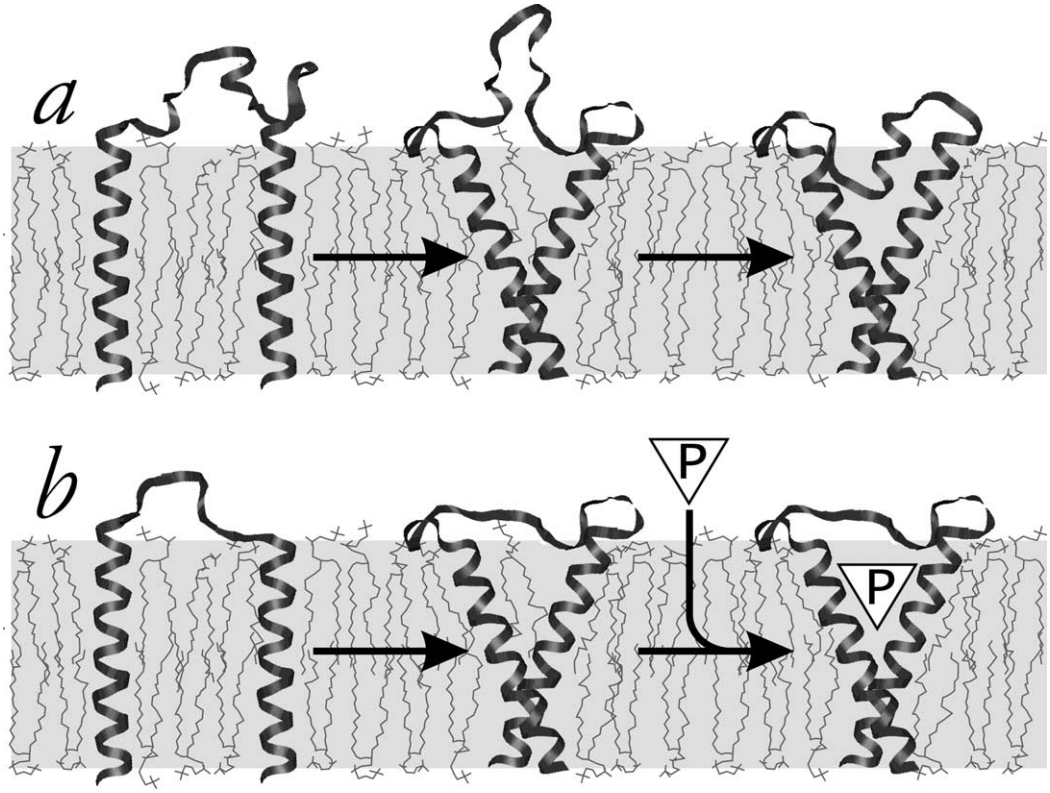

Fig. 1. In the two stage model of membrane protein folding, independently stable helices form across the membrane bilayer (stage I) and then interact with one another to form higher order structures (stage II). In a possible third stage, these higher order structures can facilitate partitioning of (a) additional polypeptide regions such as coil regions or short helices or (b) prosthetic groups into the membrane.

sured [8] as 1.15 kcal/mol per peptide bond. By providing unsatisfied carbonyl hydrogen bond acceptors, the backbone can facilitate helix interactions (see below), or aid in the hydrophobic partitioning of additional polypeptide or prosthetic groups into the trans-bilayer region. Cases in point are seen in the glycerol facilitator and potassium channel, where the structures reveal short helices and extended polypeptide in the trans-bilayer structure. As shown in Fig. 3, these structural elements are stabilized by having their hydrogen bonding groups satisfied within the context of the bilayer, so that the net hydrophobic effect can place them in the membrane [9,19]. We suggest that the polypeptide loops between helices can exploit the hydrophobic effect to enter the membrane by using the framework of the previously inserted, interacting helices to satisfy hydrogen bond potentials.

3. Partitioning of space away from the lipid

Given helices across a bilayer, we ask: which interactions can stabilize their association to form higher order structure? Our work with the dimerization of the transmembrane helix of human glycophorin A has given us several insights. Packing of the helix surfaces is a key feature, and small interfacial changes such as G79A destabilize the association significantly [10,11]. Part of the stability may come from α-carbon hydrogen bonds [12]. Hydrogen bonding generally contributes to helix association, and can be strong [13,14]. The free energy of glycophorin helix dimerization in a detergent environment, which should be weaker than in a bilayer, is about 9 kcal/mol [15]. This energy can drive otherwise unfavorable processes. For instance, the transmembrane channel of the acetylcholine receptor is formed by largely hydrophobic helices, whose surface becomes partly exposed to water when the oligomer assembles and the channel forms [16]. Based on exposed surface area, creation of the channel was estimated to be unfavorable by about 40 kcal/mol [17], so the association of a number of hydrophobic helices to partition an aqueous internal space away form lipid is clearly a feasible step on energetic grounds.

The idea of sequestering a transmembrane space from lipid contacts originates with the observation of porin structures [18], in which the β-barrel structure creates a wall, surrounding a space in which strands of polypeptide adopt irregular structures to regulate pore permeability. Similarly, helices can surround space and allow alternative internal structures to form, as argued above. For example, the structure of KcsA, a potassium channel [19], can be interpreted as a tetramer of helix pairs that surround a space. This permits the subsequent entry of the pore helices and selectivity region, as is schematically shown in Fig. 1. We suggest that helix interactions can create internal spaces that allow subsequent, less regular polypeptide folding.

4. The creation of binding surfaces

A single helix is a poor surface for prosthetic group binding, as it is convex and has few constraints on the rotamers of long side chains. Thus, helix association, which creates defined, concave cavities and clefts, is a likely antecedent to prosthetic group binding in many cases. As has been noted, there is evidence for this sequence of events in bacteriorhodopsin assembly from fragments [6], and many of the known structures show internal prosthetic groups. The idea is sche-

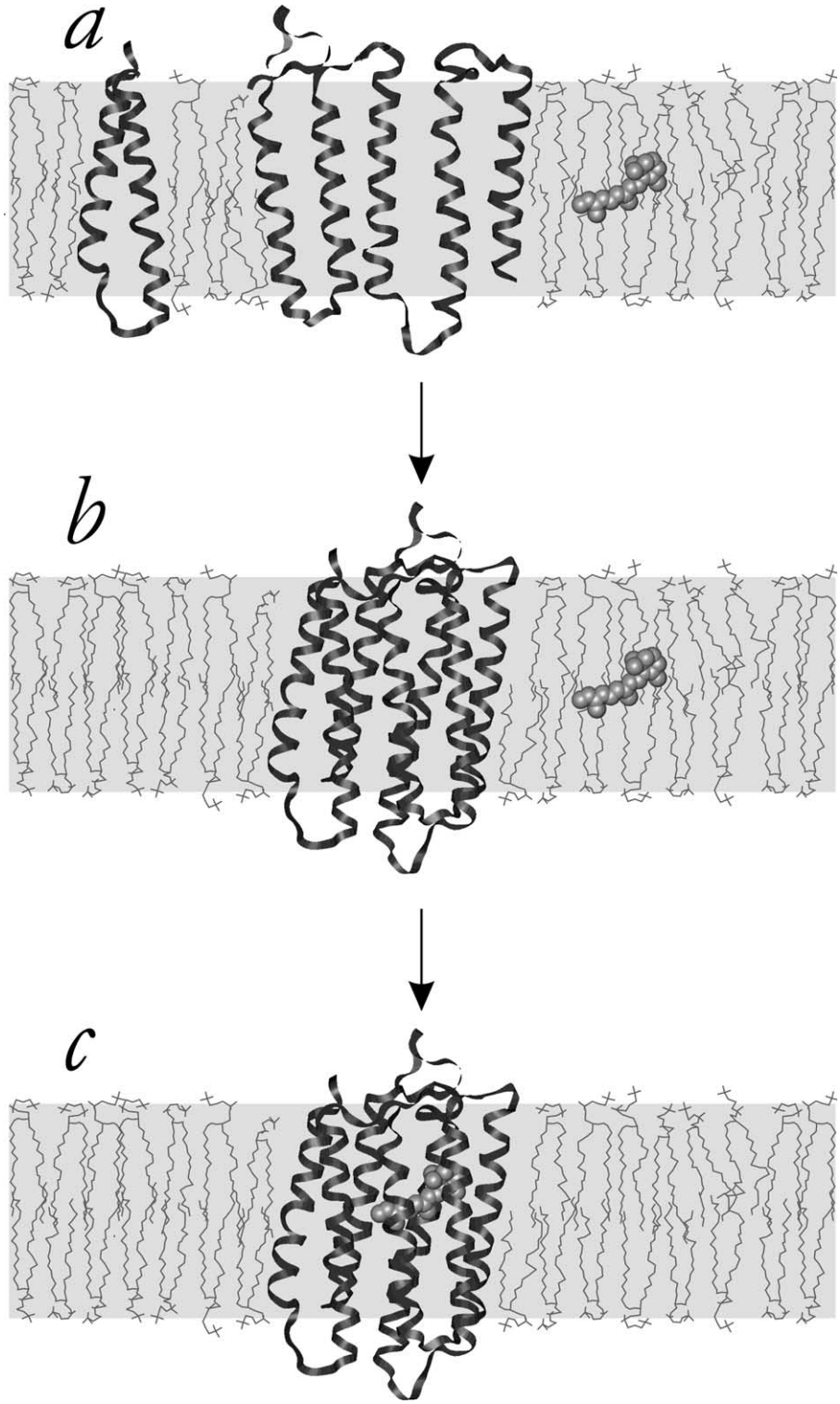

Fig. 2. Popot et al. [5] reconstituted two protein fragments of bacteriorhodopsin, comprising helices 1–2 and 3–7 respectively, into a lipid bilayer where they (a) formed independent transmembrane helices which then (b) associated into the globular apoprotein. Experimental evidence suggested a third stage (c) of the folding process, initiated by the binding of retinal to the helical framework.

matically shown in Fig. 1. Although there are examples in which prosthetic groups appear to stabilize oligomerization, such as in LH2 [21], even these cases may involve prior helix association (helix 1 interactions in LH2). We suggest that helix association occurs before prosthetic group binding in most cases.

By examining the consequences of helix association in the two stage model, we are able to extend our folding concepts to subsequent stages. Interestingly, the process of oligomerization seems permitted at any stage. In the case of the potassium channel, the four subunits seem likely to associate prior to the binding of the loops at the interior, whereas bacterio-

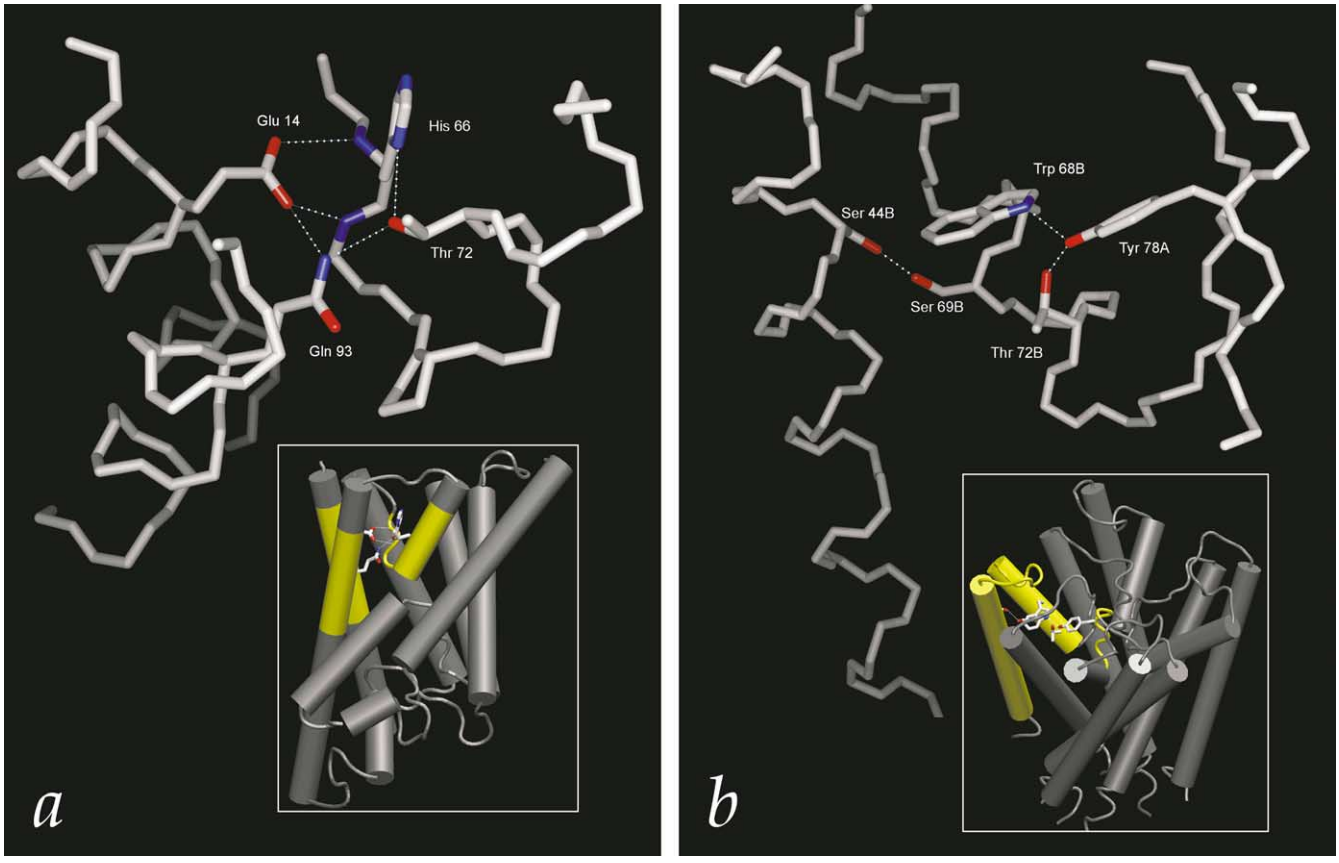

Fig. 3. The proposed third stage of membrane protein folding is illustrated in the hydrophobic partitioning of short helices and extended polypeptide into the trans-bilayer region of (a) the glycerol facilitator [9] and (b) the KcsA potassium channel [19]. Rearrangement of these regions into the bilayer satisfies hydrogen bond potentials of previously inserted helices. a: Glu 14 and Gln 93 from transmembrane helices 1 and 4 of the glycerol facilitator interact with Thr 72 and His 66 of a short helix and a coil region. b: The short pore helix interacts with transmembrane helix 1 through a hydrogen bond between two Ser residues, and with the coil region of the adjacent subunit via hydrogen bonds between Trp, Thr and Tyr residues.

rhodopsin probably trimerizes after retinal binding (reviewed in [20]). It is hoped that formulation of the folding pathway in a specific sequence of events will stimulate further experimental tests.

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