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Current Opinion in Structural Biology

Vol. 5, No. 4, August 1995

Characterization and modeling of membrane proteins using sequence analysis

[Review article]

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Current Opinion in Structural Biology 1995, **5**:491-500.

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Abstract

The current libraries of amino acid sequences of membrane proteins are a valuable resource for the analysis of elements common to these proteins. Multiple-sequence alignment techniques and the identification of conserved features of transmembrane segments have improved the prediction of membrane protein topology. Molecular modeling in combination with structural studies or site-directed mutagenesis is proving to be a powerful link

between theory and experiment. Unfortunately, the number of high-resolution structures of intrinsic membrane proteins, although increased recently, presents a restricted and perhaps biased view of membrane protein structure.

Abbreviations

ER—endoplasmic reticulum;
FTIR—Fourier transform infrared spectroscopy;
LH2—*Rhodopseudomonas acidophila* light-harvesting complex 2;
LHCII—plant light-harvesting complex;
TM—transmembrane.

Introduction

The amino acid sequences of membrane proteins can be readily obtained by the application of molecular biological techniques. The 1994 SWISS-PROT protein data bank lists over 3,000 proteins under the heading 'membrane' and about 300 proteins under 'G-protein-coupled receptor' or 'transporter'. These numbers stand in stark contrast to the handful (now 6) of high-resolution membrane-protein structures determined by X-ray diffraction or electron microscopy. The abundance of membrane protein sequences and the difficulty in obtaining high-quality crystals has resulted in a great deal of effort aimed at predicting the topography and even the three-dimensional structure of membrane proteins. This review will focus on recent progress in predicting membrane protein structure, with an update on the structural front. Information on the structure of membrane proteins and references to important earlier studies can be found in [1••][2•][3].

Topology of membrane proteins

Transmembrane α helices

The hydrophobic core of the lipid bilayer is about 30 Å thick and can be delineated by the average position of the carbonyl group of the fatty acyl chain [4••]. A protein segment containing 20 amino acids can span the 30 Å thickness of a bilayer in an α-helix conformation (Fig. 1). In order to maintain a fully hydrogen bonded network within this hydrophobic core, the α helix must extend for one turn on either side of the membrane. These boundary turns of helix pass through the interface region of the lipid bilayer. Membrane proteins are built from bundles of transmembrane (TM) α helices, each helix varying in the degree of exposure to lipid.

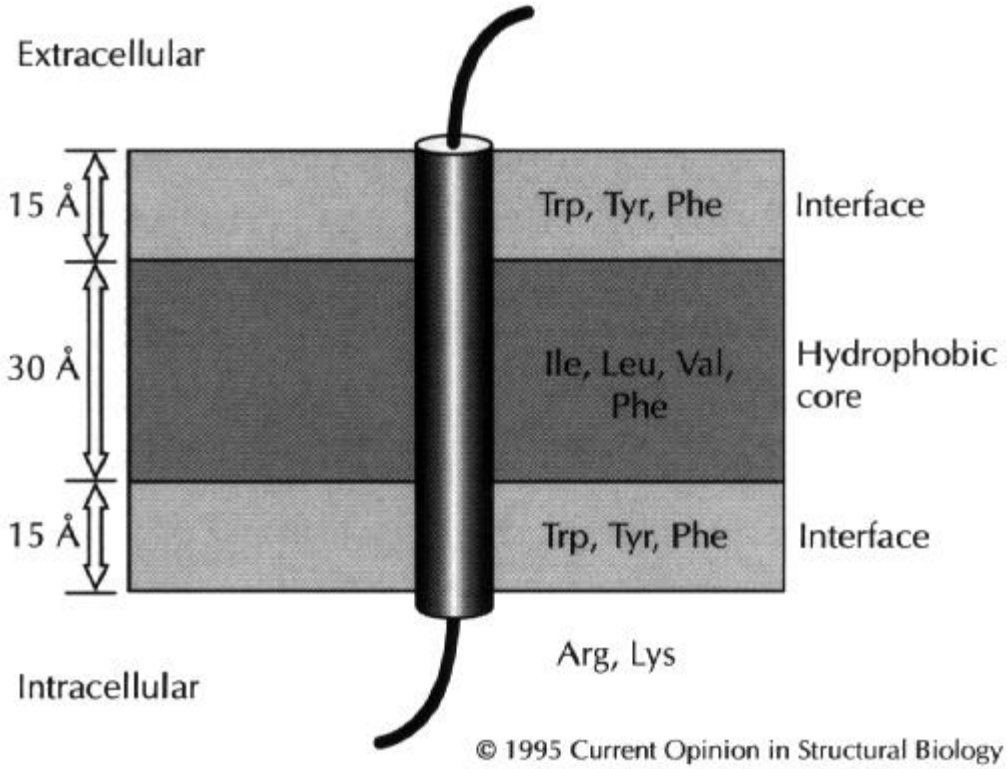

Fig. 1. Cartoon illustrating the average dimensions of a phosphatidylcholine lipid bilayer and a single TM α helix. The bilayer consists of a central hydrophobic core, 30 Å thick, bounded on both sides by an interface region, about 15 Å thick, containing a complex mixture of water, phosphocholine, glycerol, carbonyl and methylene groups [4••]. The cylinder represents a TM α helix (diameter = 10 Å). Twenty amino acid residues arranged as an α helix are required to span the hydrophobic core and an additional ten residues to span each interface region. Isoleucine, leucine, valine and phenylalanine are enriched in the central core while tryptophan, tyrosine and phenylalanine residues are localized to the interface region. A helix lying parallel to the bilayer surface can be accommodated within the interface region.

Return to text reference [1]

Some basic steps in establishing the topology of a membrane protein are listed in Table 1. As a first step, searching a novel protein sequence for hydrophobic regions that might potentially span a lipid bilayer is a necessary, but not sufficient, pursuit for the membrane molecular biologist [1••]. The Kyte–Doolittle hydropathy analysis [5] continues to be the most widely applied search method for the identification of membrane-spanning elements. Other hydrophobicity scales designed specifically for membrane proteins [6], however, work as well, if not marginally better. Searches using a window of 21 amino acids will locate hydrophobic stretches sufficiently long to span the bilayer as an α helix, although closely spaced TM segments and short hydrophilic connecting segments cannot easily be distinguished using such a wide window. A second analysis using a narrower window (e.g. 11 residues) is useful in defining the ends of TM segments.

Table 1. Modeling of membrane proteins.

Step	Action
1	Locate putative TM segments using hydropathy profiles.
2	Calculate reverse-turn potentials for connecting segments.
3	Determine topology from endogenous markers ('positive inside rule', *N*-glycosylation sites, cleaved signal sequences etc.) and experimental evidence.
4	Position TM segments relative to the bilayer using aromatics at interface, change in polarity etc.
5	Define lipid-exposed face of segments from sequence periodicity.
6	Pack helices together using constraints such as short connecting loops.
7	Use homologies at all steps to improve predictions and to identify protein family.
8	If reasonable, use energy minimization and molecular dynamics to refine structure.

Return to table reference [1]

Connecting segments

TM segments are connected by hydrophilic reverse turns, loops, or larger, more elaborate structures. Because the connecting segments are exposed to water, propensity rules for the prediction of secondary structure (α helix, β sheet, reverse turns, coil) that have been derived from water-soluble proteins [7] can be applied to these regions of membrane proteins. Segments linking TM segments can often be located by searching the sequence for four residue amino acid segments with a high propensity to form reverse turns [8].

Post-translational modifications

The position of an *N*-linked oligosaccharide within a eukaryotic membrane protein defines a domain of the protein, containing an Asn-X-Ser/Thr sequon, that faces the lumen of the endoplasmic reticulum (ER) during biosynthesis. *N*-linked oligosaccharide chains in polytopic membrane proteins are localized to single loops greater than 30 residues in size [9•]. The acceptor site must be located greater than 10 residues from the end of a TM segment to be efficiently glycosylated [10][11•]. The presence of a cleaved N-terminal signal sequence [12] places the N terminus of the mature membrane protein on the extracytosolic side of the membrane. Identified sites of phosphorylation provide markers for cytosolic domains. Modification of cysteine side chains by fatty acids localizes this amino acid precisely [13•] to the interface region on the cytosolic side of the membrane. Disulfide bonds linking two regions of a membrane protein constrain possible folding models.

Experimental approaches

Molecular biological techniques have not only provided abundant sequence data but can also be used to probe membrane protein folding [1••]. Functional expression in combination with mutagenesis can confirm that the alteration has not seriously affected protein folding. Fusion proteins have been used to assay the translocation ability of specific TM segments [1••][14•]. The insertion of epitopes [15][16] and consensus glycosylation sites [17][18] within loops provides information concerning the location of the loops with respect to the membrane. The introduction of cysteine residues into specific locations within membrane proteins [1••] has been used to assay topography [19], to define helix–helix interactions [20], to characterize the environment of the probe [21], to map interactions between labelled sites [1••][21], and to determine the nature of channel linings [22•] — a valuable approach indeed.

Aromatic residues localize to the membrane interface

Surveys of putative TM segments in single-span [23][24•] and multiple-span proteins [24•][25•] have shown that the central 21 amino acid portions of TM segments have a unique hydrophobic amino acid composition and that a sharp break in composition occurs beyond the 21st [23][25•]. In general, then, TM segments within plasma membrane proteins average 21 amino acids in length, perfectly matching the thickness of the hydrophobic core of the bilayer. Many surveys have indicated that the hydrophobic cores of TM helices are enriched in isoleucine, phenylalanine, leucine, and valine residues. Peptides containing these residues readily assume an α-helical conformation in a hydrophobic membrane environment [26•].

The boundary regions of peptide segments, located within the interface region of the lipid bilayer, are enriched in aromatic amino acids (tryptophan, tyrosine, and phenylalanine). Small peptides [27][28], single TM helices [23], amphipathic helices (prostaglandin synthase) [29], helical bundles (photoreaction center) [3], and β barrels (porins) [3], all show a localization of aromatic residues to the membrane interface. A molecular dynamics study [30••] of a gramicidin dimer and a bilayer containing 16 dimyristoylphosphatidylcholine molecules has confirmed experimental data that the interface region is at least 12 Å thick and that tryptophan side chains, located at the interface, can form hydrogen bonds with ester carbonyls of the lipid at the bilayer interface. Thus, the amphipathic nature of the side chain of amino acids such as tryptophan or tyrosine is ideally suited to position peptide segments at the membrane interface, regardless of the secondary structure context or orientation of helices.

Arginine and lysine side chains are amphipathic and can be located near the ends of TM helices, with their charged moieties exposed to solvent. They tend to be localized to the cytosolic side of the membrane (positive inside rule), especially in single-span membrane proteins [23] and bacterial inner membrane proteins [2•]. In eukaryotic multispan membrane proteins, the first TM segment usually has a net charge difference (negative out, positive in) between the extracytosolic and cytosolic flanking sequences [2•]. Changing the TM distribution of charge in a model four-helix membrane protein can change the orientation of the segments [14•]: TM segments are left out of the membrane to satisfy the positive inside rule [14•].

Improving TM-segment identification

Some improvements in the identification of TM segments have been made by considering the unique composition of amino acids present in the interface region of the lipid bilayer. Jones, Taylor and Thornton [24•] analyzed the composition of residues in the middle, outside and inside flanking regions of TM segments and connecting loops of well characterized single-span and multispan membrane proteins. Scores based on the preferential bias towards particular amino acids in the middle region and inside or outside flanking regions were assigned to all potential topologies of a target sequence. The highest value corresponded to the most likely topology, and a useful list of prediction scores for alternative topologies was calculated. The method correctly predicted 34 of 37 multispanning topologies; however, underprediction of the number of spans occurred with some multispan proteins (e.g. the last putative TM segment of G-protein-coupled receptors). The parameters defining the orientation of TM segments were weak, and were based primarily on the enrichment of positive charges on the cytosolic side of the membrane.

Persson and Argos [25•] calculated the propensity values for amino acids in the hydrophobic core (21 residues) and the flanking four-residue regions of well defined membrane proteins. The hydrophobic core propensities were used to determine the consensus location and length (limited to 15–29 residues) of TM helices in sets of homologous proteins. A similar calculation using flanking propensities helped to define the ends of the hydrophobic core helix. As the inner and outer flanking regions were grouped together to calculate propensity values, the orientations of the individual TM segments could not be determined. The use of multiple-sequence

alignment improved the accuracy of prediction of TM helices by about 10%.

The conservation of physico-chemical properties of amino acids in TM segments can also be used to locate TM segments in a protein by alignment with a sequence containing established TM segments [31]. This method is insensitive to sequence identity but is sensitive to the property of a sequence to assume a TM helical structure. Thus, TM segments can be detected in proteins regardless of their level of sequence similarity.

A neural network procedure yielded an impressive 95% accuracy in predicting TM segments in a test population of 69 membrane proteins [32•]. The input data (amino acid frequency, conservation, insertions/deletions, position of window in sequence) were derived from sequence alignments for each position in a 13-residue window. The multiple sequence alignment provided a significant improvement over predictions using single sequence information.

Finding the ends of helices

'N-cap' and 'C-cap' residues define the beginning and end of α-helical segments. As TM helices extend beyond the hydrophobic core of the lipid bilayer, the rules governing helix initiation and termination in soluble proteins may also apply to membrane proteins. Harper and Roses's model [33] for helix formation suggests that the side chains of residues flanking the helix termini can form hydrogen bonds with the initial four helix NH groups and final four C=O groups because these helical residues lack intrahelical hydrogen-bonding partners. Thus, the side chain of the N-cap residue can hydrogen bond with the backbone NH of residue N-cap +3, forming a 'capping box' motif. Common pairs include N-cap serine/threonine and N-cap +3 glutamic acid/aspartic acid. ^{1}H NMR studies of synthetic peptides containing the sequence Ser-Glu-Asp-Glu near the N terminus have revealed reciprocal hydrogen bonds formed between side-chain oxygens and the backbone amide hydrogens of serine and glutamate [34]. The motif Ser-X-X-Glu (where X is any amino acid) acts as an N-terminal capping box, initiating helix formation in the C-terminal direction but preventing helix propagation N-terminal to the capping box. One-third of all helices in soluble proteins end with glycine, and two types of C-terminal motifs involving glycine can be distinguished [35•]. A search for these types of motifs in membrane proteins may help to predict the precise ends of TM helices (see below).

Proline residues commonly occur at the N-terminal end of helices (N-cap +1 position) in soluble proteins and may display a similar preference in TM helices. Prolines also occur within reverse turns at the i+1 position, and even in the middle of a helical segment (8% of prolines). Proline residues within TM segments will cause the helix to kink, with the convex side of the kink packed away from the lipid [36]. The weakening of the backbone hydrogen bonding network would allow for conformation flexibility in TM helices containing proline. TM segments containing proline should be considered as two separate helices as a result of the kink and the weakened hydrogen-bonding interactions. The importance of proline residues in the function of membrane proteins is highlighted by site-directed mutagenesis of G-protein-coupled receptors, in which mutation of the perfectly conserved proline residue in the seventh transmembrane helix (TM7) affects receptor expression, ligand affinity and receptor activation [37•][38•].

The lipid face

Analysis of the structures of the photoreaction center protein family has revealed that the lipid-exposed surfaces of TM helices are both very hydrophobic and poorly conserved [39]. The size of the hydrophobic/variable face predicts the degree of exposure of the helix to lipid. The patch of side chains in a TM α helix that is exposed to the lipid acyl chains can be predicted by three methods. First, calculation of the hydrophobic moment of a helix (vectors pointing in the direction of maximum hydrophobicity) can be used to predict which side of the helix

faces the lipid. Helices that line an aqueous channel on one side and face the lipid bilayer on the opposite side are quite amphipathic. Helices that face the lipid bilayer and are also involved in protein contacts are less amphipathic, as both surfaces are hydrophobic. Second, a variability profile at each position in a homologous set of proteins can be used to identify the non-conserved lipid-interactive face. Such an approach has been used to predict the packing of the seven TM helices in G-protein-coupled receptors [37•][40]. Third, the substitution pattern [41] found for a particular amino acid exposed to lipid can be distinguished from the less frequent substitutions found for the same amino acid buried within a protein structure. For example, phenylalanine facing the lipid is substituted by a broad spectrum of residues in the rank order phenylalanine→isoleucine>leucine>alanine>threonine, : whereas for buried phenylalanine, the substitution order is phenylalanine→leucine>valine>tyrosine [41]. Amino acid substitution tables calculated from homologous members of a membrane protein family that contains at least one well determined structure (e.g. the photoreaction center) have allowed the discrimination of protein-buried and lipid-exposed faces of TM helices. Once the lipid face has been identified, the position at which the helix emerges from the hydrophobic core can be estimated by a change in polarity or by the presence of the first charged residue to appear on the lipid face of the helix [42].

Helix–helix interactions

Recent studies point to the paramount importance of helix–helix interactions in membrane protein structure. Functional membrane proteins can be reconstituted from complementary coexpressed fragments of the protein [43][44][45][46]. This shows that discrete regions of membrane proteins insert independently into the ER membrane and associate with each other to form a native structure. Membrane proteins contain multiple signals for membrane insertion [1••][2•][14•][47]. The integrity of the extramembranous loops is often not necessary for assembly and function; however, disruption of TM segments in lactose permease precludes functional reassembly [43]. Connecting loops play a secondary role in protein folding and stability as their structure can often be altered dramatically (e.g. by deletion, or by insertion of novel peptide epitopes, or through glycosylation sites) without seriously affecting protein biosynthesis or its function.

Sequences forming TM segments are usually highly conserved, indicating that extensive protein–protein interactions occur within the membrane domains of these proteins. In fact, the interiors of membrane proteins are as hydrophobic, closely packed and conserved as in soluble proteins [39]. In addition, motifs common in soluble proteins, such as anti-parallel and parallel helices, coiled-coils, and four helix bundles, have been observed in membrane proteins [1••]. The rules that govern helix–helix interactions in soluble proteins can therefore be applied to helical membrane proteins. For example, the modes of helix packing can be grouped into different classes depending on the tilt angles and nature of residues at the helix contact points [48]. Helix–helix interactions between the TM segments of the glycophorin A dimer are well characterized [49][50•]. The TM helices form a left-handed coiled coil using a seven-residue motif that may be unique to the protein system. Molecular dynamics studies (cited in [49][50•]) predicted this structure, which was confirmed using saturation mutagenesis — a good illustration of the complementarity of modeling and experiment.

NMR has been used extensively to study the structure of membrane proteins as small as coat proteins from filamentous bacteriophage and as large as bacteriorhodopsin [1••]. The structures of synthetic peptides corresponding to individual TM segments have been determined by NMR spectroscopy. Solution structures of the peptides corresponding to the first two TM segments of the erythrocyte anion exchanger in the helix-promoting solvent trifluoroethanol have confirmed their helical nature [51]. The central proline residue in TM1 did not disrupt the helix to any significant extent. TM7 of G-protein-coupled receptors contains an almost invariant proline-asparagine in the middle of the segment [38•]. A 15-residue peptide based on TM7 of the tachykinin receptor forms a β turn structure in organic solvents [52]. The insolubility of these peptides in micellar systems or even in some common alcoholic solvents limits the significance of these findings with respect to the native state in the lipid bilayer, as it is the conformation of the segments in the context of the

entire protein within a lipid bilayer that is relevant. It is important to establish whether TM segments predicted to pack together in the folded protein can interact specifically with each other in these solvent systems. In this regard, interactions between the first two helical TM segments in a fragment of bacteriorhodopsin (residues 1–71) have been detected by multidimensional NMR [53].

Moving into the third dimension

The formation of autonomous transmembrane helices as a prelude to assembly of helical bundle proteins simplifies the folding pathway. Transmembrane helices pack sequentially or in helical pairs. The possible folding patterns of membrane proteins can be restricted by considering the minimum lengths of connecting loops. Baldwin [54] created a consensus model of G-protein-coupled receptors based on an analysis of conserved and non-conserved residues and constrained by minimum loop size. She used this model to interpret the low-resolution two-dimensional map of rhodopsin. Locating hydrophobic segments in combination with amphipathic analysis and loop lengths helped to identify potential TM segments in the uniporter/symporter/antiporter superfamily of transport proteins and to generate a consensus 12-helix three-dimensional model [55].

Models have been built of bacteriorhodopsin [56] and rhodopsin [57] using a multistep procedure involving a hydrophilic/lipophilic variability index that predicts lipid- and water-exposed regions in a sequence and allows accurate definition of the helix boundaries. The structures were refined using energy minimization of both side chains and backbone coordinates. An automatic method to pack TM helices together to form a three-dimensional structure has been developed [58•]. Information concerning the degree of lipid exposure of each TM helix is the key to this method.

Computer models

A powerful use of computer modeling is the further refinement or elaboration of a well defined structure. In addition, the structure of a novel protein can often be modeled based on the structure of a homologous protein. Computer modeling can also provide a more realistic cartoon of membrane protein structure than can a two-dimensional topological map of a linear amino acid sequence. Such computer models are of course useful in designing experiments. If one is interested in molecular detail, however, computer-generated models can be overly simplistic, or even inaccurate. For example, bundles of identical helices can form a beautiful coiled-coil structure [59] but whether this type of structure represents the complexities of a natural channel is doubtful. Modeling individual segments without consideration of interacting elements in the protein limits the usefulness of the results. Finally, basing the membrane arrangement of G-protein-coupled receptors on the structure of bacteriorhodopsin is faulty [60]: the proteins are not homologous and the two-dimensional map of rhodopsin (see below) is significantly different from that of bacteriorhodopsin.

The packing of the seven TM helices in bacteriorhodopsin was determined by calculating the energy of interaction as a function of rotation of each helix [61••]. Modeling of bacteriorhodopsin predicted a displacement of helix D relative to the other six helices [61••]. The various models were confirmed using neutron diffraction of the protein deuterated at specific residues [62••]. These studies illustrate the complementarity of low-resolution electron microscopy structures, neutron diffraction data and modeling.

Real structures provide real answers

High-resolution structures of only a limited number of intrinsic membrane proteins have been established, and

their number is only increasing slowly [63][64][65••][66•][67••][68••]. Each new structure is greeted with considerable enthusiasm, finding prominent places in publications such as *Nature*. These proteins have served as valuable models, helping to define the general properties of TM segments, such as their conformation and the nature of their interactions with each other and with the lipid bilayer. Membrane protein structures such as the photoreaction center and bacteriorhodopsin also serve as test cases for predictive methods.

The rhodopsin family

The projection structure of rhodopsin at 9 Å can be distinguished from the density map of bacteriorhodopsin [69]. The three-dimensional structure of halorhodopsin, a chloride pump, has an arrangement of seven α helices similar to that of the homologous protein bacteriorhodopsin [63]. We await the three-dimensional map of rhodopsin and the crystallization of other members of this class of receptors, which are so important in transmembrane signaling. At that time, meaningful homology modeling of G-protein-coupled receptors can proceed with vigor.

Photosystem I

The three-dimensional structure of bacterial photosystem I, a multisubunit protein, has been solved to a resolution of 6 Å [64]. Twenty eight helices were identified, most of which are TM and contained within the two large subunits (A and B, together M_r 83 kDa).

Light-harvesting complexes

Bacteria

The light-harvesting complex 2 (LH2) from the photosynthetic bacterium *Rhodopseudomonas acidophila* has been determined to a resolution of 2.5 Å [65••]. The complex consists of two small protein subunits (α, 53 residues and β, 41 residues). The overall structure is a cylinder containing an inner ring of nine α subunits and an equal number of β subunits in the outer ring. The α subunit begins with an amphipathic helix that lies on the membrane surface, followed by a turn, a 26-residue TM helix, and a second C-terminal amphipathic helix. The β subunit is similar in structure, although the N-terminal region has an extended structure which is followed by two turns of an irregular helix that leads to the TM helix, which is then followed by a turn and a short C-terminal tail. The combination of a short amphipathic helix lying within the interface region of a bilayer followed by a TM helix is a structure common to bacteriophage coat proteins [1••] and small peptides like alamethicin, a channel-forming peptide [66•]. The C-terminal region contains aromatic residues that are directed into the membrane interface region. The TM helices do not interact with each other; rather, their interaction is mediated by pigment molecules. Models assuming direct helix–helix interactions or the formation of four-helix bundles [42] have now proven to be incorrect.

Plants

The plant light-harvesting complex (LHCII) consists of a single polypeptide containing three TM helices in the order B-C-A in the sequence (Fig. 2) [67••]. Helices A (43 Å long) and B (51 Å long) are tilted 30° with respect to the membrane normal and form a parallel left-handed coiled-coil stabilized by arginine–glutamic acid ion pairs. Helix C (31 Å long) runs straight across the bilayer. Helices A and B begin with prolines, which are preferred at the N-cap +1 position of helices. The TM helices in LHCII contain both C-terminal glycine capping

residues [35•] and an N-terminal capping box motif [33]: helix B (AspXXThr), helix A (AspXXGlu), helix C (HisXXSer) (Fig. 2). The helix termination rules that were developed from the structures of soluble globular proteins may therefore apply to membrane proteins (see above, Finding the ends of helices). The TM helices in the light-harvesting complexes provide the binding sites for prosthetic groups and therefore may not be ideal models for TM segments in other proteins that interact with a phospholipid bilayer.

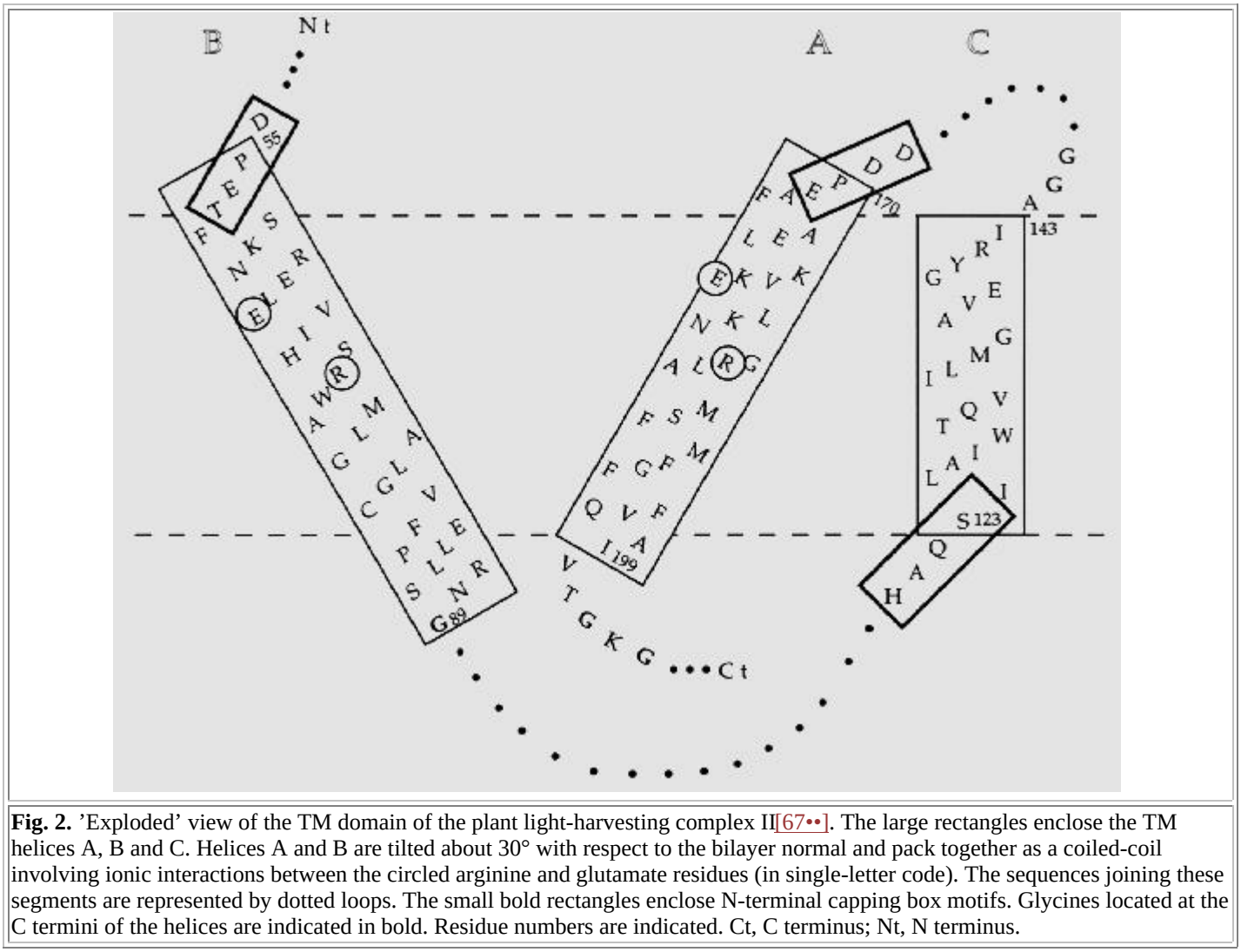

Fig. 2. 'Exploded' view of the TM domain of the plant light-harvesting complex II[67••]. The large rectangles enclose the TM helices A, B and C. Helices A and B are tilted about 30° with respect to the bilayer normal and pack together as a coiled-coil involving ionic interactions between the circled arginine and glutamate residues (in single-letter code). The sequences joining these segments are represented by dotted loops. The small bold rectangles enclose N-terminal capping box motifs. Glycines located at the C termini of the helices are indicated in bold. Residue numbers are indicated. Ct, C terminus; Nt, N terminus.

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Prostaglandin H synthase

The crystal structure of prostaglandin H synthase has revealed the presence of four amphipathic helices which lie parallel to the surface of the membrane, in the interfacial region of the lipid bilayer [68••]. Interestingly, one side of each helix facing the hydrophobic core of the bilayer contains hydrophobic residues (leucine, isoleucine, valine, phenylalanine), and together with hydrophilic residues on the opposite side of the helix, sandwiches an intermediate zone containing aromatic residues (tryptophan, tyrosine). Earlier conclusions that this enzyme contains one or more TM segments based on prediction and experimental evidence are not supported by the X-ray structure [68••][70].

The case of the acetylcholine receptor

Although structural studies have shown that the TM α helix is a common motif, it is not the only secondary structural element found in membrane proteins [1••][2•][3]. Porins found in the outer membrane of Gram-negative bacteria and mitochondria have a β-barrel structure [3]. Fischbarg *et al*. [71] have used predictive algorithms that suggest that transporters and channels are β barrels. Helical bundles and β barrels may represent two structural extremes that make up membrane proteins. The α/β motifs are found in soluble proteins such as the enzyme triosephosphate isomerase, which consists of a central core of eight parallel β strands surrounded by an equal number of helices (TIM barrel). Do similar mixtures of α helices and β strands exist in the TM domains of membrane proteins?

The acetylcholine receptor consists of five homologous subunits (α_2, β, γ, δ, ε). Each subunit is synthesized with an N-terminal signal sequence and consists of a large N-terminal extracellular glycosylated domain and four putative TM segments (M1–M4), generally assumed to be α helical [1••]. Analysis of electron microscopic images of crystalline tubes containing the receptor shows a fivefold symmetry, with each subunit contributing a single bent helix that lines the channel [72]. The images could not resolve the other TM segments as helices and the suggestion was made that the outer part of the TM domain consists of β sheet. The central helical segment is dynamic and changes its configuration upon ligand binding to the receptor [73••]. Labeling of engineered cysteine mutants has confirmed that segment M2 lines the channel in a predominantly helical conformation interrupted by a three-residue extended structure [22•]. Molecular dynamics studies of the isolated M2 helix have shown that it can assume (rarely) a kinked structure that is stabilized by side chain to backbone hydrogen bonds [74].

Spectroscopic (FTIR) data have indicated that the helical content of the intact acetylcholine receptor is high (39%) [75]. While the TM portion of the receptor generated by proteolysis is enriched in helix (50% helix), it also contains 40% β structure and turns [76]. Labeling of the lipid-exposed part of the receptor with a hydrophobic probe revealed a helical conformation for both segments M3 and M4 [77•]. Analysis of the periodic distribution of hydrophobicity in the putative TM segments (M1–M4) of the acetylcholine receptor suggested that the first three segments have a β-sheet conformation, whereas M4 is α helical [78].

Unlike porins, which assemble in a post-translational fashion, the acetylcholine receptor is inserted into the endoplasmic reticulum membrane cotranslationally and consists of multiple subunits that must come together to form a functional channel. Because isolated β strands are not stable within the bilayer if backbone amide hydrogen bonds are not made, the assembly of the acetylcholine receptor presents a considerable challenge. If higher resolution structural analyses bear out the α/β structure [79•] of the TM portion of the acetylcholine receptor , intrinsic membrane proteins may prove to be as diverse in structure as their soluble cousins.

Conclusions

The detailed elucidation of membrane protein structure will allow the understanding of the molecular mechanisms of complex functions such as transmembrane signaling and transport. The elucidation of the topologies of membrane proteins has been enhanced by results of mutagenesis experiments that move models beyond predictions based solely on hydropathy profiles. The application of molecular dynamics approaches is limited by the availability of high-resolution structures as starting points and the complexity of modeling a large membrane protein in a lipid bilayer, surrounded by water. Modeling is further complicated by the oligomeric nature of most membrane proteins. The mechanism by which TM segments are inserted into the lipid bilayer is still poorly understood and requires further study. There are no substitutes for high quality structural studies. Difficulties in the structural determination of membrane proteins include establishing an adequate source of material, purifying proteins to homogeneity with retention of activity and crystallizing membrane protein–detergent complexes. It is difficult to assess the accuracy of the predictive methods currently in use

because very few high-resolution structures of membrane proteins are available, and because the known structures also provide the parameters for the predictive methods. Funding agencies should be aware of the benefits of providing long-term financial aid for studies aimed at elucidating the structure of membrane proteins. Major breakthroughs in our understanding of the molecular basis of hormone action and diseases such as cystic fibrosis will come from the elucidation of the structure of receptors and transporters. High quality structural studies will also facilitate rational drug design.

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•• of outstanding interest.

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