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Transmembrane domains in the functions of Fc receptors

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Abstract

In the present study, we use a novel method, PHDhtm, to predict the exact locations and extents of the transmembrane (TM) domains of multisubunit immunoglobulin Fc-receptors. Whereas most previous studies have used single residue hydrophobicity plots for characterizing of these domains, PHDhtm utilizes a system of neural networks and the evolutionary information contained in multiple alignments of related sequences to predict the above. Present PHDhtm application predicts TM domains of immunoglobulin Fc-receptors that in many cases differ significantly from those derived by using earlier methods. Comparisons of helical wheel projections of the presently derived TM domains from PHDhtm with those produced earlier reveal different hydrophobic moments as well as hydrophobic and hydrophilic surfaces. These differences probably alter the character of subunit association within the receptor complexes. This new algorithm can also be used for other membrane protein complexes and may advance both understanding the principles underlying such complexes formation and design of peptides that can interfere with such TM domain association so as to modulate specific cellular responses.

Keywords: Bioinformatics; Integral membrane protein; α-Helix association

1. Introduction

Assembly of non-covalent and covalent multicomponent complexes is a ubiquitous feature of integral membrane proteins [1]. The association of different polypeptide chains, some endowed with ligand binding and some transducing the signals across the cellular membranes, has an evolutionary advantage in allowing formation of diverse signal transduction systems using already existing cellular components [2,3]. Subunit association was also shown to control the surface expression of the receptors and their protection from degradation in the ER. Structural and chemical properties of the transmembrane (TM) helices of receptor subunits are crucial for their proper association and incorporation into active receptors. However, in most reports the precise length and location of the TM regions have so far been predicted by using the Kyte and Doolittle hydropathy plots based on single residues [4]. Using the neural network system PHDhtm (PHD), we have earlier predicted

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the TM helices for B- and T-cell antigen receptor complexes [5]. These receptors are members of the family of multichain immune recognition receptors (MIRR) [6] and here we extend that analysis to the TM helices for another class of MIRR: the cell membrane receptors for Fc domains of immunoglobulins (FcRs).

Most FcRs are hetero-oligomeric complexes: the type I (so called high affinity) receptor for IgE, FcεRI, the high- and low-affinity receptors for IgG, FcγRI (CD64) and FcγRIII (CD16), and the high affinity receptor for IgA (FcαR). Exceptions are the family of FcγRII receptors that are generally found to be single-chain entities having either immunoreceptor tyrosine-based activation motif (ITAM) or immunoreceptor tyrosine-based inhibitory motif (ITIM) incorporated into their sequence [7–10], although a possibility that the FcγRIIA might associate with FcεRIγ chain has been suggested by Masuda and Roos, [11]. The multichain FcRs are also composed of a ligand-binding FcRα subunit, associated with one or two signal transduction subunits, such as the FcεRIγ chains, that contain ITAMs in their cytoplasmic tails [9,12–14]. The functions and structures of these receptors have been subject to several reviews [9,13,15–17]. It has been shown that in many cases, both expression of integral membrane proteins on the cell surface, and their association with other TM proteins are a function of their TM domains ([5], and see below). Accordingly, knowledge of precise locations and extents of TM domains of the integral proteins is required for promising areas of research in cell biology. Firstly, it provides identification of the amino acid residues in the TM domains that are crucial for functional association of receptor complexes. In addition, and related to the previous, it may contribute to the development of synthetic transmembrane peptides, designed to interact with TM domains of integral membrane proteins and thereby to modify association of TM proteins with their partners and affect the corresponding function. In the present study we demonstrate that, as was in the case of the BCR and TCR complexes [5], the TM helices of other MIRRs predicted by PHD often differ significantly from those predicted by the Kyte and Doolittle or other single residue hydrophobicity plots. We further suggest that the differences in the predicted TM helices lead to changes in characteristics of helix–helix interaction and hence, to corresponding modification of the specificity of the receptor subunit association and biological function.

2. Experimental

2.1. Predicting TM helices of integral membrane proteins with PHD

Early prediction methods for the TM domains of integral membrane proteins have been based on hydrophobicity analyses of single sequences [18]. Despite relatively high levels of accuracy, most of these simple methods have major problems. In particular, ends of TM helices are not predicted very accurately [18,19]. The breakthrough in protein structure prediction during the 1990s has originated from the use of evolutionary information as available in multiple alignments of protein families [20]. PHD improves prediction accuracy through three essential components [19,21]. Firstly, the relevant information contained in alignments of homologous proteins is extracted. Secondly, the alignment profile is fed into a system of neural networks. Thirdly, the raw output from the neural network is considered as an energy landscape of the propensity ‘membrane helix’ and the optimal path through that landscapes found with a dynamic programming method. This final step optimizes the correctness in predicting the number of membrane helices. In general, prediction accuracy is higher when more homologues and more divergent homologues are known. PHD improves prediction accuracy by about 10 percentage points (about 80% of all residues are predicted correctly as judged by the X-ray and proteolysis data). Furthermore, PHD has additional advantages over methods developed in the 1980s. (1) TM segment termini are predicted better. (2) The number and locations of most TM segments are correctly predicted: for about 80% of the tested proteins, all TM regions are correctly predicted, compared to a level of around 50% for advanced hydrophobicity-based prediction methods [C.-P. Chen, A. Kernytsky and B. Rost, Transmembrane helix predictions revisited, submitted for publication]. (3) Finally,

prediction strength correlates well with expected accuracy; this enables identification of regions that are more likely to be correct than others, i.e. are more reliably predicted. Thus, PHD is clearly a better method for expert-based predictions than are single sequence-based methods of the 1980s [22]. Despite the high level of accuracy, PHD—like all other methods predicting secondary structure for globular and membrane proteins—predicts the cores of helices more accurately than the ends. The main reason for this appears to be that helix caps are not as well conserved evolutionarily as helix cores are, i.e. the length of TM helices may differ marginally between a protein in fly and human. Nevertheless, in contrast to methods based solely on hydrophobicity-scales, PHD frequently captures strong sequence signals from TM helix ends. These signals can be captured since PHD has an additional component (neural network) that explicitly detects such signals. Overall, the advantage of PHD routes in the fact that the underlying neural networks can extract more detailed information from known membrane proteins than simple hydrophobicity scales can and that the method successfully combines the local sequence context and global information from the entire protein. PHD is available via the internet, (http://www.cubic.bioc.columbia.edu/predictprotein/submit_adv.html#top with PHDhtm (transmembrane helices) option selected.)

2.2. Helical wheel projections

The TM domains were further analyzed using helical wheel projections [24] and hydrophobic moments [25] of the corresponding proteins. The hydrophobic moment is defined as a sum over the hydrophobicity values of the residues in a helix. Individual amino acid vectors are drawn towards the central α-carbon of the amino acid residue [25]. We used 100° as the periodicity of the residues side chain orientations, and whole residue interface hydrophobicity scale [26]. The output presents the hydrophilic residues as circles, hydrophobic residues as diamonds, potentially negatively charged as triangles, and potentially positively charged as pentagons. The hydrophobicity is also color coded: the most hydrophobic residue is green, and the amount of green is decreasing proportionally to the hydrophobicity, with zero hydrophobicity coded as yellow. Hydrophilic residues are coded red with pure red being the most hydrophilic (uncharged) residue, and the amount of red decreasing proportionally to the hydrophilicity. The potentially charged residues are light blue. Unless stated otherwise, the helical wheel projections are drawn as seen from the outside of cell, and in most cases the numbering corresponds to the depth of the residue in the membrane. Other details are given in the corresponding figure legends. The use of this program is available on line: http://www.rzlab.ucr.edu/scripts/wheel/wheel.pl

3. Results and discussion

3.1. FcγR

The cell surface receptors for the G class immunoglobulins (FcγR) constitute a rather large and heterogeneous family of membrane proteins that mediate a diverse array of cellular responses to antibodies and antibody–antigen complexes [9,16,23]. They are expressed by the majority of leukocytes mediating a variety of immune responses, ranging from antibody-dependent cellular cytotoxicity, inflammation, to phagocytosis, regulation of the B cell response and endocytosis [16]. The Fcγ receptors belong to three distinct classes: I (high-affinity), II and III (low-affinity) [15]. Most FcγRs belong to the immunoglobulin superfamily and hence, are structurally-related to one another [16]. The extracellular domains of FcγRI and FcγRIII are conserved throughout the family, with the former type having three, and the latter two Ig-like domains [16]. However, the TM and the cytoplasmic domains diverged. This divergence is the base for the variety of functions they perform and hence the diverse intracellular responses triggered by these receptors. FcγRIIIA is composed of at least three polypeptide chains: α chain, responsible for the ligand binding and the associated γ and ζ chains [13]. Interestingly, γ or ζ chains are also essential components of other receptors: γ has originally been identified as a component of FcεRI [27], and ζ as a component of the TCR/CD3 complex [28]. Both FcγRIIIA

and TCRα contain structural elements in their TM domains sequences that direct them for degradation in the ER [29,30]. However, interaction with a non-covalently associated subunit masks these motifs and hence prevents the degradation, as illustrated by such a process in the TCR/CD3 assembly, where this role is played by the CD3δ chain [30].

In the following we separately address the FcγRI and FcγRIII.

3.2. FcγRI

Human FcγRI (CD64) is a 72-kDa glycoprotein (see [16] for a review), with a polypeptide core of 55 kDa [31]. It is constitutively expressed on monocytes and macrophages and can also be induced in neutrophils and eosinophils [16]. Three human FcγRI genes have been identified: A, B and C [16]. However, only human FcγRIA is an integral membrane protein, because both its B and C genes contain stop codons in the third extracellular domain and probably encode soluble receptors [16]. An alternatively spliced form of human FcγRI, FcγRIa2 has also been described [32]. This form lacks the cleavage site for the leader sequence (signal peptide) and thus is expected to be integrated in the membrane with both its leader and the TM hydrophobic regions [32]. However, Ernst et al. [32], suggest that the receptor can still be fully functional. The extra-cellular and the TM domains of mouse and human FcγRIs have an overall sequence identity level of 75%. In contrast, their cytoplasmic tails share only 25% of residues, and the mouse protein is also 23 amino acids longer [33].

As all other Fc receptors, the cytoplasmic tails of FcγRI have no intrinsic enzymatic (e.g. tyrosine kinase) activity. Rather, in order to couple with signaling cascades, they associate with auxillary molecules—the γ chains of FcεRI that contain an ITAM [34]. Significantly, though human FcγRI associates with FcεRI γ chain, it is also expressed on the cell surface independent of such association [35,36]. However, a more stable surface expression of the receptor is achieved in the presence of associated FcεRIγ [37].

The interaction between the human FcγRI and the γ chain is mediated solely via their TM domains [38]. This interaction is necessary and sufficient for the FcγRI-triggered cell activation because deletion of the cytoplasmic portion of the receptor does not impair its function [39] demonstrating that it is the γ chain that activates the cellular response.

In the mouse, two alleles of FcγRI have originally been identified, one derived from BALB/c mice and originally designated FcγRI-BALB [33,40] and the other from NOD mice, designated FcγRI-NOD [41]. Five additional alleles have recently been described [42] bringing the total to 7 alleles, including the previously described standard (designated allele *a*, protein 1) and NOD (designated allele *d*, protein 4). Receptors encoded by four of the five new alleles (*b, c, f, g*) bind IgG2a with high affinity, and IgG binding characteristics similar to those of the *a* allele. The *d* allele (previously FcγRI-NOD) and the *e* allele [derived from *Mus spretus* (SPRET/Ei)] encode receptors which showed broader specificity by binding monomeric IgG2a, IgG2b and IgG3 [42].

The TM helices of human and mouse FcγRIs predicted by PHD (Fig. 1) were shorter than those based on hydrophobicity plots [33,35]. Our results show strong conservation of both the locations and the extents of the TM regions among the full-length mouse alleles (*a, b, c, f, g*) (Fig. 1). Even the much shorter *d* and *e* alleles (336 amino acids as compared to 404 amino acids in other alleles) exhibited only a two-residue shift, and, in the case of the *e* allele, only one residue elongation, of their TM helices toward the C-terminals (Fig. 1). In this and other cases below we do not consider one residue changes predicted by PHD as significant. For the human FcγRI, PHD predicted the TM helix to be shorter and shifted toward the N-terminus relatively to the position assumed by using hydrophobicity plots (Fig. 1). This shift implied a 6 residues longer cytoplasmic region. Remarkably, there is no significant difference between the TM regions of the standard (A) and the B isoforms of the human FcγRI: both differ by only one residue shift of the TM helix of the B isoform although the two proteins differ grossly in length (280 amino acids for B isoform, 374

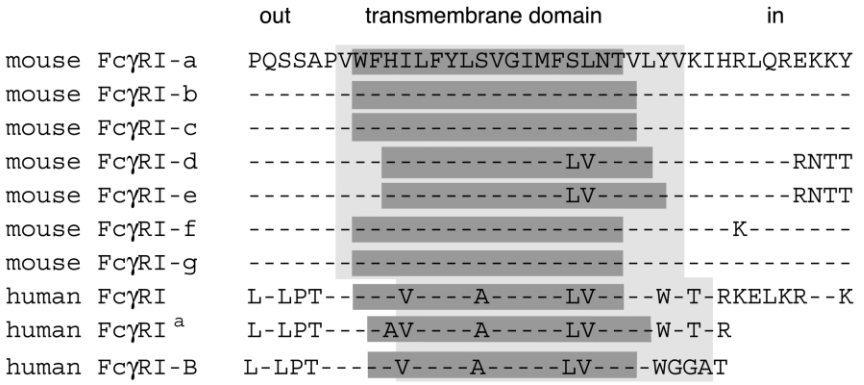

Fig. 1. Sequences of the C-terminal regions of mouse and human FcγRI. Light shade: mouse proteins TM domains according to [33]; human proteins: TM domains according to [35]. Dark shade: TM domains according to PHD. The accession numbers of these proteins are given below. Mouse alleles *a*: P26151, AAD34915; *b*: AAD34932; *c*: AAD34941; *d*: AAD34942; *e*: AAD34946; *f*: AAD34947; *g*: AAD34948. Human standard: A39878; human FcγRI-B: I55577; [a]mutated H284A [38]. The numbers of the first plotted here residues for each of the sequences from the corresponding GenBank entries are given below. Mouse alleles *a–c*, *f*, *g*: #292. Mouse alleles *d*, *e*: #293. Human standard: #283. Human B isoform: #189.

amino acids for standard A form, Fig. 1). Substituting the His 284 by an Ala does not seem to affect the function of the receptor [38]. Our results suggest that such a substitution may result in elongating the TM helix from 18 to 19 residues, and a shift towards the C-terminus resulting in the two residue shorter cytoplasmic tail of the mutated receptor.

The implications of the differences between the TMs commonly reported in the literature (hereafter referred to as TRD for 'traditional') and these presently obtained from PHD were further analyzed using helical wheel projections and hydrophobic moments. Comparison of the helical wheel projections of TMs obtained by PHD and TRD hydrophobicity plots shows dramatic differences between the corresponding hydrophobic and hydrophilic surfaces (Fig. 2). Whereas the hydrophobic surface of the PHD TM contains primarily hydrophobic residues, the corresponding surface of the TRD is more mixed, and even includes a charged residue, Asn14. The hydrophilic surface of the PHD TM contains two charged residues, His3 and Asn17.

The significance of these differences is illustrated by plotting the helical wheels of the associated subunit, FcεRIγ below the corresponding FcγRI subunits, with the hydrophilic surfaces of the associated subunits facing each other (Fig. 2). Although, generally, helix association occurs through a combination of hydrogen-bonding, electrostatic, and van der Waals interactions [43], it was noticed early that there is a tendency for apposition of the hydrophobic moments in neighboring helices [25,44], with the most probable orientation leading to association of the hydrophilic surfaces in the hydrophobic milieu of the lipid bilayers. Specifically, in the case of associations among FcεRI components, the role of Asp29 was reported to be crucial (see below). Therefore, it is significant that as a result of the PHD arrangement, Asp29 (corresponding to D4 in Fig. 2) is facing directly His3 on the hydrophilic surface of FcγRI (Fig. 2, left), whereas no such obvious partner is apparent when the TRD hydrophilic surface is examined (Fig. 2, right). Thus, in this instance, the PHD TM provides a better match and rationalizes better the association of these subunits, than the traditional method. It further predicts that the FcγRI TM residues forming the hydrophilic surface and, specifically H3, will be important for the subunit association, and, therefore, for the receptor expression and function.

In the case of the mouse FcγRI, the TRD TMs were assumed to be identical for all the alleles, whereas significant differences are obtained

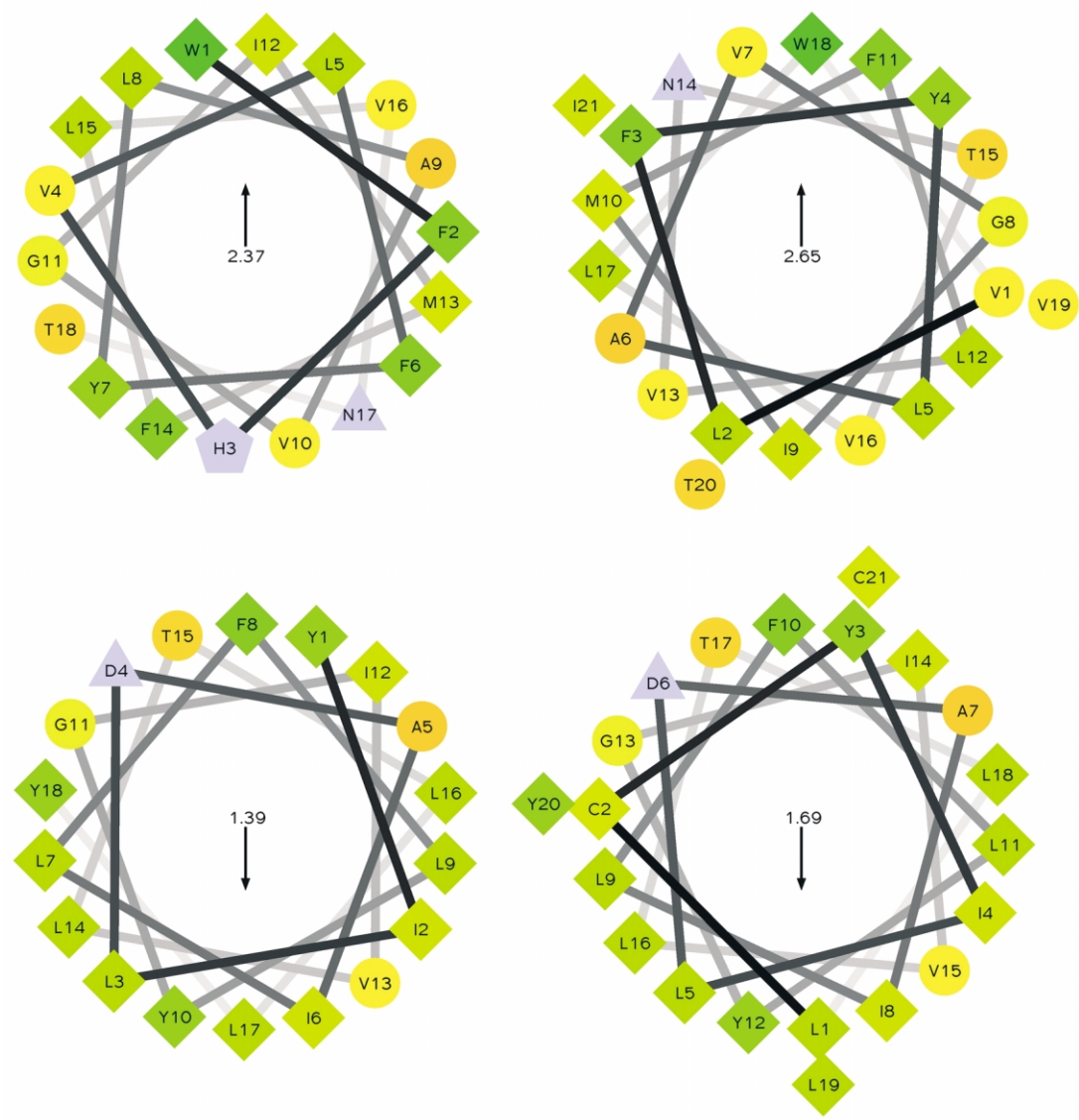

Fig. 2. Helical wheel projections of the PHD (left) and TRD (right)-derived human FcγRI (top) and FcεRIγ (bottom) TM domains. The arrows indicate the directions of the hydrophobic moments, and the numbers in the centers of the wheels are the magnitudes of the moments. The residue numbers reflect the depths of their location in the membranes. For more details see Section 2 and Section 3.

between the full length and short alleles by the PHD analysis (Fig. 1). Fig. 3 presents the helical wheel projections of the PHD TMs of the full length allele *a* (top left), and the short allele *d* (top right) with the traditional TM helical wheel projection (top middle). It is apparent that the amphipathic characters of the corresponding wheels, and the hydrophobic and the hydrophilic surfaces predicted by the PHD (Fig. 3, top left) and TRD (Fig. 3 top middle) are similar for the *a* allele. However, the PHD analysis predicts a much smaller hydrophobic moment of 0.73 for the *d*

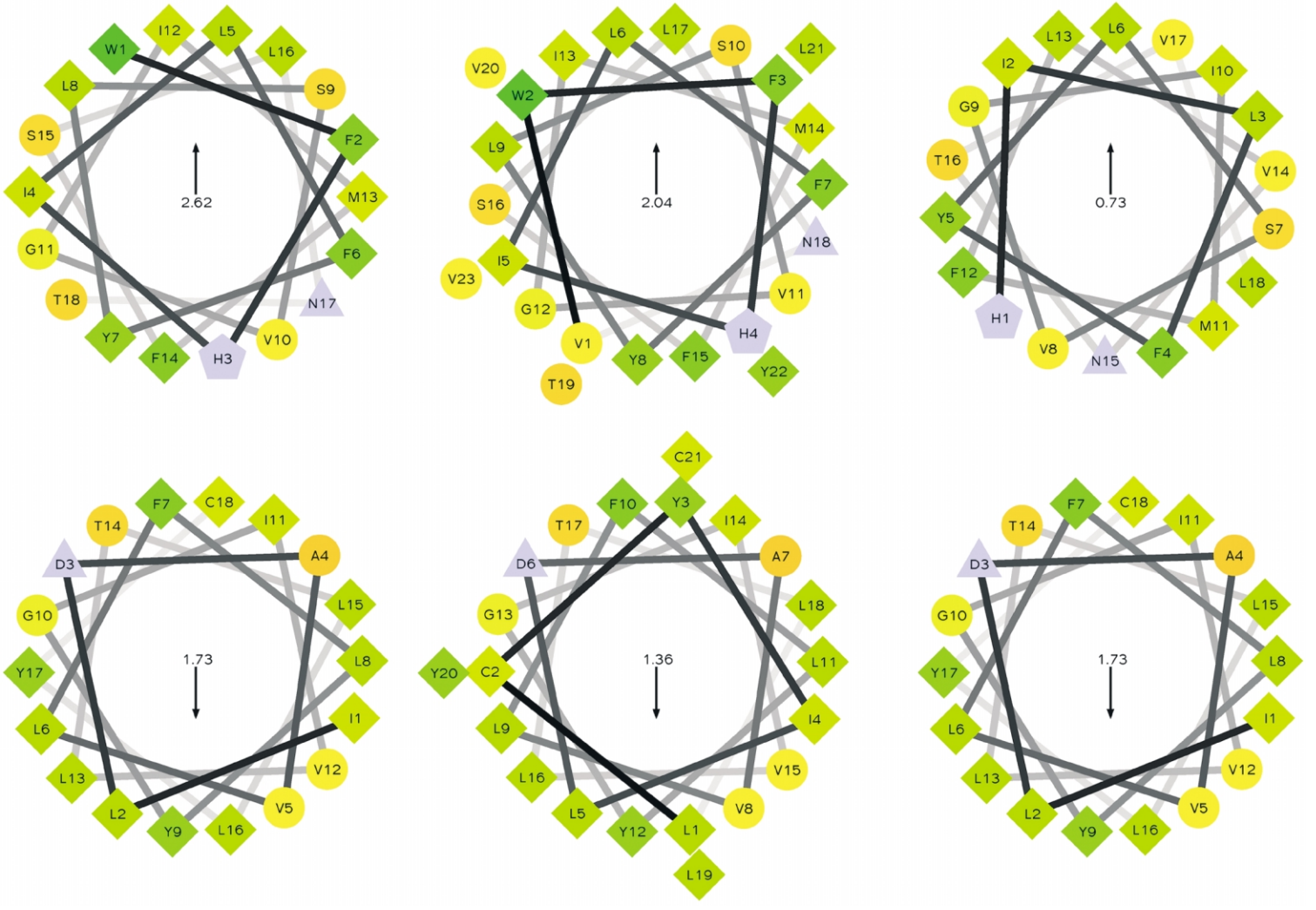

Fig. 3. Helical wheel projections of mouse FcγRI (top) and mouse FcεRIγ (bottom) TM domains. The FcγRI domains on the top row are drawn using the TM sequences obtained by PHD for the *a* allele (left) or *d* allele (right). The middle projection corresponds to the TRD TM, identical for all the alleles. In the bottom row, the left and right helical projection are identical, and correspond to the PHD-derived TM domain of the mouse FcεRIγ, whereas the middle projection is drawn using the TRD TM domain. The residue numbering corresponds to the positions of the residues relative to the outside surface of the membrane.

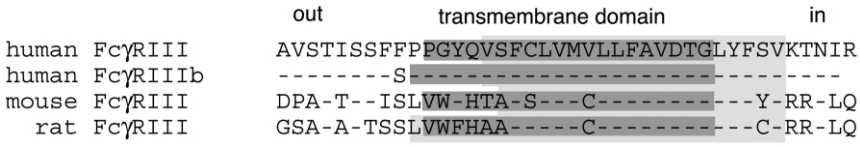

Fig. 4. Sequences of the TM regions of the FcγRIII. Light shade human receptors: TM domains according to [45,57–60]; mouse: TM domains according to [45]; rat: TM domains according to [56,61]. Dark shade: TM domains suggested by PHD. Accession numbers: human FcγRIII: JL0107; human FcγRIIIb: JU0284; mouse FcγRIII: S29360; rat FcγRIII I72882. The numbers of the first plotted here residues for each of the sequences from the corresponding GenBank entries are given below. Human receptors: #195. Mouse receptor: #201. Rat receptor: #207.

allele (Fig. 3, top right). Moreover, the potentially charged His residue in PHD TM domain of the *d* allele is located close to the membrane surface (the residue numbers in Fig. 3 correspond to the positions of the residues relative to the outside surface of the membrane), whereas it is located deeper in the membrane interior in the case of TRD TM domain (Fig. 3, top middle).

3.3. FcγRIII

Two human FcγRIII (CD16) genes are known; a and b, each encoding a single transcript that is expressed in a cell-specific manner [45]. The A isoform is an integral membrane protein, and the B isoform is GPI-anchored [16]. Both isoforms are heterogenous in size with molecular masses ranging from 50 to 80 kDa due to extensive *N*-linked glycosylation [16]. Similar size heterogeneity was exhibited by the mouse FcγRIII [46].

Efficient cell-surface expression and signal transduction of the TM form of human FcγRIII requires coexpression of associated molecules. These accessory molecules may be the FcεRI γ or TCR ζ subunit [47–51]. Human FcγRIII can also associate with FcεRIβ [52,53]. The interactions between human FcγRIII and CD3ζ or FcεRIγ are not affected by truncating the cytoplasmic regions. Thus, Lanier et al. [54] concluded that these interactions are determined by the TM domains. In the case of mouse FcγRIII, association with the FcεRI γ chain is required for both cell-surface expression and signaling [47,55]. Rat FcγRIII form a family of multiple isoforms all encoding TM proteins that require associated subunits for full function [16]. However, the rat isoforms fail to associate with rat CDζ [56]. The rat isoforms primarily differ from one another in their extracellular regions [56].

The TM domains of the FcγRIIIs are shown on Fig. 4. The predicted TRD (Fig. 4, light shade) or PHD (Fig. 4, dark shade) TM helices differ substantially for each of the considered three species. In particular, all the helices predicted by PHD were shorter and, in the cases of the human and mouse proteins, shifted four–five residues toward the N-terminal relative to the TM domains commonly assumed in the literature (Fig. 4), resulting in correspondingly longer cytoplasmic tails. It is interesting that both TM and GPI-anchored forms of this receptor have similar TM domains, as indicated by both TRD and PHD. The effects of these changes in the TM locations on the corresponding helical surfaces are illustrated in Figs. 5 and 6. In the case of the human protein, the PHD and TRD TMs result in somewhat similar hydrophobic moments and hydrophilic surfaces. However, the residues forming the hydrophilic surfaces are totally different (Fig. 5). In the case of PHD, the TM domain has a hydrophilic residue (Q208) at a depth compatible with forming hydrogen bond with D29 of FcεRIγ:, whereas there is no obvious candidate for interaction with D29 in the TRD TM (Fig. 5). The differences between the two methods of TM determination are further illustrated by the fact that the neighbors in the helical wheel projection V214 and V221 are part of the hydrophobic surface in the PHD, but are included into the hydrophilic surface of the TRD helix (Fig. 5). Thus, the PHD provides an experimentally testable prediction that Q208 is important for association

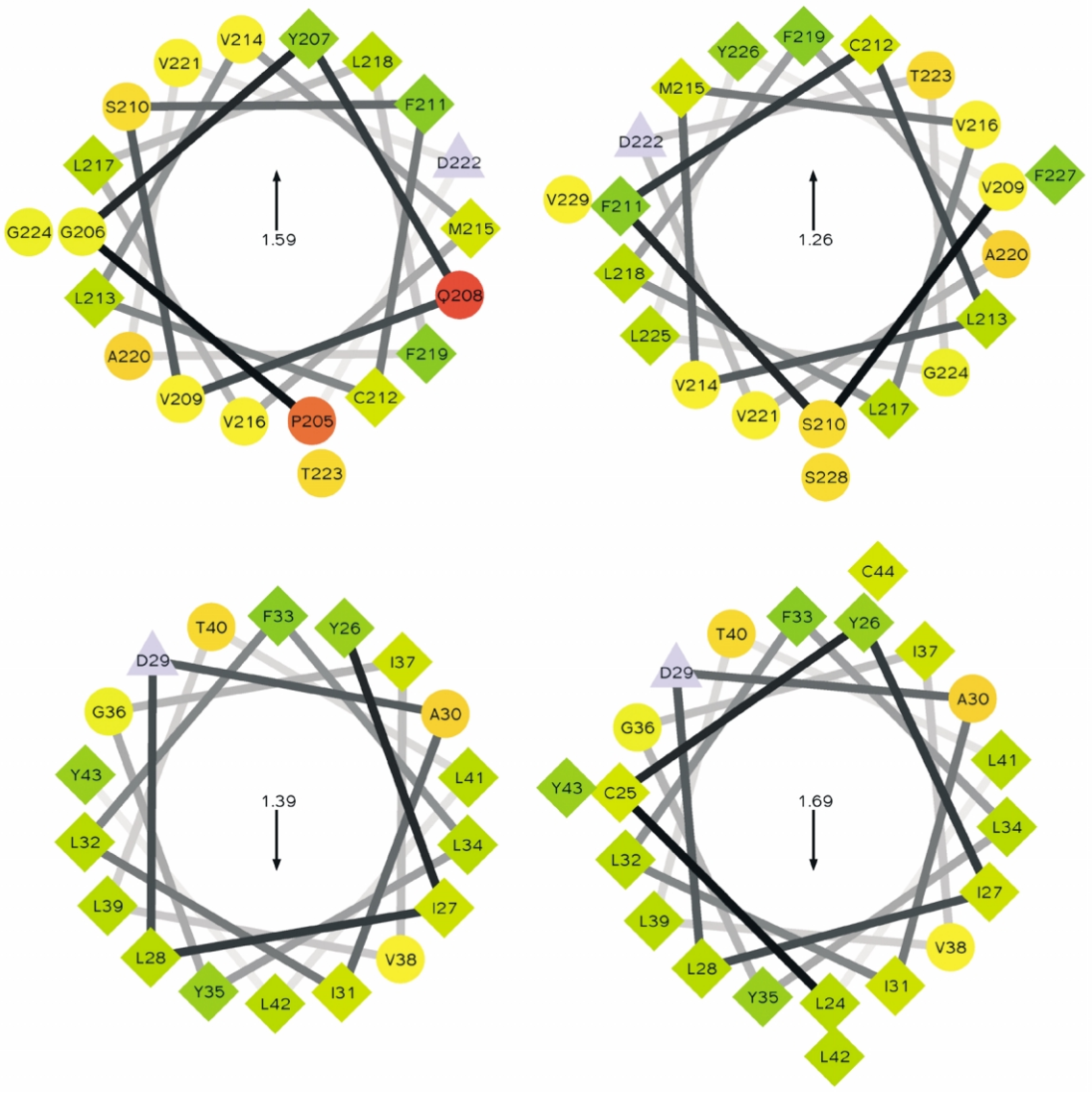

Fig. 5. Helical wheel projections of the PHD (left) and TRD (right)-derived human FcγRIII (top) and FcεRIγ (bottom) TM domains. The residue numbers correspond to the sequence numbers of the corresponding proteins in the GenBank data base; the accession numbers are given in the legend to Fig. 4.

with FcεRIγ. In addition, there remains a possibility that the subunit association is governed by van der Waals interaction.

The differences between the TM domains obtained using either the PHD or TRD methods lead to profound differences in the corresponding TM helices in the case of mouse FcγRIII (Fig. 6): whereas a significant hydrophobic moment of 2.7 is present in the PHD helix, the TRD analysis yields a helix that has only a weak amphipathic character, with a hydrophobic moment of 0.28. Furthermore, the helical surfaces are almost completely different in both cases. Specifically, the hydrophilic surface of the helix, likely to interact

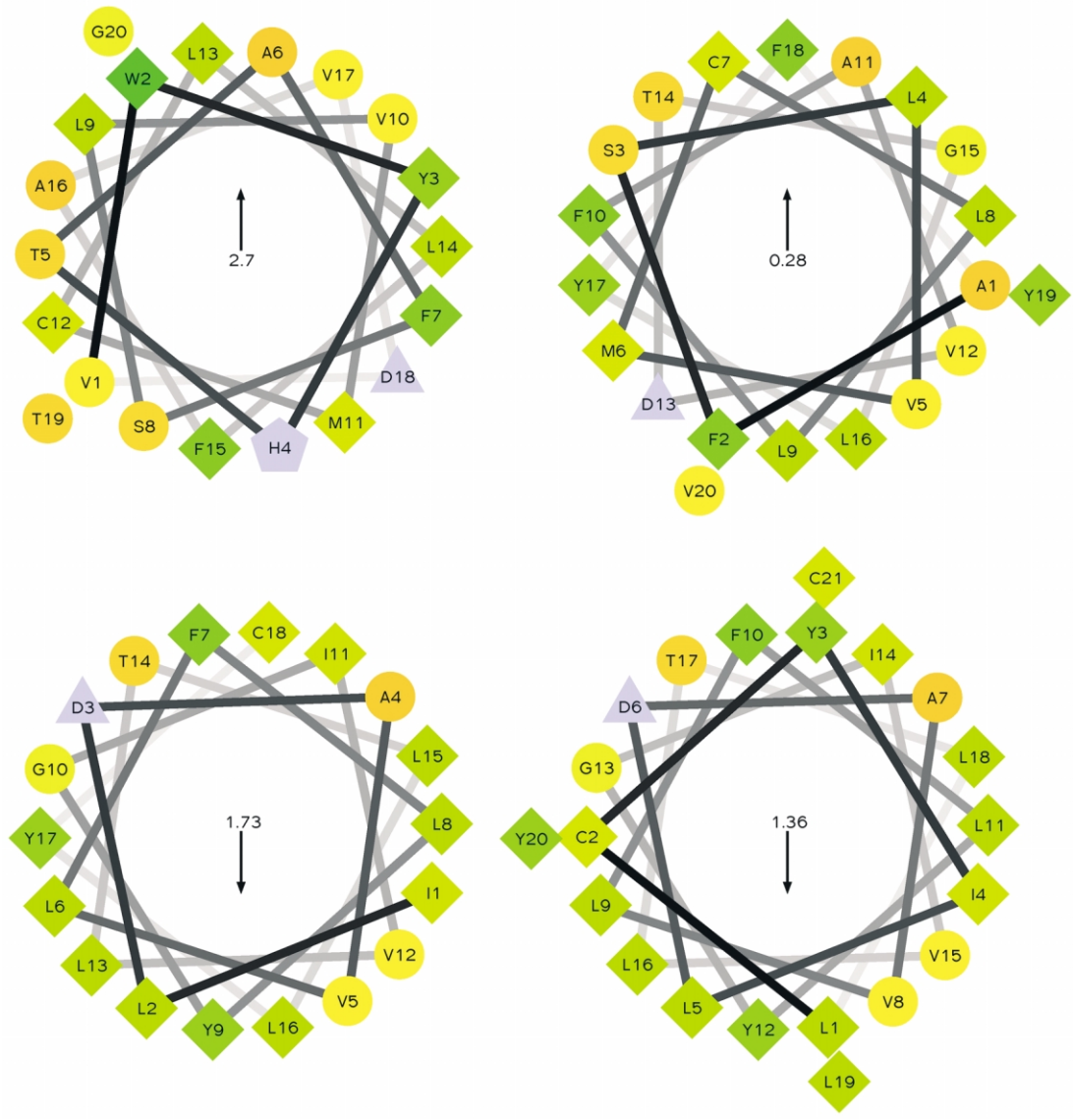

Fig. 6. Helical wheel projections of the PHD (left) and TRD (right)-derived mouse FcγRIII (top) and FcεRIγ (bottom) TM domains. The residue numbers reflect the depths of their location in the membranes.

with the FcεRIγ subunit, in the case of the PHD includes a potentially positively charged H4 located in proximity to the FcεRIγ D29 (corresponding to D3 on the figure), whereas His is not present at all in the TRD helix; and there is no suitable candidate apparent to interact with the D29 (corresponding to D6 for the TRD TM on Fig. 6). Thus, as in many other cases considered here, PHD and TRD method yield significant differences in identifying the TM residues involved in helix–helix interactions.

3.4. FcαR

Human FcαRI is expressed on monocytes, macrophages, neutrophils, eosinophils and mesangial

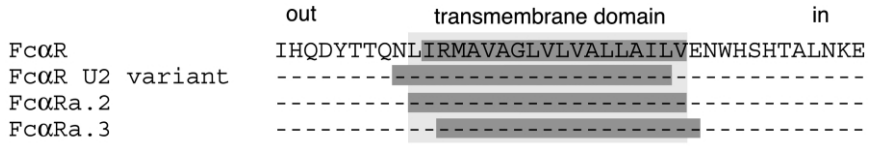

Fig. 7. Sequences of the TM regions of the FcαR. Light shade: TM domains according to [68,73]. Dark shade: TM domains according to PHD. Accession numbers: FcαR: P24071; U2 form: BAA13477; a.2 form: AAC50639; a.3 form: AAC50595. The numbers of the first plotted here residues for each of the sequences from the corresponding GenBank entries are given below. Standard receptor: #219. U2 form: #223. A.2 form: #197. A.3 form: #123.

cells [16,62]. This protein is also a member of the Ig gene superfamily and is, therefore, related both to Fcγ receptors (FcγRI, FcγRII and FcγRIII) and to the type I Fcε receptor (FcεRI) [15]. Human FcαR on monocytes/macrophages and neutrophils is a 55–75 kDa glycoprotein [63,64], whereas in eosinophils (70–100 kDa) it is larger due to heavier glycosylation [65]. Both types of receptors are recognized by specific mAb for CD89 [64,65] and bind both IgA1 and IgA2 via their Fc regions [66]. The cDNA of FcαR encodes a 30-kDa protein with two extracellular Ig-like domains, a hydrophobic TM region and a cytoplasmic tail devoid of any known signaling motifs [67,68]. Additionally, a novel form of human FcαR has been found in human eosinophils and neutrophils [69]. This isoform (FcαRb) lacks both the TM and cytoplasmic regions. Instead, in their place, it has an additional 23 amino acids sequence [69]. Although FcαRb is membrane-associated and is probably not GPI-anchored [69], the authors could not establish whether the additional peptide stretch is integrated into the membrane. PHD analysis also did not indicate any TM helix in this isoform. Thus, this isoform probably associates with the membranes as a peripheral, rather than an integral, membrane protein. Also, it neither associates with the FcεR γ chain nor is it inducing tyrosine phosphorylation or Ca^{2+} mobilization upon clustering [69], consistent with the hypothesis that the presence of a TM region is required for these functions.

In analogy with other multichain Fc receptors, FcαRI associates with FcεRI γ chain [70]. Nevertheless, it is not clear whether this association is required for this receptor's expression. Van Egmond et al. [71] have demonstrated the requirement for such association for human FcεRI, expressed in transgenic mice. However, FcαRI was shown to be expressed in vitro without the FcεRI γ chain [14]. Moreover, in a recent study Launay et al. [72] have expressed human FcαR with or without the FcεRI γ chain in rat mast cells. These authors have also generated a mutant FcαR by changing Arg^{209} (corresponding to the Arg^{230} in the GenBank data base entry #P24071) to a Leu. As expected by the authors on the basis of the lack of charge in Leu, this substitution prevented association of FcαR with the γ chain [72]. The mutant-less FcαR was expressed in the mast cell line RBL 2H3. It bound IgA, but failed to induce degranulation upon being clustered. The same cells expressed both γ-less and γ-associated FcαR, and this was assumed to constitute the basis for differential endocytosis pathways of IgA, in which γ-less receptors recycle IgA toward the cell surface whereas the γ-associated receptors undergo endo-lysosomal sorting for IgA degradation [72]. Maliszewski et al. [68] using an undefined 'hydropathy' scale determined that the TM region of the FcαR comprises 19 amino acids, residues #228–246, (the residue numbering used here corresponds to the GenBank data base entry #P24071) whereas 41 amino acids are in the cytoplasmic tail (Fig. 7, light shade). PHD predicted a similarly located TM helix of 18 residues (Fig. 7, dark shade).

As in the FcγRI, FcγRIIIA and FcεRI, the TM helix of the FcαR contains a single charged amino acid (Arg^{230}), the position of which may lead to the formation of a salt bridge or a hydrogen bond with the γ-chain TM region Asp^{29} [14]. Indeed, mutations of Arg^{230} showed that only a positively charged residue (Arginine or Histidine) enables

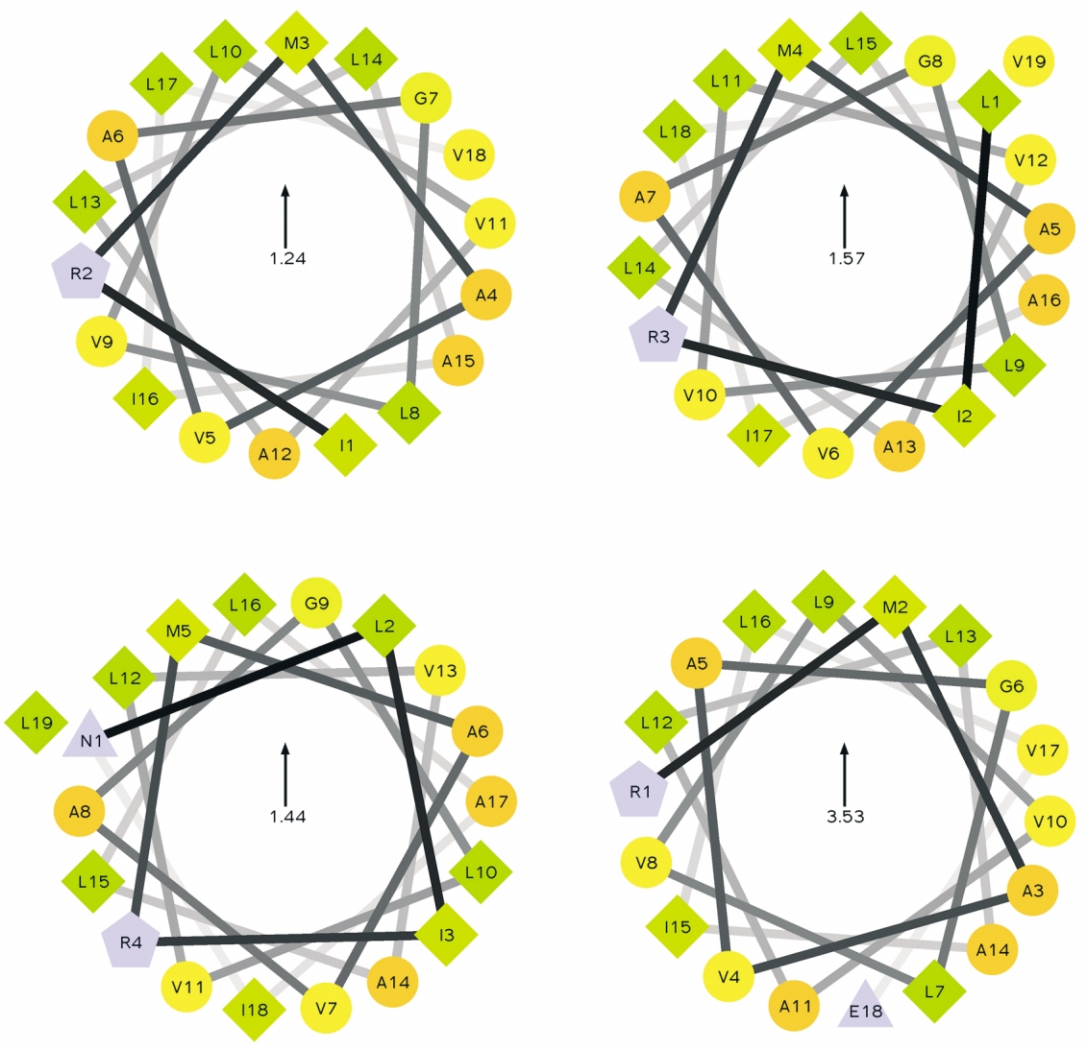

Fig. 8. Helical wheel projections of different isoforms of FcαR. Clockwise from top left: standard FcαR according to PHD; all FcαR isoforms according to TRD; FcαR a.3 isoform according to PHD; FcαR a.2 isoform according to PHD. The residue numbers reflect the depths of their location in the membranes.

functional association of FcαR with FcεRIγ and the triggering of both proximal (intracellular Ca^{2+} release and tyrosine phosphorylation), as well as distal (IL-2 release) cellular responses. PHD results suggested that indeed in the standard, a.2 and U2 isoforms, Arginine is, respectively, the second, third and fourth residue in the TM segment below the external membrane surface (Fig. 7), compatible with the fourth-residue position of Asp in the human FcεRIγ TM domain (Fig. 12). However, according to the PHD analysis, in the a.3 isoform Arginine is the first residue inside the membrane (Fig. 7). The difference between the a.3 and other isoforms is further illustrated by the corresponding helical wheel projections (Fig. 8): in addition to the different depth of the Arginine, the helix of the a.3 isoforms has also a much higher hydrophobic moment, and a different hydrophilic surface, as compared to the other isoforms, or to the TRD helix. Thus, PHD indicates that the

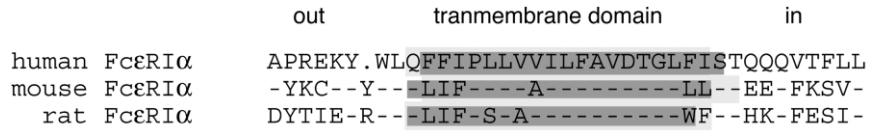

Fig. 9. Sequences of the TM regions of FcεRI receptor α chains. The proteins are aligned according to [80]. Light shade TRD TM domains; human receptor: TM domain according to [81] (the TM domain according to [82] does not include the Gln at the N-terminus side of the TM); mouse: TM domain according to [80]; rat: TM domain according to [83,84]; the TM domain according to [82] does not include the Gln at the N-terminus side of the TM. Dark shade: TM domains suggested by PHD. Accession numbers: human FcεRIα: S00682; mouse FcεRIα: A34342; rat FcεRIα: A30154. The numbers of the first plotted here residues for each of the sequences from the corresponding GenBank entries are given below. Human receptor: #197. Mouse and rat: #195.

a.3 isoform may have different parameters of association with FcεRIγ, and, consequently, an altered function. Generally, the presence of strongly polar residues forming salt bridges or hydrogen bonds with other receptor components in the TM regions, seems to be a frequently encountered and an important feature of MIRR family members [5]. The role of salt bridges or hydrogen bonds formed between strongly polar residues in the TM helices for assembly of functional membrane receptors has already been established by a number of studies [74–76].

3.5. FcεRI

The FcεRI, originally found to be expressed only on mast cells and basophils, has more recently been also found on Langerhans cells, eosinophils, and activated monocytes [16]. In most cases, the FcεRI is expressed as a four chain ($\alpha\beta\gamma_2$) complex [16]; the γ_2 dimer is linked by a disulfide bond whereas all other bonds forming this receptor complex are non-covalent. In the human system, an alternate form is also expressed, comprised of an $\alpha\gamma_2$ trimer only [77,78]. As already stated above, the FcεRI γ chain has been found to be a component of other MIRRs. Overall, the predicted human, mouse and rat FcεRIγ chains have 38% pairwise sequence identity. In contrast, their TM regions are about 62% identical to each other [80].

All of the FcεRIα and FcγRIIIA chains share a striking homology within their TM regions across three different species: a sequence comprising 8 amino acids LFAVDTGL is fully conserved [79], suggesting the conservation of an important structural and/or functional motif. The PHD and TRD TM regions of the human, rat and mouse FcεRIα are shown in Fig. 9. The previously published TM regions of the human and rat FcεRIα chains, (Fig. 9, light shade) did not differ significantly from those derived from PHD predictions (Fig. 9, dark shade). PHD predicted that the human TM helix is shifted by one position toward the C-terminus, and that of the rat chain to be one residue shorter than previously assumed. For mouse, the TM helix predicted by PHD is one residue shorter, and is shifted one position toward the N-terminus, extending the cytosolic segment by two residues. Helical wheel projections of the FcεRIα chains did not show significant differences between the PHD and the TRD helices (not shown).

Although the β subunit contains an ITAM and, therefore, has an intrinsic signaling capacity, it was proposed to serve rather as an amplifier for signals generated by the other ITAM-containing chains of FcεR, the γ chains [85,86]. Erdei and Pecht [87] have shown that the anaphylotoxin C3a binds to the β chain and causes suppression of the mast cell secretory response. Thus, the FcεRI β chain may possibly have a role of signal modulator.

Recently, an additional amplification function has been suggested for the β subunit, namely, a role in the membrane expression of the FcεR complex [88]. The β subunit appears to facilitate this expression by promoting the processing and export of the α chain to the cell surface and by enhancing stability of receptor protein complexes [88]. The β chain has four TM helices [78,80]. As for most receptors analyzed in this study, the TM helices appear to be remarkably well conserved among FcεRI γ subunits of all different species examined (Fig. 10); even the shorter TMs

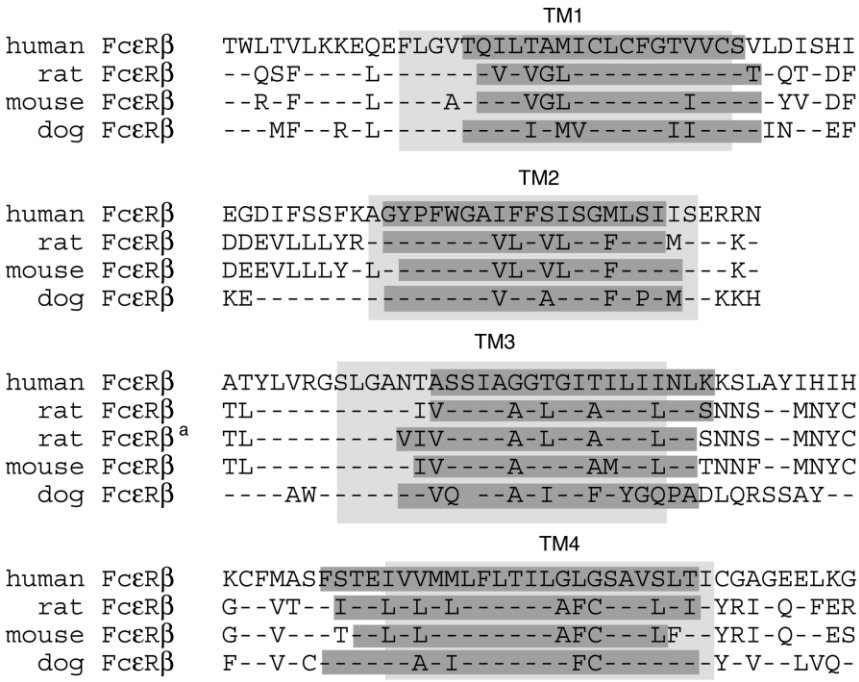

Fig. 10. Sequences of the TM regions of FcεRI receptor ? chains. The human, rat and mouse chains are aligned according to [89]; the dog chain alignment added according to [90]. Light shade human, rat and mouse chains: TMs according to [80,89,91]. Dog chain: TMs according to [90]. Dark shade: TMs according to PHD. Accession numbers: human FcεRI β chain: A42806; rat: P13386; mouse: P20490; dog: BAA78715. [a]rat chain mutated N134V [92]. The numbers of the first plotted here residue for each of the sequences from the corresponding GenBank entries are given below. Human receptor, TM1: #49; TM2: #89; TM3: #123; TM4: #171. Rat, TM1: #49; TM2: #89; TM3: #123; TM4: #170. Mouse, TM1: #41; TM2: #81; TM3: #115; TM4: #162. Dog, TM1: #49; TM2: #89; TM3: #123; TM4: #171.

of the mouse subunit are similar to those of other species. Nevertheless, contrary to the assumption made in the most publications, where the TM regions of the β chain were deduced from single residue hydropathy plots (Fig. 10, light shade), the PHD indicates the presence of some diversity (Fig. 10, dark shade). Furthermore, the PHD predictions (Fig. 10, dark shade) differed, in particular for helices 1, 3 and 4, from the commonly adopted hydrophobicity assignment (Fig. 10, light shade). These results may require re-interpreting previous analyses of the structure of the TM domains. An example for such a need for re-interpretation of previous studies can be the results in [92], which suggested that the potentially charged residues in the TMs of the FcεRI chains are buried in the membrane interior where the TMs interact with each other resulting in the sensitivity of the surface expression of the receptor to mutations in the TM regions. Varin-Blank and Metzger [92] then proceeded to estimate the depths of the charged residues in the membrane by using hydrophobic moments calculated using the commonly assumed TM regions. However, TM regions suggested by PHD differ substantially from those assumed in [92]. Thus, the depths of the TM residues and the corresponding 'hydropathy scores', calculated in [92] will have different values, requiring reconsideration of the suggested hypothesis.

We further explored the implications of the TM sequence differences between PHD and the TRD estimates by construction helical wheel projections for the human FcεRIβ (Fig. 11). In the cases of the first two TM segments, TM1 and TM2, it is interesting to note that the significant differences in the TM sequences' lengths are not necessarily

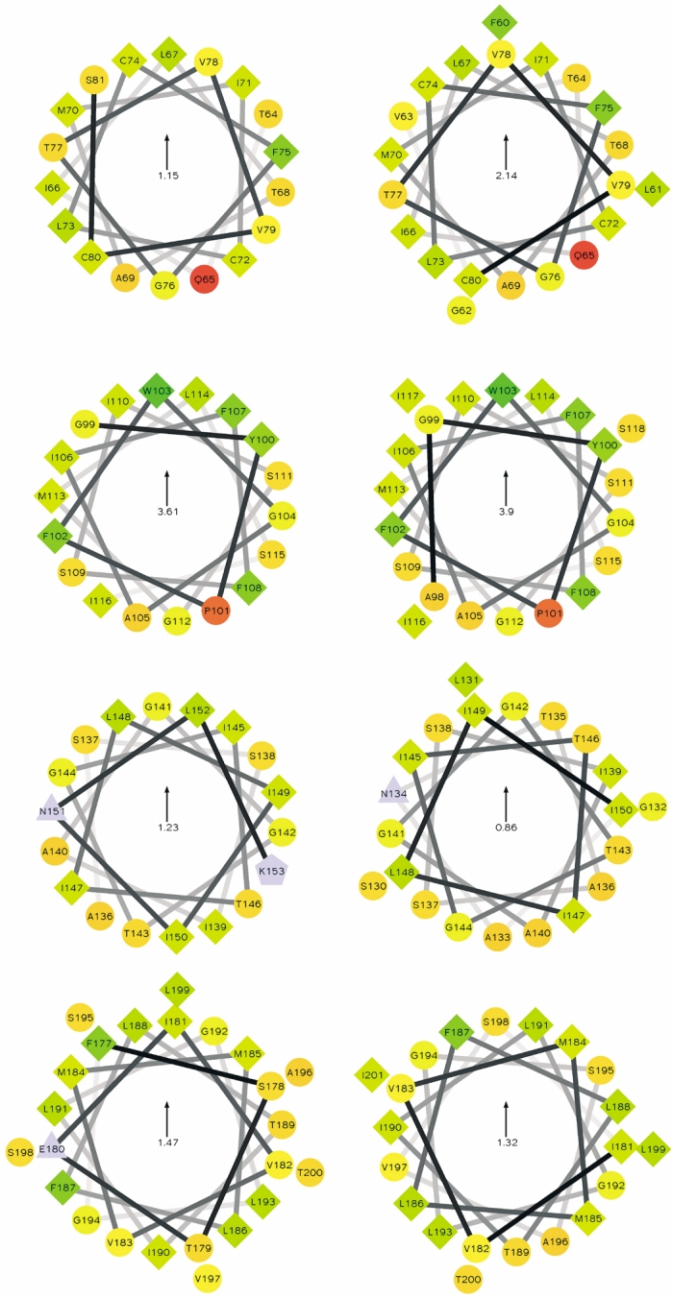

Fig. 11. Helical wheel projections of the PHD- (left) and TRD-derived (right) TM domains of human FcεRI β. The four rows correspond to the four TM domains, starting from the top row which corresponds to the TM1 on Fig. 10. The residue numbers correspond to the sequence numbers of the corresponding proteins in the GenBank database. All projections drawn as viewed from the outside of the cell; thus the helical wheels of the TM1 (top row) and TM3 (third row from the top) are drawn in reversed direction.

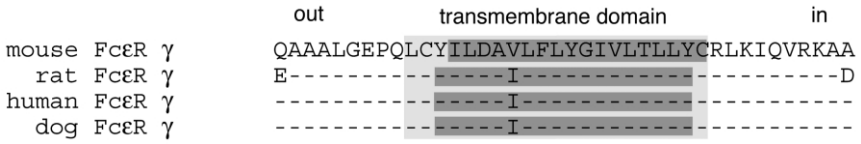

Fig. 12. Sequences of the TM regions of the FcεRI γ. Light shade: TM domains according to [27,80,78,90]. Dark shade: TM domains according to PHD. Accession numbers: mouse FcεRI γ chain: P20491; rat: P20411; human: P30273; dog: BAA78716. The first plotted here residue for each of the sequences from the corresponding GenBank entries is the residue 15.

translated into a significant helical surfaces differences. In both cases the surfaces are quite similar for PHD and the TRD helices. There are, however, dramatic differences between the helical surfaces of the PHD and the TRD TM3 and TM4 segments (Fig. 11). Thus, in the case of TM3, the PHD helix contains two charged residues (N151 and K153), located at the opposite surface of the helix, rather than only one charged residue (N134), assumed by the TRD TM sequence. In the case of TM4, the PHD predicts a charged residue, E180, whereas no charged residues are present in the traditional helix (Fig. 11). Furthermore, in both cases the hydrophilic and hydrophobic surfaces predicted by PHD and the single-residue hydrophobicity plots are quite different. A recent study modeled the structure of FcεRIβ using computer modeling, CD, NMR, lipophilicity mapping, hydrophobic moments, and docking of TM helices [93]. The original bundle arrangements of the four TM helices of FcεRIβ were chosen by Zloh et al. [93] on the basis of canceling hydrophobic moments, as determined using the single residue hydrophobicity method for TM domains, and the Eisenberg et al. [25] scale for residue's hydrophobic moments; the situation may be different if the PHD combined with Wimley and White [26] scale were used. The results presented by us here should be helpful in the future investigation of the structure of FcεRI and, especially, in the arrangements of its TM domains.

We have also considered the possibility that FcεRI β mutations observed in human populations may affect the receptor function. Mutations E237G, or I181L V183L were found in some populations and proposed to be associated with atopy [94,95]. Both of these mutations resulted in a change in the first TM from TQI...VCS (see Fig. 10) to QIL.....SVL with other TM regions being affected at most by a difference in one residue (data not shown). Although this change would shorten the first external loop by two residues, its functional and physiological implications are currently unknown.

The TM domains suggested for the FcεR γ chains by PHD are well conserved among the four species studied (Fig. 12). The largest difference between the TRD and PHD TM domains was found in the case of the mouse protein, where the PHD method yielded the TM domain three residues shorter, with all the extra TM residues of the TRD sequence being at the N-terminus side (Fig. 12). In other cases the TM domains suggested by PHD are two residues shorter than those obtained from the hydropathy plots, with one extra residue on each side of the domains. It is not clear, however, whether Asp^{29}, the single negatively charged residue, (Fig. 12) is positioned at the depth which is always compatible with its proposed proximity to the positively charged residues in the TM domains of mouse *d* and *e* alleles of the FcγRI (Fig. 3) and the a.3 variant of FcαR (Fig. 8) where the positively charged residues are located near the membrane surface. A more detailed discussion of this, including helical wheel projections of FcεRIγ was given above (Figs. 2, 3, 5 and 6).

4. Conclusions

Numerous studies have shown that TM domains of the multisubunit Fc receptors are critical for their proper surface expression and signal transduction, primarily because of the role of these domains in the association of the subunits of multichain receptors. In addition, recent studies

provided significant advances in understanding folding of membrane proteins, conformation of the TM helices, and the role of specific amino acids in the interhelix interaction-driven membrane protein oligomerization [43,96–98]. Clearly, the lengths, locations and amino acid sequences of the TM regions are critical for determining the association of the corresponding integral membrane proteins with other membrane components. In most cases considered here, the TM domains predicted by the PHD were shorter than those assumed using the single-residue hydrophobicity analyses. Among consequences of these changes are altered (usually increased) intracellular tails of the receptors, and different lengths of their extracellular loops. Length of the TM domain is also an important parameter on its own: it determines the hydrophobic mismatch of the protein with the lipid bilayer and the tilt of its helix within the membrane that can affect the protein's function [99–102]. Additionally, Osborn et al. [103] have shown that changing the length of the TM domains of, e.g. MHC I is by itself sufficient for a decrease in its cell surface stability. Lankford et al. [104] have also observed that the capacity of the charged TM residues of CD3ε to induce ER retention is affected by the length of the TM domain. In many cases demonstrated in this study, the differences in TM segments predicted from PHD, as compared with the single residue hydrophobicity methods, resulted in altered amphipathic characteristics of the corresponding helices, implying the correspondingly different hydrophobic and hydrophilic surfaces and leading to revisions of helix–helix associations assumed using earlier methods.

Notwithstanding these developments, relatively little attention has been given to the identification of the precise locations and lengths of the TM helices. In most cases, TM regions have been deduced by visual inspection of the corresponding sequences and/or by single-residue hydrophobicity analyses. However, at times of rapidly growing knowledge of evolutionary relationships, basing predictions on single sequences is not justified. The currently available computer resources allow for a more in-depth analysis of the TM sequences which, in many cases, results in significant differences from the previously assumed locations. These results can lead to modifications of the putative TM motifs and to a re-interpretation of the studies of the mutated TM domains as illustrated here. Furthermore, the TM analysis algorithms like PHD may be used to efficiently design peptides with a potential to compete with the association processes of the receptor subunits and thereby to attenuate specific immune responses. Modulation of the cellular immune functions and of murine autoimmune disorders by the peptides mimicking TM regions of the TCR have indeed been recently reported in [105,106]. Such an approach may have a rather interesting application in therapy of human autoimmune disorders.

Acknowledgments

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