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Membrane protein misassembly in disease ☆

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ABSTRACT

Helix–helix interactions play a central role in the folding and assembly of integral α-helical membrane proteins and are fundamentally dictated by the amino acid sequence of the TM domain. It is not surprising then that missense mutations that target these residues are often linked to disease. In this review, we focus on the molecular mechanisms through which missense mutations lead to aberrant folding and/or assembly of these proteins, and then discuss pharmacological approaches that may potentially mitigate or reverse the negative effects of these mutations. Improving our understanding of how missense mutations affect the interactions between TM α-helices will increase our capability to develop effective therapeutic approaches to counter the misassembly of these proteins and, ultimately, disease. This article is part of a Special Issue entitled: Protein Folding in Membranes.

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1. Introduction

Integral α-helical membrane proteins play a fundamental role in cell viability, from acting as one of the initial members in a series of cell-signaling events, to transporting and regulating cellular levels of both small and large molecules to provide the required conditions for survival. These largely hydrophobic polypeptide chains fold into various membrane-bound organizations, including single- and multi-spanning transmembrane (TM)[2] domains coupled with both extra and intra-cellular soluble domains ranging widely in number and size. The assembly of eukaryotic membrane proteins requires a level of complexity above that of their cytoplasmic counterparts, because membrane proteins are extremely hydrophobic and must undergo the additional step of being threaded into the hydrophobic lipid bilayer.

Membrane protein folding has been simplified into a two-stage model, with insertion and α-helical formation of the nascent polypeptide in the membrane comprising the first stage, followed by helix–helix association into tertiary and/or quaternary structures [1]. To facilitate this process and ensure that the membrane-integrated protein is properly assembled, the cell has evolved systems of coordination and quality control collectively known as the proteostasis network (for recent reviews, see [2,3]). However, even with these systems in place, the assembly efficiency of many of these proteins is less than 50% at body temperature [4] and may explain why a change in amino acid sequence at a single site is enough to cause misassembly, leading to disruption of the proteostasis network and, ultimately, human disease. It would seem then that many membrane proteins exist literally on the edge of aggregation [5].

The TM α-helices of membrane proteins are a frequent target for disease-causing missense mutations [6–8]. This is not surprising given their significant role in membrane protein assembly [9]. In this review, we first demonstrate the importance of both TM α-helix sequence and helicity for their interactions with lipids and/or other helices to form specific higher-ordered structures — situations that can easily be altered via a single missense mutation resulting in a disease-causing misassembly of the protein. In subsequent sections, we focus on the molecular mechanisms through which these mutations cause membrane protein misassembly by disrupting or strengthening the helix–helix interactions within the TM domain, and recent approaches as to how such misfolding might be reversed.

2. Proteins in membranes — mutual accommodation of structures

The notion of a distinct hydrophobic/hydrophilic division by the plasma membrane does not convey the actual complexity of its structure. The membrane consists mainly of glycerophospholipids with a ~30 Å hydrophobic core of lipid acyl-chains of varying lengths and a ~15 Å interfacial region on either side containing polar head groups and water molecules that gradates into the bulk water (Fig. 1A) [10]. In higher animals, lipid side chains consist predominantly of 16 or 18 carbons with between 0 and 3 *cis*-double bonds,

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[2] TM, transmembrane; GpA, glycophorin A; CFTR, cystic fibrosis transmembrane conductance regulator; MC4R, melanocortin 4 receptor; ErbB2, human epidermal growth factor receptor; EGF, epidermal growth factor.

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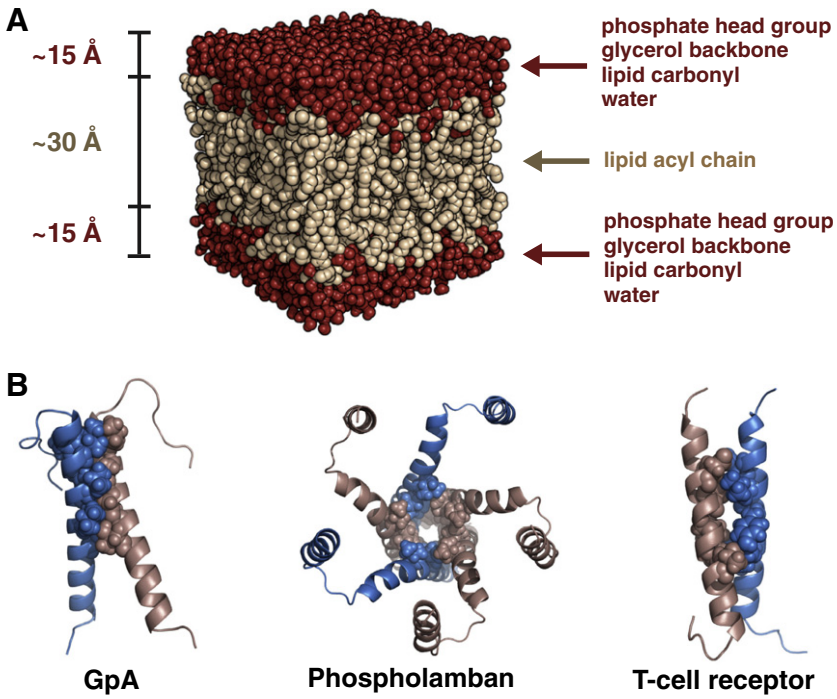

Fig. 1. Lipid structure and membrane protein helix–helix interactions. (A) A space-filling representation of the lipid bilayer. (B) Helix–helix interactions in the TM domains of GpA, phospholamban, and T-cell receptor. The TM α-helices of each protein are rendered as ribbons and the residues mediating the helix–helix interactions are highlighted as space-filling models. See Section 2.2 in the text for the identity of the residues in the interaction interfaces.

with various negative or zwitterionic polar head groups. Furthermore, the presence of cholesterol modulates the fluidity of the membrane. Interestingly, lipid composition varies in a tissue-specific manner and likely plays a significant role in protein folding which makes the understanding of membrane protein folding more challenging.

2.1. Sequence dictates membrane insertion

The principal requirement for a membrane protein TM domain is a stretch of ~18–25 amino acids that is energetically satisfied in the hydrophobic lipid bilayer. While soluble proteins generally consist of a melange of secondary structures including α-helices, β-sheets, and random-coiled regions, membrane proteins are limited by the hydrophobic environment of the lipid bilayer to α-helices or β-barrels since these structures can self-satisfy the H-bonding potential of the polar peptide backbone [11].

A TM domain does not explicitly contain only hydrophobic residues: approximately 20% of residues in the membrane are polar [12]. In this case, the energetic cost of introducing a polar or charged residue into a hydrophobic environment is likely offset by interactions with another polar residue or the peptide backbone (as is the case in some oligomerization motifs). As well, the formation of water- or ion-lined pores, snorkeling of amino acid side chains, and deformation of the membrane or helix to interact with the aqueous phase or lipid head groups, could alleviate the cost of placing polar substituents in the hydrophobic environment [13,14]. Additionally, the remaining residues on the TM segment are often correspondingly elevated in hydrophobicity in order to yield an overall threshold hydrophobicity that is permissive for membrane insertion [15]. Although several *in vitro* hydrophobicity scales for the 20 amino acids have been presented [16], seminal work by von Heijne has led to the identification of the positional insertion requirements using an *in vivo* translocon assay [17,18]. The residues that are decidedly hydrophobic and will promote insertion into the membrane when placed in the middle of the helix are (from most to least favorable): Ile, Leu, Phe, Val, Met, and Cys. As a general trend, polar residues are typically located near the ends of the helix and are likely to interact with the polar head groups and water molecules in the interfacial region of the membrane.

2.2. Helix–helix interactions in the membrane

The insertion of a TM α-helix in the membrane is the first determinant of a membrane protein, yet the fate of the protein is dependent on the helix stability in this environment: the helix will either remain solvated by lipid as a single-spanning monomeric membrane protein, or the helix will only be partially solvated, driving two or more helices together. Although membrane protein helices are solvated by lipid, acyl chains are not the ideal solvent for hydrophobic side chains that protrude irregularly into the cross section of the lipid bilayer. This relative instability promotes helix–helix interactions that will form at faces that are the least lipid-accessible [19]. These interactions occur largely via van der Waals or polar/electrostatic interactions, and involve specific sequence motifs that either fold the protein into its tertiary structure or allow for quaternary oligomerization. Outlined in Table 1 are the known eukaryotic interaction motifs, among which the more common are described below.

2.2.1. GG4, GAS_{right}, or right-handed motifs

It was noted that in nature, there is an abundance of Gly residues in TM α-helices that are found in GxxxG sequence motifs [20]. The right-handed packing of helices is mediated by Gly's that are separated four residues apart (hence GG4), placing them on the same helix face; in a side view of such a helix, the Gly residues are located one above the other due to the 3.6 residues per turn geometry of an α-helix [21] (Fig. 1B).

The prototypical dimer, glycophorin A (GpA), is a single-spanning human erythrocyte glycoprotein associated with blood group determination that employs a LIxxGVxxGVxxT dimerization sequence [22–25]. The Gly residues appear as 'holes' in the helix surface and dimerization occurs due to 'knobs-into-holes' packing, with the adjacent Val 'knob'

Table 1
Experimentally-determined eukaryotic transmembrane oligomerization motifs.

Protein[a]	Transmembrane sequence[b]	Oligomer state	Ref
GG4 (GAS_{Right}/Right-handed)			
GpA	^{72}EITLIIFGVMAGVIGTILLISYGIRRL^{98}	Dimer	[22,24,25]
MPZ	^{125}YGVVLGAVIGGVLGVVLLLLLGYVV^{150}	Dimer	[73]
ErbB-2	^{651}LTSIVSAVVGILLVVVLGVVFGILI^{675}	Dimer	[33,74,75]
BNIP3	^{164}VFLPSLLLSHLLAIGLGIYIGR^{185}	Dimer	[76]
NRP1	^{857}ILITIIAMSALGVLLGAVCGVVLY^{880}	Dimer	[77]
EphA1	^{548}IVAVIFGLLLGAALLLGILVF^{568}	Dimer	[78]
RPTP DEP1	^{976}VICGAVFGCIFGALVIVTVGGFIFW^{1000}	Dimer	[79]
APP[c]	^{700}GAIIGLMVGGVVIATVIVITLVML^{723}	Dimer	[80]
APP[c]	^{700}GAIIGLMVGGVVIATVIVITLVML^{723}	Dimer	[81]
Kitl TMD	^{215}WTAMAFPALISLVIGFAFGAFYW^{237}	Dimer	[82]
AdipoR1 TM5	^{268}AGVFLGLGLSGVVPTMHFTIA^{288}	Dimer	[83]
Na,K-β	^{36}LLFYVIFYGCLAGIFIGTIQVMLLTI^{61}	Dimer	[84]
ABCG2 TM1	^{394}ASIAQIIVTVVLGLVIGAIYFGL^{416}	Dimer	[85]
Syn TM2	^{145}VLAAVIAGGVIGFLFAIFLILLLVY^{169}	Dimer	[86]
Syn TM3	^{385}VLVAVIVGGVVGALFAAFLVTLLIY^{409}	Dimer	[86]
Syn TM4	^{146}VLAALIVGGIVGILFAVGLILLLMY^{170}	Dimer	[86]
hCTR1[d]	^{136}TVLHIIQVVISYFLMLIFM^{154}	Trimer	[87]
Heptad (GAS_{Left}/Left-handed)			
PLB	^{31}LFINFCLILICLLLICIIVMLL^{52}	Pentamer	[30,31,88]
PDGFβR[e]	^{531}KVVVI**S**AI**L**AL**L**VVLTVI**S**LII**L**IMLW^{556}	Dimer or trimer	[89]
EphA2	^{535}LAVIGGVAVGVVLLLVLAGVGFFI^{558}	Dimer	[90]
SR-BI	^{9}WVALGLGALGLIFAALGVVMILMVPSLI^{36}	Dimer	[91]
DDR1	^{418}ILIGCLVAIILLLLIIALMLW^{439}	Dimer	[92]
ANTXR1	^{318}ILAIALLILFLLLALALLWW^{337}	≥Dimer	[93]
Polar			
TNF5	^{26}YLLTVFLITQMIGSLFAVYL^{46}	≥Dimer	[94]
TCR ζ-chain	^{28}DSKLCYLLDGILFIYGVILTALFLRVKFSR^{57}	Dimer	[32]
Ii	^{30}GALYTGFSILVTLLLAGQATTAYFLY^{55}	Trimer	[95]
RET	^{635}RTVIAAAVLFSFIVSVLLSAFCI^{657}	≥Dimer	[96]
DAP12	^{7}GVLAGIVMGDLVLTVLIALAVYFL^{30}	Dimer	[97]
FUT3	^{17}LAALLFQLLVAVCFFSYL^{34}	≥Dimer	[94]
Hetero-oligomers			
αIIb	^{968}WVLVGVLGGLLLLTILVLAMK^{988}	Dimer, reciprocal large-small motif	[98]
β3	^{693}ILVVLLSVMGAILLIGLAALLIWK^{716}		[98]
ErbB-1	^{645}SIATGMVGALLLLLVVALGIGLFM^{677}	Dimer	[99]
ErbB-2	^{651}LTSIVSAVVGILLVVVLGVVFGILI^{675}		[33,74,75]
DAP12	^{7}GVLAGIVMGDLVLTVLIALAVYFL^{30}	Trimer	[97]
NKG2C	^{61}ELFPILVITKLVTAVLVISIIGLV^{38}	(DAP12 dimer + NKG2C)	

[a] GpA, human erythrocyte protein glycophorin A; MPZ, myelin protein zero; ErbB-2, epidermal growth factor receptor tyrosine kinase 2; BNIP3, Bcl-2/19 kDa interacting protein 3; NRP1, neuropilin-1; EphA1, erythropoietin-producing hepatocellular receptor A1; RPTP DEP1, human receptor-like protein tyrosine phosphatase; APP, Alzheimer precursor protein; Kitl, mouse stemcell factor Kit-ligand; AdipoR1 TM5, human adiponectin receptor; Na,K-β, Na,K-ATPase β-subunit; ABCG2 TM1, human ABC multidrug transporter G subfamily; Syn TM2, syndecan; Syn TM3, syndecan; Syn TM4, syndecan; hCTR1, human copper transporter 1; PLB, cardiac phospholamban; PDGFβR, platelet-derived growth factor β-receptor; EphA2, erythropoietinproducing hepatocellular receptor A2; SR-BI, HDL Scavenger receptor class B type I N-terminal TMD; DDR1, human discoidin domain receptor tyrosine kinase 1; ANTXR1, anthrax toxin receptor 1; TNF5, tumor necrosis factor 5/CD40-ligand; TCR ζ-chain, T-cell surface glycoprotein CD3; Ii, class II MHC receptor Ii protein; RET, RET receptor tyrosine kinase; DAP12, TYRO protein tyrosine kinase-binding protein; FUT3, fucosyltransferase 3; αIIb, human platelet membrane glycoprotein αIIb; β3, human platelet membrane glycoprotein β3; ErbB-1, epidermal growth factor receptor tyrosine kinase 1; NKG2C, natural killer cell-activating receptor.

[b] The numbering indicates the start and end residue number in the natural TM domain. Residues determined to stabilize TM association are underlined.

[c] Two dimer sites have been proposed for APP.

[d] hCTR1 utilizes a unique MxxxM right-handed motif.

[e] Bolded residues are used in addition to the underlined residues by PDGFβR to form a trimer.

residues supporting the structure through van der Waals interactions. Recent work suggests that GG4 interactions may be more complex than previously thought, with the identities of the 'knob' residues playing an important role in the oligomerization strength of this interaction [26]. In the latter study, it was determined that the adjacent Val residues can be replaced with Ile, but not Leu or Ala, in order to maintain dimerization, a result which suggests a requirement for β-branched residues at this location. Other possible interactions in the motif are weak H-bonding between Cα-H groups on one helix and the carbonyls of the opposite helix [27,28]. Nevertheless, the specific role(s) of Leu and Ile residues in this GpA local sequence are not fully understood, yet likely form further van der Waals interactions as mutation of these residues affect dimerization. The remaining Thr forms polar interactions with the backbone of the opposite monomer.

These sequences are also referred to as GAS_{right} since they have right-handed crossing angles and other small residues such as Ala and Ser can replace the Gly in the GG4 motif [21]. Although only a handful of GG4 motifs have been structurally characterized, these oligomerization sequences likely play a major role in both tertiary and quaternary helix–helix interactions in membrane proteins due to the many possible combinations of Gly, Ala, and Ser that occur four residues apart in TM sequences.

2.2.2. Heptad, GAS_{left}, or left-handed motifs

As with the GG4 motif, Gly, Ser, and Ala residues can accommodate the close approach of helices in a typical heptad repeat which leads to left-handed crossing angles [21,29]. Examples of the GAS_{left} motif are not as common as the GAS_{right} motif. However, due to the increased tendency of small residues to situate in heptad motifs, it is likely that these motifs are commonly found in nature, especially in tertiary contacts.

The heptad repeat can also contain large residues, as in the case of the Leu-Ile zipper, which drives the oligomerization of the phospholamban pentamer [30,31] (Fig. 1B). Phospholamban is found in cardiac sarcoplasmic reticulum and is associated with the regulation of calcium pump activity. This membrane protein utilizes an IxxxLxxIxxxLxxIxxxL heptad repeat (typified with letters *abcdefg*), with the Leu residues at the *a* positions and the Ile at the *d* positions, resulting in the large hydrophobic residues lining a single face of a helix while mediating helix–helix contacts via van der Waals packing interactions.

2.2.3. Polar motifs

Polar residues in the membrane often lead to hydrogen bonding between the side chains of two or more helices. These categories of oligomerization sequences are highly variable and can arise from an interaction between a single polar residue on each helix or from interactions between multiple residues on each helix face. An example is the T-cell receptor ζ-chain, which serves as a portion of the T-cell receptor complex that is responsible for recognizing antigens bound to the major histocompatibility complex. The T-cell receptor ζ-chain utilizes polar residues in a DxxLxxYxxxLT motif to form an H-bonded dimer in the membrane that includes an Asp-Asp inter-monomer interaction and Tyr-Thr, Thr-Tyr interactions [32] (Fig. 1B). Other interactions that have been determined to mediate helix–helix interactions in the membrane via polar/electrostatic mechanisms include cation–π and π–π between aromatic and/or basic amino acids [33–36].

3. Missense mutations and disease — molecular mechanisms

In this section of the review, we focus on the molecular mechanisms through which missense mutations in the TM domain of membrane proteins lead to misassembly. We define misassembly here as broadly encompassing both defects in tertiary (misfolding) and quaternary (aberrant oligomerization) structure formation. Misassembled species may ultimately cause disease by affecting the normal trafficking of the protein, resulting in, but not limited to, decreased amounts of functional protein at the destination membranes; entrapment of toxic gain-of-function protein in the ER; and/or

overwhelming the ER quality control components which would then invoke the unfolded protein response and apoptosis [37]. Alternatively, misassembled membrane proteins may arrive at their destined membranes but their abnormal functions lead to disease [38].

3.1. Disrupting helix–helix interactions

Membrane protein assembly is highly dependent on TM α-helices interacting in a sequence-specific manner (as exemplified by the numerous interaction motifs listed in Table 1). These interactions can loosely be categorized into those that allow for optimal van der Waals packing and/or polar/electrostatic interactions. It follows that non-conservative missense mutations to the residues in these motifs (*i.e.*, polar to non-polar, large to small) would impose a folding burden on a TM domain, and potentially cause membrane protein misassembly (Fig. 2). For example, conversion of glycine's side chain volume from small to that of a larger residue would interrupt the tight "knobs into holes" packing found at GG4 interfaces. Similarly, mutating away the H-bonding capacity of a residue at an interaction interface would be disruptive if the α-helices are held together by H-bonds.

The strength of association mediated by a helix–helix interaction motif is heavily influenced by variables such as the context of surrounding residues and position of the motif relative to the center of the bilayer [39–41], which suggests that disruptive missense mutations would have wide-ranging effects on misassembly depending on the location of the motif. This might be a factor in why different disease-causing mutations in the TM domain of membrane proteins can show variation in the severity of disease-phenotype [42].

3.1.1. Examples of disease-causing mutations that cause misassembly via disrupted helix–helix interactions

There are several known membrane protein-linked diseases that are a result of non-conservative mutations that disrupt folding and/or oligomerization. For example, the mutation V509A in the thyrotrophin receptor alters the tertiary interactions of the membrane protein by disrupting the tight van der Waals packing between TM3 and TM5. This disruption imbues the helices with more conformational flexibility and increases the activity of the receptor, which in turn leads to congenital hyperthyroidism [43].

In the case of quaternary structure disruption, the G163R and G167R mutations in the TM α-helix of myelin protein zero — a cell adhesion membrane protein found in the cellular membrane of Schwann cells — are associated with Charcot–Marie–Tooth disease and Dejerine–Sottas syndrome [44]. These mutations target the small residues of the glycine-zipper motif found in the helix of this protein,

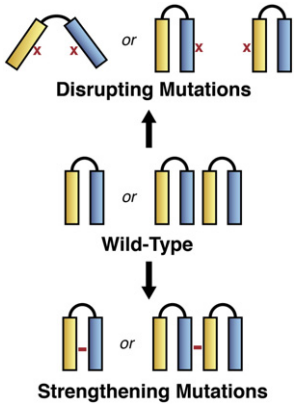

Fig. 2. Effects of missense mutations on membrane protein structure. The hairpins represent the TM domain of a simple membrane protein where the yellow and blue cylinders are individual TM α-helices linked together via a loop. Missense mutations can affect helix–helix interactions within a protein monomer or between oligomers by changing the strength of the interactions. Red Xs and bars represent disrupting and strengthening mutations, respectively.

weakening the tight packing interactions and likely disrupting proper tetramer formation [45]. Similarly, in connexin26, the mutation T135A abolishes the H-bonding capacity of TM3 and destabilizes the hexameric oligomer, leading to hereditary deafness [46].

Disruption of helix–helix interactions is not limited to non-conservative missense mutations. Mutations that are traditionally considered conservative may also have an effect if the specific property modulating the interaction is targeted. For example, Glu325 in TM10 of the *E. coli* lactose permease normally interacts with Arg302 in an adjacent helix. However, mutating this residue to a similarly charged side chain, Asp, reduces transport activity. This phenomenon is attributed to the shortened side chain in the mutant, which apparently weakens the H-bonding interaction with the partner residue [47]. As seen in this example, non-conservative missense mutations could foreseeably be disruptive in disease-associated membrane proteins.

3.2. Strengthening helix–helix interactions

Missense mutations can also strengthen or create helix–helix interactions that can lead to membrane protein misassembly (Fig. 2). In the case of an existing helix–helix interaction site, mutation of residues at or near the interaction interface may further stabilize or enhance the interaction. For example, in the classic dimerization motif of GpA, mutating the Val residues immediately adjacent to Gly79 and Gly83 to other large hydrophobic residues enhances the extent of dimerization relative to the wild-type protein [26]. A similar effect is seen if a Phe is added to the −3 position relative to Gly79 [48]. Alternatively, a missense mutation could ostensibly generate a novel helix–helix interaction site in the TM α-helix and drive non-native helix associations; thus, the introduction of polar residues to a hydrophobic peptide can drive helix–helix interactions in the membrane [49]. The strengthening/creation of helix–helix interactions could be deleterious for membrane protein assembly, because they could promote non-native conformations within the TM domain of a protein chain and/or aberrant oligomerization between protein chains.

3.2.1. Mutations that cause misassembly via strengthened helix–helix interactions

Cystic fibrosis has been attributed to ER trafficking defects caused by misassembly of the cystic fibrosis transmembrane conductance regulator (CFTR). Our lab previously showed that the mutation V232D in a hairpin construct containing the TM3 and 4 α-helices of CFTR could potentially lock the two helices into a compact conformation through the formation of a non-native interhelical H-bond [50]. The tightly interacting helices may restrict the dynamics/flexibility required for channel function and/or reorient the helices, changing the overall protein fold. A similar scenario is seen for the melanocortin 4 receptor (MC4R) — a GPCR that plays a role in energy homeostasis. Several missense mutations in the TM domain increase the helix packing and/or H-bonding interactions between the helices, effectively locking the receptor in an inactive conformation, leading to dominantly inherited human obesity [51].

Strengthening of helix–helix interactions can also affect membrane protein oligomerization. An I655V mutation in the TM α-helix of ErbB2 (human epidermal growth factor receptor) has been shown to increase the risk of breast cancer. This is likely due to the stabilizing effect of the mutated residue on the nearby SxxxG interaction motif, which increases the population of the active conformation of the receptor dimer [38]. Additionally, in the rat ErbB2 homologue neu, a mutation of V664E also leads to permanent dimerization and activation of the receptor, resulting in the formation of tumors [52]. This phenomenon has been mainly attributed to H-bonding between Glu residues of the monomers [53]. However, other workers presented evidence that the Glu residues do not interact directly, and the stabilization of the active conformational form of the dimer is a result

of the polar residue shifting the TM α-helices to favor one adjacent helix–helix interaction motif over another [54]. In either case, the outcome of the mutation is an increase in helix–helix interactions that leads to aberrant oligomerization.

3.3. Misassembly due to missense mutations changing helix–lipid interactions

The helix–helix interactions that assemble membrane proteins can be greatly affected by the lipid bilayer in which the TM α-helices reside. Depending on the difference in property between helix and membrane, the effect can strengthen or disrupt helix–helix interactions. One such property is the hydrophobic length or thickness of a TM α-helix relative to that of the lipid bilayer. If the lengths do not match, the helix might change its conformation in order to accommodate this hydrophobic mismatch [55]. In the situation where the helix is interacting with another helix, this change in conformation could either enhance or disrupt the interaction. For example, when the dimerization of GpA is examined in lipid bilayers of varying thicknesses, the extent of dimerization decreases when there is hydrophobic mismatch [56]. This effect has been attributed to a change in the tilt angle of the TM helices, resulting in a less efficiently packed helix interaction interface. Other lipid–protein interaction properties that might affect the assembly of TM α-helices could include lipid solvation of the helix [19], and occurrence of interfacial-lipid anchoring residues in the helix termini [57,58]. Missense mutations that alter these properties could have foreseeable consequences on helix–helix interactions, resulting in membrane protein misassembly.

3.4. Missense mutations and misassembly in multi-spanning membrane proteins

Helix–helix interactions are important in mediating the assembly of both single-spanning and multi-spanning membrane proteins. The main difference is that when a membrane protein is comprised of a single TM α-helix, any helix–helix contacts would mediate only quaternary structure, whereas for membrane proteins containing multiple helices, these contacts can mediate both tertiary and quaternary structure [59]. This situation indicates that the effect of missense mutations on membrane protein misassembly would be considerably more complex in multi-spanning vs. single-spanning membrane proteins. For example, a mutation in the TM domain of a polytopic species could hypothetically affect its oligomerization state by (1) directly affecting the helix–helix interaction sites mediating the oligomer; or (2) affecting the helix–helix interactions mediating the fold of the monomer that would then indirectly affect the interactions mediating the oligomer. It is possible that a missense mutation that disrupts the monomer fold of a multi-spanning membrane protein might allow interaction sites that are normally masked by the properly folded protein to become surface-accessible, leading to aberrant assembly of the protein oligomer [5]. This type of mechanism may underlie disease-causing point mutations in the myelin proteolipid protein that initiate premature oligomerization and results in Pelizaeus–Merzbacher disease [8,60]. In an analogous manner, the L16P *Trembler-J* mutation in the TM1 (and, perhaps, the G150D *Trembler* mutation in TM4) of peripheral myelin protein 22 likely results in disease by disrupting the protein's tertiary fold, which exposes the TM1 α-helix such that calnexin aberrantly binds to it, leading to the retention and toxic accumulation of the misassembled protein in the ER [61].

4. Can disease-phenotypic misassembly be reversed?

Membrane protein misassembly causes disease either by the protein's inability to traffic to the proper membrane or by its improper function once successfully at the membrane. In either case, we can conceivably use pharmacological molecules to regain trafficking to the membrane or to alter the misfolded product to restore function. Furthermore, an exogenous molecule could be used to inhibit or modulate a properly functioning protein that is giving an undesired effect in a given scenario.

A protein mutation that is conceivably functional, yet gives a diseased phenotype, often occurs due to proteins being flagged as misfolded by the ER quality control system. This results in ER-trapped proteins that cannot successfully traffic to the cell surface for function. A potential treatment for this condition came in the form of small, non-peptide molecules (termed 'pharmacoperones'), originally identified as GPCR antagonists, that were able to selectively rescue cell surface expression and function of mutant GPCRs [62,63]. The general mechanism of action by these pharmacoperones is thought to consist of the molecule penetrating the plasma and ER membranes to bind to the partially folded receptor. This binding shifts the thermodynamic equilibrium towards the correctly folded conformation and results in an increased amount of protein that is then able to escape the stringent quality control system of the ER and reach the cell surface [64] (Fig. 3A). To-date, the trafficking of four misfolded GPCRs that cause disease has been rescued by the use of these pharmacoperones: rhodopsin causing retinitis pigmentosa (*e.g.*, 9-cis-retinal) [65]; vasopressin type 2 receptor causing nephrogenic diabetes insipidus (*e.g.*, satavaptan) [66]; gonadotropin-releasing hormone receptor causing hypogonadotropic hypogonadism (*e.g.*, indoles) [67]; and calcium-sensing receptor causing familial hypocalciuric hypercalcemia (*e.g.*, NPS R-568, N-(3-[2-chlorophenyl]propyl)-(R)-α-methyl-3-methoxybenzylamine) [68]. Given the high number of membrane protein misfolding leading to improper trafficking, and ultimately disease, the numbers of pharmacoperones could be greatly increased.

Misassembled proteins that successfully traffic to the membrane can also lead to disease by a loss or gain of function and/or oligomerization. Small molecules that could interact with either the TM domains or the loop regions of such proteins would be ideal in correcting this misassembly. A further possibility would be the use of TM peptide fragments that could interact with the helix of a naturally occurring defective membrane protein. Endogenous TM segments often modulate function of membrane proteins, and peptides that mimic this control could be employed in disease treatment (Fig. 3C). A study of the Na/K-ATPase sodium pump determined that its enzymatic activity — which is normally regulated by a γ-subunit — was modulated by synthetic peptides mimicking the γ-subunit [69]. Similarly, the calcium channel γ_6 subunit inhibits Cav3.1 low voltage-activated calcium current in human embryonic kidney cells and cardiomyocytes using a critical GxxxA motif in TM1. Thus, with an eight-residue peptide where the native sequence contains this GG4 motif, specific inhibition was obtained of the Cav3.1 calcium current, a finding that has implications for future use as in a therapeutic context after myocardial infarctions [70].

Inhibiting homo-oligomerization by peptides may also be an area of disease treatment (Fig. 3B). As proof of principle, this has been accomplished using peptides that target the Class II G-protein coupled secretin receptor, which functionally oligomerizes at TM4. It was found that by synthesizing TM4 peptides of an identical sequence to the native GPCR and treating cells expressing the GPCR with such peptides, the function of the GPCR was reduced [71]. Furthermore, the single-spanning membrane protein ErbB2 is a tyrosine kinase receptor for the epidermal growth factor (EGF) that via a mutation can be constitutively activated as a dimer leading to cancer. Both synthetic peptides and short vector-encoded fusion peptides with the native TM sequence specifically inhibit the ErbB2 dimerization, thus abolishing the signaling pathway [72]. Although the examples of peptides targeting TM domains are few, it is a promising area of study if the proper conditions for treatment by largely hydrophobic peptides can be devised.

5. Concluding remarks

The amino acid sequences of membrane protein TM α-helices have been shown to play a demonstrably significant role in directing the

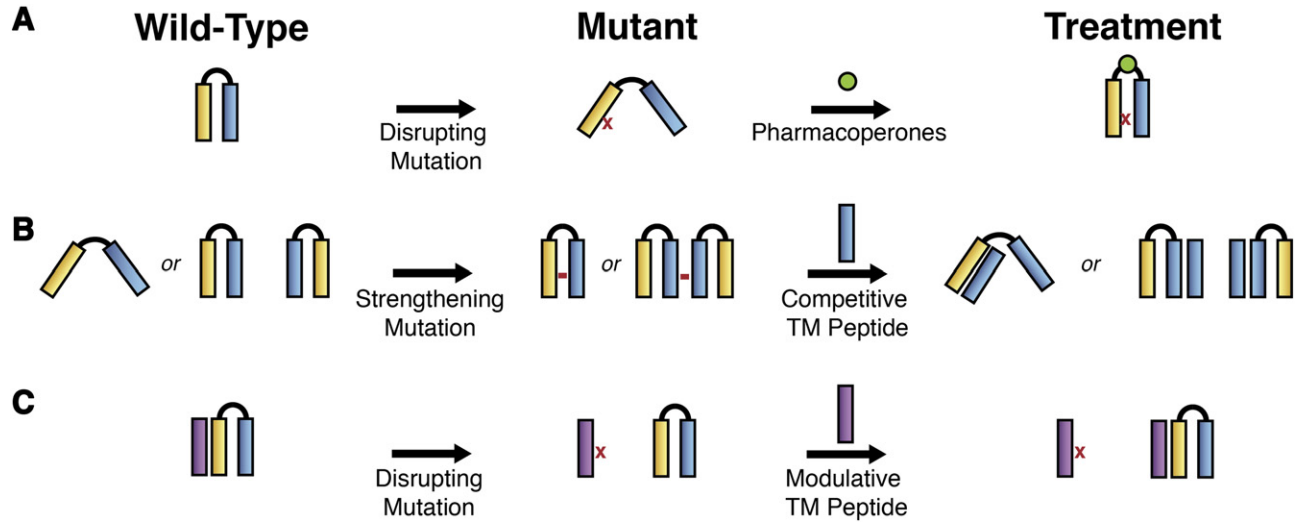

Fig. 3. Reversing membrane protein misfolding through a pharmacological approach. The cylinders represent the TM α-helices of membrane proteins. (A) Pharmacoperones (green) correct misfolded membrane proteins and allow them to escape the ER quality control system. (B) Mutations that increase the strength of helix–helix interactions or create novel interaction sites in the TM domain of a membrane protein may be corrected in a competitive manner by a TM peptide containing the wild-type sequence of the helix partner. (C) The helix–helix interactions of two membrane proteins that are disrupted by mutations may be rescued through the use of exogenous peptides that mimic the wild-type TM α-helix sequence(s) of the mutated protein.

helix–helix interactions that lead to correct folding and assembly of these proteins. As such, even a single missense mutation at a critical site on a helix can be detrimental to the folding and/or function of the protein. Our increasing knowledge of the mechanisms through which a membrane protein assembles into its native structure is now providing new insights as to how mutations can compromise this process. Improvement of our understanding of these concepts will allow us to increase our arsenal of approaches to counter membrane protein misassembly — and ultimately human disease.

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