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Helix–helix interactions inside lipid bilayers

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Far from being simple hydrophobic anchors, it is now clear that the transmembrane α-helices of integral membrane proteins can participate in strong, specific interactions that are important in their folding and oligomerization. Crystallographic studies of 21 such helices have indicated that these interactions are similar to those described for soluble proteins. Helix–helix interactions are also important in the oligomerization of a number of proteins that have a single transmembrane α-helix. The interactions are rather specific, involving interhelical salt bridges, hydrogen bonds or precise packing interactions. In some cases, such oligomerization is required for exit from the endoplasmic reticulum. The transmembrane helices of some Golgi-residing proteins also contain sufficient information to ensure their retention in this compartment. Finally, interactions between transmembrane α-helices may be important in the mechanism of transmembrane signalling by a number of membrane-bound receptors.

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Introduction

While the general notion that membrane proteins often contain largely hydrophobic transmembrane helices has been accepted for more than a decade, the specificity of their mutual interactions and the diversity of their roles are only now becoming apparent. Far from being simple hydrophobic anchors which locate proteins at a membrane, or weakly interacting structural elements which are stabilized by interactions outside the bilayer, it is now clear that transmembrane helices can participate in highly specific interactions. These interactions involve sufficient energy to drive folding or oligomerization in some cases, and are being shown to contribute to an increasingly diverse set of functional roles. In this review, we highlight work of the past year in light of the conceptual and experimental background that went before. A great deal of progress has been made and the pace is quickening.

A useful conceptual framework for the consideration of helix–helix interactions within lipid bilayers is provided by the two-stage model for the thermodynamics of folding of integral membrane proteins [1]. In stage I, independently stable α-helices are established across the lipid bilayer, and in stage II these interact to form functional transmembrane structures. Thus, the energetics of helix formation can be separated conceptually from those of the interactions between these helices to form higher-order structures. The model can be applied equally well to the helix–helix interactions occurring within polytopic membrane proteins and to those involved in oligomerization.

Structural studies have characterized 21 transmembrane α-helices

Most data concerning the structure of α-helical integral membrane proteins comes from X-ray crystallography of bacterial photosynthetic reaction centers (RCs; for reviews, see [2,3]), electron crystallographic [4] and neutron diffraction [5] studies of the structure of bacteriorhodopsin, and, most recently, electron crystallography of the plant light-harvesting complex [6••], which together have a total of 21 transmembrane helices. The detailed consideration of helix–helix interactions in these cases is complicated by the influences of the abundant cofactors. Nonetheless, a view emerges from these structures that the packing of the interior of integral membrane proteins is as efficient as that generally observed for water-soluble proteins. It is noteworthy that no interhelical salt bridges are seen, in contrast with what is proposed below for interactions between transmembrane α-helices in the oligomerization of some bitopic membrane proteins. Furthermore, each helix contains, on average, less than one interhelical hydrogen bond. In the case of bacteriorhodopsin, for example, the map obtained from electron crystallographic studies [4] suggests that Asp212 in helix G is involved in hydrogen bonding to Tyr57 of helix B, Trp86 of helix C and to Tyr185 of helix F. Residues buried in the interior of these proteins are, on average, more hydrophilic than those which are lipid-exposed, and are also found to be the most well conserved between species. From the analysis of Rees *et al.*

Abbreviations

CD—circular dichroism; **EGF**—epidermal growth factor receptor; **EGF-R**—EGF factor; **ER**—endoplasmic reticulum; **GpA**—glycophorin A; **GT**—β1,4-galactosyltransferase; **NGF-R**—nerve growth factor receptor; **RC**—photosynthetic reaction center; **SDS**—sodium dodecyl sulfate; **ST**—α2,6-sialyltransferase; **TCR**—T-cell receptor.

[7], this appears to be a general phenomenon for polytopic α-helical integral membrane proteins (e.g. [8]).

In the 6 Å structure of the trimeric plant light-harvesting complex [6••], two of the three transmembrane α-helices are longer than those seen in bacteriorhodopsin or the RC, and are associated as in a right-handed supercoil [9], separated from one another by a contact distance of 10 Å. The subunit boundaries in the trimeric complex are not unambiguous, but it is clear that both helix–helix and helix–chromophore contacts stabilize the structure of the individual subunits as well as the interactions between them.

One aspect of the interaction between α-helices in lipid bilayers that has received attention during the past year is the role of proline residues, which occur frequently in the transmembrane domains of transport proteins. A proline residue in the middle of a transmembrane α-helix results, in many (but not all) cases, in a kink in that helix. Analysis of such kinked helices in the RC and bacteriorhodopsin structures has suggested that they tend to bury their convex sides against other helices [10•]. The exception is helix C of bacteriorhodopsin, for which the convex, most hydrophobic, face is in contact with lipid. Consideration of a number of proline-containing transmembrane α-helices predicted from sequence analysis has suggested that, in general, the postulated convex side is the most polar face of the helix, which is predicted to be disposed towards the protein interior or towards the pore of a channel [10•,11•]. Indeed, where charged residues appear in such transmembrane domains, they tend to lie on the expected convex face. In contrast, in water-soluble proteins, the convex side of proline-kinked α-helices tends to be exposed to the solvent. It has also been proposed that proline residues in transmembrane α-helices may provide structural rigidity which optimizes the positioning of important side chains (for a review, see [12]).

Helix-containing fragments of membrane proteins can reassociate

Chymotryptic cleavage of bacteriorhodopsin yields two fragments: C1, which contains five of the seven transmembrane α-helices; and C2, which contains the remaining two helices. The two fragments have been isolated and reconstituted into separate populations of lipid vesicles, and were seen by circular dichroism (CD) to retain their α-helical structure. Upon mixing of the two populations of vesicles, followed by vesicle fusion, the fragments reassociate. The resulting complex binds retinal and acquires spectroscopic properties characteristic of native bacteriorhodopsin, as well as the lattice formation characteristic of purple membrane [13,14]. Recently, this approach has demonstrated a three-way association of C1, helix A and helix B (the two helices of C2) to form bacteriorhodopsin [15••]. Circular dichroism and Fourier transform infrared spectroscopy studies of the isolated A and B helices have shown them to be independently stable transbilayer α-helices (JF Hunt, O Bousche, KM Meyers, KJ Rothschild, DM Engelman, abstract W-AM-K2, 35th Annual Meeting of the Biophysical Society, San Francisco, February 1991). These data demonstrate that the covalent linkages between helices A and B and between helices B and C are not required for the correct folding of bacteriorhodopsin, and show that interactions between transbilayer α-helices are a major determinant in the folding of this integral membrane protein.

Similar experiments have also been reported for *lac* permease from *Escherichia coli*, which has 12 putative transmembrane α-helices. Co-expression of a two-helix fragment with a 10-helix fragment [16] or of two six-helix fragments [17] resulted in restoration of lactose transport, where none of the individual fragments was capable of transporting lactose. It should, however, be noted that the individual fragments appear to be degraded when expressed alone. Functional β_2 adrenergic receptor has also been obtained in *Xenopus* oocytes by the co-expression of two fragments corresponding to helices 1–5 and helices 6–7, respectively [18]. These results provide further support for the two-stage model for folding of integral membrane proteins [1].

Helix–helix interactions in oligomerization

There has been much study over the past year of the role of intramembraneous helix–helix interactions in the assembly of oligomeric complexes of proteins containing single transmembrane α-helices. In the absence of oligomerization, degradation of subunits can occur in the endoplasmic reticulum (ER). The main system for such studies is that of the T-cell receptor (TCR; for a review, see [19]). It has been shown that the single transmembrane domain of TCRα, which includes two basic amino acid side chains, can target this protein for degradation in the ER (for a review, see [20]). Furthermore, a nine-amino-acid segment from the transmembrane domain of TCRα, which includes these two basic residues, contains all of the information necessary for its association with CD3δ, which has one acidic residue in its single transmembrane domain [21]. The interaction of TCRα with CD3δ masks determinants in both of these proteins for ER degradation, i.e. in the absence of CD3δ, TCRα is degraded, whereas in the absence of TCRα, CD3δ is degraded. Thus, it appears that the transmembrane determinants for association of TCRα with CD3δ and for ER degradation are co-localized [22], such that if oligomerization occurs, degradation is blocked.

Reports published this year have delineated further the roles of the potentially charged residues in the transmembrane domains of these receptor subunits. Bonifacino *et al.* [23••] reported that a single arginine or aspartic acid residue can cause targeting for retention and degradation in the ER when placed at central positions within the transmembrane domain of the Tac antigen, which is normally transported to the cell surface. One hypothesis that arises from these data is that interaction of these polar groups with a transmembrane domain of a protein of the ER degradation apparatus may occur. The same laboratory also reported further studies concerning the role of these potentially charged residues in the assembly of the TCR complex [24••]. In a chimeric protein consisting of the extramembraneous portion of Tac and the trans-

membrane domain of TCRα, mutation of either one of the basic residues has relatively little effect upon interaction with CD3δ, whereas mutation of both of them completely abrogates the association. The TCR clonotypic δ chain, which has the same two basic residues in its transmembrane domain as TCRα, was also able to interact with CD3δ. Mutation of one residue in the transmembrane domain of Tac to an arginine could cause this protein to interact with CD3δ in much the same way as such mutations cause Tac to be degraded in the ER [23••]. Mutation of the single aspartic acid residue in the transmembrane domain of CD3δ abrogated the interactions of this protein with TCRα and TCRδ as well as the Tac mutants bearing a basic residue in the transmembrane domain. By altering the position of this aspartic acid residue within the transmembrane domain, the influence of the position of the arginine residue within transmembrane domain of Tac upon its association with CD3δ was altered. Most effective interaction was seen when the acidic residue of CD3δ and the basic residue introduced into Tac were such that these residues would be at approximately the same level in the lipid bilayer. These results were interpreted as suggesting that interhelical salt bridges, which are expected to be very strong in the low dielectric environment of the membrane [25–27], are important in the assembly of the TCR complex. It should be noted that the intramembraneous interaction may either occur between formal charges or involve strong hydrogen bonding of the uncharged groups [25–27]. In contrast with this case, no interhelical salt bridges are seen in the RC structures determined at high resolution, or in bacteriorhodopsin.

A situation similar to that for TCR assembly is also seen for the assembly of the transmembrane form of the Fcγ receptor (FcγRIII or CD16) (for reviews, see [28,29]). The amino acid sequence of the single transmembrane domain of this receptor is highly conserved between species, as is also true for other FcγRs [28]. In order for the α-subunit of FcγRIII to reach the plasma membrane, it must associate with either the γ-subunit of FcεRI or the ζ-subunit of the TCR/CD3 complex or both (as $\alpha\gamma_2$, $\alpha\zeta_2$ or αγζ) [30••]. The γ- and ζ-subunits both have a single transmembrane domain, which is almost identical in the two cases, and contains one aspartic acid residue. In a manner analogous to that described for the TCRα–CD3δ case, γ or ζ protects FcγRIIIα from degradation in the ER. Studies of chimeric proteins [30••] suggest, as for TCRα, that the signal that determines the degradation of FcγRIIIα in the ER resides in its transmembrane domain, which contains an aspartic acid residue. Interaction between the transmembrane domain of the α-subunit and that of γ or ζ appears to mask this determinant. This hypothesis is strengthened by the finding that substitution of an isoleucine for a leucine residue in the transmembrane domain of human ζ reduces the extent of its interaction with FcγRIIIα by 65 %. This leucine is conserved in human γ, but is an isoleucine in mouse ζ. Mutation of this isoleucine in mouse ζ to leucine led to a fivefold increase in its association with FcγRIIIα. No such effects were seen when mutations were made at the few other positions not conserved in mouse ζ. Thus, these data clearly suggest that specific interactions between transmembrane α-helices are important in this assembly process. Each of the aspartic acid residues in the transmembrane domains of α, ζ and γ is important for the interhelical interactions.

There exist other examples of ER retention signals in transmembrane domains which do not involve potentially charged residues. For example, membrane IgM is retained in the ER of non-B cells. Its transmembrane domain contains a number of well conserved amino acids with hydroxyl side chains, the mutation of which to aliphatic residues abrogates this ER retention [31]. Some of these residues are also important in the mechanism of transmembrane signalling of IgM in B cells [32].

In addition to these studies of ER retention and degradation signals within transmembrane domains, there have also been reports over the past year of Golgi retention signals in the transmembrane domains of a number of proteins. Through the construction of a series of chimeric molecules and analysis by immunofluorescence microscopy, it has been shown that the transmembrane domain of N-glucosaminyltransferase I is sufficient to confer Golgi retention on several heterologous proteins [33••]. Similar results have been obtained for the transmembrane domains of α2,6-sialyltransferase (ST) [34••] and β1,4-galactosyltransferase (GT) [35•,36••,37••]. In the latter case, a 10-amino-acid region from the lumenal half of the transmembrane domain was sufficient for retention of a heterologous protein in the *trans* Golgi cisternae [36••]. That the lumenal half of this domain may not be the sole determinant is suggested by a separate study [37••], which identified a cysteine and a histidine residue in the cytoplasmic half of the transmembrane domain that are important for the Golgi retention of GT. There is no apparent sequence similarity between the transmembrane domains of these glycosyltransferases.

Golgi retention of a coronavirus E1 protein has also been found to be determined by the first of its three membrane-spanning domains [38••]. Replacing the transmembrane domains of two proteins normally destined for the plasma membrane with this domain leads to their retention in the Golgi. Polar uncharged residues in this transmembrane domain, which would line up on one face of an α-helix, are well conserved among coronaviruses. Mutation of these residues to isoleucine, or insertion of two isoleucines to disrupt the helical periodicity, results in the transport of some of the protein to the plasma membrane. These data should be compared with those obtained with the E1 protein of the mouse hepatitis virus A59 [39••]. In this case, the data obtained suggest that the Golgi retention signal is a more general property of the molecule. The reasons for this difference are unclear.

Another intriguing case of potential interactions between intramembraneous domains is that involving the 44-amino-acid E5 oncogene product of fibropapillomaviruses. The amino-terminal two thirds of E5 has a sequence suggesting a transmembrane α-helix, whereas the 14-amino-acid hydrophilic carboxy-terminal tail extends into the lumen of the Golgi apparatus (for a review, see [40]). A conserved glutamine residue within the hydrophobic domain is important for association with the hydrophobic 16 kD subunit of the vacuolar H^+-ATPase [41•]. However, there appear to be no spe-

cific sequence requirements in the remainder of the hydrophobic region for this association to occur. The glutamine residue is also important for the transforming ability of E5. For this activity, however, there are specific sequence requirements in the hydrophobic domain of E5 [42••]. The transmembrane domain of E5 may also serve as its Golgi retention signal.

The formation of bundles of transmembrane α-helices appears to occur in some other cases. One such example is the 52-amino-acid protein phospholamban, which is a regulator of the Ca^{2+}-ATPase of cardiac muscle sarcoplasmic reticulum. Phospholamban forms pentamers that are stable in sodium dodecyl sulfate (SDS), although the relevance of this to its function is not yet clear. Residues within the predicted transmembrane α-helix have been shown to be involved in pentamer formation [43]. In the case of ion-conducting channel proteins, the pore itself may consist of a bundle of amphipathic α-helices. Specific peptide sequences, which it has been predicted would form the channel-lining helices, have been synthesized, and shown to reproduce a number of properties of the channels from which their sequences were derived (for a review, see [44]).

Perhaps the best characterized example of interactions between transmembrane α-helices is the human erythrocyte sialoglycoprotein glycophorin A (GpA), which forms a dimer that is stable in SDS. Bormann *et al.* [45] showed that the GpA dimer was disrupted upon addition of a synthetic peptide corresponding in sequence to its transmembrane domain. The addition of a number of heterologous transmembrane peptides did not disrupt the dimer. Further studies using a chimeric protein have shown that the GpA transmembrane domain alone contains all of the information required for this specific association [46••]. In addition, mutational analysis of the chimera shows that even very subtle alterations in the nature of certain side chains can significantly disrupt the interaction. For example, mutation of a valine to a leucine in the transmembrane domain disrupts the dimerization, and therefore this residue is proposed to lie at the dimer interface. If the α-helix geometry is canonical as CD data would seem to suggest, this valine would lie on the same face of the helix as several of the glycine residues in the transmembrane domain. There are no highly polar residues in the transmembrane domain of GpA, which thus serves as a contrast to the cases mentioned above.

Intramembraneous helix–helix interactions in transmembrane signalling by receptors

It is now widely accepted that the primary event in transmembrane signalling by receptors such as the epidermal growth factor receptor (EGF-R), is ligand-stimulated receptor dimerization (for a review, see [47,48••]. Since it was found that the mutation of a valine to glutamic acid in the transmembrane domain of the *neu* oncogene product causes this EGF-R-like receptor to become constitutively active as a tyrosine kinase [49] and increases the proportion of the receptor existing as a dimer [50], there has been much speculation concerning the possible role of interactions between transmembrane α-helices in this signalling mechanism [51••].

A number of experiments have been reported in which transmembrane domains of related proteins have been swapped, resulting in the production of inactive receptors. For example, Yan *et al.* [52•] made chimeric receptors in which the ectodomain was derived from EGF-R, and the intracellular domain was from the low-affinity nerve growth factor receptor (NGF-R) represented by the $p75^{NGF\text{-}R}$ protein. A morphological response to epidermal growth factor (EGF) stimulation was seen for PC12 cells expressing a chimera in which the transmembrane domain was derived from the $p75^{NGF\text{-}R}$, but not if it was derived from the EGF-R. The low-affinity $p75^{NGF\text{-}R}$ may interact with the *trk* oncogene product ($p140^{prototrk}$), another low-affinity NGF-R, to form high-affinity binding sites for NGF [53•]. The experiments of Yan *et al.* [52•], and the fact that the sequence of the transmembrane domain of $p75^{NGF\text{-}R}$ is highly conserved between species [54], suggest that this predicted transmembrane α-helix may be involved in interactions with other proteins, such as $p140^{prototrk}$.

Based upon consideration of the amino acid sequence surrounding the activating Val→Glu mutation in the transmembrane domain of the *neu* oncogene product, and comparison with a similar region of other related receptors, a model has been proposed in which a five-amino-acid motif is responsible for the specific dimerization of transmembrane α-helices in a number of receptors of this type [55]. A recent mutational analysis of the *neu* oncogene product has confirmed that a subdomain within its transmembrane domain, consistent with the nature of this motif, is required for activation of transformation by the Val→Glu mutation mentioned above [56•]. In contrast with the conclusions drawn from this study, analysis of the effects of deletions and mutations in the transmembrane domain of the insulin receptor [57•] and EGF-R [58•,59] seems to indicate a passive role for these domains in signal transduction by these receptors. Furthermore, the extracellular and transmembrane domains of v-*erb*B, the truncated and constitutively active form of the EGF-R, can be replaced by a myristyl anchor without affecting transformation potency or specificity [60•]. This argues against the dimerization of the transmembrane domain of EGF-R being important in activating the tyrosine kinase domain of this receptor.

Whatever their exact role, transmembrane domains must be important in signal transduction, as they comprise the only connection between the ligand-binding domain of the receptor and the effector region in the cytosol. Recent studies of the chemotactic aspartate receptor (Tar) of *E. coli* may shed some light upon this issue. The structure of the ectodomain of this dimeric receptor, both with and without ligand, was reported last year [61••], and showed a small ligand-induced rotation of the subunits about an axis parallel to the membrane. It is proposed that this rotation is translated to a relatively large alteration in the relative disposition of the endodomains of the dimer, resulting in their activation. An extensive analysis of disulfide crosslinking of cysteine-substituted proteins within the membrane region of this receptor by Pakula and Simon [62••] has provided data for the construction of a

model in which the four transmembrane domains (two from each transmembrane subunit; TM1 and TM2) form a distorted four-helix bundle, the two TM1 helices interacting the most extensively. Residues capable of participating in crosslink formation were found to be restricted to one face of each helix, implying that the TM1–TM1′ interaction is axially symmetric. The face of each helix thus shown to be involved in helix–helix interactions was identified as the most hydrophilic and most conserved face [63••], using the analytical method of Rees *et al.* [7]. For example, there are glutamine and serine residues in the TM1–TM1′ interface, which may participate in interhelical hydrogen bonding. It will be very interesting to see how the pattern of disulfide crosslinking changes in the aspartate-stimulated receptor. Preliminary data suggest that the two TM1 helices are closer together in the activated receptor [63••].

Conclusions and perspectives

Many generalizations have been put forward for membrane helix properties during the past decade. Some of these apply relatively broadly, others more specifically; few describe the full range of properties without exception. A number of notions that are useful from a structural perspective now exist. The side chains of a transmembrane α-helix are largely hydrophobic. A limited number of potentially charged groups may be included, although it is not clear that they would be ionized within the bilayer. Proline residues occur more commonly in the transmembrane α-helices of polytopic membrane proteins than in α-helices of soluble proteins, and may cause a kink in many cases. Where transbilayer helices are significantly amphipathic, the more polar surface is likely to be involved in interactions with other helices and prosthetic groups rather than with the lipids. Association of α-helices within the bilayer may be driven by strong polar interactions and/or detailed van der Waals fits. These interactions can be highly specific, and can have sufficient energy to drive association between helices without covalent linkages outside the membrane.

On the functional side, a rapidly expanding list is becoming established, including roles in defining channels and transmembrane transport pathways, signals for oligomerization and for degradation if oligomerization fails, positioning of prosthetic groups for electron-transfer reactions, signals for selective localization in specific membrane compartments, and as mediators of transmembrane signalling. It is clear that transmembrane helices are more than mere hydrophobic anchors. It seems a reasonable hope that the future will allow an understanding of the function of these important structural elements that will permit a chemical understanding of the myriad functions that they perform.

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24. •• COSSON P, LANKFORD SP, BONIFACINO JS, KLAUSNER RD: **Membrane Protein Association by Potential Intramembrane Charge Pairs.** *Nature* 1991, 351:414–416.

Two basic residues in the transmembrane domain of TCRα are shown to be of prime importance for its association with CD3δ, which has an aspartic acid residue in its transmembrane domain. The TCR clonotypic δ chain, which contains the same basic residues as TCRα, is also shown to associate with CD3δ. Furthermore, a mutant of Tac, in which an arginine had been introduced into its transmembrane domain, is shown to interact with CD3δ. This mutant is degraded in the ER [23••]. Mutation of the aspartic acid residue in CD3δ abrogates all of these associations. These data suggest that interhelical salt bridges and/or strong hydrogen bonds are involved in the assembly of the TCR complex.

25. ENGELMAN DM: **An Implication of the Structure of Bacteriorhodopsin. Globular Membrane Proteins Are Stabilized by Polar Interaction.** *Biophys J* 1982, 37:187–188.

26. ENGELMAN DM, STEITZ TA, GOLDMAN A: **Identifying Nonpolar Transbilayer Helices in Amino Acid Sequences of Membrane Proteins.** *Annu Rev Biophys Biophys Chem* 1986, 15:321–353.

27. HONIG BH, HUBBELL WL: **Stability of Salt Bridges in Membrane Proteins.** *Proc Natl Acad Sci USA* 1984, 81:5412–5416.

28. KINET JP: **Antibody–Cell Interactions: Fc Receptors.** *Cell* 1990, 57:351–354.

29. RAVETCH JV, KINET JP: **Fc Receptors.** *Annu Rev Immunol* 1991, 9:457–492.

30. •• KUROSAKI T, GANDER I, RAVETCH JV: **A Subunit Common to an IgG Receptor and the T-cell Receptor Mediates Assembly Through Different Interactions.** *Proc Natl Acad Sci USA* 1991, 88:3837–3841.

This paper presents a demonstration that the ζ-subunit of TCR/CD3 associates through interactions mediated by its transmembrane domain with the α-subunit of the FcγRIII receptor (CD16) of human natural killer cells. CD16 is thus spared from degradation in the ER. An aspartic acid residue in each of the transmembrane domains is important for this interaction, as is a leucine residue in the transmembrane domain of ζ. Mutation of this leucine to isoleucine reduces the extent of interaction by 65 %.

31. WILLIAMS GT, VENKITARAMAN AR, GILMORE DJ, NEUBERGER MS: **The Sequence of the μ Transmembrane Segment Determines the Tissue Specificity of the Transport of Immunoglobulin M to the Cell Surface.** *J Exp Med* 1990, 171:947–952.

32. SHAW AC, MITCHELL RN, WEAVER YK, CAMPOS-TORRES J, ABBAS AK, LEDER P: **Mutations of Immunoglobulin Transmembrane and Cytoplasmic Domains: Effects on Intracellular Signalling and Antigen Presentation.** *Cell* 1990, 63:381–392.

33. •• TANG BL, WONG WH, LOW SH, HON H: **The Transmembrane Domain of N-Glucosaminyltransferase I Contains a Golgi Retention Signal.** *J Biol Chem* 1992, 267:10122–10126.

Different amino-terminal portions of N-acetylglucosaminyltransferase I are attached to the ectodomains of a plasma membrane protein via a single transmembrane helix. When the transmembrane domain alone is placed in the chimeric construct, the chimeric proteins are retained in the Golgi, as observed by immunofluorescence staining.

34. •• MUNRO S: **Sequences Within and Adjacent to the Transmembrane Segment of α-2,6-Sialytransferase Specify Golgi Retention.** *EMBO J* 1991, 10:3577–3588.

A chimeric protein in which amino-terminal portions of ST are fused to chicken lysozyme is used to identify regions of ST involved in Golgi retention. It is clear that the single transmembrane domain contains targeting information, although there is also a contribution from the flanking sequence. Replacing the transmembrane domain of full-length ST with that of a plasma membrane protein results in the appearance of ST at the cell surface. The most striking feature of the transmembrane domain of ST is that it has four phenylalanine residues which would fall on one face of a canonical α-helix.

35. • RUSSO RN, SHAPER NL, TAATJES DJ, SHAPER JH: **β1,4-Galactosyltransferase: a Short NH_2-terminal Fragment that Includes the Cytoplasmic and Transmembrane Domain is Sufficient for Golgi Retention.** *J Biol Chem* 1992, 267:9241–9247.

Using chimeric proteins and localizing them by immunofluorescence staining, the Golgi retention signal of GT is shown to reside in an amino-terminal fragment that includes the cytoplasmic and transmembrane domains.

36. •• NILSSON T, LUCOCQ JM, MACKAY D, WARREN G: **The Membrane Spanning Domain of β1,4-Galactosyltransferase Specifies *trans* Golgi Localization.** *EMBO J* 1991, 10:3567–3575.
Portions of GT are placed in the context of the human invariant chain (Iip31). When the transmembrane and cytosolic domains, just the transmembrane domain, or just 10 amino acids from the lumenal side of the transmembrane domain of GT replace the corresponding region of Iip31 (also a type II membrane protein), retention in the *trans* Golgi cisternae is confirmed by immuno-fluorescence and immuno-electron microscopy. A model is proposed whereby oligomerization of the transmembrane domains might be important in the mechanism of Golgi retention (see also [37••]).

37. •• AOKI D, LEE N, YAMAGUCHI N, DUBOIS C, FUKUDA MN: **Golgi Retention of a *trans*-Golgi Membrane Protein, Galactosyltransferase, Requires Cysteine and Histidine Residues Within the Membrane-anchoring Domain.** *Proc Natl Acad Sci USA* 1992, 89:4319–4323.
Immunofluorescence microscopy is used to study the location in COS cells of variants of a chimera between GT and the human chorionic gonadotrophin α-subunit. A variety of replacement mutations have no effect upon subcellular localization; however, the cytoplasmic half of the transmembrane domain of GT does seem to be important in this regard. In particular, a cysteine and a histidine residue in this region are found to be important for Golgi retention. This result should be compared with that obtained in [36••], where the lumenal half of the transmembrane domain is identified as being the region most important for Golgi retention.

38. •• SWIFT AM, MACHAMER CE: **A Golgi Retention Signal in a Membrane-spanning Domain of Coronavirus E1 Protein.** *J Cell Biol* 1991, 115:19–30.
The first of the three transmembrane domains of the E1 protein of the avian coronavirus infectious bronchitis virus is substituted for the transmembrane domains of two other transmembrane proteins which are normally transported to the cell surface. Both proteins are consequently retained in the Golgi apparatus. Point mutations within this transmembrane domain which alter the periodicity of conserved polar uncharged residues, which would usually line up on the same face of an assumed canonical α-helix, abrogate Golgi retention. Disruption of the relative spacing of these residues by insertion of two isoleucine residues has the same effect. The results from this study should be compared with those in [39••].

39. •• ARMSTRONG J, PATEL S: **The Golgi Sorting Domain of Coronavirus E1 Protein.** *J Cell Sci* 1991, 98:567–575.
In the E1 protein of the coronavirus mouse hepatitis virus A59, studies of the signals for Golgi retention give rather different results from those described in [38••] for the avian infectious bronchitis virus. In this case, the retention signal appears to comprise several portions of the E1 protein, rather than a single peptide sequence. The discussion in this paper also highlights the problems inherent in interpreting studies of localization signals through the construction of chimeric proteins.

40. PETTI L, NILSON L, KULKE R, LEPTAK C, RIESE DJ, ZIBELLO T, DIMAIO D: **The E5 Mini-oncogene of Bovine Papillomavirus: Biological Activities, Genetic Analysis, and Proposed Mechanisms of Action.** In *Origins of Human Cancer: a Comprehensive Review.* Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1991:707–713.

41. • GOLDSTEIN DJ, KULKE R, DIMAIO D, SCHLEGEL R: **A Glutamine Residue in the Membrane-associating Domain of the Bovine Papillomavirus Type 1 E5 Oncoprotein Mediates its Binding to a Transmembrane Component of the Vacuolar H^+-ATPase.** *J Virol* 1992, 66:405–413.
Immunoprecipitation and gel filtration are employed to study the association of E5 with the hydrophobic 16 kD subunit of the vacuolar H^+-ATPase (16K). Each disulfide-linked E5 dimer binds two molecules of 16K, an absolute requirement for this binding being a hydrophilic residue in the middle of the E5 hydrophobic domain. Binding of E5 to 16K is not sufficient for cell transformation.

42. •• KULKE R, HORWITZ BH, ZIBELLO T, DIMAIO D: **The Central Hydrophobic Domain of the Bovine Papillomavirus E5 Transforming Protein Can Be Functionally Replaced by Many Hydrophobic Amino Acid Sequences Containing a Glutamine.** *J Virol* 1992; 66:505–511.
Variants of E5 with a variety of sequences in their hydrophobic domain are analyzed for their ability to transform C127 cells. Most variants have transforming activity if a glutamine residue is inserted at its normal position relative to the carboxyl terminus. However, three variants are found which will not transform cells even with a glutamine at this position. In addition, three variants show significant transforming activity in the absence of glutamine. Thus, there must be features of the hydrophobic domain other than the presence of a hydrophilic residue that are important for the transforming activity of E5.

43. FUJII J, MARUYAMA K, TADA M, MACLENNAN DH: **Expression and Site-specific Mutagenesis of Phospholamban: Studies of Residues Involved in Phosphorylation and Pentamer Formation.** *J Biol Chem* 1989, 264:12950–12955.

44. MONTAL M: **Molecular Anatomy and Molecular Design of Channel Proteins.** *FASEB J* 1990, 4:2623–2635.

45. BORMANN BJ, KNOWLES WJ, MARCHESI VT: **Synthetic Peptides Mimic the Assembly of Transmembrane Glycoproteins.** *J Biol Chem* 1989, 264:4033–4037.

46. •• LEMMON MA, FLANAGAN JM, HUNT JF, ADAIR BD, BORMANN BJ, DEMPSEY CE, ENGELMAN DM: **Glycophorin A Dimerization Is Driven by Specific Interactions Between Transmembrane α-Helices.** *J Biol Chem* 1992, 267:7683–7689.
This paper demonstrates that the transmembrane domain of GpA is sufficient, when fused to a normally monomeric protein (staphylococcal nuclease), to direct the specific dimerization of this protein. Addition of a synthetic peptide corresponding to the transmembrane domain of glycoprotein A disrupts this dimer, with concomitant formation of a peptide–protein heterodimer. Heterologous transmembrane peptides have no such effect. This behaviour is identical to that seen for native GpA [45]. In addition, mutagenesis studies show that dimerization is exquisitely sensitive to the sequence of the transmembrane domain. Mutation of a valine residue to leucine, for example, leads to significant disruption of the dimer of this chimeric protein. This work is the most direct biochemical demonstration of dimerization driven by transmembrane α-helices.

47. ULLRICH A, SCHLESSINGER J: **Signal Transduction by Receptors with Tyrosine Kinase Activity.** *Cell* 1990, 61:203–212.

48. •• HENDRICKSON WA: **Receptor Structure: Modes of Transduction.** *Curr Biol* 1992, 2:57–59.
This brief review considers mechanisms for signal transduction across bilayers in the light of the recent publication of X-ray crystal structures for the ectodomains of two receptors.

49. BARGMANN CI, HUNG MC, WEINBERG RA: **Multiple Independent Activations of the *neu* Oncogene by a Point Mutation Altering the Transmembrane Domain of p185.** *Cell* 1986, 45:649–657.

50. WEINER DB, LIU J, COHEN JA, WILLIAMS WV, GREEN MI: **A Point Mutation in the *neu* Oncogene Mimics Ligand Induction of Receptor Aggregation.** *Nature* 1989, 339:230–231.

51. •• BORMANN BJ, ENGELMAN DM: **Intramembrane Helix–Helix Association in Oligomerization and Transmembrane Signalling.** *Annu Rev Biophys Biomol Struct* 1992, 21:233–242.
This review discusses the two-stage model for integral membrane protein folding as it applies to those proteins with a single transbilayer α-helix. It further considers models for transmembrane signalling by such proteins. A model involving receptor oligomerization is argued to be most reasonable, and evidence supporting such a model, and the role of transmembrane domains in oligomerization, is reviewed.

52. • YAN H, SCHLESSINGER J, CHAO MV: **Chimeric NGF-EGF Receptors Define Domains Responsible for Neuronal Differentiation.** *Science* 1991, 252:561–563.
Chimeric receptor molecules are constructed in which the endodomain is derived from the $p75^{NGF-R}$ and the ectodomain from EGF-R. Responses similar to those usually seen with NGF are observed upon EGF stimulation of cells expressing these chimerae only when the transmembrane domain of the receptor is derived from $p75^{NGF-R}$. Thus, it is considered that this domain may be involved in interaction with other NGF-responsive signal-transducing molecules, such as the *trk* oncogene product.

53. • HEMPSTEAD BL, MARTIN-ZANCA D, KAPLAN DR, PARADA LF, CHAO MV: **High-affinity NGF Binding Requires Coexpression of the *trk* Proto-oncogene and the Low-affinity NGF Receptor.** *Nature* 1991, **350**:678–683.

This paper describes experiments demonstrating that $p75^{NGF\text{-}R}$ and $p140^{prototrk}$, each of which form low-affinity binding sites for NGF when present alone, form a high-affinity NGF binding site when present together. Fusion of membrane preparations from cells expressing just $p140^{prototrk}$ with similar preparations from cells expressing just $p75^{NGF\text{-}R}$ led to the appearance of both high- and low-affinity NGF binding sites, whereas the individual preparations had just low-affinity binding sites. Transient transfection of COS cells with plasmids expressing both proteins also indicated that co-expression of the two proteins is required for high-affinity NGF binding. Thus, it is proposed that $p75^{NGF\text{-}R}$ and $p140^{prototrk}$ interact to form the high-affinity NGF-R.

54. LARGE TH, WESKAMP G, HELDER JC, RADEKE MJ, MISKO TP, SHOOTER EM, REICHARDT LF: **Structure and Developmental Expression of the Nerve Growth Factor Receptor in the Chicken Central Nervous System.** *Neuron* 1989, **2**:1123–1134.

55. STERNBERG MJE, GULLICK WJ: **A Sequence Motif in the Transmembrane Region of Growth Factor Receptors with Tyrosine Kinase Activity Mediates Dimerization.** *Protein Eng* 1990, **3**:245–248.

56. • CAO H, BANGALORE L, BORMANN BJ, STERN DF: **A Subdomain in the Transmembrane Domain is Necessary for $p185^{neu}$ Activation.** *EMBO J* 1992, **11**:923–932.

A number of mutations are made in the transmembrane domain of the *neu* oncogene. In the context of the activating Val→Glu mutation at position 664, it is found that the transforming ability of the oncogene product is in general resilient to alterations. However, alteration of residues at positions 661–665 inhibits transforming ability. Furthermore, replacement of the carboxy-terminal two thirds of the *neu* transmembrane domain with that of CD4 abrogates transformation. Although it is difficult to draw conclusions from such a limited set of mutations, this work clearly shows that there are determinants within the transmembrane domain that are important for the transforming ability of this protein. This result contrasts with those obtained for EGF-R [58•,59] and the insulin receptor [57•]. It should be noted, however, that, in the absence of an available ligand for the *neu* oncogene, it was not possible to determine the ability of the mutants in this study to transduce signals across the membrane.

57. • FRATTALI AL, TREADWAY JL, PESSIN JE: **Evidence Supporting a Passive Role for the Insulin Receptor Transmembrane Domain in Insulin-dependent Signal Transduction.** *J Biol Chem* 1991, **266**:9829–9834.

A series of mutations are made in the transmembrane domain of the insulin receptor, and their effect upon insulin-stimulated receptor autophosphorylation are analyzed. Deletion of up to five amino acid residues from within the transmembrane domain is shown to have no effect upon insulin-stimulated signalling. Furthermore, the introduction of a mutation analogous to that which activates the *neu* oncogene has no detectable effect. Thus, these data, although rather limited, lead to the opposite conclusion from that drawn in [56•].

58. • CARPENTER CD, INGRAHAM HA, COCHET C, WALTON GM, LAZAR CS, SOWADSKI JM, ROSENFELD MG, GILL GN: **Structural Analysis of the Transmembrane Domain of the Epidermal Growth Factor Receptor.** *J Biol Chem* 1991, **266**:5750–5755.

Mutations are made in the transmembrane domain of EGF-R, and their effects upon EGF binding, EGF-stimulated tyrosine kinase activity, and EGF-dependent dimerization are analyzed. Shortening of the transmembrane domain by six residues from its carboxyl terminus, by 10 from its amino terminus, or the insertion of three proline residues separated from one another by four residues, has no effect upon these activities of the EGF-R. Furthermore, mutations analogous to those found to activate the *neu* oncogene are found to have no such effect upon the EGF-R. This work complements that reported in [59], drawing the similar conclusion that the transmembrane domain of the EGF-R plays a passive role in signal transduction across the bilayer. Although limited, these data suggest that the situation with EGF-R may be different from that seen for the *neu* oncogene product [56•].

59. KASHLES O, SZAPARAY D, BELLOT F, ULLRICH A, SCHLESSINGER J, SCHMIDT A: **Ligand-induced Stimulation of Epidermal Growth Factor Receptor Mutants with Altered Transmembrane Regions.** *Proc Natl Acad Sci USA* 1988, **85**:9567–9571.

60. • MCMAHON M, SCHATZMAN RC, BISHOP JM: **The Amino-terminal 14 Amino Acids of v-*src* Can Functionally Replace the Extracellular and Transmembrane Domains of v-*erbB*.** *Mol Cell Biol* 1991, **11**:4760–4770.

It is shown that the transmembrane and extracellular domains of v-*erb*B are not necessary for its transforming ability. Rather, what seems to be necessary is that the cytosolic domain is anchored in the membrane. In order to achieve this, the amino-terminal 14 amino acids of v-*src*, which contain a myristylation signal, are used to replace these domains. The resultant myristyl-anchored v-*erb*B cytosolic domain retains the transforming properties of v-*erb*B.

61. •• MILBURN MV, PRIVÉ GG, MILLIGAN DL, SCOTT WG, YEH J, JANCARIK J, KOSHLAND DE JR, KIM S-H: **Three Dimensional Structures of the Ligand Binding Domain of the Bacterial Aspartate Receptor With and Without a Ligand.** *Science* 1991, **254**:1342–1347.

The X-ray crystal structure of a disulfide-crosslinked dimer of the ectodomain of Tar is reported with and without bound ligand (at 2.0 and 2.4 Å resolution, respectively). The protein is essentially a dimer of four-helix bundles, which suggests that a four-helix bundle transverses the lipid bilayer. One aspartate molecule binds to the dimer, causing a conformation change which can be described as a 4° rotation between the subunits about a pivot axis perpendicular to both the dimer interface and the dimer twofold axis. Such a change could be transmitted to the cytosolic domain via movement of the transmembrane α-helices with respect to one another, leading to activation of that domain.

62. •• PAKULA AA, SIMON MI: **Determination of Transmembrane Protein Structure by Disulfide Cross-linking: the *Escherichia coli* Tar Receptor.** *Proc Natl Acad Sci USA* 1992, **89**:4144–4148.

In this rather elegant study, cysteine substitutions are introduced at 48 positions within the transmembrane region of the Tar receptor. Through an extensive analysis of the positions within the transmembrane region between which disulfide crosslinks will form, data are generated for modelling of the relative disposition of the four transmembrane helices of the receptor dimer. The positions which crosslink most readily are found to lie on one face of each helix of each subunit (TM1 and TM2). The two TM1 helices of the dimer interact extensively with one another in a distorted four-helix bundle structure suggested by the modelling. The TM2 helices interact less extensively. The most highly conserved transmembrane residues are found to occur at or near the helix–helix interfaces inferred from the crosslinking study.

63. •• LYNCH BA, KOSHLAND DE: **Disulfide Cross-linking Studies of the Transmembrane Regions of the Aspartate Sensory Receptor of *Escherichia coli*.** *Proc Natl Acad Sci USA* 1991, **88**:10402–10406.

A rather more limited set of cysteine substitutions is studied in this paper, yielding results which are consistent with those of [61••]. Of particular interest is the finding that the rate of crosslink formation for two TM1–TM1′ crosslinks is increased in the presence of aspartate. This indicates that these helices are drawn closer or have a different relative orientation in the activated receptor. An analysis of TM1 and TM2 according to the method of Rees *et al.* [7] is also presented, from which it appears that the most hydrophilic and most conserved face of each helix is that most intimately involved in helix–helix interactions.

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