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Review

Are specific nonannular cholesterol binding sites present in G-protein coupled receptors?

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ABSTRACT

The G-protein coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major drug targets in all clinical areas. Membrane cholesterol has been reported to have a modulatory role in the function of a number of GPCRs. Interestingly, recently reported crystal structures of GPCRs have shown structural evidence of cholesterol binding sites. Two possible mechanisms have been previously suggested by which membrane cholesterol could influence the structure and function of GPCRs (i) through a direct/specific interaction with GPCRs, which could induce a conformational change in the receptor, or (ii) through an indirect way by altering the membrane physical properties in which the receptor is embedded or due to a combination of both. We discuss here a novel mechanism by which membrane cholesterol could affect structure and function of GPCRs and propose that cholesterol binding sites in GPCRs could represent ‘nonannular’ binding sites. Interestingly, previous work from our laboratory has demonstrated that membrane cholesterol is required for the function of the serotonin_{1A} receptor, which could be due to specific interaction of the receptor with cholesterol. Based on these results, we envisage that there could be specific/nonannular cholesterol binding site(s) in the serotonin_{1A} receptor. We have analyzed putative cholesterol binding sites from protein databases in the serotonin_{1A} receptor, a representative GPCR, for which we have previously demonstrated specific requirement of membrane cholesterol for receptor function. Our analysis shows that cholesterol binding sites are inherent characteristic features of serotonin_{1A} receptors and are conserved over evolution. Progress in deciphering molecular details of the nature of GPCR-cholesterol interaction in the membrane would lead to better insight into our overall understanding of GPCR function in health and disease, thereby enhancing our ability to design better therapeutic strategies to combat diseases related to malfunctioning of GPCRs.

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Abbreviations: 5-HT_{1A} receptor, 5-hydroxytryptamine_{1A} receptor; 7-DHC, 7-dehydrocholesterol; CCM, cholesterol consensus motif; CRAC, cholesterol recognition/interaction amino acid consensus; FRET, Fluorescence Resonance Energy Transfer; GPCR, G-protein coupled receptor; MbCD, methyl- β -cyclodextrin; SLOS, Smith–Lemli–Opitz Syndrome; SSD, sterol-sensing domain

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1. G-protein coupled receptors and cholesterol

The G-protein coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes [1–3]. GPCRs are prototypical members of the family of seven transmembrane

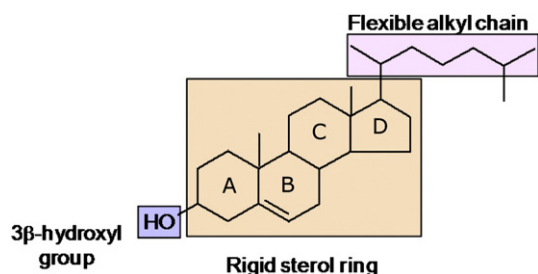


Fig. 1. Chemical structure of cholesterol showing the individual rings (A–D). Three structurally distinct regions are shown as shaded boxes: 3β-hydroxyl group, the rigid steroid ring, and the flexible alkyl chain. 3β-hydroxyl moiety is the only polar group in cholesterol thereby contributing to its amphiphilic character and helps it to orient and anchor in the membrane. Adapted and modified from [71].

domain proteins and include >800 members which together constitute ~1–2% of the human genome [4]. They are involved in the generation of cellular responses to a diverse array of stimuli that include biogenic amines, peptides, lipids and even photons. As a result of this, these receptors mediate multiple physiological processes such as neurotransmission, cellular differentiation, growth, inflammatory and immune responses. GPCRs therefore have emerged as major targets for the development of novel drug candidates in all clinical areas [5–8]. Interestingly, although GPCRs represent 30–50% of current drug targets, only a small fraction of all GPCRs are presently targeted by drugs [9]. This points out the exciting possibility that the receptors which are not recognized yet could be potential drug targets for diseases that are difficult to treat by currently available drugs.

Cholesterol is an essential and representative lipid (see Fig. 1) in higher eukaryotic cellular membranes and is crucial in the organization, dynamics, function, and sorting of membranes [10,11]. Cholesterol is often found distributed nonrandomly in domains in biological and model membranes [10–14]. Many of these domains (sometimes termed as ‘lipid rafts’) are believed to be important for the maintenance of membrane structure and function. The idea of such specialized membrane domains assumes significance in cell physiology since important functions such as membrane sorting and trafficking [15], signal transduction processes [16], and the entry of pathogens [17,18] have been attributed to these domains.

2. Cholesterol and nonannular lipids in the function of membrane proteins

Cholesterol plays a vital role in the function and organization of membrane proteins and receptors [19,20]. The effect of cholesterol on the structure and function of integral membrane proteins and receptors has been a subject of intense investigation [19,20]. It has been proposed that cholesterol can modulate the function of GPCRs in two ways: (i) through a direct/specific interaction with GPCRs, which could induce a conformational change in the receptor [21,22], or (ii) through an indirect way by altering the membrane physical properties in which the receptor is embedded [23,24] or due to a combination of both. There could be yet another manner in which membrane cholesterol could affect structure and function of membrane proteins. For example, it has been reported that for the nicotinic acetylcholine receptor (which requires cholesterol for its function), cholesterol is proposed to be present at the ‘nonannular’ sites around the receptor [25]. The first evidence for the presence of nonannular lipids came from experiments monitoring effects of cholesterol and fatty acids on $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase [26,27].

The role of dynamic lipid–protein interactions in regulating the structure and functional activity of membrane proteins has been extensively studied using a variety of spectroscopic approaches [28]. Integral membrane proteins are surrounded by a shell or annulus of lipid molecules which mimics the immediate layer of solvent

surrounding soluble proteins [29,30]. These are termed ‘annular’ lipid around the membrane protein. After several years of moderate controversy surrounding the interpretation of spectroscopic data, it later became clear that the annular lipids are exchangeable with bulk lipids [28]. The rate of exchange of lipids between the annular lipid shell and the bulk lipid phase was shown to be approximately an order of magnitude slow than the rate of exchange of bulk lipids resulting from translational diffusion of lipids in the plane of the membrane. It therefore appears that exchange between annular and bulk lipids is slightly slower since lipid–protein interaction is favorable compared to lipid–lipid interaction. However, the difference in interaction energy is modest, consistent with the observation that lipid–protein binding constants (affinity) depend weakly on lipid structure [30]. Interestingly, the two different types of lipid environments (annular and bulk) can be readily detected using electron spin resonance (ESR) spectroscopy [31]. In addition to the annular lipids, there is evidence for other lipid molecules in the immediate vicinity of integral membrane proteins. These are termed as ‘nonannular’ lipids. Nonannular sites are characterized by lack of accessibility to annular lipids, i.e., these sites cannot be displaced by competition with annular lipids. This is evident from analysis of fluorescence quenching of intrinsic tryptophans of membrane proteins by phospholipid or cholesterol covalently labeled with bromine [25,26], which acts as a quencher due to the presence of the heavy bromine atom [32]. These results signify that nonannular lipid binding sites remain vacant even in the presence of annular lipids around the protein [33]. Although not shown experimentally yet, the exchange of lipid molecules between nonannular sites and bulk lipids would be relatively slow compared to the exchange between annular sites and bulk lipids, and binding to the nonannular sites is considered to be more specific compared to annular binding sites [30].

The location of the postulated nonannular sites represents an intriguing question. It has been suggested that the possible locations for the nonannular sites could be either inter or intramolecular (interhelical) protein interfaces, characterized as deep clefts (or cavities) on the protein surface [26,33]. For example, in the crystal structure of the potassium channel KcsA from *S. lividans*, one of the best understood ion channels, a negatively charged lipid molecule was found to be bound as ‘anionic nonannular’ lipid at each of the protein–protein interface in the homotetrameric structure [34]. These nonannular sites show high selectivity for anionic lipids over zwitterionic lipids, and it has been proposed that the change in the nature of the nonannular lipid results in a change in packing at the protein–protein interface which modulates the open channel probability and its conductance. Interestingly, the relationship between open channel probability of KcsA and negative phospholipid content exhibits cooperativity. This is consistent with a model in which the nonannular sites in the KcsA homotetramer have to be occupied by anionic lipids for the channel to remain open [33]. This example demonstrates the crucial requirement of nonannular lipids in the function of membrane proteins and the stringency associated with regard to specificity of nonannular lipids.

3. Presence of specific (nonannular?) cholesterol binding sites in the crystal structures of GPCRs

3.1. Rhodopsin

Rhodopsin, the photoreceptor of retinal rod cells, undergoes a series of conformational changes upon exposure to light. The light activated receptor exists in equilibrium with various intermediates collectively called metarhodopsins. The state of equilibrium is sensitive to the presence of cholesterol in the membrane [35–37]. An increase in the amount of cholesterol in the membrane shifts this equilibrium toward the inactive conformation of the protein. Direct interaction between rhodopsin and cholesterol has been monitored

utilizing fluorescence resonance energy transfer (FRET) between tryptophan residues of rhodopsin (donor) and a fluorescent cholesterol analogue, cholestatrienol (acceptor) [38]. In this work, replenishment of cholesterol or ergosterol into cholesterol-depleted rod outer segment disk membranes was carried out and their ability to inhibit the quenching of donor tryptophan fluorescence was monitored. Interestingly, cholesterol was able to inhibit tryptophan quenching, whereas in presence of ergosterol, quenching was observed due to energy transfer between tryptophan residues of rhodopsin and cholestatrienol, indicating a specific interaction between rhodopsin and cholesterol. In addition, it was postulated

that one cholesterol molecule per rhodopsin monomer would be present at the lipid–protein interface [38]. This has been recently supported by the crystal structure of a photo-stationary state, highly enriched in metarhodopsin I, which shows a cholesterol molecule between two rhodopsin monomers, which could possibly represent a nonannular site for cholesterol binding [39, see Fig. 2a]. In addition, cholesterol was reported to improve the reliability and yield of crystallization. In this structure, cholesterol is shown to be oriented with its tetracyclic ring aligned normal to the membrane bilayer. Interestingly, these authors proposed that some of the tryptophans in transmembrane helices would be able to interact with the cholesterol

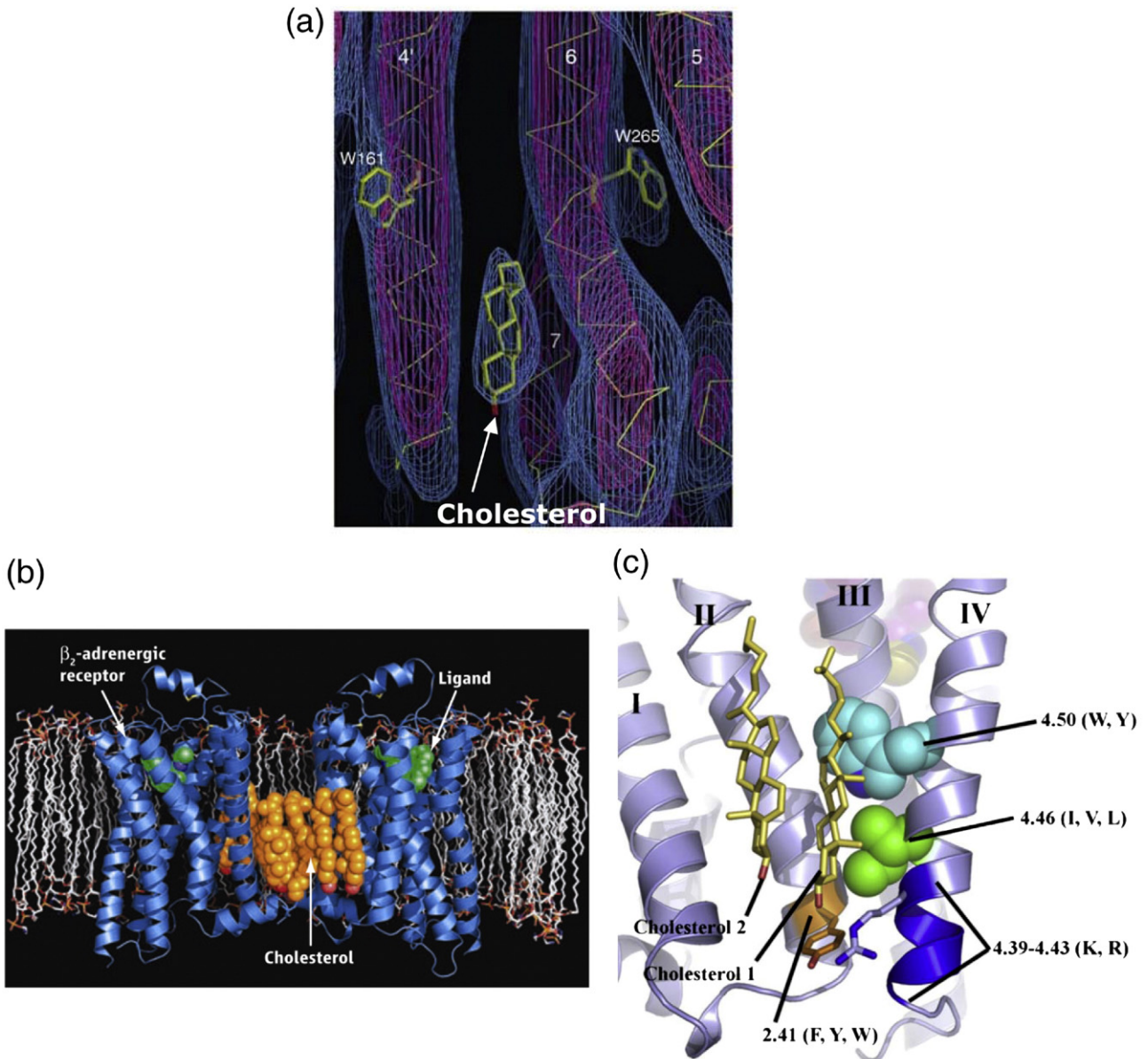


Fig. 2. Presence of tightly bound cholesterol molecules in the transmembrane regions in the recently reported crystal structures of metarhodopsin I (panel a) and human β_2 -adrenergic receptor (panels b and c). Panel (a) shows side view of metarhodopsin I showing cholesterol between transmembrane helices. Notice the close proximity of tryptophan residues (W161 and W265) to cholesterol, independently confirmed by FRET studies (see text for more details). Reproduced from [39] with permission from Macmillan, license number 1973080185756. Panel (b) depicts the structure of the human β_2 -adrenergic receptor (shown in blue) bound to the partial inverse agonist carazolol (in green) embedded in a lipid bilayer. Cholesterol molecules between two receptor molecules are shown in orange (reproduced from [42], with permission from AAAS and the corresponding author). Panel (c) shows the Cholesterol Consensus Motif (CCM) in the β_2 -adrenergic receptor (bound to the partial inverse agonist timolol) crystal structure. The side chain positions of the β_2 -adrenergic receptor and two bound cholesterol molecules are shown. Residues at positions 4.39–4.43 fulfill the CCM requirement (if one or more of these positions contains an arginine or lysine residue) and constitute site 1 (shown in blue) toward the cytoplasmic end of transmembrane helix IV. Site 2 (in cyan) represents the most important site at position 4.50 on transmembrane helix IV since it is the most conserved site with tryptophan occupying this position in 94% of class A GPCRs. The other choice of amino acid for this site is tyrosine. Site 3 (in green) at position 4.46 on transmembrane helix IV satisfies the CCM requirement if isoleucine, valine, or leucine occupy the position. Site 4 (in orange) on transmembrane helix II is at position 2.41 and can be either phenylalanine or tyrosine (reproduced from [43], with permission from Elsevier, license number 1975300141913).

tetracyclic ring. Recently reported crystallographic structures of the β_2 -adrenergic receptor have shown similar interactions (see below).

3.2. β_2 -adrenergic receptor

In general, lipid molecules that are resolved in crystal structures of membrane proteins are tightly bound. These lipid molecules, which are preserved even in the crystal structure, are often localized at protein–protein interfaces in multimeric proteins and belong to the class of nonannular (sometimes termed as ‘co-factor’) lipids [30,40]. Cholesterol has been shown to improve stability of GPCRs such as the β_2 -adrenergic receptor [41], and appears to be a necessary component for crystallization of the receptor since it is believed to facilitate receptor–receptor interaction and consequent oligomerization [42]. The cholesterol analogue, cholesterol hemisuccinate, has recently been shown to stabilize the β_2 -adrenergic receptor against thermal inactivation [43]. Since a possible location of the nonannular sites is interprotein interfaces [see above; 25,26], it is possible that cholesterol molecules located between individual receptor molecules (Fig. 2b, see later) occupy nonannular sites and modulate receptor structure and function. Importantly, the recent crystal structure of the β_2 -adrenergic receptor has revealed structural evidence of a specific cholesterol binding site (Fig. 2c, [43]). The crystal structure shows a cholesterol binding site between transmembrane helices I, II, III and IV with two cholesterol molecules bound per receptor monomer. The cholesterol binding site appears to be characterized by the presence of a cleft located at the membrane interfacial region. Both cholesterol molecules bind in a shallow surface groove formed by segments of the above mentioned helices (I, II, III and IV), thereby providing an increase in the intramolecular occluded surface area, a parameter often correlated to the enhanced thermal stability of proteins [44]. Calculation of packing values of various helices in the β_2 -adrenergic receptor which are involved in the cholesterol interacting site showed that the packing of transmembrane helices IV and II increases upon cholesterol binding. This increased packing would restrict their mobility rendering greater thermal stability to the protein [43].

Several structural features of proteins that are believed to result in preferential association with cholesterol have been recognized [45]. In many cases, proteins interacting with cholesterol have a characteristic stretch of amino acids, termed the cholesterol recognition/interaction amino acid consensus (CRAC) motif [46]. Another important cholesterol interacting domain is the sterol-sensing domain (SSD). SSD is relatively large and consists of five transmembrane segments and is involved in cholesterol biosynthesis and homeostasis [47,48]. It has been recently proposed that cholesterol binding sequence or motif should contain at least one aromatic amino acid which could interact with ring D of cholesterol [43] and a positively charged residue [49,50] capable of participating in electrostatic interactions with the 3 β -hydroxyl group. In the crystal structure of the β_2 -adrenergic receptor, three amino acids in transmembrane helix IV, along with an amino acid in transmembrane helix II, have been shown to constitute a cholesterol consensus motif (CCM, see Fig. 2c). The aromatic Trp 158^{4.50} (according to the Ballesteros–Weinstein numbering system [51]) is conserved to a high degree (~94%) among class A GPCRs and appears to contribute the most significant interaction with ring D of cholesterol [43]. In this structure, the hydrophobic residue Ile154^{4.46} would interact with rings A and B of cholesterol and is largely conserved (~60%) in class A GPCRs. The aromatic residue Tyr70^{2.41} in transmembrane helix II could interact with ring A of cholesterol and with Arg151^{4.43} of transmembrane helix IV through hydrogen bonding. The criterion of specific residues in CCM (as described above) could be somewhat broadened by conservative substitutions of amino acids (see legend to Fig. 2c).

The above description of CCM in the recently reported crystal structure of the β_2 -adrenergic receptor raises the interesting

possibility of the presence of putative nonannular binding sites in transmembrane interhelical locations in GPCRs. Interestingly, it was previously proposed from quenching analysis of intrinsic tryptophan fluorescence in the nicotinic acetylcholine receptor by brominated lipids and cholesterol analogues, that there could be 5–10 nonannular sites per ~250,000 Da monomer of the receptor [25]. This is consistent with the above proposal of two putative nonannular sites per ~50,000 Da monomer of the β_2 -adrenergic receptor.

4. The serotonin_{1A} receptor: a representative member of the GPCR superfamily in the context of membrane cholesterol dependence for receptor function

The serotonin_{1A} (5-HT_{1A}) receptor is an important member of the large family of serotonin receptors [52,53]. Serotonin receptors have been classified into at least 14 subtypes on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization, and second messenger coupling pathways [54]. The serotonin_{1A} receptor is a crucial neurotransmitter receptor and is the most extensively studied of the serotonin receptors for a variety of reasons [52,53]. The serotonin_{1A} receptor is the first among all the types of serotonin receptors to be cloned as an intronless genomic clone (G-21) of the human genome which cross-hybridized with a full length β -adrenergic receptor probe at reduced stringency [52,55]. Sequence analysis of this genomic clone (later identified as the serotonin_{1A} receptor gene) showed considerable (~43%) amino acid similarity with the β_2 -adrenergic receptor in the transmembrane domain [53]. The serotonin_{1A} receptor was therefore initially discovered as an ‘orphan’ receptor and was identified (‘deorphanized’) later [56]. The human gene for the receptor encodes a protein of 422 amino acids (see Fig. 3). Serotonergic signaling plays a key role in the generation and modulation of various cognitive, developmental and behavioral functions. Interestingly, mutant (knockout) mice lacking the serotonin_{1A} receptor exhibit enhanced anxiety-related behavior, and represent an important animal model for genetic vulnerability to complex traits such as anxiety disorders and aggression in higher animals [57,58].

Seminal work from our laboratory has comprehensively demonstrated the requirement of membrane cholesterol in the function of the serotonin_{1A} receptor ([59], reviewed in [20]). We demonstrated the crucial modulatory role of membrane cholesterol on the ligand binding activity and G-protein coupling of the hippocampal serotonin_{1A} receptor using a number of approaches such as treatment with (i) M β CD, which physically depletes cholesterol from membranes [59,60] (ii) the sterol-complexing detergent digitonin [61], and (iii) the sterol-binding antifungal polyene antibiotic nystatin [62]. Interestingly, while treatment with M β CD physically depletes cholesterol from membranes, treatment with other agents merely modulates the availability of membrane cholesterol without physical depletion. The unifying theme of these findings is that it is the *non-availability of membrane cholesterol*, rather than the manner in which its availability is modulated, is crucial for ligand binding of the serotonin_{1A} receptor. Importantly, replenishment of membranes with cholesterol using M β CD-cholesterol complex led to recovery of ligand binding activity to a considerable extent. It was, however, not clear from these results whether the effect of membrane cholesterol on the function of the serotonin_{1A} receptor is due to specific interaction of membrane cholesterol with the receptor or general effect of cholesterol on the membrane bilayer, or a combination of both.

In order to further explore the mechanism of cholesterol-dependent function of the serotonin_{1A} receptor and examine the stringency of the process, membranes were treated with cholesterol oxidase, which catalyzes the oxidation of cholesterol to cholestenone. These results showed that oxidation of membrane cholesterol led to inhibition of the ligand binding activity of the serotonin_{1A} receptor

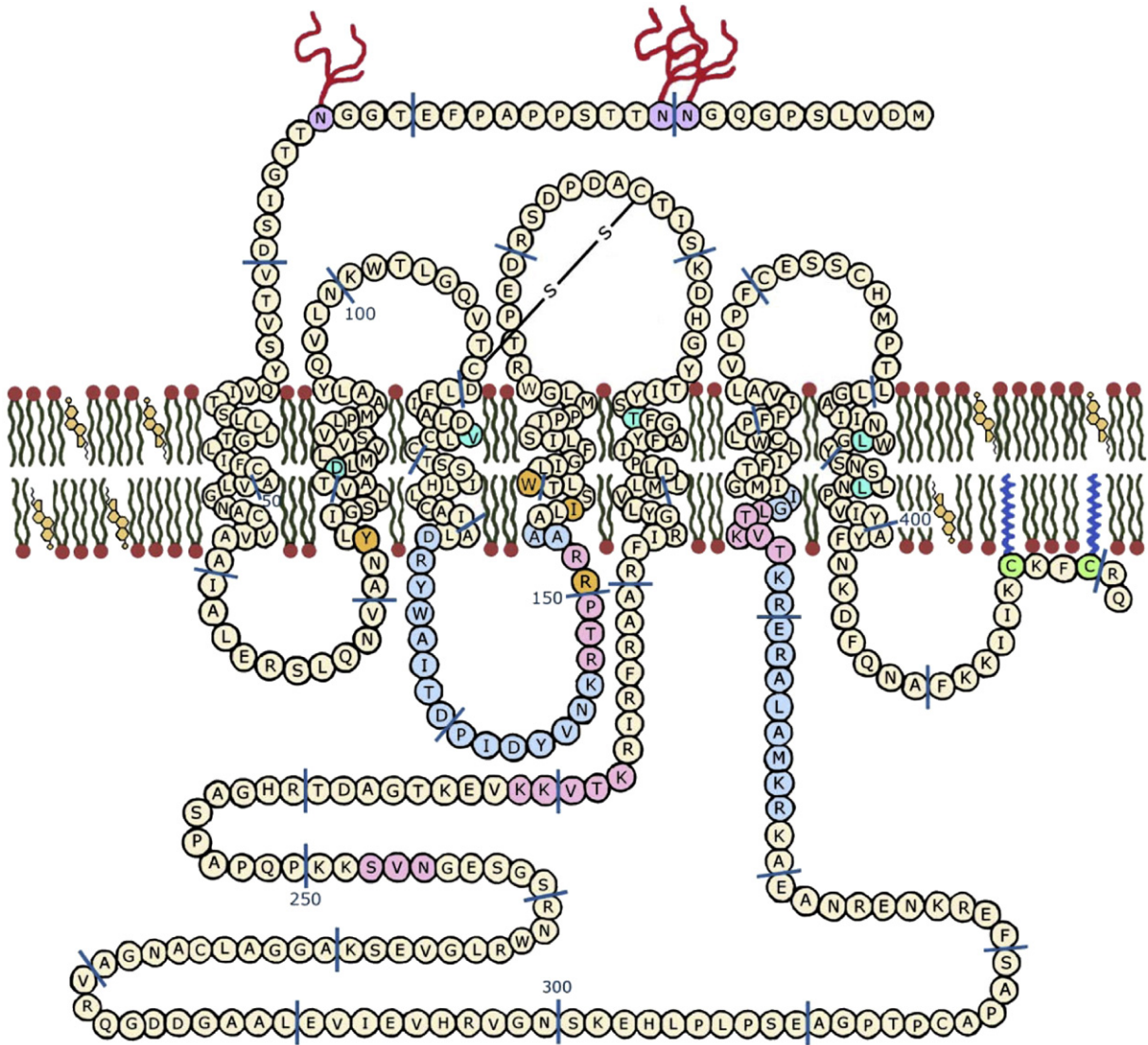


Fig. 3. A schematic representation of the membrane embedded human serotonin_{1A} receptor showing its topological and other structural features. The membrane is shown as a bilayer of phospholipids and cholesterol, representing typical eukaryotic membranes. The transmembrane helices of the receptor were predicted using TMHMM2. The predictions were confirmed by other programs such as MEMSTAT, SPLIT4 and HMMTOP2 (it has been earlier reported that these four programs were among the best and perform equally well in predicting transmembrane helices [72]). Seven transmembrane stretches, each composed of ~22 amino acids, are depicted as putative α -helices. The exact boundary between the membrane and the aqueous phase is not known and therefore the location of the residues relative to the membrane bilayer is putative. The amino acids in the receptor sequence are shown as circles and are marked for convenience. The potential sites (shown in lavender) for N-linked glycosylation (depicted as branching trees in red) on the amino terminus are shown. A putative disulphide bond between Cys¹⁰⁹ and Cys¹⁸⁷ is shown. The transmembrane domains contain residues (shown in cyan) that are important for ligand binding. The putative cholesterol binding site (see text) is highlighted (in orange). The receptor is stably palmitoylated (shown in blue) at residues Cys⁴¹⁷ and/or Cys⁴²⁰ (shown in green). Light blue circles represent contact sites for G-proteins. Light pink circles represent sites for protein kinase mediated phosphorylation. Further structural details of the receptor are available in [20] and [52]. Adapted and modified from [20]. It is probable, based on comparison with known crystal structures of similar GPCRs such as rhodopsin and β_2 -adrenergic receptor, that there are motionally restricted water molecules that could be important in inducing conformational transitions in the transmembrane portion of the receptor.

without altering overall membrane order [63]. Based on these results, we proposed that there could be specific interaction between membrane cholesterol and the serotonin_{1A} receptor. Toward this effect, we have recently generated a cellular model of the Smith-Lemli–Opitz Syndrome (SLOS) using cells stably expressing the human serotonin_{1A} receptor [64]. SLOS is a congenital and developmental malformation syndrome associated with defective cholesterol biosynthesis in which the immediate biosynthetic precursor of cholesterol (7-dehydrocholesterol or 7-DHC) is accumulated [65]. The cellular model of SLOS was generated by metabolically inhibiting the biosynthesis of cholesterol, utilizing a specific inhibitor (AY 9944) of the enzyme required in the final step of cholesterol biosynthesis. SLOS serves as an appropriate condition to ensure the specific effect of membrane cholesterol in the function of the serotonin_{1A} receptor,

since the two aberrant sterols that get accumulated in SLOS, *i.e.*, 7- and 8-DHC, differ with cholesterol only in a double bond. Our results show a progressive and drastic reduction in specific ligand binding with increasing concentrations of AY 9944 used [64]. In addition, our results show that the G-protein coupling and downstream signaling of serotonin_{1A} receptors are impaired in SLOS-like condition, although the membrane receptor level does not exhibit any reduction. Importantly, metabolic replenishment of cholesterol using serum partially restored the ligand binding activity of the serotonin_{1A} receptor under these conditions. Interestingly, we have recently shown that 7-DHC does not support the function of the serotonin_{1A} receptor without change in overall membrane order [66,67], and the effects of 7-DHC and cholesterol on membrane organization and dynamics are considerably different [68]. The requirement for

maintaining ligand binding activity therefore appears to be more stringent than the requirement for maintaining membrane order [66,67]. Taken together, these results indicate that the molecular basis for the requirement of membrane cholesterol in maintaining the ligand binding activity of serotonin_{1A} receptors could be due to specific interaction. In the light of these results, it is indeed interesting to note that there are reported cholesterol binding sites (possibly nonannular) in the crystal structure of a closely related receptor *i.e.*, the β_2 -adrenergic receptor, as discussed above.

5. Cholesterol binding motif(s) in serotonin_{1A} receptors ?

In the overall context of the presence of CCM in the recently reported crystal structure of the β_2 -adrenergic receptor [43], it is an appropriate time now to consider whether there is similar CCM(s) present in the serotonin_{1A} receptor and if present, whether it is conserved during the evolution of the receptor. This is particularly relevant in view of the similarity between the serotonin_{1A} and β_2 -adrenergic receptors (~43% amino acid similarity in the transmembrane domain) [53], and the reported cholesterol dependence of serotonin_{1A} receptor function [20]. In order to examine the evolution of specific cholesterol binding site(s) of the serotonin_{1A} receptor over various phyla, we analyzed amino acid sequences of the serotonin_{1A} receptor from available databases (see Fig. 4). Partial, duplicate and other non-specific sequences were removed from the set of sequences obtained. The amino acid sequences used for the analysis belong to diverse taxa that include insects, fish and other marine species,

amphibians and extending up to mammals. Initial alignment was carried out using ClustalW. It is apparent from this alignment that the cholesterol binding motif, which includes Tyr73 in the putative transmembrane helix II and Arg151, Ile157 and Trp161 in the putative transmembrane helix IV (see Fig. 3 and Fig. 4), is conserved in most species. Realignment with ClustalW (after eliminating the relatively divergent parts of the receptor) resulted in conservation of the motif across all phyla analyzed, except in organisms such as *T. adhaerens* and *S. purpuratus*. Interestingly, pairwise alignment of the human serotonin_{1A} receptor with the human β_2 -adrenergic receptor and rhodopsin exhibited conservation of the motif in all sequences (data not shown). It therefore appears that cholesterol binding sites represent an inherent characteristic feature of serotonin_{1A} receptors which is conserved during the course of evolution. It is interesting to note here that cholesterol binding sites appear to be present even in organisms which are not capable of biosynthesis of cholesterol. Organisms which lack cholesterol biosynthesis could, however, acquire cholesterol through diet [70]. Organisms such as insects possess sterols that are different from cholesterol which have diverged from cholesterol during the sterol evolution pathway [69]. The presence of CCM in these organisms could be due to binding of closely related sterols or dietary cholesterol to CCM.

6. Conclusion and future perspectives

Previous work from our laboratory has demonstrated that membrane cholesterol is required for the function of the serotonin_{1A}

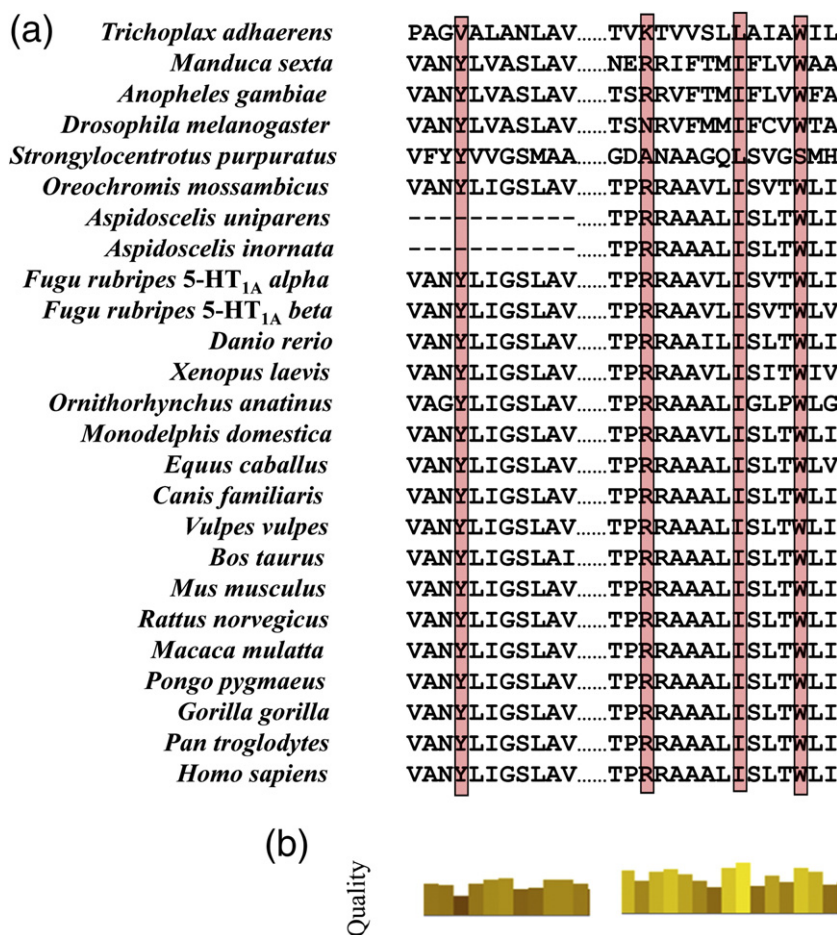


Fig. 4. Multiple alignment of the serotonin_{1A} receptor around the CCM of interest with the conserved residues highlighted. As evident from the panel (a), Trp161 is the most conserved residue, except in *S. purpuratus*. The sequences of *T. adhaerens*, *M. sexta* and *A. gambiae* are putative serotonin_{1A} receptors whereas those of *S. purpuratus*, *B. taurus*, *O. anatinus*, *D. rerio*, *M. domestica* and *M. mulatta* are predicted by homology. Panel (b) is a graphical representation displaying the quality of alignment, with lighter shades representing higher quality. Amino acid sequences of serotonin_{1A} receptors are from NCBI and ExPASy databases.

receptor, which could be due to specific interaction of the receptor with cholesterol. Based on these results, we envisage that there could be specific/nonannular cholesterol binding site(s) in the serotonin_{1A} receptor. Our future work will focus on mutating the amino acid residues involved in the cholesterol binding site of the serotonin_{1A} receptor followed by functional and organizational (in the context of membrane localization and domains) analyses of the receptor, in order to gain further insight into membrane cholesterol dependence of receptor function.

As mentioned earlier, GPCRs are involved in a multitude of physiological functions and represent important drug targets [6–8]. Although the pharmacological and signaling features of GPCRs have been studied widely, aspects related to their interaction with membrane lipids have been addressed in relatively few cases. In this context, the realization that lipids such as cholesterol could influence the function of GPCRs has remarkably transformed our idea regarding the function of this important class of membrane proteins. With progress in deciphering molecular details on the nature of this interaction, our overall understanding of GPCR function in health and disease would improve significantly, thereby enhancing our ability to design better therapeutic strategies to combat diseases related to malfunctioning of these receptors.

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