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Review

Ion channels gated by acetylcholine and serotonin: structures, biology, and drug discovery

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The nicotinic acetylcholine receptors (nAChRs) and the 5-HT_3 receptors (5-HT_3Rs) are cation-selective members of the pentameric ligand-gated ion channels (pLGICs), which are oligomeric protein assemblies that convert a chemical signal into an ion flux through postsynaptic membrane. They are critical components for synaptic transmission in the nervous system, and their dysfunction contributes to many neurological disorders. The diverse subunit compositions of pLGICs give rise to complex mechanisms of ligand recognition, channel gating, and ion-selective permeability, which have been demonstrated in numerous electrophysiological and molecular biological studies, and unraveled by progress in studying the structural biology of this protein family. In this review, we discuss recent insights into the structural and functional basis of two cation-selective pLGICs, the nAChR and the 5-HT_3R, including their subunit compositions, ligand binding, and channel gating mechanisms. We also discuss their relevant pharmacology and drug discovery for treating various neurological disorders. Finally, we review a model of two alternative ion conducting pathways based on the latest 5-HT_{3A} crystal structure.

Keywords: nAChR; 5-HT_3R; structure biology; subunit composition; channel activation; channel gating; ion selectivity; neurological disorders

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Introduction

The nicotinic acetylcholine receptors (nAChRs) and the 5-hydroxytryptamine type 3 receptors (5-HT_3Rs) are cation-selective members of the pentameric ligand-gated ion channels (pLGICs), which also include the anion-selective GABA and glycine receptors, the cation channel homologs in prokaryotes (including the bacterial *Erwinia chrysanthemi* ligand-gated ion channel (ELIC) and the *Gloebacter violaceus* ligand-gated ion channel (GLIC)) and the anion-selective homolog in invertebrates (the glutamate-gated chloride channel (GluCl)) (Figure 1).

Both nAChRs and 5-HT_3Rs comprise five subunits arranged around a central ion-conducting pore that is permeable to cations including Na^+, K^+ and Ca^{2+} when the receptors are activated[1-3]. These receptors exist in different inter-convertible conformational states that are triggered by the binding of agonists, antagonists, or allosteric modulators[4] (Figure 2).

The nAChRs and 5-HT_3 receptors are expressed throughout the central nervous system (CNS)[5, 6] and peripheral nervous system (PNS)[7] and mediate a variety of physiological functions. The nAChRs are potential therapeutic targets for multiple central nervous system disorders such as schizophrenia, Alzheimer's disease, Parkinson's disease and nicotine addiction[8-11]. Moreover, ligands that target the 5-HT_3Rs are powerful therapeutic agents for the control and treatment of drug and alcohol dependence, schizophrenia, anxiety, and cognitive dysfunction, as well as chemotherapy-induced and post-operative nausea and vomiting[12-15]. As such, both nAChRs and 5-HT_3Rs have been the targets of drug discovery efforts for many years. Some of these efforts have gone beyond clinical evaluation and led to marketed drugs[16]. A more comprehensive understanding of the connection between the structure and function of these receptors could facilitate ongoing drug discovery efforts. In this review, we summarize the current structural and functional knowledge of the nAChRs and the 5-HT_3Rs.

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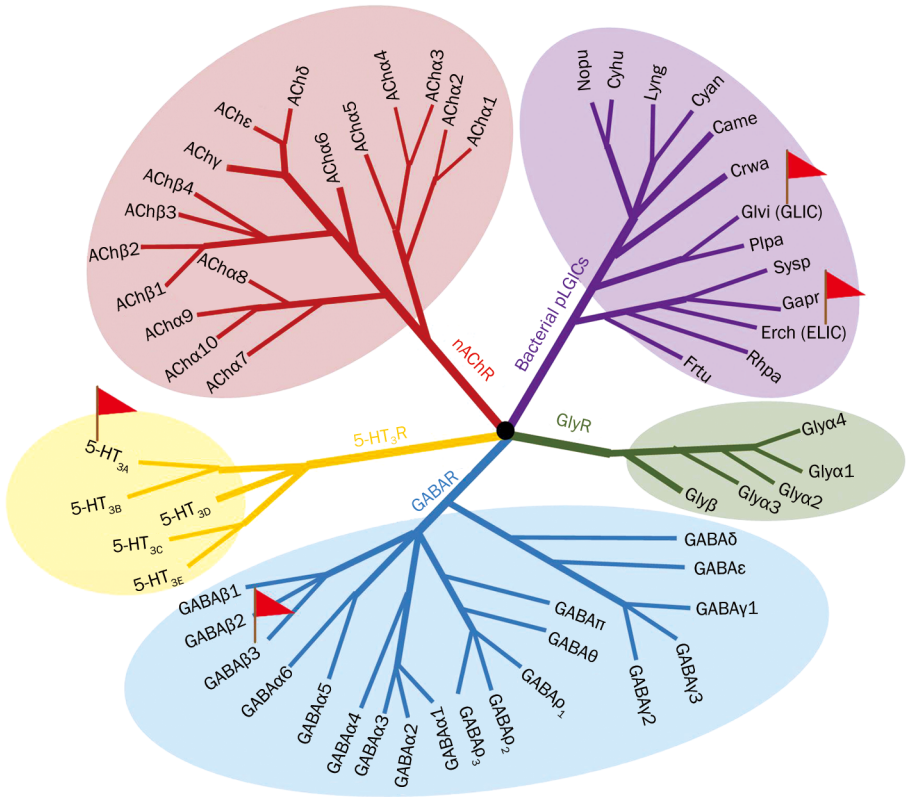

Figure 1. Phylogenetic tree showing the relationship between the pentameric ligand-gated ion channels (pLGICs). Red: the nicotinic acetylcholine receptors (nAChRs); yellow: the 5-HT_3Rs; blue: gamma-aminobutyric acid receptors (GABARs); army green: glycine receptors (GlyRs); and purple: bacterial pLGICs. The red flags indicate the members whose X-ray structure has been determined.

Receptor subunits and components

To date, seventeen nAChR subunits and five 5-HT_3R subunits have been identified. The nAChR subunits include multiple α (α1−α10) and β subunits (β1−β4) as well as δ, γ, and ε subunits, and the 5-HT_3R subunits include A, B, C, D, and E subtypes[17]. These subunits have been highly conserved through evolution and each single subunit has more than 80% amino acid identity across vertebrate species. The nAChR subunits can be divided into four subfamilies (I–IV) based on similarities in protein sequence, and the classification of 5-HT_3R subunits is relatively simple[18] (Figure 3).

The diversity in subunit composition may influence the characteristics of nAChRs and 5-HT_3Rs, including their agonist sensitivity, channel kinetics, Ca^{2+} permeability, assembly, interactions with chaperones, trafficking and cell localization[19-23]. Muscle-type nAChRs are composed of α1, β1, γ, and δ subunits in a 2:1:1:1 ratio or composed of α1, β1, δ and ε subunits in a 2:1:1:1 ratio. Neuronal-type receptors are homomeric or heteromeric combinations of twelve different nicotinic receptor subunits, α2−α10 and β2−β4, such as $(α4)_3(β2)_2$, $(α4)_2(β2)_3$, or $(α7)_5$[24]. A functional 5-HT_3 receptor may be composed of five identical 5-HT_{3A} subunits (homopentameric) or a mixture of 5-HT_{3A} and one of the other four 5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} subunits (heteropentameric)[25].

The homomeric nAChR and 5-HT_3Rs have five identical ligand binding sites located at the interface between two adjacent subunits[26]. Each heteromeric nAChR contains two agonist binding sites with different affinities. Although the subunit stoichiometry of the heteromeric 5-HT_3Rs is not clearly studied, it was demonstrated that agonists bind to an interface between two adjacent 5-HT_{3A} subunits in the heteromeric 5-HT_{3AB} receptor[27], which may explain why the 5-HT_{3A} subunit is essential to form functional 5-HT_3 receptors. Because the binding sites cooperate, all sites need to be occupied with agonist to fully activate the ion channel. Elucidation of the influence of subunit composition on ligand binding and channel function will be an important topic of future research on these two receptors.

Definition and physiological functions

In the human nervous system, nicotinic cholinergic signals are extended throughout the system, where the neurotransmitter acetylcholine (ACh) plays a key role in activating ligand-gated ion channels, which is one of the most important and oldest modulatory neurotransmitter systems[28]. ACh is synthesized in specific neurons by choline acetyltransferase from choline and acetyl-CoA. The enzyme acetylcholinesterase converts ACh into inactive metabolites choline and acetic acid in the intercellular space. The degradation products can be transported back into the nerve cells by specific transporters[29]. Importantly, ACh is released from presynaptic neurons and binds to the nAChRs that modulate the flow of ions across the

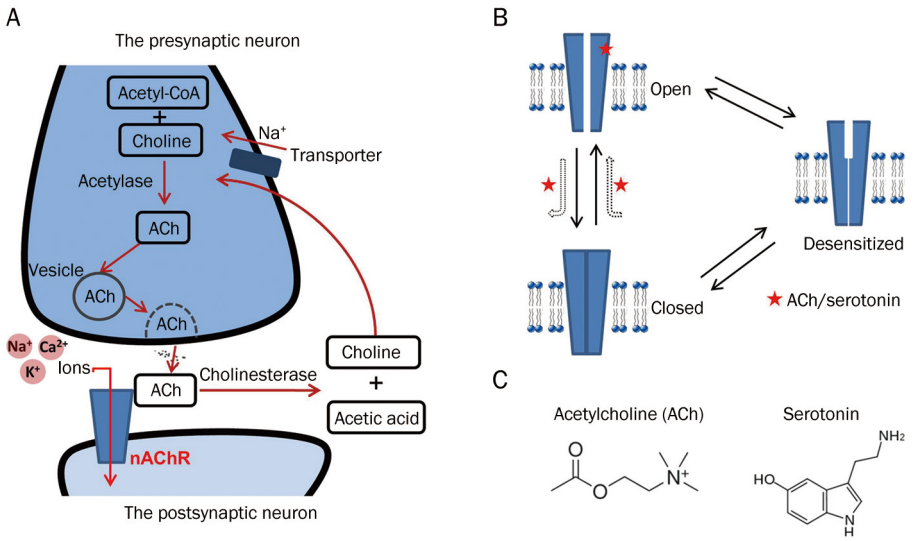

Figure 2. Mechanism of nAChR activation. (A) Acetylcholine (ACh) is synthesized, stored and released by cholinergic neurons. ACh is released from presynaptic neurons and binds to the nAChR of postsynaptic neurons to modulate the flow of ions across the cell membrane. (B) Three distinct functional states of the receptor exist: the closed, open and desensitized states; however, only the open state permits ion flux. (C) Chemical structures of the endogenous neurotransmitters ACh and serotonin (agonists).

cell membrane similarly to all cell-surface ligand-gated ion channels (Figure 2). In the nervous system, cholinergic stimulation mediated through nAChRs controls pathways such as transmitter release and cell sensitivity, which can influence physiological activity including sleep, anxiety, pain processing and cognitive functions[30-33]. A net influx of cations through the associated channel pore depolarizes the cell membrane and increases neuronal excitability. In particular, calcium entry through the nAChRs triggers a series of intracellular signaling cascades[23].

On the molecular level, the activation of presynaptic 5-HT_3Rs also induces Ca^{2+} influx and modulates the vesicular release of various neurotransmitters and neuropeptides[34-36]. The activation of postsynaptic 5-HT_3Rs leads to depolarization by the opening of a channel permeable to Ca^{2+} as well as Na^+ and K^+. On the systemic level, the 5-HT_3Rs in the CNS are involved in the integration of the vomiting reflex, pain processing, anxiety control and the reward system, while peripheral receptors participate in the regulation of autonomic functions and sensory transmission[37]. 5-HT_3R agonists lead to unpleasant feelings, such as nausea and anxiety, thus are rarely used clinically. However, 5-HT_3R antagonists are widely used for relieving chemotherapy-induced vomiting as well as radiotherapy-induced and post-operative nausea and vomiting. The 5-HT_3Rs also regulate gastrointestinal (GI) functions including secretion and motility, while their antagonists are effective in the management of post-infectious irritable bowel syndrome and severe diarrhea-predominant irritable bowel syndrome, although they present adverse gastrointestinal effects. More recently, involvement of the 5-HT_3Rs was found in psychiatric indications such as drug addiction, cognitive function, schizophrenia, satiety control, and the regulation of inflammatory and immune responses[38-40].

nAChRs					
Neuronal-type subunits					Muscle-type subunits
I	II	III			IV
α9, α10	α7, α8	1	2	3	α1, β1, δ, γ, ε
		α2, α3, α4, α6	β2, β4	β3, α5	
5-HT_3Rs					
Homopentameric		Heteropentameric			
5-HT_{3A}		5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, 5-HT_{3E}			

Figure 3. Subunits of the nAChRs and 5-HT_3Rs.

The physiological processes regulated by the nAChRs and the 5-HT_3Rs are dependent on the specific ligand bound[41]. As illustrated in Figure 2, the binding of exogenous agonists to the orthosteric site substantially influences the transition rates between three distinct functional states of the receptor: the closed, open and desensitized states[42]. The rate constants between different functional states are highly dependent on the specific combination of subunits and the chemical nature of the agonist bound at the receptor[43]. The transition rates between the conformational states can also be modulated by endogenous or exogenous allosteric modulators[44]. Essentially, the conformational states of the receptor influence the activity of the target cells through the selective transportation of Na^+, K^+, and Ca^{2+} into cells to regulate various physiological processes[45].

Overall structure

With the development of new technologies in structural biology, structural studies of ion channels have been progressing steadily, providing insights into the three-dimensional struc-

ture of pentameric ligand-gated ion channels, especially the cation-selective channels (Figure 4). The first high-resolution cryo-EM structure (4 Å) of the nAChR from the *Torpedo marmorata* electric organ was reported in 2005, revealing important functional and pharmacological characteristics of this receptor[46]. Based on the X-ray structures of the acetylcholine binding protein (AChBP) and the serotonin binding protein (5-HTBP), an atomic model of the extracellular domain (ECD) of nAChR has been available for 15 years[47-49]. AChBP and 5-HTBP are water-soluble proteins that have a high degree of sequence similarity to the ECD of the nAChR and the 5-HT_3Rs, respectively. In 2008 and 2009, X-ray structures of two prokaryotic pLGICs, ELIC[50] and GLIC[51], were determined. Both are cation-selective ion channels that show high sequence and structure similarity to the nAChRs and the 5-HT_3Rs. In 2011, the first X-ray structure of a eukaryotic pLGIC was determined[52]. The crystal structure of the GluCl from *Caenorhabditis elegans* revealed an open conformation of the pLGIC. In 2014, two groups presented the X-ray structures of two mammalian pLGICs, the human $GABA_A$β3 receptor[53] and the mouse 5-HT_{3A} receptor[54]. These two structures offered the latest insights into the signaling mechanisms of pLGICs. Specifically, the serotonin 5-HT_3R and the nAChR are closest among the pLGICs and show very high sequence similarity (Figure 5A), suggesting that they may share structural characteristics and regulatory mechanisms. Notably, both the GluCl and 5-HT_{3A} structures were determined in complex with an antibody or a nanobody, which facilitates the crystallization of transmembrane channels.

Comparison of the aforementioned structures reveals that the overall structure appears to be conserved among the pLGICs, particularly the eukaryotic cation pLGICs, the nAChRs and the 5-HT_3Rs. The different nAChR and 5-HT_3R subunits share a basic scaffold composed of an ECD, a four-transmembrane helix domain (TMD), an intracellular domain (ICD) between the third and fourth transmembrane helix and a short extracellular C-terminus. The receptors are therefore built from modular units with an extracellular domain containing the agonist/antagonist binding pocket, a transmembrane domain containing the allosteric modulatory sites and a large cytoplasmic domain involved in receptor trafficking and regulation (Figure 5B and 5C). Comparison of available structures revealed that the overall structure of cation-selective pLGICs (nAChRs and 5-HT_3Rs) is different from anion-selective pLGICs ($GABA_A$Rs and GlyRs), with the presence of a relatively large ICD that may play an important role in cation conduction.

Extracellular domain and binding pocket

The organization of the ligand binding pocket of the nAChRs and the 5-HT_3Rs has been confirmed by many structures. A major step toward this achievement was the determination of the high-resolution crystal structures of the soluble proteins that bind ACh or serotonin, AChBP and 5-HTBP, respectively. These soluble proteins share key structural elements with the ECDs of nAChRs and 5-HT_3Rs, which display similar ligand binding signatures, and their respective structures have become models for the ECDs of the nAChR and the 5-HT_3R. The 5-HTBP was engineered from the weakly 5-HT-binding AChBP by a series of mutations. Single-point mutations of the nAChR and the 5-HT_3R are sufficient to switch agonist actions of serotonin and acetylcholine, respectively, to antagonists[55, 56]. These mutations confirm the ligand recognition sites and reveal that the nAChR and the 5-HT_3R use the same structural elements for ligand binding.

Based on the modeling of the pentameric structure of AChBP, each nAChR ECD monomer consists of an N-terminal α-helix and a core of ten β-strands that form an αβ-sandwich structure. The inner β-sheet is formed by β1, β2, β3, β5, β6 and β8 and the outer β-sheet by β4, β7, β9 and β10. The N- and C-termini are located at the top and bottom of the pentamer fold, respectively. The C-terminus of β10 is connected to the N-terminus of TM1. The linker between strands β6 and β7 forms the signature Cys-loop found in all members of the Cys-loop receptor (CLR) family. This Cys-loop is close to the transmembrane domain and may play a role in the propaga-

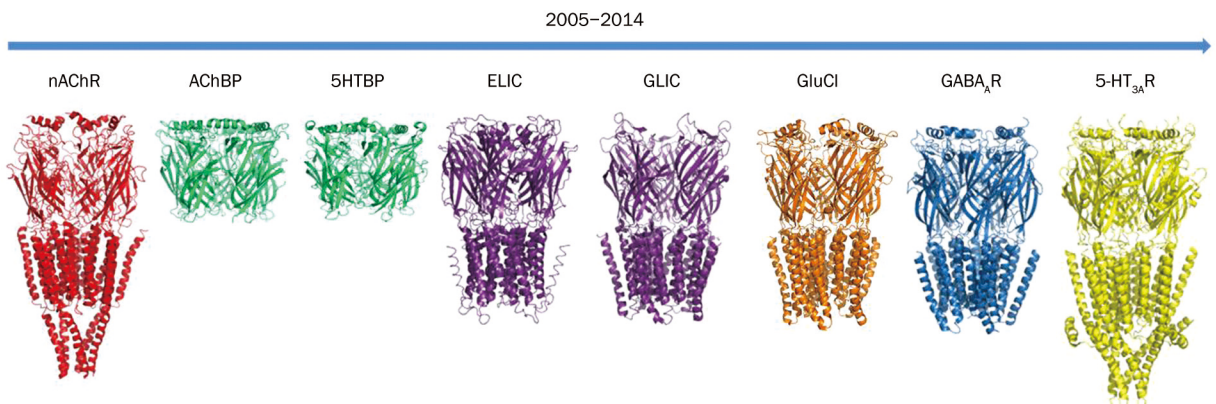

Figure 4. Overview of the published pLGIC structures (2005–2014). From left to right: the electron-microscopic structure of the *Torpedo marmorata* nAChR and the X-ray structures of ACh Binding Protein (AChBP), 5-HT Binding Protein (5-HTBP), the *Erwinia chrysanthemi* ligand-gated ion channel (ELIC), the *Gloeobacter violaceus* ligand-gated ion channel (GLIC), the *Caenorhabditis elegans* glutamate-gated chloride channel (GluCl), the human GABA receptor ($GABA_A$R), and the mouse serotonin 5-HT_{3A} receptor (5-HT_{3A}R).

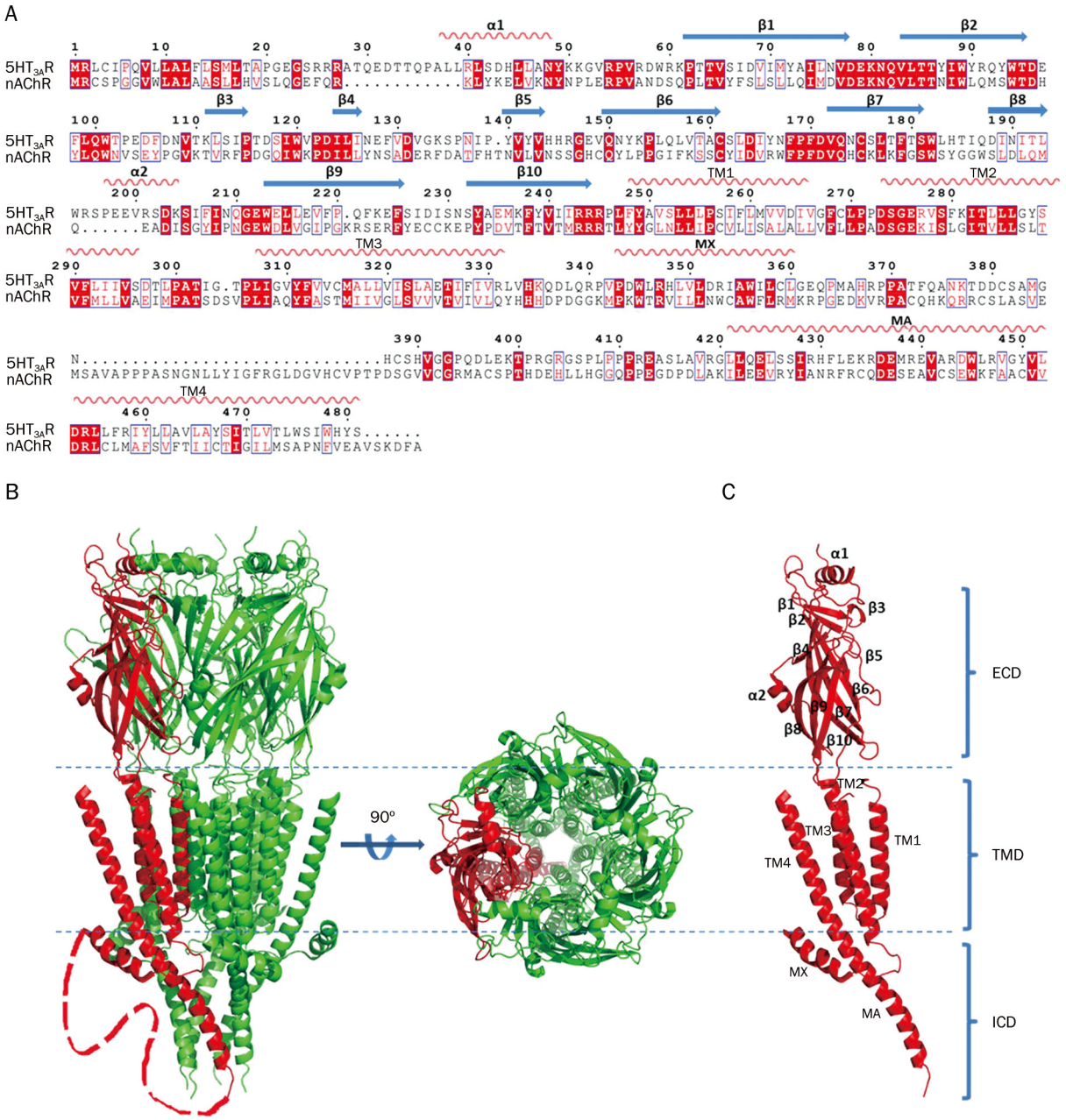

Figure 5. Basic structure of the cation-selective pentameric ligand-gated ion channels. (A) Structure-based sequence alignment of the human nAChR and the mouse 5-HT_{3A} receptor. (B) Schematic view of the X-ray structure of the 5-HT_3 receptor (side and top view). The dashed line represents a long fragment of the cytoplasmic loop (shown only for one front subunit) that was not resolved in the X-ray structure. (C) Structure of one subunit of the 5-HT_3R model. A side view showing the extracellular domains (Cys-loop, α1–2 and β1–10), the transmembrane domains (TMD, TM1–4), and the intracellular domain (ICD).

tion of conformational changes from the ECD to the TMD[57] (Figure 5C). High resolution crystal structures of AChBPs in complex with several nicotinic receptor ligands revealed the orthosteric binding sites for agonists and antagonists in detail. The ligand binding sites are situated at the interface between two neighboring subunits, the principal (*ie*, two constant α-subunits) and the complementary non-α subunits (Figure 6A). Residues from loops A–C of the principal subunit as well as β-strands D/E and loop F of the complementary subunit contribute to ligand recognition. The key residues involved in ACh binding are Trp86, Tyr93 (loop A), Trp149, Gly153 (loop B), Tyr190, Cys192, Cys193, and Tyr198 (loop C) from the principal subunit, and Trp55, Asp57 (β strand D), Leu109, Arg111, Thr117, Leu119 (β strand E), Asp174, and Glu176 (loop F) from the complementary subunit[58] (Figure 6A). Although the subunits of mammalian pLGIC are generally conserved, these pocket amino acids are not identical in nAChRs and 5-HT_3Rs, providing a basis of their ligand binding selectivity.

Transmembrane domain and allosteric modulatory sites

The transmembrane domain of the nAChRs and the 5-HT_3Rs is composed of four helices, TM1 to TM4, which perpendicularly span through the membrane bilayer. The TM1, TM2, and TM3 helices are a closely packed bundle and are ordered in two concentric circles. The assembly of the five TM2 helices forms the inner circle and the ion channel pore, which is an important segment of the ion conduction pathway. Transmembrane helices TM1 and TM3 form the outer circle that stabilizes the pore. TM4, located at the periphery of the transmembrane domain, is relatively loosely packed[59-61] (Figure 6B).

The transmembrane domains of the nAChRs and the 5-HT_3Rs contain binding sites for various allosteric modulators. General anesthetics are small hydrophobic compounds that allosterically inhibit the receptors by binding to a small cavity formed by specific residues located between TM3 and TM4. Crystal structures of the GluCl in complex with a hydrophobic ligand enabled visualization of this allosteric binding site. These allosteric modulators act by binding to regions called allosteric sites, which are separate from the ACh/serotonin binding sites (orthosteric sites) (Figure 6B). The allosteric modulators have either positive (positive allosteric modulators, PAMs) or negative (negative allosteric modulators, NAMs) effects[62]. PAMs/NAMs typically exhibit little intrinsic activity but provide selective potentiation/inhibition of physiological activity without directly interfering with the ongoing signaling processes. Ivermectin, 5-hydroxyindole, NS-1738, SB-206553 and PNU-120596 have been reported to function as PAMs at the nAChRs[63]. The development of PAMs is of great significance, as they may effectively avoid receptor desensitization processes.

Channel activation

The overall architecture of the channel complex assembly reveals that the ligand binding site in ECD is far above the channel pore in the TMD region (Figure 4). The mechanism of how ligand binding at the ECD can control (by gating) the opening and closing of the distal channel pore at the TMD is very intriguing. At their resting states, the channels of the nAChRs and the 5-HT_3Rs are closed. The binding of agonist to the ECD triggers rapid conformational changes, which leads to the opening of the transmembrane pore, a process referred to as gating isomerization.

The crystal structures of a chimeric α7 nicotinic recep-

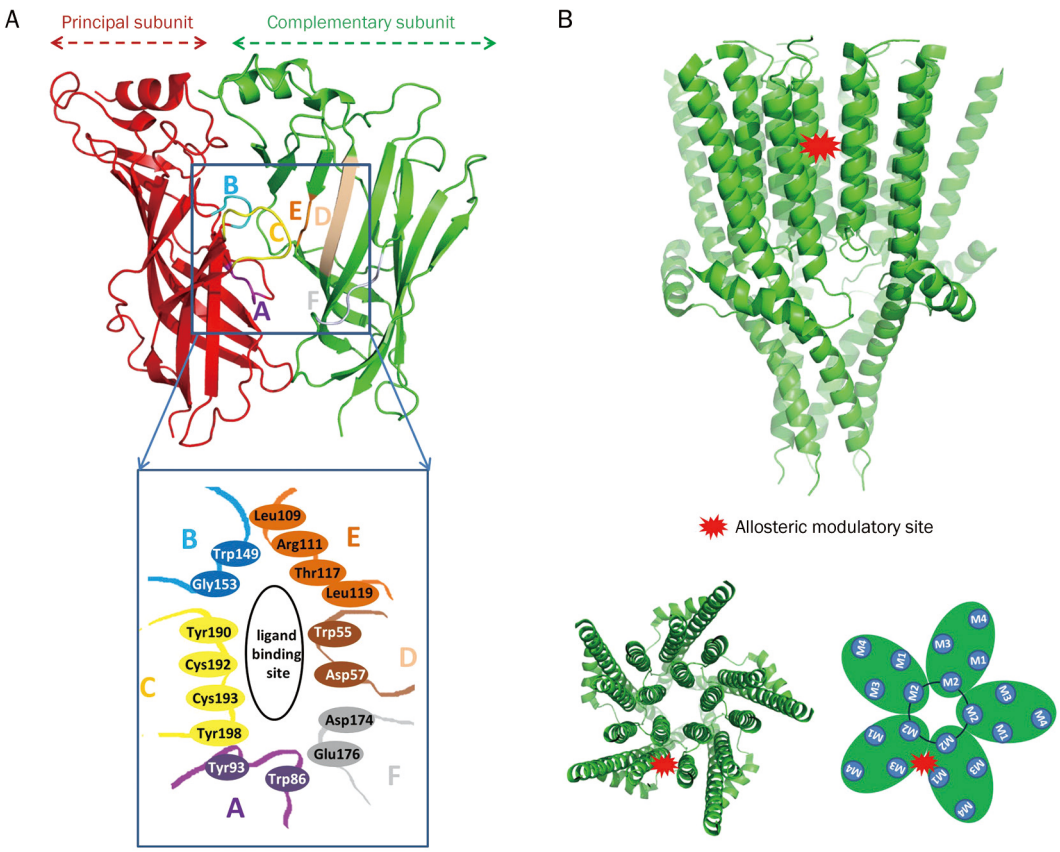

Figure 6. Structure of the ligand binding and allosteric modulatory sites. (A) Close-up view of a ligand binding site showing the amino acid residues in the loops that participate in its formation. Loops A, B, and C are provided by the principal subunit and loops D, E, and F by the complementary subunit. (B) The X-ray structure of the transmembrane domain of the 5-HT_3 receptor (side/top view and schematic chart). Each subunit of the TMD contributes four helices (TM1–4), which approach one another at the intracellular membrane surface, creating a tapered central pore. View of the side of the TMD showing a potential binding site for allosteric modulators (marked by a red asterisk). The intersubunit allosteric modulatory site is modeled based on the crystal structure of ivermectin bound GluCl. The site is located in the transmembrane domain between the four transmembrane segments (TM1–4).

tor ECD with its agonist epibatidine[64] and its antagonist α-bungarotoxin[65] enabled observation of this process. Structural comparison of the agonist- and antagonist-bound conformations revealed a large movement of loop C, which was also observed in a similar comparison of the agonist- and antagonist-bound 5-HTBP structures[66]. By further investigation of these structures, a twisting and blooming model was initially suggested to be directly involved in ion channel activation (Figure 7A). The relatively small conformational changes at the ligand binding site suggest a highly efficient gating mechanism.

Structures of the open state GLIC and the closed state ELIC elucidate how the conformational changes induced by agonist binding are propagated from the ECD to the TMD (Figure

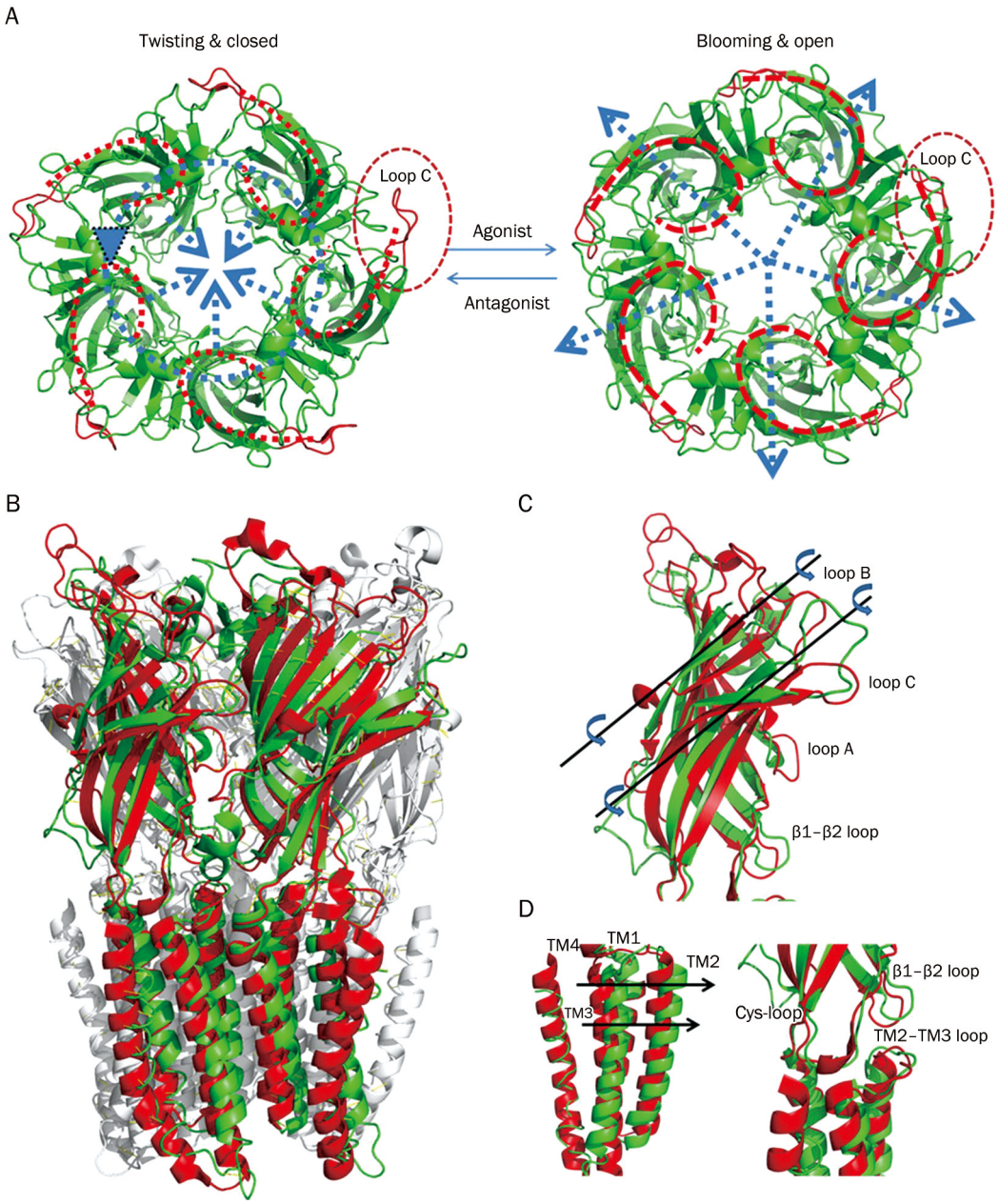

Figure 7. The proposed gating mechanism of the receptors. (A) The twisting and blooming model. On top, the twisting and blooming transition is shown. The conformation of the open and closed states is captured by the crystal structures of the ligand binding domain of a pentameric α7 nicotinic receptor chimera with its agonist epibatidine (right) and its antagonist α-bungarotoxin (left). When agonist is bound, loop C is repositioned towards the structural subunit to cap the agonist-binding site, and it extends away from the agonist binding pocket when the site is occupied by an antagonist. The blue dashed arrows illustrate the direction of the twisting and blooming. (B) Open GLIC and closed ELIC structure comparison. The two subunits in the foreground are colored green for GLIC and red for ELIC, and the other subunits are shown in grey. (C) A rotation of the extracellular domain. (D) Close-up view of the TMD and the interface between the ECD and the TMD. Concerted downward motion of the β1–β2 loop and outward motion of the TM2–TM3 segment causes pore opening.

7B). The proposed model of gating is a stepwise isomerization process that starts from the orthosteric binding site (loops A, B, and C) (Figure 7C), then propagates to the ECD/TMD interface (β1–β2 loop and Cys-loop) via a rigid-body rearrangement of the extracellular β-sandwiches, which in turn induces outward movement of the loop between transmembrane helices TM2 and TM3, ultimately resulting in the opening of the gate that is formed by the TM2 helix (Figure 7D). This structural rearrangement was best described as a concerted opposite-twist rotation of the ECD relative to the TMD around the five-fold symmetry axis.

In 2011 and 2014, two structures of the *C. elegans* GluCl were published. One structure is in complex with the allosteric partial agonist ivermectin, which provided insights into the structure of a potentially open state, and the other is the apo state, a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)-bound conformation[67]. These two structures of eukaryotic Cys-loop receptors further answered questions regarding the mechanism of channel opening and closing. The TM2–TM3 loop can shift away from the ion channel pore, as visualized by the movement of Pro268 from the TM2–TM3 loop passing beneath Val45 on the β1–β2 loop. The closed pore is most constricted at Leu254 on TM2, suggesting that Leu254 forms the shut gate of the ion channel pore (Figure 8A). Further-

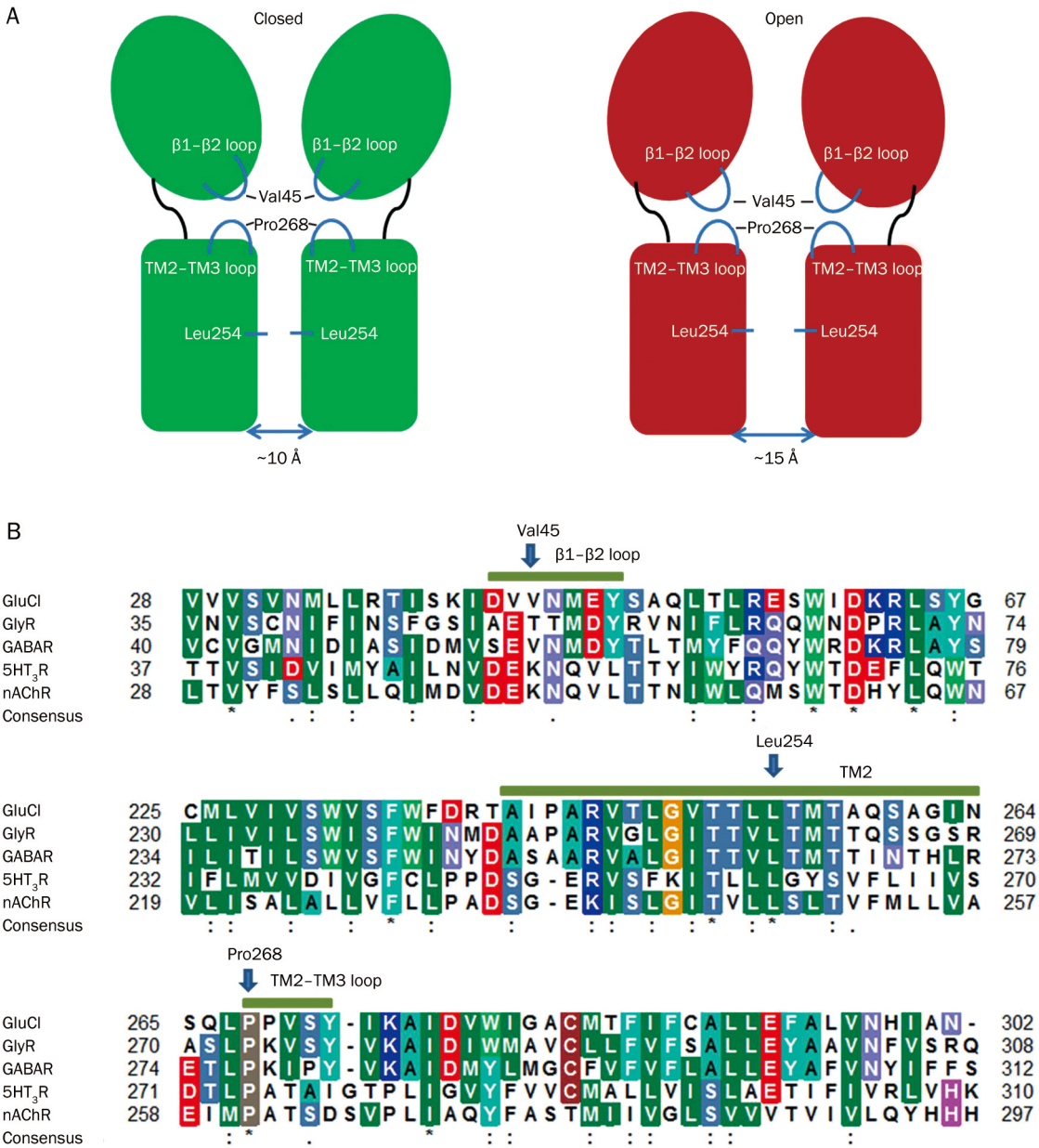

Figure 8. Model of the transitions between the closed and open states. (A) Schematic illustration of the conformations of the closed and open states. The figure has been redrawn from the work of THOMPSON AJ *et al*[68]. (B) Sequence alignment of the Cys-loop receptors. Key residues are indicated by the arrow. GluCl: *C. elegans* GluCl α; GlyR: human glycine α1; GABAR: human GABAβ3; 5-HT_3R: mouse 5-HT_{3A}; and nAChR: human nAChR α7.

more, Pro268 and Leu254 are strictly conserved throughout the family of Cys-loop receptors (Figure 8B), and they could play a similar role in the opening and closing of nAChRs and 5-HT_3Rs.

Taken together, the available structures present a common ligand gating mechanism, where ligand binding at the ECD induces a stepwise isomerization of the subunit interface, which is then propagated to the ECD-TMD interface, resulting in tilting of the TM2 helix that leads to the opening of the channel pore (Figure 7). Interestingly, the allosteric ligand binding site is located in the TMD that is close to the channel pore but far from the ligand binding site in the ECD. We speculate that PAMs and NAMs modulate the channel activities by reducing (PAM) or increasing (NAM) the energy barrier of channel opening.

Cation selectivity and ion conduction in the ICD

The cation selectivity of nAChRs and 5-HT_3Rs is determined by the charge properties along the ion permeation pathway (Figure 9A). The width of the extracellular vestibule is approximately 20 Å, which is appropriate for electrostatic interactions between the charged groups lining the vestibule and for cations to pass through. The charge below the conserved constriction (D105 in the mouse 5-HT_{3A} receptor) is predominantly negative, thus providing an environment to stabilize cations and increase the local concentrations of cations within the lower part of the ECD vestibule. From the bottom of the ECD, cations enter the pore lined by the TM2 helices, and the residues that form the pore lumen surface are almost identical in nAChRs and 5-HT_3Rs (Figure 9B). The upper portion of the pore is a hydrophobic constriction that may serve as the channel gate, while the lower half contains two polar rings that are negatively charged. The negatively charged residues at the bottom of the transmembrane pore may facilitate cations entry into the intracellular vestibule, which has been identified as a major determinant of ionic selectivity and conductance[68-70] (Figure 9B).

The intracellular vestibule is lined by the post-TM3 loop and the C-terminal amphipathic membrane-associated (MA) helices, which were revealed in the EM structure of the Torpedo acetylcholine receptor and the X-ray structure of the mouse serotonin 5-HT_{3A} receptor. The structure of the Torpedo acetylcholine receptor revealed that there are solvent-exposed channels in the upper part of the MA helices for ion exit and entry. The corresponding solvent-exposed channels appear to be blocked by the post-TM3 loops in the mouse serotonin 5-HT_{3A} receptor structure. Moreover, in the 5-HT_{3A} structure, the lower part of the MA helical bundle forms a central channel that is too narrow for ions to pass through unless conformational changes occur. To confirm the exit or entry of ions in the ICD, the conformation of intact receptors in the active state requires further investigation. However, two possible pathways for ion conductance in the ICD are shown in Figure 10. Mutagenesis studies support that the upper channels, not the lower MA helix, are involved in ion conductance[71].

Diseases and drug discovery

In parallel with advances in our understanding of the pharmacology of nAChRs and 5-HT_3Rs, there has been an increasing interest in these receptors as potential drug targets for a number of psychiatric, neurological, and peripheral disorders[72] (Table 1). During the past 10 years, a growing number of

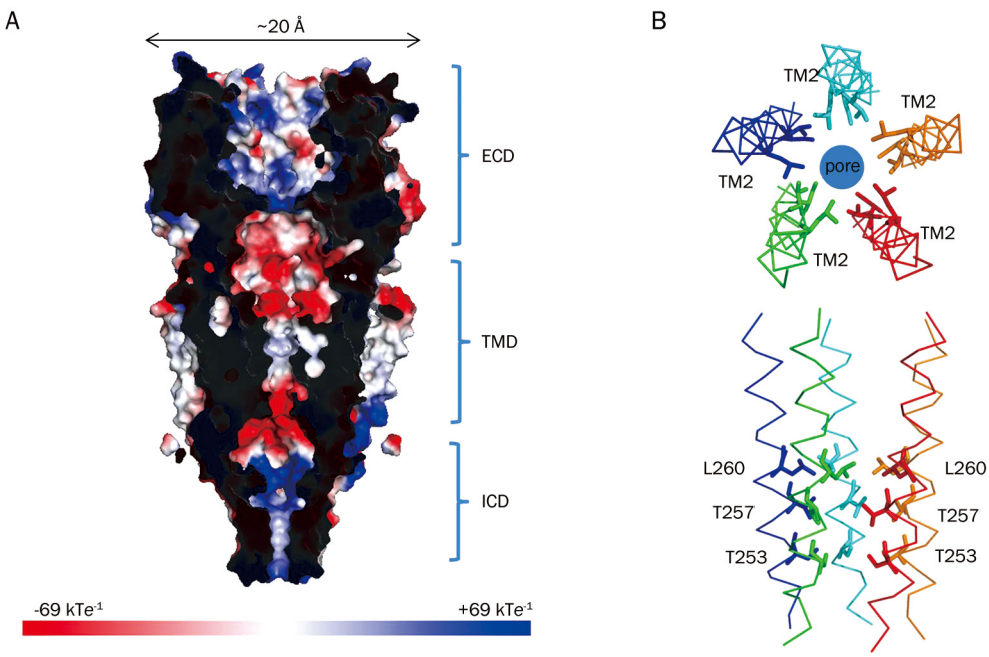

Figure 9. Cation-selectivity. (A) The front of the mouse 5-HT_3 receptor is cut away to reveal the interior surface of the pore, colored by electrostatic potential from −69 kTe^{-1} to 15 kTe^{-1} (red to blue). (B) The side chains of key pore-lining residues, numbered according to their position in the TM2 helix.

Table 1. Clinical development pipeline of nAChR and 5-HT_3R ligands.

nAChR				
Compound	Classification	Indications	Development status	Reference
Nicotine	agonist	Smoking cessation	Marketed	[73]
Cytisine	agonist	Smoking cessation	Marketed	[74]
Bupropion	antagonist	Smoking cessation	Marketed	[75]
Varenicline	agonist	Smoking cessation	Marketed	[76]
CP-601927	agonist	Smoking cessation	Phase 2	[77]
Pozanicline	agonist	ADHD	Phase 3	[78]
Sofinicline	agonist	ADHD	Phase 2	[79]
Altinicline	agonist	Parkinson	Phase 2	[80]
GTS-21	antagonist	Alzheimer	Phase 2	[81]
5-HT_3R				
Compound	Classification	Indications	Development status	Reference
Alosetron	antagonist	IBS	Marketed	[82]
Granisetron	antagonist	Antiemetic	Marketed	[83]
Tropisetron	antagonist	Antiemetic	Marketed	[84]
Ondansetron	antagonist	Antiemetic	Marketed	[85]
Metoclopramide	antagonist	Antiemetic	Marketed	[85]

ADHD, attention deficit hyperactivity disorder; IBS, irritable bowel syndrome.

ligands have been discovered that selectively alter the function of nAChR and 5-HT_3R subtypes, which have been characterized in a variety of expression systems, native cells, tissues and model animals[86]. In most cases, these compounds have been designed to be agonists, antagonists, or allosteric modulators for treating a specific disease. The main obstacles in the development of new compounds are unsatisfactory clinical efficacy and a high incidence of adverse events with a narrow therapeutic window. The most common side effects associated with nAChR and 5-HT_3R ligands occur in the gastrointestinal and central nervous systems. Despite these challenges, several drugs targeting the nAChRs and the 5-HT_3Rs have moved beyond clinical trial stages into medical application. Further structural studies of the nAChR and the 5-HT_3R will enhance the discovery of small molecule modulators of these two important receptors for therapeutic purposes.

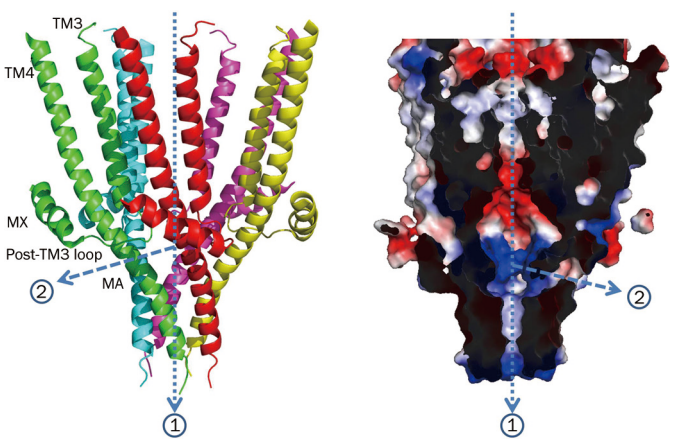

Figure 10. Two possible modes of ion conduction in the ICD of the cation-selective pLGIC: through the enlarged tunnel surrounded by the lower MA helices ① or through the open windows ② between the upper MA helices.

Conclusions and future directions

In the past few decades, the nAChRs and the 5-HT_3Rs have been a focus of intense research. These efforts have greatly increased our understanding of the structural and functional basis of these two important ion channels, which is predominantly a result of technological advances in high-throughput screening and crystallization. Significant progress has also been made in recent years on receptor expression, distribution, and physiological function. However, the molecular mechanisms of cation selectivity, channel gating and interaction with downstream effector proteins remain to be elucidated through the determination and analysis of high resolution structures of these two channels in various conformational states.

Additionally, the nAChRs and the 5-HT_3Rs are implicated in a range of neurological and psychiatric diseases. Significant drug discovery efforts have been devoted to these two receptors and several promising ligands targeting these two receptors have been developed over the past few years. However, the discovery of potent ligands that interact more selectively with the nAChRs and the 5-HT_3Rs and display minimal or no side effects is urgent. To avoid receptor desensitization and to increase ligand efficacy and selectivity, the development of

PAMs has garnered significant interest.

Taken together, the structural information of cation-selective pentameric ligand-gated ion channels has been steadily improved because of the increasing availability of relevant structures over the past decade. These advancements have also led to a better understanding of the disorders and physiological functions associated with nAChRs and 5-HT_3Rs, and they have started to provide a rational basis for ligand design and drug discovery. With recent technological breakthroughs in structure determination by cryo-EM and femtosecond X-ray laser, one would expect that structural information of these two receptors in various functional states (*eg*, closed and open states) will provide direct mechanisms of ligand gating and activation and further promote structure-based design of specific ligands to modulate their physiological function and modify the disease states. The recent expression and purification of the nAChR and the human 5-HT_{3A} receptor will further facilitate the structural studies of these two receptors in various conformational states[87, 88].

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