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Structural Basis of Activation of Cys-Loop Receptors: the Extracellular–Transmembrane Interface as a Coupling Region

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Abstract Cys-loop receptors mediate rapid transmission throughout the nervous system by converting a chemical signal into an electric one. They are pentameric proteins with an extracellular domain that carries the transmitter binding sites and a transmembrane region that forms the ion pore. Their essential function is to couple the binding of the agonist at the extracellular domain to the opening of the ion pore. How the structural changes elicited by agonist binding are propagated through a distance of 50 Å to the gate is therefore central for the understanding of the receptor function. A step forward toward the identification of the structures involved in gating has been given by the recently elucidated high-resolution structures of Cys-loop receptors and related proteins. The extracellular–transmembrane interface has attracted attention because it is a structural transition zone where β-sheets from the extracellular domain merge with α-helices from the transmembrane domain. Within this zone, several regions form a network that relays structural changes from the binding site toward the pore, and therefore, this interface controls the beginning and duration of a synaptic response. In this review, the most recent findings on residues and pairwise interactions underlying channel gating are discussed, the main focus being on the extracellular–transmembrane interface.

Keywords Cys-loop receptors · Gating · Nicotinic receptor · 5-HT_3 receptor · Ion channels · Synaptic transmission

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Introduction

The human brain is a vast and complicated network, where billions of nerve cells use signals to communicate with each other. Chemical synaptic transmission is the main process by which nerve cells signal one another. It offers the advantages of signal amplification, reversal of polarity, and great potential for modulation, all important properties for higher brain function. At chemical synapses, the neurotransmitter is released into a narrow synaptic gap after depolarization of the presynaptic terminal and binds to a postsynaptic receptor. Neurotransmitter-gated ion channels are a family of synaptic receptors that convert the chemical signal into an electrical one by rapidly opening a channel that allows the flux of ions through the membrane. Just as important, the channel closes within a few milliseconds as the transmitter dissociates to terminate the synaptic event. Thus, moment-to-moment communication relies on rapid on and off responses of synaptic receptors.

Neurotransmitter-gated ion channels of the Cys-loop superfamily play key roles in chemical synapses throughout the nervous system and include receptors activated by acetylcholine (ACh), γ-aminobutyric acid (GABA), glycine, and serotonin (5-HT) [1–4]. They are known as Cys-loop receptors because all family subunits contain a pair of disulfide-bonded cysteines separated by 13 residues which form a loop located at the interface between extracellular and transmembrane domains. Their vital role in converting chemical recognition into an electrical impulse makes these receptors prime loci for learning, memory, and disease processes, as well as targets for clinically relevant drugs. Cys-loop receptors are targets of widely prescribed drugs, such as neuromuscular blockers, barbiturates, and benzodiazepines. In the last years, an ever increasing number of human and animal diseases have been found to be caused

by defective function of Cys-loop receptors, such as Alzheimer's and Parkinson's disease, schizophrenia, hereditary epilepsies, attention-deficit, hyperactivity disorder, autoimmune autonomic neuropathy, autism, myasthenia gravis, and congenital myasthenic syndromes [5].

In vertebrates, Cys-loop receptors can be cation-selective, as nicotinic ACh (nAChRs) and 5-hydroxytryptamine type 3 (5-HT_3) receptors, or anion-selective channels, as $GABA_A$, $GABA_C$, and glycine receptors. The repertoire of invertebrate Cys-loop receptors is larger, also including a GABA-gated cation channel [6] and serotonin-, ACh-, glutamate-, histamine-, and biogenic amine-gated anionic channels [7–12]. The selectivity of the channels for cations or anions governs the sign of the current and, in most of the cases, the type of response: inhibitory, for anionic channels because they hyperpolarize the cell, and excitatory, for cationic channels because they induce membrane depolarization by allowing a net influx of Na^+ ions into the cell.

Nicotinic receptors have been object of attention since Claude Bernard investigated the action of the Central American arrow poison, curare [13]. The muscle nAChR was the first to be identified and purified and the first to be characterized biochemically and electrophysiologically. The nAChR is widely distributed throughout the animal kingdom, from nematodes to human [14]. It is expressed in many regions of the central and peripheral nervous system. It plays a major role in neuromuscular transmission, where it begins the action potential that ends in muscle contraction. This receptor is the target of competitive blockers, such as curare, and other muscle relaxants used in surgery, and it is modulated by a great variety of compounds [15]. In nematodes, muscle nAChRs are targets for anthelmintic chemotherapy [16]. In neuronal tissues, nAChRs contribute to a wide range of brain activities and influence a number of physiological functions [5]. nAChRs are also present in various nonneuronal tissues, such as glia, blood cells [17], keratinocytes [18], endothelial cells [19], multiple cell types of the digestive system, and lung cells [5, 20, 21].

5-HT_3 receptors are found in the central and peripheral nervous system. They are involved in sensory processing, nociception, emesis, cardiovascular regulation, and gut function [22, 23]. Selective antagonists are used as antiemetic agents during antineoplastic therapy. To date, five different subunits are known in human, and all subunits show splice variants (A–E) [24–29]. Only 5-HT3A subunits are able to form functional homomeric channels in heterologous expression systems and probably in native cells [29–31].

$GABA_A$ and glycine receptors are mainly involved in inhibition in the central nervous system (CNS), with the $GABA_A$ receptor distributed throughout the CNS and the glycine receptor found predominantly in the brainstem and spinal cord. The activity of GABA receptors is allosterically enhanced by benzodiazepines, barbiturates, intravenous anesthetics, alcohols, steroids, and volatile anesthetics, and it is blocked by picrotoxin [32–35]. Glycine receptors are targets of the plant alkaloid strychnine, which by acting as competitive antagonists leads to agitation, muscle spasms, and convulsions; the development of therapeutic agents against these receptors may therefore have significant utility as muscle relaxants and analgesic agents [36].

The essential function of these receptors is to couple the binding of the agonist to the opening of the ion channel. Given that this process governs synaptic transmission, elucidation of its mechanism and the structures involved has been a long-standing challenge.

Overall Structure

Cys-loop receptors are composed of five identical (homopentamers) or different (heteropentamers) polypeptide chains arranged around an axis perpendicular to the membrane (Fig. 1). Subunits are classified in two types, α and non-α, with the α-type subunits containing a disulfide bridge in the binding site. A wide number of α and non-α subunits have been cloned for all members of the superfamily (Ligand-Gated Ion Channel database, http://www.ebi.ac.uk/compneur-srv/LGICdb/cys-loop.php).

All subunits share a basic scaffold composed of (1) a large N-terminal extracellular domain of ~200 amino acids, (2) three transmembrane domains separated by short loops, (3) a cytoplasmic loop of variable size and amino acid sequence, and (4) a fourth transmembrane domain with a relatively short and variable extracellular COOH-terminal sequence [37]. The receptors are therefore built on a modular basis, with the extracellular domain containing the agonist binding sites and the transmembrane domain containing the pore, the selectivity filter, and the channel gate [2, 38] (Fig. 1).

Recent structural studies have provided an insight into the three-dimensional structure of nAChRs and all members of the superfamily. In particular, a high-resolution structural model (4 Å) of the nAChR from the marine ray *Torpedo* [38] has revealed important information and has been invaluable in the interpretation of functional and pharmacological data (Protein Data Bank (PDB) code 2BG9; Fig. 1). Although no atomic resolution structural information is available for any Cys-loop receptor, an atomic resolution (1.94 Å) structure of the extracellular domain of the nAChR α1 subunit has been determined [39]. In addition, high resolution structural information has become available from studies of proteins which show close sequence similarity to nAChRs, namely (a) the high-resolution structures of soluble ACh binding proteins (AChBP) from the freshwater snail *Lymnaea stagnalis*

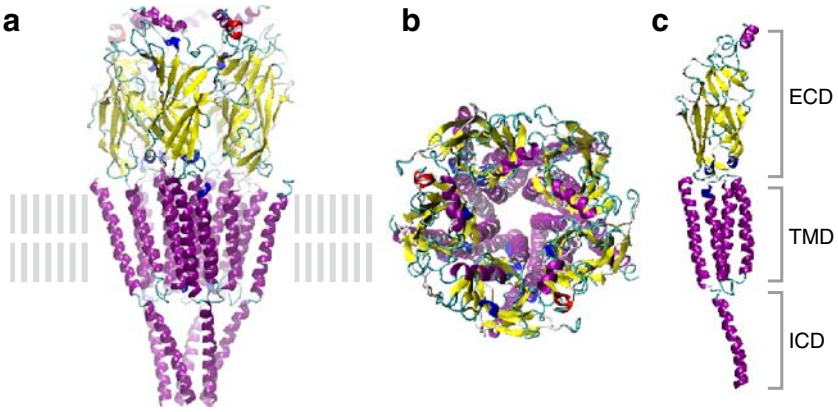

Fig. 1 Structure of the nAChR. Cartoon diagrams for the *Torpedo* nAChR (2BG9.pdb) viewed parallel to the membrane (**a**) and from the synaptic cleft (**b**). **c** Diagram of one subunit with the pore axis on the *right*, showing its three principal domains: extracellular (*ECD*), transmembrane (*TMD*), and part of the intracellular domains (*ICD*)

(2.7 Å; PDB 1I9B [40, 41]), the sea snail *Aplysia californica* (1.96–3.4 Å; PDB 2BYN [42]), and the freshwater snail *Bulinus truncatus* (2.0 Å; PDB 2BJ0 [43]) and (b) X-ray structures of prokaryotic ligand-gated ion channels from the bacterium *Erwinia chrysanthemi* (ELIC; 3.3 Å; PDB 2VL0 [44]) and *Gloebacter violaceus* (GLIC; 3.1 Å; PDB 3EHZ [45]; 2.9 Å; PDB 3EAM [46]).

The Extracellular Domain: Location of the Agonist Binding Sites

Our knowledge of the structure of the extracellular domain of Cys-loop receptors took a giant step forward with the solution of the high-resolution structure of the AChBP from *L. stagnalis* [40]. This soluble protein is produced and stored in glial cells and is released in an ACh-dependent manner in the synaptic cleft where it regulates synaptic transmission. AChBP lacks the transmembrane region but contains many of the structural cornerstones that give nAChRs their unique signature and has therefore become a functional and structural model of the extracellular domain of Cys-loop receptors [47]. AChBP has been used to create homology-based models of the extracellular domains of 5-HT_3, nAChR, $GABA_A$, and glycine receptors [26, 48–50]. It contains 210 amino acids and shares ~15–24% sequence identity to aligned sequences of the amino-terminal, extracellular halves of Cys-loop receptor subunits. Each AChBP monomer consists of an N-terminal α-helix, two short 3_{10} helices, and a core of ten β-strands that form a β-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-terminals are located at top and bottom of the pentamer, respectively. In Cys-loop receptors, the end of β10 connects to the start of M1. Located at the bottom of the subunit, the linker between β6 and β7 strands is the signature Cys-loop found in all members of the superfamily.

Each agonist binding site is found in a cavity at an interface between two adjacent subunits. One interface, called the principal or "positive" face, contributes three loops that span β strands and harbor predominantly key aromatic residues; these regions have been named as loop A (which corresponds to β4β5 loop), loop B (β7β8 loop), and loop C (β9β10 loop). The adjacent subunit, which forms the complementary or "negative" face, contributes three β strands with residues clustered in segments called loops D–F. Thus, key residues from the principal face come from loop A (Trp86 and Tyr93), loop B (Trp149 and Gly153), and loop C (Tyr190, Cys192, Cys193, and Tyr198; residues correspond to *Torpedo* α subunit). The complementary face is formed by residues from loops D (Trp55 and Asp57), E (Leu109, Arg111, Thr117, and Leu119), and F (Asp174 and Glu176; residues from δ or γ *Torpedo* subunits) [40, 47, 51, 52].

The ancestral Cys-loop receptor was likely homomeric and contained five identical binding sites, similarly to present day homomeric receptors, such as α7 and 5-HT_3A receptors [53–55]. Evolution led to the appearance of new subunits which lost the ability to form agonist binding sites, giving rise to heteromeric receptors with fewer than five binding sites. The prototypic heteromeric receptors, muscle nAChR and $GABA_A$, contain only two agonist binding sites. For some types of these receptors, a third positive allosteric modulatory site located at noncanonical subunit interfaces has been identified, such as the site for benzodiazepines in $GABA_A$ [48] and for morantel in neuronal AChRs [56]. Because homomeric receptors contain five identical transmitter binding sites, the long-standing question has been how many of these sites are occupied by the agonist during activation. Recent kinetic modeling studies for homomeric 5-HT_3A receptors suggest that three agonist binding sites are required for optimal activation [57, 58]. A direct evidence of the relationship between agonist occupancy and activation was obtained by applying an electrical fingerprinting strategy in homomeric

α7–5HT$_3$A chimeric receptor [59]. The results revealed that occupancy of two agonist binding sites allows proper activation, but only when three agonist molecules are bound to the receptor in a nonconsecutive array and channel lifetime is maximal. Thus, the third site resembles the positive allosteric site found in heteromeric receptors. Macroscopic recording studies reveal that the availability of more binding sites (five) than those required for maximal activation (three) enhances agonist sensitivity [59]. The enhanced sensitivity, together with the benefit of a single self-assembling gene product, could have been important as far back as prokaryotes [55] and before the appearance of structurally efficient synapse. In present day homomeric receptors, high agonist sensitivity could be important in the function of extra- and presynaptic receptors [60].

The Transmembrane Domain: the Region that Comprises the Ion Pore and the Gate

Structural and electrophysiological studies have shown that the ion channel is largely lined by the M2 domains of the five subunits. A model of the closed pore of *Torpedo* nAChR at 4 Å was first obtained by cryo-electron microscopy [61]. This model confirmed the long-held view that the pore is shaped by an inner ring of five α-helices (M2 segments). An outer ring of 15 α-helices (M1, M3, and M4 segments) shields the inner ring from the lipids. Some years later, the complete picture of the nAChR structure was provided by Unwin [38]. Since the model was obtained in the absence of agonist, it is considered to depict the closed resting state, although this remains a matter of controversy. Despite lacking atomic resolution, this model has become a cornerstone for structure–function studies. This refined 4-Å resolution electron microscopy structure shows that the N-terminal extracellular portion is built around a β-sandwich core consisting mainly of ten β-strands from each subunit, similar to AChBP, resulting in a whole domain that contains two binding sites for ACh. The membrane-spanning portion, composed of the four α-helical segments from each subunit, is joined covalently to the extracellular domain at the N-terminal end of M1 (Fig. 1). The high level of amino acid sequence conservation implies that all members of the Cys-loop superfamily are constructed around the same three-dimensional framework and function according to the same global principles [38].

The pore is maximally constricted in the middle of the membrane due to side-to-side interactions between hydrophobic residues of neighboring helices (positions 9′ and 13′). This tight hydrophobic girdle creates an energetic barrier to ions across the membrane, and it has been suggested that it corresponds to the gate [38, 61]. The ion pore contains the filter selectivity, which is the structure that determines which types of ions are able to pass through the channel. Point mutations in the M2 region supported the role of positions −2′ to 2′ as main determinants of the selectivity filter of all Cys-loop receptors [62].

The long intracellular region between M3 and M4 contains a short α-helix. It is thought to be associated with cytoskeletal proteins, such as rapsyn for nAChR [63, 64], gephyrin for glycine receptors, and GABARAP and MAP1B for GABA$_A$ and GABA$_C$ receptors, respectively [65]. These proteins allow the clustering of the receptors at appropriate regions of the membrane [66–68]. This intracellular region has been shown to contribute to channel kinetics in muscle nAChR [69, 70] and contains determinants of channel conductance in 5-HT$_3$A receptors [71–73]. It also contains phosphorylation sites, and it has been demonstrated that phosphorylation modulates expression, upregulation, desensitization, and interaction with cytoskeleton proteins of nAChRs [74–80].

The structure of the nAChR resembles those of the homologous pentameric ligand-gated ion channels recently identified in bacterial sources [55, 81]. The first characterization of a pentameric ion channel from *G. violaceus* (GLIC) showed that it forms a cation-selective channel that it is activated by protons and that currents do not decay during activation, suggesting no desensitization [81]. The first X-ray structure at 3.3 Å of a prokaryotic receptor was obtained from *E. chrysanthemi* (ELIC) [44]. This receptor shows 16% sequence identity to the α nAChR subunit. The extracellular domain is very similar to its eukaryotic counterpart and to AChBP, but lacks the N-terminal α-helix. The putative binding site and several of the aromatic residues found in nAChR are conserved. The central part of the Cys-loop is also conserved, but lacks the flanking disulfide-bridge cysteine residues. The transmembrane region is equivalent to that of nAChR, with four α-helices from each subunit, but lacks the long intracellular loop between M3 and M4. Unlike in the structure of the nAChR, which shows a narrow but continuous pore, in ELIC, the hydrophobic residues physically obstruct the pore. The highly conserved key structures suggest that the basic mechanisms of ion permeation and gating are preserved across the prokaryotic–eukaryotic specie boundary [44]. The structure of GLIC was solved at low pH, and because it is activated by protons and currents do not decay in the presence of the protons, it might represent the receptor in a potentially open state [45, 46]. However, it should be taken into account that the absence of current decay during a short period of time may not unequivocally indicate lack of desensitization since slow opening or fast recovery from desensitization may also lead to a similar behavior, and also, desensitization may require longer times than the recording ones. Assuming that the structures of ELIC and GLIC do depict closed and open conformations,

respectively, the comparison of both structures has allowed suggestions of the structural changes occurring during channel opening [45, 46].

Channel Activation

The gating reaction couples changes in structure at the binding sites with changes in the channel that allow the increase in conductance. The identification of residues that transduce neurotransmitter binding into channel gating is now possible by combining the information of residue locations within functionally crucial regions of the receptors from the high-resolution structures with electrophysiological, pharmacological, and computational studies. Conclusions from the latter are limited by the resolution of the structural models, the uncertainty of the functional state that they represent, the environment used, and of course, the complexity of gating.

The first model of channel activation was introduced by del Castillo and Katz [82]. In their kinetic model (Scheme 1), binding and gating steps were written as two separate events, with R being the unliganded receptor and AR being the liganded one.

On the basis of the allosteric Monod–Wyman–Changeux model [83] extended to these receptors, the channels exist spontaneously in reversible equilibrium between closed and open states, which are stabilized by agonists [84, 85]. A relevant step was given when Neher and Sakmann [86] first recorded currents through single AChR channels and after the development of the giga-ohm seal method [87]. Further significant advances in the understanding of the mechanisms of activation and their relation to structure were achieved in the 1990s with the introduction of single-channel kinetic analysis applied to high-temporal resolution data of wild-type and mutant channels (see the description of the pioneer work in reviews [88] and [89]). Two programs are mainly used for the analysis, HJCFIT [90] and QUB software [91]. This analysis has allowed the generation of kinetic schemes that explain activation of different Cys-loop receptors, the estimation of the microscopic rates, and the elucidation of the contribution of specific amino acids to these rates ([58, 92–96] and see reviews in [3] and [88]).

Recent high-resolution single-channel kinetic analysis in glycine and muscle AChR allowed the identification of an earlier conformational change from the closed liganded state (R) to an intermediate, preopen state [97, 98] that precedes the open state (R*). In this intermediate state, named flipped or primed (F), the receptor is closed but shows high agonist affinity. Moreover, the differential responses between partial and full agonists can be explained by the different kinetics of entry and exit into this preopen state [97]. Thus, in contrast to the del Castillo and Katz's original model, transitions from the closed to open states do not occur in a single conformational step, which can be represented in the most simple way by the inclusion of an intermediate closed state (F) between the liganded receptor and the open state (Scheme 2 and see full schemes in [97] and [98]).

The classical method for measuring the efficacy of channel activation has been the determination of EC_{50} values from the relationship between macroscopic peak currents and agonist concentration, usually for receptors expressed in oocytes. The latter ensure high expression, which cannot be achieved for some types of receptors or mutants in mammalian cells. Also, single channels cannot be detected for some Cys-loop receptors due to their low conductance [71]. However, it should be kept in mind that the interpretation from these experiments is limited because EC_{50} values are a composite of binding, gating, desensitization, and even blocking events, in addition to the fact that for accurate measurements, rapid agonist application is required [99].

An interesting approach to understanding global motions underlying gating of the nAChR has been developed by Auerbach and colleagues based on the rate-equilibrium free-energy relationship analysis (REFER) [100]. AChR gating appears as a Brownian conformational cascade between the agonist binding site and the gate, with motions of lots of residues clustered into rigid-body domains [101–103]. REFER compares changes in the forward closed–open rate to changes in the gating equilibrium constant between closed and open states, when a specific amino acid residue is mutated to several types. The slope of the log–log plot is called Φ and, according to this model, implies the relative timing of that residue's gating motion, from 0 which corresponds to the closed state to 1 that corresponds to open state. Higher Φ values indicate earlier, lower Φ values indicate later, and equivalent Φ values indicate synchronized movements during gating. REFER analyses of hundreds of residues showed that the nAChR is organized into several domains (blocks) within which Φ values are indistinguishable, ranging from ~1 in the vicinity of the transmitter

$$\underset{\text{Closed}}{\text{R} \leftrightarrow \text{AR}} \quad \leftrightarrow \quad \underset{\text{Open}}{\text{AR}^*}$$

Scheme 1 The del Castillo and Katz's original model of channel activation

$$\text{R} \leftrightarrow \text{AR} \leftrightarrow \text{AF} \leftrightarrow \text{AR}^*$$

Scheme 2 Inclusion of an intermediate closed state (F)

binding site to ~0 in the middle of the transmembrane domain, during channel opening [104]. REFER analyses for different regions of the mouse muscle AChR show similar Φ values for unliganded versus diliganded gating. This finding suggests that the conformational pathway of the structural change underlying gating is the same in the presence or absence of agonist, and therefore, agonist binding may change the energy but not the mechanism of the conformational change [105].

By applying normal mode analysis, Taly and coworkers [106–108] explored protein flexibility on a three-dimensional model of α7 and proposed the quaternary twist model for channel gating. Normal mode analysis approximates the surface of the conformational landscape and gives a decomposition of the movements into discrete modes. This method was found to adequately describe the slow transitions of proteins, notably compared to experimental X-ray structures of different conformations [52, 107]. The model describes that channel opening occurs mainly due to a symmetrical reorganization of the quaternary structure of the entire protein complex with opposing rotations of the extracellular and transmembrane domains. A global twisting motion in which the extracellular domain and the corresponding transmembrane helices undergo global rotations in opposite directions has been also proposed by normal mode analysis in α7 [109] and muscle nAChR [110] and by the comparison of ELIC (presumably representing the closed state) and GLIC (potentially open state) structures [46].

Structural Changes Underlying Channel Activation

Binding of the agonist and the resulting conformational changes have been well studied in nAChR. Stabilization forces of the agonist at the binding site include π-cation, dipole-cation, hydrogen bonding, and van der Waals interactions [111–113]. The superposition of crystal structures of AChBP with a variety of agonists and antagonists shows that the C-loop from the principal face is in an "open" conformation in the resting state of the receptor. In the presence of the agonist, the C-loop caps the entrance to the binding cavity, trapping the agonist [114, 115]. Molecular dynamics simulation also revealed a time-dependent change of C-loop from uncapped or open to a capped or closed conformation [116, 117]. Loop C is connected directly with M1 via β10, and therefore, it might propagate conformational changes occurring after agonist binding to the interface between the extracellular and transmembrane domains. During activation, the conserved tyrosine (Tyr190 from nAChR) in the C-loop is drawn closer to Lys145 in β7 strand, breaking or weakening a previous interaction between this lysine and Asp200 in β10 strand [3, 118]. By monitoring disulfide bond formation between cysteines substituted at a critical intrasubunit salt bridge between conserved charged residues, it was demonstrated that agonist activation proceeds via restricting C-loop mobility in the $GABA_A$ receptor [119]. Thus, the movement of this loop seems to be the initial conformational change underlying channel activation after binding of the agonist in Cys-loop receptors [120, 121]. Interestingly, by performing cysteine substitutions at the C-loop of each binding site and at each of the two juxtaposed subunits of the muscle nAChR and by further recording single-channels from the mutant receptors before and after oxidation, it was shown that capping of the C-loop is involved in the transition of the closed receptor to the activated preopen intermediate state (priming, see Scheme 2) [98]. It was also shown that simultaneous capping at both agonist binding sites is required to evoke long-lived openings.

Mutagenesis and electrophysiological studies have shown that residues from other agonist binding site loops are also involved in binding and gating. For example, Trp55 in loop D has been shown to be involved in channel gating and desensitization of muscle and α7 AChRs [122, 123]. In the same loop, Gln57 has been shown to govern the high potency of morantel to activate α7 AChRs [124]. Position 153 in the nAChR (B-loop) has been shown to be associated with a slow-channel syndrome [92], to govern the strong activation of nematode AChRs by anthelmintic drugs [125], and to affect gating by interacting with C-loop in neuronal nAChRs [126].

The endpoint of the activation process is the transient removal of the barrier to ion flow. Various molecular rearrangements have been proposed to underlie channel opening but the fundamental motion of M2 that opens the pore remains unknown. It was first proposed that channel opening proceeds by a rotation of the pore-lining helices around their helix axis [61, 117], whereas subsequent studies suggested rigid-body tilting of M2 [127], a subtle rearrangement of transmembrane segments [128, 129], or a mixed picture. For example, the rotation of M2 was not supported by single-channel electrophysiological experiments in which residues that line the pore in the open state were detected by measuring the ability of protons to block the channel after lysine substitution [128, 129]. These studies suggested that the pore dilation that underlies channel opening involves only a subtle rearrangement of M2, M3, and M1 transmembrane helices and that the rotation of M2, if any, is minimal [128, 129]. This subtle movement is fully consistent with the concept of "hydrophobic gating" proposed for the nAChR [61, 130, 131]. Here, the large hydrophobic residues located at positions 9′, 13′, and 17′ of M2 [132, 133] act as a desolvation barrier for ions instead of a steric one. In this mechanism, the gate

is so narrow that an ion has to shed at least some water molecules from its hydration shell to pass the constriction. Simulations of the nAChR have suggested that increasing the radius of this hydrophobic girdle of the closed-state pore by as little as ~1.5 Å is enough to increase the computed conductance to values similar to the experimental ones [130, 134]. However, the hydrophobic girdle hypothesis is still a matter of controversy [127].

Computational simulations using homology models also suggested different mechanisms by which the channel is opened, such as twisting motion of the extracellular domain combined with tilting of the M2 which, in turn, disrupts the hydrophobic girdle [135], rotation of M2 [109], rotation with bending motions of M2 [110], and the above-described quaternary twist motion [52, 107]. A detailed analysis of the energy and structural changes of M2 muscle AChR during gating showed that the residues that experience larger energy changes (positions 12′, 13′>9′, 16′, 17′>2′–6′) move later and are in general the most exposed to the channel lumen. The relationship between the polarity of the side chain and the effects on energy changes suggests that the environment around them increases in polarity between closed and open states. A possible interpretation to this finding is that some of the energy change is associated with a change in hydration, and therefore, a change in the configuration of water inside the pore may contribute to the overall closed versus open energy change [136].

A different mechanism for channel gating has been recently postulated by a thermodynamic study. In this theory, ion channels use an air bubble in their pore to control single-channel current in a switch-like manner. The idea is that the current flow through an open channel is blocked when a bubble forms spontaneously in a hydrophobic region of the channel pore. The channel becomes a conducting column when its bubbles break and ions and water suddenly fill the channel. In the light of this theory, bubbles would be localized and controlled by the rings of nonpolar amino acids of the nAChR pore [137].

Structural models are limited in part by the uncertainty of the functional state that they represent. Comparison of the structures of the presumably closed (ELIC) and open channels (GLIC) shows that whereas the transmembrane pore of ELIC is constricted on its extracellular side, the equivalent region of GLIC shows a funnel-shaped opening with a linearly decreasing diameter that places its narrowest part at the intracellular entry of the channel. This shows a clear difference between what could be a closed or an open state. On the other hand, the electron microscopy model of muscle nAChR was proposed to correspond to the resting closed state as it was obtained in the absence of agonist [38]. Nevertheless, if this corresponds to the physiological closed resting state is still a controversy [110]. In contrast to ELIC, which shows a discrete barrier to ion permeation, the muscle nAChR structure is closer to the structure of a conducting state. However, molecular simulation approaches have assigned a negligible conductance to the derived structural model of the muscle nAChR, even though the geometry would permit permeation [130, 134].

The Extracellular–Transmembrane Interface: a Region Involved in Coupling Agonist Binding to Channel Gating

The agonist binding site projects into the synaptic cleft, whereas the region that gates ion flow localizes within the membrane [38]. Communication over the 50 Å separating the two regions is thus essential to the function of Cys-loop receptors. The binding–pore interface has attracted attention because it is a structural transition zone where β-sheets from the binding domain merge with α-helices from the pore (Fig. 2). Within this zone, several regions form a network that relays structural changes from the binding site toward the pore. Structures at the interface include β1β2 loop, Cys-loop, β8β9 loop, and the end of β10, all from the extracellular region, and the pre-M1 region, M2–M3 linker, and the C-terminal end of M4 from the transmembrane region (Fig. 2; Table 1).

A structural interplay between loops at the interface required for coupling agonist binding to channel gating was demonstrated by generating a chimeric receptor composed of the AChBP protein, which presumably evolved without the constraint of functional coupling to an ion pore and the pore domain from the 5-HT_3A receptor [138]. Although the chimeric receptor expresses on the cell surface and shows high affinity for ACh, it is not functional. However, if amino acid sequences of three loops (β1β2, Cys-, and β8β9) in AChBP are changed to their 5-HT_3A counterparts, ACh binds with low affinity characteristic of activatable receptors. Moreover, ACh is capable of triggering opening of the ion pore. Since the efficacy for channel opening is low, it is possible that additional loops at the interface have to be exchanged to achieve high-efficacious activation. Nevertheless, the findings reveal that this region mediates a bidirectional allosteric interaction between the binding sites and the pore domain and that the functional coupling process is mediated by a network of loops from both domains [138].

Further insights into the role of the interface in synaptic responses mediated by homomeric Cys-loop receptors emerged from studies of the chimeric receptor containing the extracellular region of α7 and the transmembrane region of 5-HT_3A (α7–5HT_3A; Fig. 3). This chimera has served as a model for studying the pharmacology of α7 receptors because it shows high expression in mammalian cells [72, 139]. Interestingly, although it carries a mixed

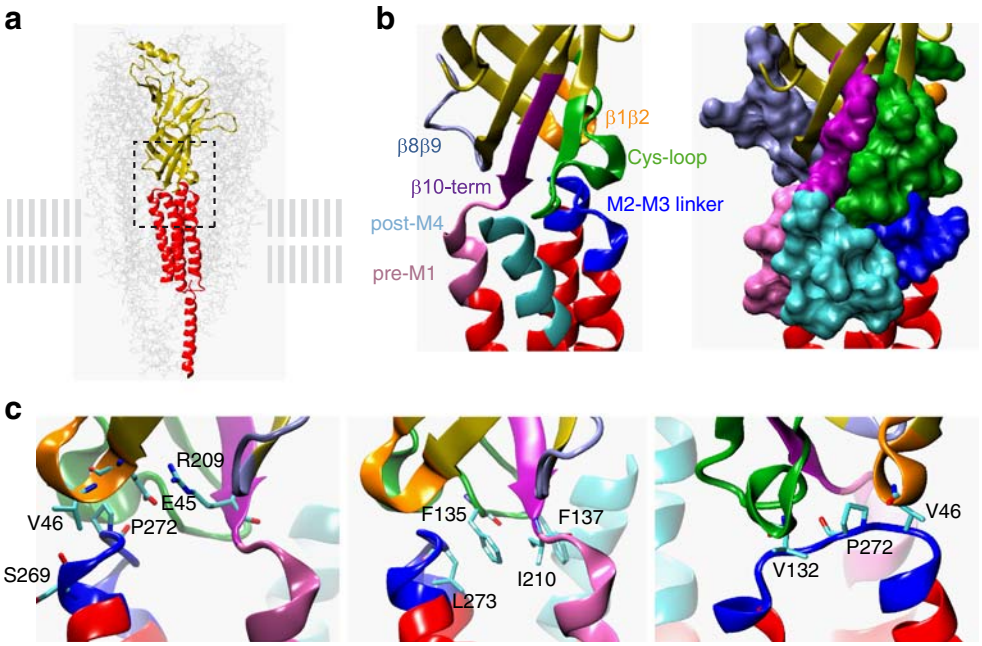

Fig. 2 The interface between extracellular and transmembrane domains. **a** Structure of the *Torpedo* nAChR with one of its subunits highlighted with the extracellular domain in *yellow* and the transmembrane and intracellular domains in *red*. The interface is shown in the *dashed square*. **b** View of the structures at the interface. *Left*: The different segments are colored as follows: *orange* (β1β2 loop), *ice blue* (β8β9 loop), *green* (Cys-loop), *purple* (β10-terminal), *pink* (pre-M1), *blue* (M2-M3 linker), and *cyan* (post-M4). *Right*: Surface representation of the interface loops. **c** Different views of the interface with key residues labeled. Ile210 in *Torpedo* nAChR corresponds to Leu210 in the human receptor

α7/5-HT$_3$A interface, it functions as a neurotransmitter-gated ion channel. Thus, it is a good model to study how the interface is involved in channel activation [73]. The interface of the starting α7–5HT$_3$A chimera carries α7 sequences in β1β2 (whose sequence is identical to that of 5-HT$_3$A), Cys-loop, β8β9, and 5-HT$_3$A sequences in M2–M3 linker, β10, and pre-M1 region (Table 1). The comparison of the kinetics of the chimera with that of the parent receptors has provided further insights into the contribution of the interface loops to channel function [73].

Macroscopic currents of wild-type α7 channels decay very rapidly supporting extremely fast desensitization (<1 ms), and channel activation occurs mainly as isolated brief events (~300 μs; Fig. 3). By contrast, the decay rate of 5-HT$_3$A currents is slow (~1 s), and activation of the high-conductance 5-HT$_3$A receptor occurs in many long openings (~100 ms) grouped in bursts, which in turn, coalescence in long clusters (Fig. 3). The decay rate of currents from the chimeric α7–5HT$_3$A receptor, which shows structures of both α7 and 5-HT$_3$A receptors at the interface (Fig. 3), is fast but not as fast as that of α7 (~10 ms), channels show intermediate open durations (~6 ms), and activation occurs mainly in bursts of few openings and not in long clusters as in 5-HT$_3$A or single openings as in α7. Thus, the chimera shows an intermediate kinetic profile between that of the parent receptors. The contribution of the mixed α7/5-HT3A interface to this intermediate profile was studied by generating two additional chimeras starting from the α7–5HT$_3$A chimera: an all-5HT$_3$A chimera in which major loops within the binding–pore interface contain residues solely from the 5-HT$_3$A receptor (all-5HT$_3$A) and an all-α7 chimera in which these loops contain residues solely from the α7 receptor. Macroscopic and single channel currents from all-5HT$_3$A approach those of the 5-HT$_3$A receptor (Fig. 3), exhibiting slow desensitization and clusters of many long single-channel openings. Analogously for the all-α7

Table 1 Subunit sequences at the receptor interface

		β1β2	Cys-loop	β8β9	β10 + pre-M1	M2M3	post-M4
nAChα1	torpedo	D**E**VNQI	C**E**IIVTHF**P**F**D**QQN**C**	MESGE**W**	IMQ**R**IPLYFVVN	SSAVPLIGKY	FAGR--LIELSQ
nAChα1	human	D**E**VNQI	C**E**IIVTHF**P**F**D**EQN**C**	MESGE**W**	VMQ**R**LPLYFIVN	SSAVPLIGKY	FAGR--LIELNQ
nAChα7	human	D**E**KNQV	C**Y**IDVRWF**P**F**D**VQH**C**	IPNGE**W**	TMR**R**RTLYYGLN	SDSVPLIAQY	LMSAPNFVEAVS
5HT3A	human	D**E**KNQV	C**S**LDIYNF**P**F**D**VQN**C**	MNQGE**W**	VIR**R**RPLFYVVS	AIGTPLIGVY	VMLWSIWQYA--
GABAα1	human	S**D**HDME	C**P**MHLEDF**P**M**D**AHA**C**	EDGSRL	HLK**R**KIGYFVIQ	A-YATAMDWF	WATYLNREPQLK
GABAβ2	human	S**E**VNMD	C**M**MDLRRY**P**L**D**EQN**C**	VTKIEL	KLK**R**NIGYFILQ	P-YVKAIDMY	WLYYVN------
Glyα1	human	A**E**TTMD	C**P**MDLKNF**P**M**D**VQT**C**	ADGLTL	HLE**R**QMGYYLIQ	S-YVKAIDIW	WIIYKIVRREDV

Sequences were aligned with ClustalW. The sequences shown for β8β9 and β10 correspond to those located at the interface. Highly conserved residues are shown in bold

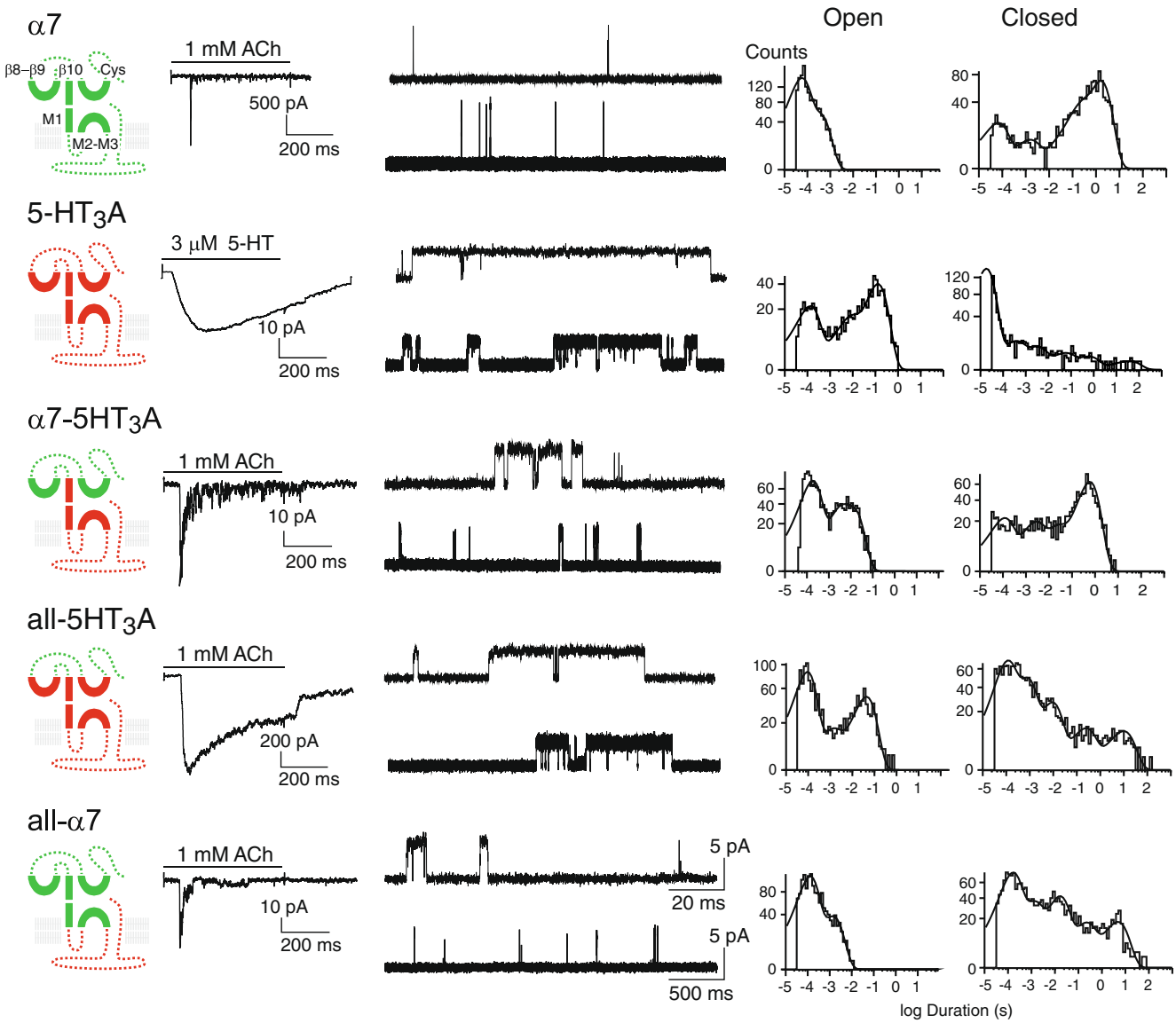

Fig. 3 Macroscopic and single-channel recordings from different α7–5HT$_3$A chimeric receptors. *Left*: Schematic diagrams representing one subunit of each homomeric receptor with α7 sequences in *green* and 5-HT$_3$A in *red*. *Middle*: Macroscopic currents from outside-out patches elicited by rapid perfusion of the agonist at the indicated concentration. Membrane potential, −50 mV; filter, 5 kHz. *Right*: Single-channel recordings at two different temporal scales obtained in the cell-attached configuration with the corresponding open and closed histograms. Single channels were activated by 100 μM ACh (*α7*), 3 μM 5-HT (*5-HT$_3$A*), and 500 μM ACh (*α7–5HT$_3$A* and *all-5HT$_3$A*). For the all-α7 chimera, single-channel events were recorded in the outside-out patch configuration activated by 1 mM of ACh. Openings are represented as upward deflections. Membrane potential, −70 mV; filter, 9 kHz

chimera, the kinetics of macroscopic and single-channel currents approach those of the α7 receptor, exhibiting fast desensitization and brief channel openings (Fig. 3). Thus, substitution of residues from the parent receptors into the extracellular–transmembrane interface of the α7–5HT$_3$A chimera reconstitutes the fundamental activation and desensitization properties of the parent homomeric receptors. The replacement of individual loops one at a time revealed that kinetics depends on the interplay between all loops, giving further support to the idea of the interface as a complex network of loops which couples conformational changes at the binding site to those at the ion pore [73].

The finding that the extracellular–transmembrane interface is involved in the rate of fast desensitization is of relevant significance. Desensitization seems not to affect normal muscular transmission through nAChR, but it has a role in synaptic transmission in pathologies underlying gain-of-function mutations of the muscle nAChR [140]; it contributes to the termination of responses mediated by α7 and 5-HT$_3$A receptors [57, 58, 73], and it may be important

under the presence of drugs, endogenous ligands, or phosphorylation [141–144]. Despite its relevance in controlling synaptic efficacy, understanding the structural movements underlying desensitization has lagged behind. This is due to the fact that the extent of desensitization results not only from its onset rate but also from the kinetics of recovery from desensitization and gating. Also, the accurate measurement of desensitization rate can be achieved only under optimal time-resolution systems, and finally, desensitization probably involves many different conformational states.

Taken together, the emerging picture shows that the extracellular–transmembrane interface is a key element for coupling agonist binding to channel opening and for determining the open channel lifetime and the rate of desensitization. Therefore, the interface is involved in the beginning, duration, and refractory period of a synaptic response.

Key Residues at Different Loops of the Extracellular–Transmembrane Interface

The important role of the interface loops, β1β2, Cys, β8β9, the end of β10, pre-M1 region, M2–M3 linker, and post-M4, in coupling agonist binding to channel gating has been shown in numerous studies for all members of the Cys-loop superfamily [123, 145, 146]. A large number of studies identified residues within these regions with crucial roles in channel gating.

The β1β2 loop is essential for gating. This loop is highly charged across the superfamily, with an overall negative charge. Val46 in this loop has been initially proposed to be the key, "pin," residue in the "pin-into-socket" mechanism for channel gating ([61]; see below). A great number of different reports have shown that several other residues of this loop are essential for gating in different family members. For example, point mutations combined with measurements of EC_{50} in muscle nAChRs showed that introducing a positive charge at four consecutive residues, Asp44, Glu45, Val46, and Asn47, decreases the EC_{50} [145]. Cysteine substitutions of residues Ile51, Glu53, Thr55, and Asp57 of the glycine α1 subunit increase EC_{50}, thus, demonstrating that the whole loop plays a key role ([146, 147] and see Table 1). Charged residues in glycine α1 [50] and GABA α1 subunits [148] and αGlu45 of the muscle nAChR [149] have been shown to be implicated in channel gating (see below).

The Cys-loop, which is the signature of the family, inserts between the pre-M1 region (close to its C-terminal half) and the M2–M3 domain (close to its N-terminal half). It is essential for nAChR assembly [150, 151]. Its crucial role in channel gating has been widely demonstrated for all family members [50, 145, 148, 152–156].

The role of β8β9 loop is less understood, but computational and experimental evidence confirm that it is involved in channel gating. This loop is long and relatively unstructured, and it is the region of most sequence variation among family members ([117], see Table 1). A glutamate residue in β8β9 loop (Glu172) [157] was found to undergo agonist-dependent movements during receptor activation [3, 158]. By using fluorescence anisotropy decay to study the segmental motion of side chains in AChBP, Hibbs et al. [159] demonstrated that agonists (but not antagonists) induced changes in conformational dynamics in the β8β9 linker. Simulations of a homology model of α7 showed that β8β9 moves inward toward its subunit. This motion occurs in all of the subunits, but it occurs to the greatest degree in the subunits adjacent to those whose C-loops move out the most [117].

The pre-M1 region, which connects β10 to M1, contains several cationic residues, including arginines that are conserved in several Cys-loop receptors. One of the arginine residues, Arg209, which is present in all family members, has been shown to have a fundamental role in gating of the human muscle nAChR [149]. In GABA receptors, this arginine (Arg216 of the β_2 subunit) is also involved in coupling neurotransmitter binding to channel gating [160]. Mutations in the equivalent residue in glycine receptors (Arg218) decrease significantly the efficacy and abolish responses by alanine and taurine [161]. The adjacent arginine residue in 5-HT_3 receptors (Arg222) has also been implicated in channel gating [162].

Several lines of experimental evidence reveal that M2–M3 linker and the flanking regions play a key role in channel gating in nAChR [100, 155, 163, 164], 5-HT_3 [165], GABA [166], and glycine receptors [167]. The importance of this region is also supported by the identification of mutations that lead to human diseases [3, 168, 169].

The M4 domain is the least conserved among the transmembrane domains, is the most hydrophobic, and has been extensively labeled by hydrophobic probes [170, 171]. The C-terminal region of M4 (post-M4; Fig. 2) is located at the interface. Potential interactions between residues in this portion of M4 and residues in the extracellular domain, including the Cys-loop, have been determined by computational studies of α7 [106], in agreement with large experimental evidence showing that this segment contributes to gating kinetics [58, 94, 172, 173] and that it moves during channel gating [174].

Crosstalk Among Loops at the Interface Associated with Channel Gating

After the identification of key residues, efforts have continued to identify key pairwise interactions involved in the structural changes that occur during gating.

Based on the structure of the *Torpedo* nAChR, Miyazawa et al. [61] first hypothesized that gating involves a "pin-into-socket" interaction between αVal46 at the tip of β1β2 loop and the M2–M3 linker. Further studies show that although these loops are essential for channel gating, the mechanism is more complex, and it involves more than a single pairwise interaction. In this regard, the hydrophobic pocket is not conserved in all Cys-loop receptors. By site-directed mutagenesis of the equivalent "pin" residue in glycine and GABA receptors, Kash et al. [166] postulated that activation of these receptors was not consistent with the pin-into-socket hypothesis. Studies of GABA receptors show that optimal gating depends on electrostatic interactions between the charged residues in β1β2 and Cys-loop (Asp57 and Asp149) and Lys279 in M2–M3 linker [148]. By mutant cycle analysis and disulfide cross-linking, it was determined that Asp149 moves closer Lys279 during gating. Equivalent charged residues in β1β2 and Cys-loop of the glycine receptor have also been shown to be implicated in channel gating of this receptor, though electrostatic interactions between them seem to not occur [50]. Reeves et al. [175] also tested with point mutations in 5-HT_3 subunits the interaction proposed for the pin-into-socket mechanism. Their results showed that the equivalent residue in 5-HT_3 receptors (Lys81 in loop β1β2 of 5-HT3A subunit) is not buried in a hydrophobic pocket, as expected from by the pin-into-socket hypothesis, though it is close to residues in the extracellular end of M2 (Ala304 (26′) and Ile305 (27′)). Electrophysiological recordings show that this residue plays a critical role in channel opening and return from fast desensitization [175].

Identification of pairwise interactions at the interface that control channel gating can be achieved by combining mutagenesis, single channel kinetic analyses, and thermodynamic mutant cycle analyses. Such an approach provides an avenue for estimating the energetic interactions (independent or dependent) between residues and allows one to infer the degree to which residues in different sites of a protein are functionally coupled [176]. Using this strategy, Lee and Sine [149] identified a transduction pathway in which the pre-M1 domain is coupled to the M2–M3 linker through the β1β2 loop in the human muscle nAChR. The authors proposed that agonist binding leads to the disruption of a salt bridge between the arginine located at the end of β10 in the pre-M1 region (Arg209) and a glutamate residue (Glu45) in β1β2 of the α1 subunit (Fig. 2c). The key glutamate and flanking valine (Val46) residues energetically couple to conserved proline (Pro272) and serine residues (Ser269) at the top of M2, and this may be a main point at which the binding domain triggers opening of the channel. The positioning of key elements of this pathway, such as the buried salt bridge formed by Arg209 and Glu45, has been later verified by high-resolution structures of the isolated α-subunit extracellular domain [39] and the bacterial channel GLIC [44]. By single-channel kinetic analysis of mouse muscle nAChRs mutants activated by choline or ACh, Purohit and Auerbach [101] showed that Arg209 moves early during gating, approximately at the same time as Glu45 in loop β1β2, and confirmed that pre-M1 participates in gating. However, results from their analysis of single and double mutants, in which the formation of the salt bridge is affected, discarded the Arg209–Glu45 salt bridge as essential for activation of the mouse muscle nAChR [101]. It would be interesting to understand the origin of these apparently controversial results, which could arise from measuring activation by a partial or a full agonist, from differences in the specific interactions between human and mouse nAChRs, in the ionic environment, or from a combination of all of them. The salt bridge between residues equivalent to Glu45 and Arg209 has also been found to be important in $GABA_A$ [177] and $GABA_C$ receptors, but it does not appear to exist in 5-HT_3A receptors [178].

Another pathway in which the pre-M1 region is coupled also to the M2–M3 linker through the Cys-loop was further identified in the human muscle nAChR [179]. The studies reveal energetic coupling among αLeu210 from the pre-M1 region, αPhe135, and αPhe137 from the Cys-loop and αLeu273 from the M2–M3 linker (Fig. 2c). Thus, the studies in the human muscle nAChR show that the extracellular β1β2 and Cys-loops bridge the pre-M1 region and M2–M3 linker to transduce agonist binding into channel gating.

A highly conserved proline is found in the M2–M3 loop of nAChR (Pro272) and 5-HT_3 receptors but not in the anionic $GABA_A$ and glycine receptors. The key role of this proline in channel gating was first studied by Lummis et al. [165] in 5-HT_3A receptors (Fig. 2c). Nonsense suppression in *Xenopus* oocytes was used to incorporate unnatural, proline analogs, amino acids. It was observed that gating, measured by the EC_{50} values, is impaired by amino acids that prefer the *trans*-conformation, whereas it is facilitated by amino acids that prefer the *cis*-conformation. This result led to propose that isomerization of this proline into the *cis*-conformation may bend the M2–M3 loop, which may in turn move M2 and allow channel opening [165]. The time scale of isomerization could be consistent with the slow activation rate of 5-HT_3A receptors [57, 58]. The possibility that proline isomerization can act as a switch for channel gating was recently supported by theoretical analysis using classical molecular dynamics and the metadynamics method [180]. However, other studies do not support the mechanism of isomerization as a switch for channel opening in 5-HT_3 receptors [181]. Here, the hypothesis was discarded since mutations of the proline to histidine or tryptophan show no profound changes in the magnitude of the currents and in EC_{50} values for 5-HT activation. However, the currents show altered calcium dependence and decay more rapidly, which

was interpreted as increased desensitization, suggesting a functional role of this proline.

Proline 272 has also an important role in channel gating of the muscle nAChR. However, the isomerization hypothesis for channel gating was discarded for this receptor since substitutions to different side chains still allow channel function [155, 156]. Also, the fast activation rate of the muscle nAChR is not compatible with the time scale of isomerization. By single-channel kinetic analysis of mutant muscle nAChRs, Lee et al. [156] showed that αPro272 functionally couples to the flanking valine residues from β1β2 (Val46) and Cys-loop (Val132), serving as an anchor that joins the hydrophobic residues from both loops. The functional contributions of the three residues may depend jointly on proper steric fit and hydrophobicity [156] (Fig. 2c).

The large body of experimental evidence shows that several fundamental structures are conserved throughout the family, which in turn, suggests a gating mechanism conserved throughout the family. However, although the overall roles of the loops seem to be conserved for all Cys-loop receptors, the specific interresidue couplings among them vary for each receptor subtype. An alternative hypothesis for the lack of specific interactions conserved across all the family was presented by Xiu et al. [145]. The overall analysis of charged residues within the interface across the entire superfamily showed a conserved charging pattern, without conserved specific interacting ion pairs [145]. The conclusion is that the overall charging pattern of the interface and not any specific pairwise electrostatic interactions controls gating.

REFER analyses of hundreds of residues of the mouse muscle nAChR allowed the construction of a Φ map which may represent the sequencing of movements of different domains. The map suggests that during channel opening, the presence of the agonist triggers motions of the binding site, which, next, moves Cys- and β1β2 loops and then M2–M3 linker, some M2 residues, and finally the gate [153, 155, 182, 183].

In addition to the identification of residues involved in channel gating, it has been recently proposed that the carbohydrate may be also involved in gating [39]. The crystal structure of the extracellular domain of the mouse α1nAChR subunit bound to α-BTX shows a long carbohydrate chain linked to Asn141, which is part of a consensus site for glycosylation at the Cys-loop that is conserved in the muscle nAChRs and some other Cys-loop receptor subunits. The oligosaccharide chain links the Cys-loop with the backside of Loop C, suggesting that it may have a role in coupling agonist binding to channel gating [39]. This hypothesis is supported by experimental studies showing that the removal of the carbohydrate affects functional properties [39, 184]. However, a weak point is the lack of conservation of the location of the sugar among members of the family. In this regard, the consensus site for glycosylation is not present in the Cys-loop of α7, α glycine, and α GABA subunits, and the different sugar location is not compatible with a common mechanism.

The overall findings reveal that it is unlikely that the entire responsibility of gating rests on only a few amino acids. A variety of functional and computational evidence over the last years suggests that movements around the binding site propagate through the β-strands to cause rearrangements of the interface. The emerging view indicates that β1β2 loop, Cys-loop, M2–M3 linker, and pre-M1 region act jointly to allow the increase in ion conductance that follows the binding of the agonist. It is less known how other interface regions, such as the post-M4 region and β8β9 loop, participate in this mechanism.

Summary

Cys-loop receptors act as chemical to electrical converters. Thus, the essence of their function relies on coupling the binding of the neurotransmitter at the extracellular domain to the opening of the ion channel. How the structural changes elicited by agonist binding are propagated through a distance of 50 Å to the gate is therefore central for the understanding of the receptor function. The interface between the extracellular and transmembrane domains is a structural transition zone where β-sheets from the binding domain merge with α-helices from the pore domain, and it has emerged as the coupling zone. Within this zone, several loops converge to form a network of interdependent residues that relay structural changes from the agonist binding site toward the pore. This network also controls channel lifetime and rate of desensitization. Thus, the interface affects the beginning, duration, and refractory period of a synaptic response mediated by Cys-loop receptors. Structural models have been useful to provide a framework on which it is possible to develop testable hypotheses of the changes that underlie channel function. However, since they represent a rigid but not a dynamic molecule, they are not enough for understanding channel gating at the molecular level, which involves both enthalpy and entropy changes. Moreover, the protein is part of a whole system which includes water, ions, and a lipid membrane, which also contribute to energy changes. Several fundamental structures have been shown to be conserved throughout the family, which in turn suggests a gating mechanism conserved throughout the family. However, it is now evident that different subtypes of Cys-loop receptors use different combinations of residues to tailor postsynaptic responses according to physiological needs. Further studies are needed to decipher all these key

interactions. Comparison of crystal structures in the open and closed state will provide valuable information to develop experiments aimed at identifying interresidue contacts that either form or break upon transitions between open and closed states. However, this is even more complex since a common feature of all family members is the capacity of suffering desensitization after prolonged exposure to agonist, a process which is still a mystery.

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