Eur Biophys J (2009) 39:37–49
DOI 10.1007/s00249-009-0452-y

REVIEW

Gating mechanisms in Cys-loop receptors

Jennie M. E. Cederholm · Peter R. Schofield · Trevor M. Lewis

Received: 30 January 2009 / Revised: 23 March 2009 / Accepted: 27 March 2009 / Published online: 29 April 2009

Abstract The Cys-loop receptor superfamily of ligand-gated ion channels has a prominent role in neuronal signalling. These receptors are pentamers, each subunit containing ten β-strands in the extracellular domain and four α-helical transmembrane domains (M1–M4). The M2 domain of each subunit lines the intrinsic ion channel pore and residues within the extracellular domain form ligand binding sites. Ligand binding initiates a conformational change that opens the ion-selective pore. The coupling between ligand binding in the extracellular domain and opening of the intrinsic ion channel pore located in the membrane is not fully understood. Several loop structures, such as loop 2, the Cys-loop, the pre-M1 region and the M2–M3 loop have been implicated in receptor activation. The current "conformational change wave" hypothesis suggests that binding of a ligand initiates a rotation of the β-sheets around an axis that passes through the Cys-loop. Due to this rotation, the Cys-loop and loop 2 are displaced. Movement of the M2–M3 loop then twists the M2 domain leading to a separation of the helices and opening of the pore. The publication of a crystal structure of an acetylcholine binding protein and the refined structure of the *Torpedo marmorata* acetylcholine receptor have improved the understanding of the mechanisms and structures involved in coupling ligand binding to channel gating. In this review, the most recent findings on some of these loop structures will be reported and discussed in view of their role in the gating mechanism.

Keywords Ligand-gated ion channels · Cys-loop receptor · Gating · Coupling · Review

Abbreviations

AChBP	Acetylcholine binding protein
Cu:Phen	Copper phenanthroline
ECD	Extracellular domain
$GABA_AR$	γ-aminobutyric acid type A receptor
GlyR	Glycine receptor
$5\text{-}HT_3R$	Serotonin type 3 receptor
LBS	Ligand binding site
LGIC	Ligand-gated ion channel
MTS	Methanethiosulfonate
nAChR	Nicotinic acetylcholine receptor
NT	Neurotransmitter
P4S	Piperidine 4-sulfonate
REFER	Rate-equilibrium free energy relationship
SCAM	Substituted cysteine accessibility method
TMD	Transmembrane domain
WT	Wild type

"Proteins, membranes and cells: the structure-function nexus". Contributions from the annual scientific meeting (including a special symposium in honour of Professor Alex Hope of Flinders University, South Australia) of the Australian Society for Biophysics held in Canberra, ACT, Australia, September 28–October 1, 2008.

Electronic supplementary material The online version of this article (doi:10.1007/s00249-009-0452-y) contains supplementary material, which is available to authorized users.

J. M. E. Cederholm · P. R. Schofield · T. M. Lewis (✉)
School of Medical Sciences,
The University of New South Wales,
Sydney, NSW 2052, Australia
e-mail: t.lewis@unsw.edu.au

J. M. E. Cederholm · P. R. Schofield
Prince of Wales Medical Research Institute,
Randwick, NSW 2031, Australia

Introduction

Signalling between neurons occurs through the release of neurotransmitters (NT) from pre-synaptic neurons into the synaptic cleft. These NTs can then bind and activate ligand-gated ion channels (LGIC) found in the membrane of post-synaptic neurons. Ligand binding to the extracellular domain (ECD) of LGICs initiates a conformational change that opens an ion-selective pore located within the transmembrane domain (TMD). This generates an excitatory or inhibitory post-synaptic current depending on the ion selectivity of the LGIC in the membrane. The most prominent family of LGICs is the Cys-loop family, so called because of a characteristic loop formed by a disulfide bond between two cysteine residues in the ECD. The Cys-loop family comprises the glycine receptor (GlyR), the γ-aminobutyric acid type A receptor ($GABA_AR$), the nicotinic acetylcholine receptor (nAChR) and the serotonin type 3 receptor ($5\text{-}HT_3R$). A cartoon illustrating the general topology of Cys-loop receptors is shown in Fig. 1. GlyR and $GABA_AR$ are anion-selective channels and allow for chloride ions to pass through the pore, leading to an inhibitory response in the post-synaptic neuron. In contrast, the nAChR and the $5\text{-}HT_3R$ are cation-selective channels and allow the passage of sodium and potassium ions, generating an excitatory post-synaptic response. Given that the distance between the ligand binding site (LBS) and the ion channel pore is about 40 Å (Unwin 2005), many studies have concentrated on identifying the structures that link these two separate domains to facilitate channel gating. Four loop structures found at the interface between the ECD and the TMD, loop 2, the Cys-loop, the pre-M1 region and the M2–M3 loop, have been the focus of numerous studies. The specific roles of these loops in linking agonist binding to gating have been investigated using a variety of experimental techniques.

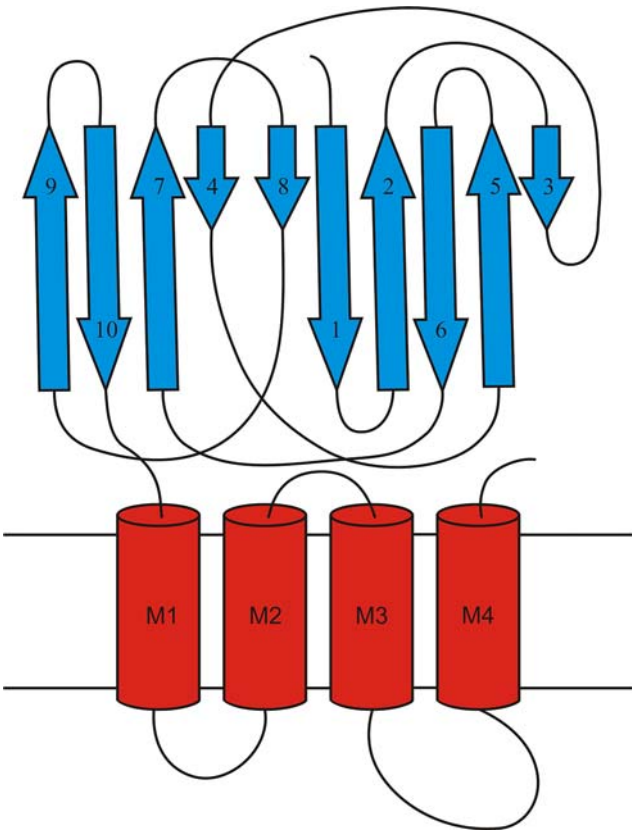

Fig. 1 A cartoon of a Cys-loop receptor subunit showing the extracellular domain (ECD), with its β-strands, and the transmembrane domain (TMD) with its four α-helices, *M1–M4*. The *M2* helices from each subunit contribute to forming the channel pore. The inner β-sheet (β-strands *1*, *2*, *3*, *5*, *6* and *8*) interacts with the outer β-sheet (β-strands *4*, *7*, *9* and *10*), forming a β-sandwich

The task of identifying the specific pathway of Cys-loop receptor activation has not been easy, mainly due to the lack of a high-resolution crystal structure of a Cys-loop receptor in both the open and closed states. However, a major breakthrough came when the crystal structure of the snail *Lymnaea stagnalis* acetylcholine binding protein (AChBP) was published in 2001 (Brejc et al. 2001). It is a homologue to the ECD of the nAChR, with the ability to bind nAChR ligands and therefore is a potential tool to provide insight into the mechanism and structures involved in ligand binding. The next milestone was the publications of the refined structure of the *Torpedo marmorata* nAChR by Miyazawa et al. (2003) and Unwin (2005). The structure published in 2003 confirmed much of the existing data on ligand binding and greatly helped our understanding of the mechanisms involved in coupling ligand binding to channel gating. The structure published in 2005 allowed for a more comprehensive description of the whole receptor in the closed-channel form.

In this review we will report on and discuss the most recent findings into the molecular mechanisms of channel gating.

Structure of Cys-loop receptors

Cys-loop receptors consist of five subunits that are arranged around a central ion channel pore within the membrane. Each subunit contains a large ECD that is made up of ten β-strands. β-strands 1, 2, 3, 5, 6 and 8 make up the inner β-sheet. The outer β-sheet (β-strands 4, 7, 9 and 10) and the inner β-sheet interact and are organised as a β-sandwich. Each subunit also contains four α-helical membrane spanning domains (M1–M4) (Fig. 2). The pore is shaped by the M2 helices from each subunit and conformational changes of this region upon ligand binding allow for ion flow through the pore. A large intracellular loop is located between domains M3 and M4, and includes

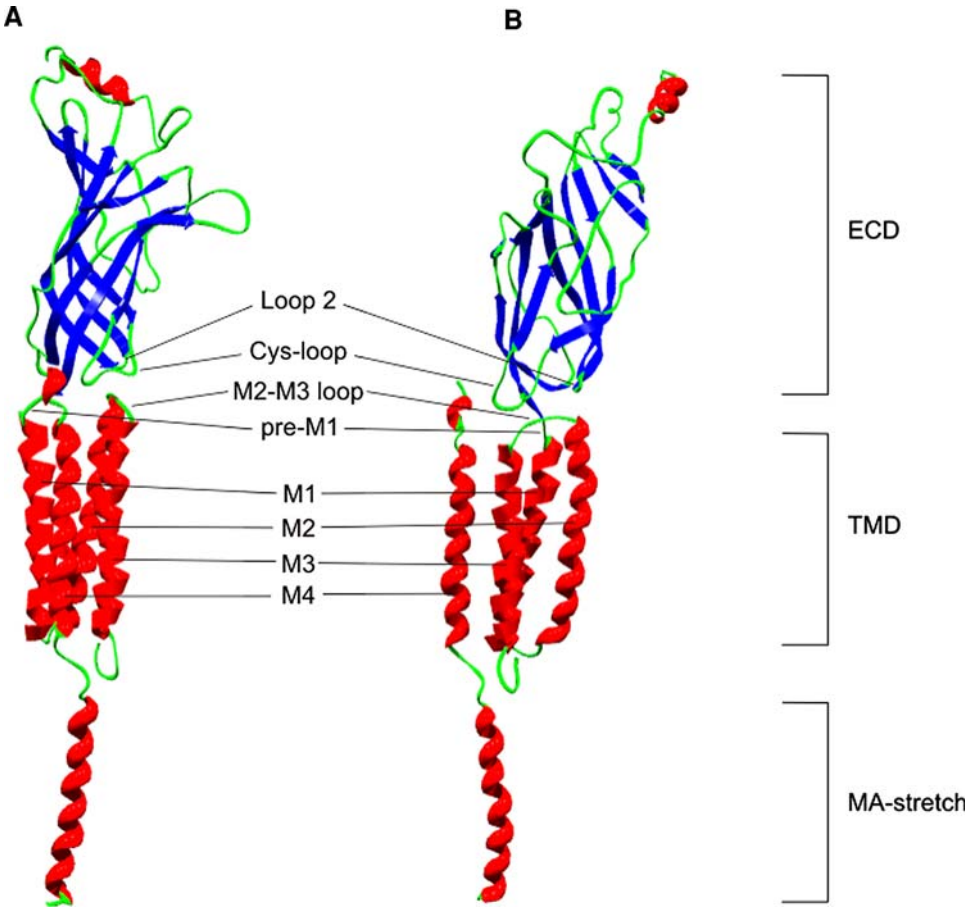

Fig. 2 Ribbon diagrams of a single nAChR α-subunit (PDB entry 2GB9) viewed parallel with the membrane plane. In **a** the *M2* pore-lining domain is at the back and **b** the subunit has been rotated clockwise so that the *M2* is towards the side. The *β*-strands composing the *β*-sandwich are in *blue*; the membrane spanning α-helices are in *red*. The *M3–M4* loop is missing

an amphipathic α-helix known as the membrane-associated (MA) stretch. The MA-stretch has been shown to be important for single-channel conductance in cation-selective (Deeb et al. 2007; Gee et al. 2007; Hales et al. 2006; Jansen et al. 2008; Kelley et al. 2003), as well as anion-selective ion channels (Carland et al. 2009). The ECD and the TMD interact through the connection at the start of M1 (pre-M1), loop 2, the conserved Cys-loop (loop 7) and the M2–M3 loop (Unwin 2005) (Fig. 3).

Gating of Cys-loop receptors

The conformational changes that occur upon ligand binding are transferred away from the binding site through the ECD towards the interface with the TMD and then to the M2 channel gate thought to be formed, in nAChRs at least, by a girdle of hydrophobic residues. This is referred to as a "conformational change wave" (Grosman et al. 2000b). This conformational wave has been extensively studied in the nAChR by Chakrapani et al. (2004), Grosman et al. (2000b), Jha et al. (2007), Purohit and Auerbach (2007a,b), Purohit et al. (2007) and provides a dynamic description of nAChR gating, which builds upon the static information that crystal structures can offer. According to this hypothesis, ligand binding initiates a rotation of the inner *β*-sheet around an axis that passes through the Cys-loop (Lester et al. 2004; Miyazawa et al. 2003; Unwin et al. 2002). At the interface, the Cys-loop and loop 2 are displaced due to the rotation of the *β*-sheets. Movement of the M2–M3 loop then twists the M2 domain leading to a separation of the helices and widening of the hydrophobic girdle. Thus, the channel is opened and ions can pass from one side of the membrane to the other.

Involvement of the ligand binding site in gating

The LBS of Cys-loop receptors is formed at the interface between two subunits in the ECD. Before any crystal structure was available, the key residues involved in ligand binding were proposed to be located in six "loops" that come together to form the LBS. These loops are referred to as loops A–F. However, it is now evident that some of the key binding residues are in fact located on *β*-strands, suggesting that a change in terminology is needed. Until a

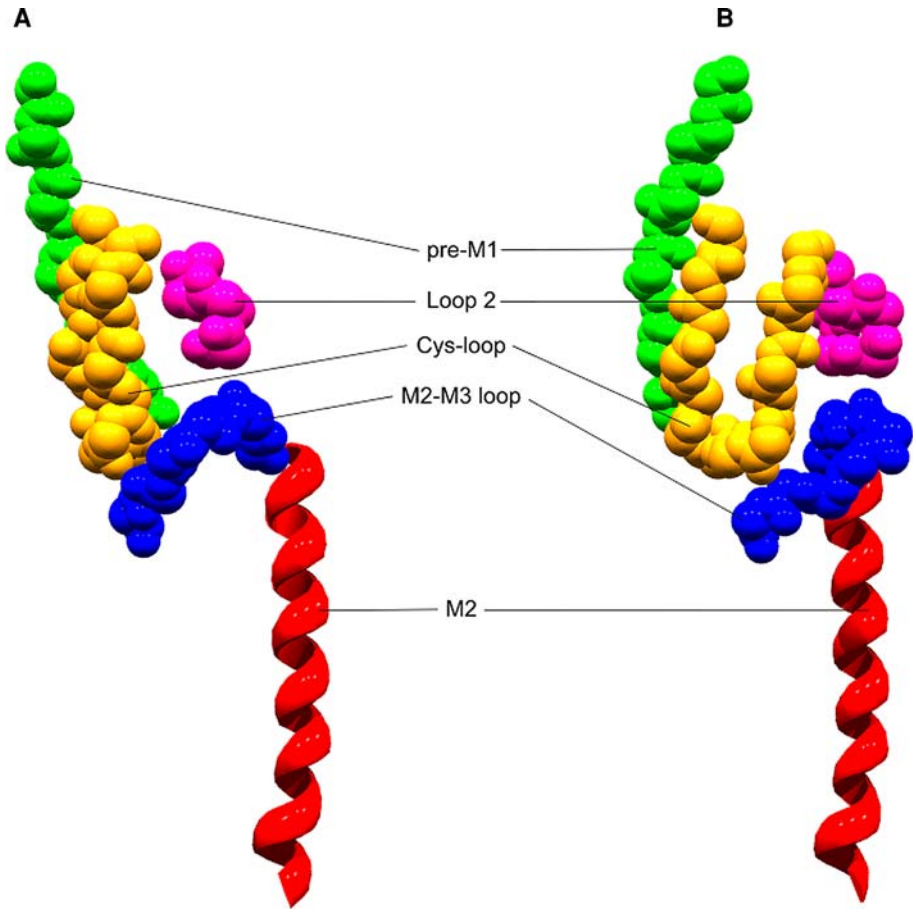

Fig. 3 Diagrams of a single nAChR α-subunit (PDB entry 2GB9) viewed parallel with the membrane plane. In **a** the M2 pore-lining domain is at the side and **b** the subunit has been rotated anti-clockwise so that the *M2* is towards the back. The diagrams show the location of the M2 domain (*red*), loop 2 (*pink*), the Cys-loop (*yellow*) and the pre-M1 region (*green*) in relation to each other

new terminology is widely accepted, and to avoid misunderstandings, this review will still refer to the binding sites as loops A–F.

Ligand binding is best understood in nAChRs. Each binding site is formed between the principal subunit and the adjacent complementary subunit (in the anticlockwise direction when looking at the receptor from the synaptic cleft). Here, loops A, B and C from the principal subunit interact with loops D, E and F from the complementary subunit (Gay and Yakel 2007). These loops contain aromatic and hydrophobic residues in the nAChR (Gay and Yakel 2007; Unwin et al. 2002), but these residues are not conserved across all Cys-loop members.

Of the loops contributing to the LBS, loop C shows the greatest movement upon agonist binding, appearing to close over the LBS (Celie et al. 2004; Gao et al. 2005). This loop, between β-strands 9 and 10, is continuous with the M1 domain via β-strand 10. Thus, a conformational change in loop C is expected to be propagated along β-strand 10 to M1, which is known to contribute to channel gating (see below). Molecular dynamics simulations also predict coupling between loop C and rotation of the M1 and M2 domains (Liu et al. 2008). In addition, β-strand 10 is close to the Cys-loop, which has been shown to interact with the M2–M3 loop in $GABA_A$Rs (Kash et al. 2003). The M2–M3 loop is another of the key structures that contribute to opening of the pore (see below). Thus, it appears that a conformational change in loop C is one of the early steps that contribute to the gating process.

Single-channel kinetic analysis and structural modelling of the muscle nAChR show that three conserved residues of the α-subunit, Lys145 in the Cys-loop, Asp200 and Tyr190 in loop C, play a significant role in the initial conformational changes involved with gating (Mukhtasimova et al. 2005). Mutating each of these residues impairs gating and double mutant studies demonstrate that these residues are interdependent. It is suggested that in the resting state a salt bridge exists between Lys145 from β-strand 7 (Cys-loop) and Asp200 from β-strand 10 (loop C). When an agonist binds, Lys145 can be pulled away from Asp200 by electrostatic force from Tyr190 in the highly mobile loop C. These local changes might be transmitted to the channel via β-strand 7 and 10. A salt bridge between residues Glu153 and Lys196 in the $GABA_A$R β2-subunit (equivalent to Lys145 and Tyr190 in muscle nAChR) has also been found (Venkatachalan and Czajkowski 2008). This salt bridge is critical for GABA receptor activation, and crosslinking experiments confirm

that these two residues (Glu153 and Lys196) are in close proximity. Interestingly, data also suggest that loop C is highly mobile in the resting state and that Glu153 and Lys196 serve to stabilise the ligand-bound state of the receptor upon activation (Venkatachalan and Czajkowski 2008). Furthermore, residue Arg207 in loop C (equivalent to Asp200 in the muscle nAChR) is believed to play an important role in the resting, unliganded state of the $GABA_AR$ by interacting with Glu153 in the Cys-loop.

A second "triggering structure" adjacent to the LBS has also been observed in the muscle nAChR. Conserved residues Tyr127, Asn39 and Asn41 (in the α-, ε- and δ-subunits, respectively) all contribute to channel gating and it seems they are interdependent (Mukhtasimova and Sine 2007). However, these two pairs (Asn39/Tyr127 and Asn41/Tyr127) of residues are intersubunit, rather than intrasubunit, and their role is only conserved in muscle nAChRs. This suggests a specialised function for the muscle receptor. The authors do suggest the possibility of similar "triggering structures" in the other Cys-loop receptors based solely upon sequence homology. This has yet to be tested experimentally.

The important role of Tyr127 and Lys145 in the mouse nAChR α1-subunit has also been highlighted in a rate-equilibrium free energy relationship (REFER) study (Purohit and Auerbach 2007b). REFER employs a simplified kinetic scheme of a single open and closed state to generate a statistical value (Φ) that estimates whether the effect of a number of mutations at a particular site is temporally closer to the open state ($\Phi = 1$) or the closed state ($\Phi = 0$) transition. From this analysis, three conformational blocks have been described for the gating of the nAChR α1-subunit. Φ values in the LBS are around 0.93, decreasing to 0.78 in the Cys-loop and loop 2 and to 0.64 in the M2–M3 loop (Purohit and Auerbach 2007b). The REFER study demonstrates that Tyr127 and Lys145 belong to two different conformational blocks, with Lys145 moving with the ligand binding (Φ of 0.96) followed by Tyr127 (Φ of 0.77), which occurs almost synchronously with residues in the Cys-loop and loop 2. This indicates that if there is a rotation of the β-strands of the α-subunit, it will be of a more flexible nature since Lys145 from the outer β-sheet and Tyr127 from the inner β-sheet belong to two different conformational blocks. Furthermore, this study also found that Tyr127 is energetically coupled to residues in the δ- or ε-subunits, and less so to those in the α-subunits. Thus, their results support the idea proposed by Mukhtasimova and Sine (2007), that there is a link between the α-subunits and the δ- or ε-subunits (described above) that is important for the gating conformational cascade.

In the $5\text{-}HT_{3A}R$ several tyrosine residues from, or around, the LBS loops have been implicated in triggering channel gating upon ligand binding (Price and Lummis 2004; Venkataraman et al. 2002). Incorporating both natural and unnatural amino acids into several tyrosine positions of the mouse $5\text{-}HT_{3A}R$ allowed Beene et al. (2004) to define the roles of these residues. Removal of the aromatic group and/or displacement of the hydroxyl group of Tyr143 in loop E led to an increase of the measured EC_{50} compared to wild-type (WT) (Beene et al. 2004), but did not have an effect on binding of competitive antagonist. This suggests that aromatic and/or hydroxyl groups at Tyr143 are not required for ligand binding, but are important for efficient gating. Furthermore, modelling data suggest that the hydroxyl group of Tyr143 can form a hydrogen bond with the backbone carbonyl of Trp183 from the opposite side of the binding site (Beene et al. 2004). Removal of the hydroxyl group, and hence the hydrogen bond, by introduction of the unnatural amino acid may then be the cause for inefficient gating. Another tyrosine in loop E, Tyr153, was shown to be important for both gating and binding. Mutation of this Tyr153 resulted in large increases in measured EC_{50}, and changes in both receptor kinetics and antagonist binding affinity. Similar to Tyr143, both an aromatic ring and a hydroxyl group at position Tyr153 seem to be critical for proper receptor function. The location of the hydroxyl group in Tyr153 suggested to the authors that it is critical for the binding of 5-HT (Beene et al. 2004). Furthermore, removal of the aromatic ring of the conserved Tyr234 in loop C resulted in non-functional receptors showing that it is essential for receptor function. Such data, when considered in the light of the *T. marmorata* nAChR work (2002), led to the suggestion of a gating model in which 5-HT enters the binding site and forms a cation-π bond with Trp183, leading to the displacement of Tyr143 and Tyr153. These two residues are located on separate β-sheets linked by a turn; thus their movements might lead to a rotation of the $5\text{-}HT_{3A}R$ structure.

Long before the crystal structure of the AChBP was available, a study of the *Torpedo californica* nAChR showed that the loop F residue, Asp174/Asp180 (in the γ- and δ-subunit, respectively), can come within 9 Å of the disulfide on loop C (Czajkowski and Karlin 1991). By introducing unnatural amino acids, it has since been confirmed that loop F interacts with loop C in muscle nAChRs (Gleitsman et al. 2008). Mutating the backbone of the α-subunit residue Ser191 in loop C to α-hydroxyserine (Sah) (giving it more flexibility and also replacing the hydrogen bond-donating N–H group with a non-hydrogen bond-donating O), leads to a 40-fold increase in EC_{50}. In addition, alanine and α-hydroxyalanine (Aah) mutations were also made at this site. In contrast to the Ser191Ala mutation, but similar to Ser191Sah, the Ser191Aah mutation significantly increased the EC_{50}. Thus, it is clear that it is the backbone of Ser191, and not the side-chain, that is important for receptor function. Mutant cycle analysis was

then used to look at possible interactions between loops C and F of the binding site. Results show that there is a large energetic coupling between Ser191Sah (loop C) and the previously studied Asp174/Asp180 in loop F (Gleitsman et al. 2008). All mutations at Asp174/Asp180 that have a major impact on function also show strong coupling to the Ser191Sah backbone mutation (Gleitsman et al. 2008). Thus, the movement of loop F closer to loop C during agonist binding very likely contributes to the gating pathway.

Pre-M1 region

The pre-M1 region connects β-strand 10 of loop C to the M1 domain. The involvement of this region in coupling ligand binding to channel gating has been studied in the rat $GABA_AR$ α1- and β2-subunits using the substituted cysteine accessibility method (SCAM) (Mercado and Czajkowski 2006). This region contains several cationic residues including an arginine that is conserved in several Cys-loop receptors. An alignment of the pre-M1 residues is shown in Fig. 4. Mutating this Arg216 in the $GABA_AR$ β2-subunit to a cysteine (Arg216Cys) abolished channel gating without modifying the binding of the GABA agonist [^{3}H] muscimol. Substitution of a cysteine at position Arg220 in the α1-subunit of this receptor was not tolerated since there was no [^{3}H] muscimol binding nor channel activation by GABA or pentobarbital. Thus, this shows that this residue plays a critical role in coupling GABA binding to gating (Mercado and Czajkowski 2006). Furthermore, by observing the effects of methanethiosulfonate (MTS) reagents to several cysteine-substituted mutations in both the closed and open states it was shown that the pre-M1 region moves during channel gating.

The Arg216/Arg220 residues in rat $GABA_AR$ subunits are highly conserved among other receptors of the Cys-loop family. The equivalent residue in the GlyR α1-subunit, Arg218, has been found to be mutated in some cases of hyperekplexia (Arg218Gln). The effect of this mutation has been studied in whole-cell patch clamp experiments and the results explain much of the observed phenotype (Castaldo et al. 2004). It was shown that the Arg218Gln mutation causes a 200-fold decrease in the apparent affinity of glycine and abolishes any channel activation by maximal concentrations of β-alanine and taurine. The observed effects are unlikely to reflect a binding site change as the competitive antagonist strychnine was still effective. Furthermore, at very high glycine concentrations both activation and desensitization rates were faster in WT compared to mutant channels, with no change in deactivation rates. All of these drastic effects support the idea that the mutation has a deleterious effect on channel gating. It is notable that Arg218 lies close to several residues that have been implicated in channel gating, such as Arg271 (Rajendra et al. 1994) and Lys276 (Lewis et al. 1998) in the M2–M3 loop and Asp148 in the Cys-loop (Schofield et al. 2003).

Arg222, in the pre-M1 region of the $5\text{-}HT_{3A}R$ has also been implicated in channel gating (Hu et al. 2003). This arginine is only found in $5\text{-}HT_{3A}Rs$ and α7 nAChRs, but is adjacent to the highly conserved Arg218 in the GlyR α1-subunit and Arg216/Arg220 in the $GABA_AR$ subunits. Thus, these residues might play the same role in the different Cys-loop receptors.

Lys215 in the pre-M1 region of the $GABA_AR$ β2-subunit is adjacent to the highly conserved Arg216 and also seems to be important for coupling ligand binding to channel gating (Kash et al. 2004a). The sensitivity to GABA and the relative efficacy to the two agonists, piperidine 4-sulfonate (P4S) and taurine, was reduced in receptors containing the the mutant β2-subunit Lys215Asp. However, the charge reversal double mutant, Asp146Lys/Lys215Asp, almost completely restored the sensitivity to GABA. By mutating the Asp146 (Cys-loop) and Lys215 to cysteine, the oxidising reagent copper phenanthroline (Cu:Phen) could be used to examine the proximity and mobility of these residues. Results demonstrate that Cu:Phen alone had no effect, whereas Cu:Phen together with GABA significantly inhibited receptor function. This suggests that the Cys-loop is only in close proximity to the pre-M1 region during the open or desensitised state.

An alanine scan of 12 residues in β-strand 10 and the pre-M1 region of human $GABA_AR$ α1-subunits identified two mutations that reduced the potency of GABA, Val212Ala in β-strand 10 and Lys220Ala in the pre-M1 region (Keramidas et al. 2006). Both mutants had a four- to fivefold increase in their respective EC_{50} value. The close location of Lys220 to loop 2, the Cys-loop and the M2–M3 loop prompted the authors to do further investigations. Results showed that removal or reversal of charge at Lys220 (Lys220Asp) did not have a great effect on the function of the receptor. These results support the hypothesis that as long as the charging pattern within the gating zone is preserved, individual changes of charge are tolerated (Xiu et al. 2005).

Loop 2 and the Cys-loop

At the interface between the ECD and the TMD, both loop 2 and the Cys-loop are predicted to be close to the top of the ion channel and the M2–M3 loop (Brejc et al. 2001; Unwin 2005) (Figs. 2 and 3). When a 4 Å resolution structure of the *T. marmorata* nAChR was published (Miyazawa et al. 2003), the authors suggested that loop 2 is in a position that allows it to dock into a hydrophobic

	Loop 2		Cys-loop	
Tc nAChR δ	46	KETDET 51	130	CPINVLYFPFDWQNC 144
h nAChR δ	46	KEVEET 51	130	CPISVTYFPFDWQNC 144
Tc nAChR γ	44	NEKEEA 49	128	CPIAVTYFPFDWQNC 142
h nAChR ε	44	NEKEET 49	128	CAVEVTYFPFDWQNC 142
Tc nAChR β	44	NEKIEE 49	128	CTIKVMYFPFDWQNC 142
Tm nAChR α	44	DEVNQI 49	128	CEIIVTHFPFDQQNC 142
Tc nAChR α	44	DEVNQI 49	128	CEIIVTHFPFDQQNC 142
m nAChR α_1	44	DEVNQI 49	128	CEIIVTHFPFDEQNC 142
h nAChR α_1	44	DEVNQI 49	128	CEIIVTHFPFDEQNC 142
h nAChR α_7	44	DEKNQV 49	128	CYIDVRWFPFDVQHC 142
r nAChR α_7	44	DEKNQV 49	128	CYIDVRWFPFDVQQC 142
c nAChR α_7	44	DEKNQV 49	128	CYIDVRWFPFDVQKC 142
h 5-HT_{3A}R	51	DEKNQV 56	134	CSLDIYNFPFDVQNC 148
m 5-HT_{3A}R	55	DEKNQV 60	138	CSLDIYNFPFDVQNC 152
h $GABA_AR$ β_2	51	SEVNMD 56	136	CMMDLRRYPLDEQNC 150
r $GABA_AR$ β_2	51	SEVNMD 56	136	CMMDLRRYPLDEQNC 150
h GlyR α_1	52	AETTMD 57	138	CPMDLKNFPMDVQTC 152
h $GABA_AR$ α_1	54	SDHDME 59	139	CPMHLEDFPMDAHAC 153
r $GABA_AR$ α_1	53	SDHDME 58	138	CPMHLEDFPMDAHAC 152
Ls AChBP	42	NEITNE 47	122	CCVSGVDTESG-ATC 136
p ELIC	27	NTLEQT 32	112	NDMDFRLFPFDRQQF 126
p GLIC	31	DDKAET 36	111	SPLDFRRYPFDSQTL 125

	pre-M1		M2-M3 loop	
Tc nAChR δ	215	VTFYLIIRRK 224	275	SQRLPETALAVPLI 288
h nAChR δ	215	ITFYLIIRRK 224	275	SKRLPATSMAIPLI 288
Tc nAChR γ	209	IIFFLIIQRK 218	270	AQKVPETSLNVPLI 283
h nAChR ε	210	VIYSLIIRRK 219	271	AQKIPETSLSVPLL 284
Tc nAChR β	207	VTFYLIIQRK 216	267	ADKVPETSLSVPII 280
Tm nAChR α	201	ITYHFIMQRI 210	261	VELIPSTSSAVPLI 274
Tc nAChR α	201	ITYHFIMQRI 210	261	VELIPSTSSAVPLI 274
m nAChR α_1	201	ITYHFVMQRL 210	261	VELIPSTSSAVPLI 274
h nAChR α_1	201	ITYHFVMQRL 210	261	VELIPSTSSAVPLI 274
h nAChR α_7	198	VTFTVTMRRR 207	258	AEIMPATSDSVPLI 271
r nAChR α_7	198	VTYTVTMRRR 207	258	AEIMPATSDSVPLI 271
c nAChR α_7	198	ITFTVTMRRR 207	258	AEIMPATSDSVPLI 271
h 5-HT_{3A}R	209	MKFYVVIRRR 218	269	SDTLPATAIGTPLI 282
m 5-HT_{3A}R	213	MKFYVIIRRR 222	273	SDTLPATIG-TPLI 286
h $GABA_AR$ β_2	208	LSLSFKLKRN 217	269	RETLPKIPYVKAID 282
r $GABA_AR$ β_2	208	LSLSFKLKRN 217	269	RETLPKIPYVKAID 282
h GlyR α_1	210	IEARFHLERQ 219	271	RASLPKVSYVKAID 284
h $GABA_AR$ α_1	213	MTTHFHLKRK 222	274	RNSLPKVAYATAMD 287
r $GABA_AR$ α_1	212	MTTHFHLKRK 221	273	RNSLPKVAYATAMD 286
Ls AChBP	162	SEYFSQYSRF 171	185	YSCCPEAYEDVEVS 198
p ELIC	190	ITVRIDAVRN 199	249	SNILPRLPYTTVID 262
p GLIC	183	LDYQLRISRQ 192	242	ETNLPKTPYMTYTG 255

Fig. 4 Aligned amino acid sequences of different subunits from five different Cys-loop receptors with their respective numbering. Alignment is only showing the amino acids that form loop 2, the Cys-loop, the pre-M1 region and the M2–M3 loop. Residues conserved across different subunits are shown in grey background and homologous residues are shown in *bold*. *Ls Lymnaea stagnalis, Tc Torpedo californica, Tm Torpedo marmorata, h* human, *m* mouse, *r* rat, *c* chick, *p* prokaryote

pocket made by the end residues (Ser269–Pro272 in the α-subunit) of the M2 domain. They referred to this as a "pin-into-socket" interaction. The "pin" in this structure was proposed to be Val46 of loop 2 in the α-subunit. An alignment of the loop 2 residues is shown in Fig. 4.

This interaction has also been studied in the $GABA_AR$ and GlyR (Kash et al. 2004b). However, results show that the equivalent "pin" residues (Val53 and His56 in $GABA_AR$ β2- and α1-subunit, respectively; Thr54 in GlyR α1-subunit) in these receptors are not likely to have a critical impact on the gating process. This suggests that the hydrophobic interaction observed in the nAChR is not a characteristic shared by $GABA_ARs$ and GlyRs. Indeed, a gating dynamic study of the mouse nAChR α1-subunit

highlighted that more than one loop 2 residue is critical for channel gating (Chakrapani et al. 2004). Subsequently, cysteine substitutions of the odd-numbered loop 2 residues, Ile51, Glu53, Thr55 and Asp57, of the GlyR α1-subunit increased glycine EC_{50}, while Thr54 decreased glycine EC_{50} (Crawford et al. 2008). The impact of Thr54 on EC_{50} suggests that this residue is involved in the gating process, which is in contrast to Kash et al. (2004b), who found that six Thr54 mutant GlyR α1-subunits (Thr replaced with Ala, Glu, His, Lys, Phe, or Ser) were not significantly different to WT. Increases of EC_{50} for the odd-numbered loop 2 residues also demonstrate that in the GlyR α1-subunit the whole loop structure plays a vital role (Crawford et al. 2008). This is supported by the spasmodic mutation in the mouse GlyR α1-subunit, in which Ala52 is replaced with a Ser, and the mutation results in an increase in the GlyR α1-subunit EC_{50} (Ryan et al. 1994). Single channel analysis of the spasmodic mutation suggests that it impairs the transition to a pre-opening conformation of the receptor (Plested et al. 2007) further supporting the role of the loop 2 as an important intermediate between ligand binding and channel opening. Interestingly, in contrast to the odd-numbered residues of loop 2 in the GlyR α1-subunit (Crawford et al. 2008), mutation of loop 2 residues in the chick nAChR α7-subunit that are equivalent to the even-numbered Ala52, Thr54 and Met56 in the GlyR α1-subunit, resulted in a significant increase in EC_{50} (McLaughlin et al. 2007). This suggests that the loop 2 structure differs between nAChRs and GlyRs.

It is notable that in the GlyR α1-subunit and $GABA_AR$ α1-subunit the Lys276/Lys279 residue located in the M2–M3 loop is conserved, but is absent in the nAChR M2–M3 loop. The $GABA_AR$ α1-subunit Lys279 has been proposed to interact with Asp57 in loop 2 (Kash et al. 2003). This suggests that equivalent interactions between the even-numbered loop 2 and M2–M3 loop residues might occur in the nAChR. Perhaps the Arg266 residue in the nAChR α7-subunit M2–M3 loop that has been found to be important for coupling (discussed below) somehow interacts with one of these even-numbered loop 2 residues. More experiments are needed to test this proposal.

Many other residues in loop 2 have also been shown to be critical for channel gating. Other charged loop 2 residues in both the GlyR α1-subunit (Absalom et al. 2003) and the $GABA_AR$ α1-subunit (Kash et al. 2003) have been implicated in receptor activation, and a specific salt bridge between Glu45 in loop 2 and Arg209 in the pre-M1 region of the nAChR α-subunit appear to be critical for proper channel gating (Lee and Sine 2005). In fact, this salt bridge has been suggested to be the key pathway linking agonist binding to channel gating. The same salt bridge has also been found to be important in the $GABA_AR$ (Price et al. 2007; Wang et al. 2007), although it does not appear to exist in the $5\text{-}HT_{3A}R$ (Price et al. 2007). The notion that such specific electrostatic interactions are critical to gating has, however, also been disputed. Xiu et al. (2005) propose that it is the global charge of the gating interface and groups of interacting ionic residues that are essential for receptor function.

The role of Glu45 and Arg209 in gating of nAChR has also been challenged by Purohit and Auerbach (2007a). In their study they mutated all five residues of the pre-M1 region and Glu45 in loop 2 and examined the effect on gating kinetics. They found that perturbation of a salt bridge between Glu45 and Arg209 was unlikely to be the main event in the gating of nAChRs. Several pieces of evidence point towards this conclusion. Firstly, there is no significant difference between the open- and closed-state energy for the different Arg209 mutations. Secondly, there is a very weak energetic coupling between Glu45 and Arg209. Thirdly, several double mutant constructs that are not able to form a salt bridge are still functional. Compared to the Cys-loop, loop 2 and the M2–M3 loop, it appears that the pre-M1 region has only a minor role in the energy transfer between the ECD and the TMD. Considering all of this, the authors suggest that a combination of side-chain interactions at several positions between loop 2 and the M2 domain, and the Cys-loop and the M2–M3 loop allows for energy to be transferred from the ECD to the TMD (Purohit and Auerbach 2007a). Overall, it is evident that different members of the Cys-loop receptor family use different molecular interactions within the same structures during gating. More work is needed to clarify these differences.

The involvement and importance of the Cys-loop in GlyR gating was first examined by Schofield et al. (2003). A highly conserved aspartate residue within the GlyR α1-subunit Cys-loop, Asp148, was reported to be critical for receptor activation. The equivalent residue in the $GABA_AR$ α1-subunit, Asp149, is also important (Kash et al. 2003) and mutant cycle analysis of double reverse charge mutations show that a direct electrostatic interaction exists between Asp149 and Lys279 (equivalent to Lys276 in the GlyR α1-subunit) in the M2–M3 loop. However, the same group found no evidence for coupling between the equivalent residues (Asp146 and Lys274) in the $GABA_A$ β2 subunit (Kash et al. 2004a). Instead they found that Asp146 interacts with Lys215 in the pre-M1 region. Single mutations of these residues significantly reduced GABA sensitivity, but it was almost completely restored in the double reverse charge mutant, Asp146Lys/Lys215Asp. Thus, not only do these findings demonstrate the importance of the Cys-loop they also show that the same structure in different subunits has different molecular interactions that are critical to channel gating.

Possible interactions between Asp148 and Lys276 have also been tested in the GlyR α1-subunit (Absalom et al.

2003; Schofield et al. 2003), but the lack of a full recovery from double reverse charge mutation experiments suggest that while these residues are involved in the gating process, they do not form a direct salt bridge in the GlyR. Further studies using different chimeras, molecular modelling and resolving the crystal structures of a Cys-loop receptor in an open versus closed state might provide some of the answers to these differences in mechanism between the LGICs.

A chimeric receptor composed of the AChBP and the pore domain from the 5-HT_{3A}R (Bouzat et al. 2004) revealed that even though binding and pore domains were folded correctly and the receptors could be expressed on the cell surface, the receptor was not functional as the correct molecular interactions were absent. Mutating residues in the Cys-loop, loop 2 and loop 9 of the AChBP to the 5-HT_{3A}R counterparts restored functional coupling, highlighting the importance of the specific interactions between these loops in the transduction mechanism between the ECD and TMD. Changing only one or two of the loops to the 5-HT_{3A} sequence produced receptors with impaired function, indicating that correctly matching loops 2, 9 and the Cys-loop with the M2–M3 loop are all required.

M2–M3 loop

One of the first structures implicated in the channel gating mechanism of Cys-loop receptors was the M2–M3 loop of the GlyR α1-subunit (Lynch et al. 1997). Several naturally occurring mutations that result in human hereditary hyperekplexia (or startle disease) have been located in this region (Elmslie et al. 1996; Shiang et al. 1993, 1995). Large increases in the EC_{50} value of two M2–M3 loop mutations (Lys276Glu and Tyr279Cys), and the conversion of *β*-alanine and taurine from full agonists to competitive antagonists, with little disruption to their binding affinities, suggested that these startle disease mutations alter the coupling of ligand binding to channel gating (Lynch et al. 1997). A complete alanine scan of the GlyR α1-subunit M2–M3 loop revealed that several residues in this loop are important for proper channel gating (Lynch et al. 1997). The importance of residues in the M2–M3 loop for linking agonist binding to Cys-loop receptor gating has been confirmed by numerous studies over the last decade (Bera et al. 2002; Campos-Caro et al. 1996; Grosman et al. 2000a; Lewis et al. 1998; Lynch et al. 1995; Rajendra et al. 1995; Rovira et al. 1999). As indicated earlier, chimera studies have highlighted the need for compatible residues in the ECD loops and the M2–M3 loop for functional gating (Bouzat et al. 2004; Castillo et al. 2006). Asp266 in the nAChR α7-subunit M2–M3 loop (Campos-Caro et al. 1996) and an equivalent residue in other neuronal nAChRs (Rovira et al. 1999) are involved in coupling binding to gating. An alignment of the M2–M3 residues is shown in Fig. 4. Mutating individual residues throughout the M2–M3 loop of the rat α7 nACh/mouse 5-HT_{3A}R chimera to aspartate increased receptor function. The Thr266Asp mutation had the most improved function, showing the smallest EC_{50} value (Castillo et al. 2006). Thus, charged residues are critical for the "correct" interaction of loop structures to enable channel gating. These results support the hypothesis of Xiu et al. (2005) that it is the overall electrostatic environment rather than specific interactions that is key to correct channel gating.

A proline residue in the M2–M3 loop, which is only found in the 5-HT_{3A}R and the nAChR, has been shown to be critical to the gating mechanism through a *cis–trans* isomerisation of the protein backbone (Lummis et al. 2005). A gating model for the 5-HT_{3A}R was proposed in which Pro308 (Pro272 in the nAChR α-subunit) is in the *trans* conformation when the channel is closed. When the ligand binds to the receptor, and a conformational change is initiated (possibly via loop 2), Pro308 in the M2–M3 loop undergoes a *cis–trans* isomerisation leading to a rotation of the M2 domain and thus opening the channel pore. In the nAChR α-subunit Pro272 has been shown to be part of two energetically coupled triads, the first one with Val46 and Glu45 (both in loop 2) and the second with Val46 and Ser269 (in the M2–M3 loop) (Lee and Sine 2005). Coupling energies between residues were estimated from changes in the gating equilibrium constants (from single channel analysis) measured with individual mutations and combinations of two or three mutations of the residues that interact. Individually mutating Val46Ala and Pro272Gly reduced the gating equilibrium constant and the combination of these mutations reduced the gating constant further, although it was 20-fold more efficient than expected from the sum of the two mutations. This indicates a significant coupling energy between these residues. Analysis of introducing a third mutation, Glu45Ala, revealed a further increase of the coupling energy between Val46 and Pro272. The authors speculate that the increase in coupling energy is made possible by the release of Glu45 from Arg209, which then gives Val46 the freedom to interact more strongly with Pro272. In a second triad (Val46, Ser269 and Pro272) the gating equilibrium constant was reduced by Val46Ala, but increased by Ser269-Leu. The double mutant revealed a significant coupling energy, and this decreased with the introduction of the third mutation Pro272Gly. Interestingly, this coupled triad involves residues that were proposed to form a "pin-into-socket" interaction in nAChRs (Miyazawa et al. 2003). Since these findings, an additional "pin-into-socket" assembly linking the ECD to the TMD has been found in the nAChR α-subunit (Lee et al. 2008). It comprises Val46,

Val132 (in the Cys-loop) and Pro272, with Val46 serving as the pin and the other two residues as the socket. Using the REFER method, all three of these residues were shown to move almost simultaneously (Φ ranging from 0.87 to 0.93). Findings also show that these three residues contribute to channel gating in an interdependent manner. Thus, several lines of evidence point to the importance of a proline in the M2–M3 loop for coupling ligand binding to gating in 5-HT_{3A}Rs and nAChRs. Given that this proline is not conserved among other members of the Cys-loop family, it yet again shows that although the overall gating mechanism of these receptors might be similar, individual interactions contributing to this mechanism differ.

Recent structures of prokaryotic ligand-gated ion channels

Very recently, X-ray structures of prokaryotic LGICs from *Erwinia chrysanthemi* (ELIC) (Hilf and Dutzler 2008) and *Gloebacter violaceus* (GLIC) (Bocquet et al. 2009; Hilf and Dutzler 2009) were published. The major difference between these two structures is in the pore geometry. While the ELIC structure represents a non-conducting conformation, the GLIC structure is likely to represent an open conformation. Superimposing these two structures reveal that although they only show moderate sequence conservation (20% identical residues), they are structurally very similar (Hilf and Dutzler 2009). Furthermore, the superimposed structure reveals that GLIC has undergone structural rearrangements compared to ELIC. The most prominent movements are of the inner β-sheet, with smaller changes observed for loop 2 and the Cys-loop (Hilf and Dutzler 2009). Compared to the ELIC structure, loop 2 appears to have moved downward (towards the pore) in the GLIC structure, while a small movement away from the pore axis is seen for the Cys-loop (Bocquet et al. 2009; Hilf and Dutzler 2009). Surprisingly, the pre-M1 region does not seem to have moved much at all. In contrast, the M2–M3 loop appears to move in the same plane as the membrane, swivelling away from the M2 domain and the pore axis. In addition to these movements, the β-sandwich of the ECD has rotated by approximately 5°–8° (Bocquet et al. 2009; Hilf and Dutzler 2009). A similar degree of rotation of the inner β-sheet has also been determined in the nAChR (Unwin 2005).

Global conformational changes are also suggested by comparison of the ELIC and GLIC structures. The receptor complex appears to undergo a twisting motion between the closed state (ELIC structure) and the open state (GLIC); the ECD rotates in a clockwise direction, while the TMD rotates in an anticlockwise direction (Bocquet et al. 2009). Similar concerted twisting motions, described to be like wringing out a washcloth (Samson and Levitt 2008), have been observed with normal mode analysis (Bahar and Rader 2005) of a number of different models of Cys-loop LGICs. Analysis of both homomeric α7 nAChR (Cheng et al. 2006; Taly et al. 2005) and α1 GlyR (Bertaccini et al. 2007), and heteromeric AChR (Liu et al. 2008; Samson and Levitt 2008) all reveal a similar concerted twisting motion that generally occurs as the lowest normal mode. This suggests that the twisting motion is not simply a consequence of a homomeric (symmetrical) receptor complex. Further, this twisting motion mode does not occur within the lowest 25 modes of the AChR model when α-bungarotoxin (a competitive inhibitor at AChRs) is docked into the LBS (Samson and Levitt 2008). Results from molecular dynamics simulations also support the rotations of the ECD and TMD seen with normal mode analysis (Cheng et al. 2007; Liu et al. 2008). These predicted conformational changes now need to be experimentally tested.

In the pre-M1 region of GLIC, Arg191 forms a pair of salt bridges with Asp31 in loop 2 and Asp121 in the Cys-loop. By aligning 228 full-length sequences of cation-selective LGIC subunits from the Uniprot database (S1) we observed that the Arg191 (pre-M1) and Asp121 (Cys-loop) in GLIC are completely conserved. Similarly, alignment of 109 anion-selective LGIC subunits (S1) revealed that homologous aspartate and arginine residues are also completely conserved. These conserved residues are also indicated in Fig. 4. As described earlier, the equivalent residues in the GlyR α1-subunit (Ala52, Asp148 and Arg218), the $GABA_AR$ α1-subunit (Asp149 and Arg220) and β2-subunit (Asp146 and Arg216) and the nAChR α-subunit (Arg209) have been shown to be critical for channel gating. Some of these residues have experimentally been found to interact with residues in loop 2, the Cys-loop and the pre-M1 region (Castaldo et al. 2004; Kash et al. 2004a; Kash et al. 2003; Lee and Sine 2005; Mercado and Czajkowski 2006; Ryan et al. 1994; Schofield et al. 2003). Considering that the arginines are conserved the effect they have on gating is perhaps not surprising. However, although these residues have been individually characterised as important in Cys-loop receptors, a triad similar to the one found in the GLIC structure, has not yet been reported.

Conclusions

The task of identifying the structures linking ligand binding in the ECD to opening of the channel pore in the membrane was made easier when the crystal structure of the AChBP (Miyazawa et al. 2003) and the refined structure of the *T. marmorata* nAChR (Unwin 2005) were published. Many studies have since shown important roles of structures such

as loop 2, the Cys-loop, the M2–M3 loop and the pre-M1 region in the gating mechanism of Cys-loop receptors. However, even though the gating mechanism is now clearly understood many questions remain to be resolved. For example, why do specific residue interactions and mechanisms seem to differ between different receptor subtypes? Future structural and functional studies and perhaps a crystal structure of a Cys-loop receptor in both the open and closed states, will help to answer many of these questions.

Acknowledgment This work was supported by the National Health and Medical Research Council of Australia (Project Grant 455310).

References

Absalom NL, Lewis TM, Kaplan W, Pierce KD, Schofield PR (2003) Role of charged residues in coupling ligand binding and channel activation in the extracellular domain of the glycine receptor. J Biol Chem 278:50151–50157. doi:10.1074/jbc.M305357200

Bahar I, Rader AJ (2005) Coarse-grained normal mode analysis in structural biology. Curr Opin Struct Biol 15:586–592. doi:10.1016/j.sbi.2005.08.007

Beene DL, Price KL, Lester HA, Dougherty DA, Lummis SC (2004) Tyrosine residues that control binding and gating in the 5-hydroxytryptamine$_3$ receptor revealed by unnatural amino acid mutagenesis. J Neurosci 24:9097–9104. doi:10.1523/JNEUROSCI.2429-04.2004

Bera AK, Chatav M, Akabas MH (2002) GABAA receptor M2–M3 loop secondary structure and changes in accessibility during channel gating. J Biol Chem 277:43002–43010. doi:10.1074/jbc.M206321200

Bertaccini EJ, Trudell JR, Lindahl E (2007) Normal-mode analysis of the glycine alpha1 receptor by three separate methods. J Chem Inf Model 47:1572–1579. doi:10.1021/ci600566j

Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux JP, Delarue M, Corringer PJ (2009) X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature 457:111–114. doi:10.1038/nature07462

Bouzat C, Gumilar F, Spitzmaul G, Wang HL, Rayes D, Hansen SB, Taylor P, Sine SM (2004) Coupling of agonist binding to channel gating in an ACh-binding protein linked to an ion channel. Nature 430:896–900. doi:10.1038/nature02753

Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, Der Oost J, Smit AB, Sixma TK (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411:269–276. doi:10.1038/35077011

Campos-Caro A, Sala S, Ballesta JJ, Vicente-Agullo F, Criado M, Sala F (1996) A single residue in the M2–M3 loop is a major determinant of coupling between binding and gating in neuronal nicotinic receptors. Proc Natl Acad Sci USA 93:6118–6123. doi:10.1073/pnas.93.12.6118

Carland JE, Cooper MA, Sugiharto S, Jeong HJ, Lewis TM, Barry PH, Peters JA, Lambert JJ, Moorhouse AJ (2009) Characterization of the effects of charged residues in the intracellular loop on ion permeation in α1 glycine receptor channels. J Biol Chem 284:2023–2030. doi:10.1074/jbc.M806618200

Castaldo P, Stefanoni P, Miceli F, Coppola G, Del Giudice EM, Bellini G, Pascotto A, Trudell JR, Harrison NL, Annunziato L, Taglialatela M (2004) A novel hyperekplexia-causing mutation in the pre-transmembrane segment 1 of the human glycine receptor α_1 subunit reduces membrane expression and impairs gating by agonists. J Biol Chem 279:25598–25604. doi:10.1074/jbc.M311021200

Castillo M, Mulet J, Bernal JA, Criado M, Sala F, Sala S (2006) Improved gating of a chimeric α7-5HT3A receptor upon mutations at the M2–M3 extracellular loop. FEBS Lett 580:256–260. doi:10.1016/j.febslet.2005.12.010

Celie PH, van Rossum-Fikkert SE, van Dijk WJ, Brejc K, Smit AB, Sixma TK (2004) Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures. Neuron 41:907–914. doi:10.1016/S0896-6273(04)00115-1

Chakrapani S, Bailey TD, Auerbach A (2004) Gating dynamics of the acetylcholine receptor extracellular domain. J Gen Physiol 123:341–356. doi:10.1085/jgp.200309004

Cheng X, Lu B, Grant B, Law RJ, McCammon JA (2006) Channel opening motion of α7 nicotinic acetylcholine receptor as suggested by normal mode analysis. J Mol Biol 355:310–324. doi:10.1016/j.jmb.2005.10.039

Cheng X, Ivanov I, Wang H, Sine SM, McCammon JA (2007) Nanosecond-timescale conformational dynamics of the human α7 nicotinic acetylcholine receptor. Biophys J 93:2622–2634. doi:10.1529/biophysj.107.109843

Crawford DK, Perkins DI, Trudell JR, Bertaccini EJ, Davies DL, Alkana RL (2008) Roles for loop 2 residues of α1 glycine receptors in agonist activation. J Biol Chem 283:27698–27706. doi:10.1074/jbc.M802384200

Czajkowski C, Karlin A (1991) Agonist binding site of *Torpedo* electric tissue nicotinic acetylcholine receptor. A negatively charged region of the δ subunit within 0.9 nm of the α subunit binding site disulfide. J Biol Chem 266:22603–22612

Deeb TZ, Carland JE, Cooper MA, Livesey MR, Lambert JJ, Peters JA, Hales TG (2007) Dynamic modification of a mutant cytoplasmic cysteine residue modulates the conductance of the human 5-HT$_{3A}$ receptor. J Biol Chem 282:6172–6182. doi:10.1074/jbc.M607698200

Elmslie FV, Hutchings SM, Spencer V, Curtis A, Covanis T, Gardiner RM, Rees M (1996) Analysis of GLRA1 in hereditary and sporadic hyperekplexia: a novel mutation in a family cosegregating for hyperekplexia and spastic paraparesis. J Med Genet 33:435–436. doi:10.1136/jmg.33.5.435

Gao F, Bren N, Burghardt TP, Hansen S, Henchman RH, Taylor P, McCammon JA, Sine SM (2005) Agonist-mediated conformational changes in acetylcholine-binding protein revealed by simulation and intrinsic tryptophan fluorescence. J Biol Chem 280:8443–8451. doi:10.1074/jbc.M412389200

Gay EA, Yakel JL (2007) Gating of nicotinic ACh receptors; new insights into structural transitions triggered by agonist binding that induce channel opening. J Physiol 584:727–733. doi:10.1113/jphysiol.2007.142554

Gee VJ, Kracun S, Cooper ST, Gibb AJ, Millar NS (2007) Identification of domains influencing assembly and ion channel properties in α7 nicotinic receptor and 5-HT$_3$ receptor subunit chimaeras. Br J Pharmacol 152:501–512. doi:10.1038/sj.bjp.0707429

Gleitsman KR, Kedrowski SM, Lester HA, Dougherty DA (2008) An intersubunit hydrogen bond in the nicotinic acetylcholine receptor that contributes to channel gating. J Biol Chem 283:35638–35643. doi:10.1074/jbc.M807226200

Grosman C, Salamone FN, Sine SM, Auerbach A (2000a) The extracellular linker of muscle acetylcholine receptor channels is a gating control element. J Gen Physiol 116:327–340. doi:10.1085/jgp.116.3.327

Grosman C, Zhou M, Auerbach A (2000b) Mapping the conformational wave of acetylcholine receptor channel gating. Nature 403:773–776. doi:10.1038/35001586

Hales TG, Dunlop JI, Deeb TZ, Carland JE, Kelley SP, Lambert JJ, Peters JA (2006) Common determinants of single channel conductance within the large cytoplasmic loop of 5-hydroxytryptamine type 3 and $\alpha_4\beta_2$ nicotinic acetylcholine receptors. J Biol Chem 281:8062–8071. doi:10.1074/jbc.M513222200

Hilf RJ, Dutzler R (2008) X-ray structure of a prokaryotic pentameric ligand-gated ion channel. Nature 452:375–379. doi:10.1038/nature06717

Hilf RJ, Dutzler R (2009) Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. Nature 457:115–118. doi:10.1038/nature07461

Hu XQ, Zhang L, Stewart RR, Weight FF (2003) Arginine 222 in the pre-transmembrane domain 1 of 5-HT_{3A} receptors links agonist binding to channel gating. J Biol Chem 278:46583–46589. doi:10.1074/jbc.M308974200

Jansen M, Bali M, Akabas MH (2008) Modular design of Cys-loop ligand-gated ion channels: functional 5-HT_3 and GABA ρ1 receptors lacking the large cytoplasmic M3M4 loop. J Gen Physiol 131:137–146. doi:10.1085/jgp.200709896

Jha A, Cadugan DJ, Purohit P, Auerbach A (2007) Acetylcholine receptor gating at extracellular transmembrane domain interface: the cys-loop and M2–M3 linker. J Gen Physiol 130:547–558. doi:10.1085/jgp.200709856

Kash TL, Jenkins A, Kelley JC, Trudell JR, Harrison NL (2003) Coupling of agonist binding to channel gating in the $GABA_A$ receptor. Nature 421:272–275. doi:10.1038/nature01280

Kash TL, Dizon MJ, Trudell JR, Harrison NL (2004a) Charged residues in the β_2 subunit involved in $GABA_A$ receptor activation. J Biol Chem 279:4887–4893. doi:10.1074/jbc.M311441200

Kash TL, Kim T, Trudell JR, Harrison NL (2004b) Evaluation of a proposed mechanism of ligand-gated ion channel activation in the $GABA_A$ and glycine receptors. Neurosci Lett 371:230–234. doi:10.1016/j.neulet.2004.09.002

Kelley SP, Dunlop JI, Kirkness EF, Lambert JJ, Peters JA (2003) A cytoplasmic region determines single-channel conductance in 5-HT_3 receptors. Nature 424:321–324. doi:10.1038/nature01788

Keramidas A, Kash TL, Harrison NL (2006) The pre-M1 segment of the α1 subunit is a transduction element in the activation of the $GABA_A$ receptor. J Physiol 575:11–22. doi:10.1113/jphysiol.2005.102756

Lee WY, Sine SM (2005) Principal pathway coupling agonist binding to channel gating in nicotinic receptors. Nature 438:243–247. doi:10.1038/nature04156

Lee WY, Free CR, Sine SM (2008) Nicotinic receptor interloop proline anchors β1-β2 and cys loops in coupling agonist binding to channel gating. J Gen Physiol 132:265–278. doi:10.1085/jgp.200810014

Lester HA, Dibas MI, Dahan DS, Leite JF, Dougherty DA (2004) Cys-loop receptors: new twists and turns. Trends Neurosci 27:329–336. doi:10.1016/j.tins.2004.04.002

Lewis TM, Sivilotti LG, Colquhoun D, Gardiner RM, Schoepfer R, Rees M (1998) Properties of human glycine receptors containing the hyperekplexia mutation α1 (K276E), expressed in *Xenopus* oocytes. J Physiol 507(Pt 1):25–40. doi:10.1111/j.1469-7793.1998.025bu.x

Liu X, Xu Y, Li H, Wang X, Jiang H, Barrantes FJ (2008) Mechanics of channel gating of the nicotinic acetylcholine receptor. PLOS Comput Biol 4:e19. doi:10.1371/journal.pcbi.0040019

Lummis SC, Beene DL, Lee LW, Lester HA, Broadhurst RW, Dougherty DA (2005) Cis–trans isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. Nature 438:248–252. doi:10.1038/nature04130

Lynch JW, Rajendra S, Barry PH, Schofield PR (1995) Mutations affecting the glycine receptor agonist transduction mechanism convert the competitive antagonist, picrotoxin, into an allosteric potentiator. J Biol Chem 270:13799–13806. doi:10.1074/jbc.270.23.13799

Lynch JW, Rajendra S, Pierce KD, Handford CA, Barry PH, Schofield PR (1997) Identification of intracellular and extracellular domains mediating signal transduction in the inhibitory glycine receptor chloride channel. EMBO J 16:110–120. doi:10.1093/emboj/16.1.110

McLaughlin JT, Fu J, Rosenberg RL (2007) Agonist-driven conformational changes in the inner β-sheet of α7 nicotinic receptors. Mol Pharmacol 71:1312–1318. doi:10.1124/mol.106.033092

Mercado J, Czajkowski C (2006) Charged residues in the α_1 and β_2 pre-M1 regions involved in $GABA_A$ receptor activation. J Neurosci 26:2031–2040. doi:10.1523/JNEUROSCI.4555-05.2006

Miyazawa A, Fujiyoshi Y, Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. Nature 423:949–955. doi:10.1038/nature01748

Mukhtasimova N, Sine SM (2007) An intersubunit trigger of channel gating in the muscle nicotinic receptor. J Neurosci 27:4110–4119. doi:10.1523/JNEUROSCI.0025-07.2007

Mukhtasimova N, Free C, Sine SM (2005) Initial coupling of binding to gating mediated by conserved residues in the muscle nicotinic receptor. J Gen Physiol 126:23–39. doi:10.1085/jgp.200509283

Plested AJ, Groot-Kormelink PJ, Colquhoun D, Sivilotti LG (2007) Single-channel study of the spasmodic mutation α1A52S in recombinant rat glycine receptors. J Physiol 581:51–73. doi:10.1113/jphysiol.2006.126920

Price KL, Lummis SC (2004) The role of tyrosine residues in the extracellular domain of the 5-hydroxytryptamine$_3$ receptor. J Biol Chem 279:23294–23301. doi:10.1074/jbc.M314075200

Price KL, Millen KS, Lummis SC (2007) Transducing agonist binding to channel gating involves different interactions in 5-HT_3 and $GABA_C$ receptors. J Biol Chem 282:25623–25630. doi:10.1074/jbc.M702524200

Purohit P, Auerbach A (2007a) Acetylcholine receptor gating at extracellular transmembrane domain interface: the "pre-M1" linker. J Gen Physiol 130:559–568. doi:10.1085/jgp.200709857

Purohit P, Auerbach A (2007b) Acetylcholine receptor gating: movement in the α-subunit extracellular domain. J Gen Physiol 130:569–579. doi:10.1085/jgp.200709858

Purohit P, Mitra A, Auerbach A (2007) A stepwise mechanism for acetylcholine receptor channel gating. Nature 446:930–933. doi:10.1038/nature05721

Rajendra S, Lynch JW, Pierce KD, French CR, Barry PH, Schofield PR (1994) Startle disease mutations reduce the agonist sensitivity of the human inhibitory glycine receptor. J Biol Chem 269:18739–18742

Rajendra S, Lynch JW, Pierce KD, French CR, Barry PH, Schofield PR (1995) Mutation of an arginine residue in the human glycine receptor transforms β-alanine and taurine from agonists into competitive antagonists. Neuron 14:169–175. doi:10.1016/0896-6273(95)90251-1

Rovira JC, Vicente-Agullo F, Campos-Caro A, Criado M, Sala F, Sala S, Ballesta JJ (1999) Gating of α3β4 neuronal nicotinic receptor can be controlled by the loop M2–M3 of both α3 and β4 subunits. Pflugers Arch 439:86–92. doi:10.1007/s004240051131

Ryan SG, Buckwalter MS, Lynch JW, Handford CA, Segura L, Shiang R, Wasmuth JJ, Camper SA, Schofield P, O'Connell P (1994) A missense mutation in the gene encoding the α_1 subunit of the inhibitory glycine receptor in the spasmodic mouse. Nat Genet 7:131–135. doi:10.1038/ng0694-131

Samson AO, Levitt M (2008) Inhibition mechanism of the acetylcholine receptor by α-neurotoxins as revealed by normal-mode dynamics. Biochemistry 47:4065–4070. doi:10.1021/bi702272j

Schofield CM, Jenkins A, Harrison NL (2003) A highly conserved aspartic acid residue in the signature disulfide loop of the α1

subunit is a determinant of gating in the glycine receptor. J Biol Chem 278:34079–34083. doi:10.1074/jbc.M302416200

Shiang R, Ryan SG, Zhu YZ, Hahn AF, O'Connell P, Wasmuth JJ (1993) Mutations in the α_1 subunit of the inhibitory glycine receptor cause the dominant neurologic disorder, hyperekplexia. Nat Genet 5:351–358. doi:10.1038/ng1293-351

Shiang R, Ryan SG, Zhu YZ, Fielder TJ, Allen RJ, Fryer A, Yamashita S, O'Connell P, Wasmuth JJ (1995) Mutational analysis of familial and sporadic hyperekplexia. Ann Neurol 38:85–91. doi:10.1002/ana.410380115

Taly A, Delarue M, Grutter T, Nilges M, Le Novere N, Corringer PJ, Changeux JP (2005) Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. Biophys J 88:3954–3965. doi:10.1529/biophysj.104.050229

Unwin N (2005) Refined structure of the nicotinic acetylcholine receptor at 4A resolution. J Mol Biol 346:967–989. doi:10.1016/j.jmb.2004.12.031

Unwin N, Miyazawa A, Li J, Fujiyoshi Y (2002) Activation of the nicotinic acetylcholine receptor involves a switch in conformation of the α subunits. J Mol Biol 319:1165–1176. doi:10.1016/S0022-2836(02)00381-9

Venkatachalan SP, Czajkowski C (2008) A conserved salt bridge critical for $GABA_A$ receptor function and loop C dynamics. Proc Natl Acad Sci USA 105:13604–13609. doi:10.1073/pnas.0801854105

Venkataraman P, Venkatachalan SP, Joshi PR, Muthalagi M, Schulte MK (2002) Identification of critical residues in loop E in the $5\text{-}HT_{3AS}R$ binding site. BMC Biochem 3:15. doi:10.1186/1471-2091-3-15

Wang J, Lester HA, Dougherty DA (2007) Establishing an ion pair interaction in the homomeric $\rho 1$ γ-aminobutyric acid type A receptor that contributes to the gating pathway. J Biol Chem 282:26210–26216. doi:10.1074/jbc.M702314200

Xiu X, Hanek AP, Wang J, Lester HA, Dougherty DA (2005) A unified view of the role of electrostatic interactions in modulating the gating of Cys loop receptors. J Biol Chem 280:41655–41666. doi:10.1074/jbc.M508635200