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Review

Insights into the channel gating of P2X receptors from structures, dynamics and small molecules

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P2X receptors, as ATP-gated non-selective trimeric ion channels, are permeable to Na^+, K^+ and Ca^{2+}. Comparing with other ligand-gated ion channel families, P2X receptors are distinct in their unique gating properties and pathophysiological roles, and have attracted attention as promising drug targets for a variety of diseases, such as neuropathic pain, multiple sclerosis, rheumatoid arthritis and thrombus. Several small molecule inhibitors for distinct P2X subtypes have entered into clinical trials. However, many questions regarding the gating mechanism of P2X remain unsolved. The structural determinations of P2X receptors at the resting and ATP-bound open states revealed that P2X receptor gating is a cooperative allosteric process involving multiple domains, which marks the beginning of the post-structure era of P2X research at atomic level. Here, we review the current knowledge on the structure-function relationship of P2X receptors, depict the whole picture of allosteric changes during the channel gating, and summarize the active sites that may contribute to new strategies for developing novel allosteric drugs targeting P2X receptors.

Keywords: P2X receptors; ATP; ligand-gated ion channel; channel gating; allosteric change; drug design

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Introduction

P2X receptors, a distinct family of non-selective trimeric ligand-gated channels, are mainly permeable to Na^+, K^+ and Ca^{2+}[1–3]. Binding extracellular adenosine 5'-triphosphate (ATP) from either emiocytosis or cytoclasis at the P2X receptors induces channel opening and a following ion flux[4]. Since the identification of ATP as a signal molecule of P2X[5], seven subtypes of P2X receptors have been cloned and denoted as P2X1 to P2X7, with functional channels assembled by homo- or heterotrimers (Figure 1)[6–11]. P2X receptors are widely expressed in excitatory and non-excitatory cells, such as neuron, glia, platelet, epithelia and macrophage, and participate in many important physiological and pathological processes, including synaptic transmission, pain perception, inflammation, cardiovascular modulation, immunomodulation and tumorigenesis[3, 4, 12–16]. Heritable mutations in P2X receptors are the major causes of some disorders. For example, mutations in human P2X2 lead to hearing loss[17–19]; loss of function of the P2X4 receptor is related to increased pulse pressure[20]; and many non-synonymous single nucleotide polymorphisms (NS-SNPs) in the P2X7 receptor were identified as associated with chronic lymphocytic leukemia and osteoporosis[21]. Due to their roles in a variety of physiological and pathological processes, P2X receptors have drawn attention as promising drug targets[22–27] and progress has been made toward this outcome[28, 29]. For example, AF-219, a selective P2X3 receptor antagonist, alleviated chronic coughing in a phase II clinical trial[29, 30].

However, the lead compound targeting P2X receptors could only be obtained via high-throughput screening, a rather time-consuming and costly process. Rational drug design requires knowledge of channel gating and structures of P2X receptors. In 2009, the first crystal structure of the zebrafish P2X4 (zfP2X4) receptor at the closed/*apo* state with a resolution of 3.1 Å was reported by Kawate *et al*[7]. This structural determination marked the beginning of a new era at the atomic level for P2X researches[31, 32]. In 2012, Hattori *et al* reported the open crystal structure of the zfP2X4 receptor with ATP in its binding site[33], which confirmed previous studies on ATP recognition and provided structural insight into the channel gating of P2X receptors[34]. Despite lacking obvious similarities in primary structures between P2X receptors and acid-sensing ion channels (ASICs, another member in the trimeric ligand-gated ion channel family), those two families exhibit unanticipated similarities in their three-dimensional (3D) architecture. The transmembrane (TM) domains of those two families assemble in a similar pattern, with the three extracellular domains inter-

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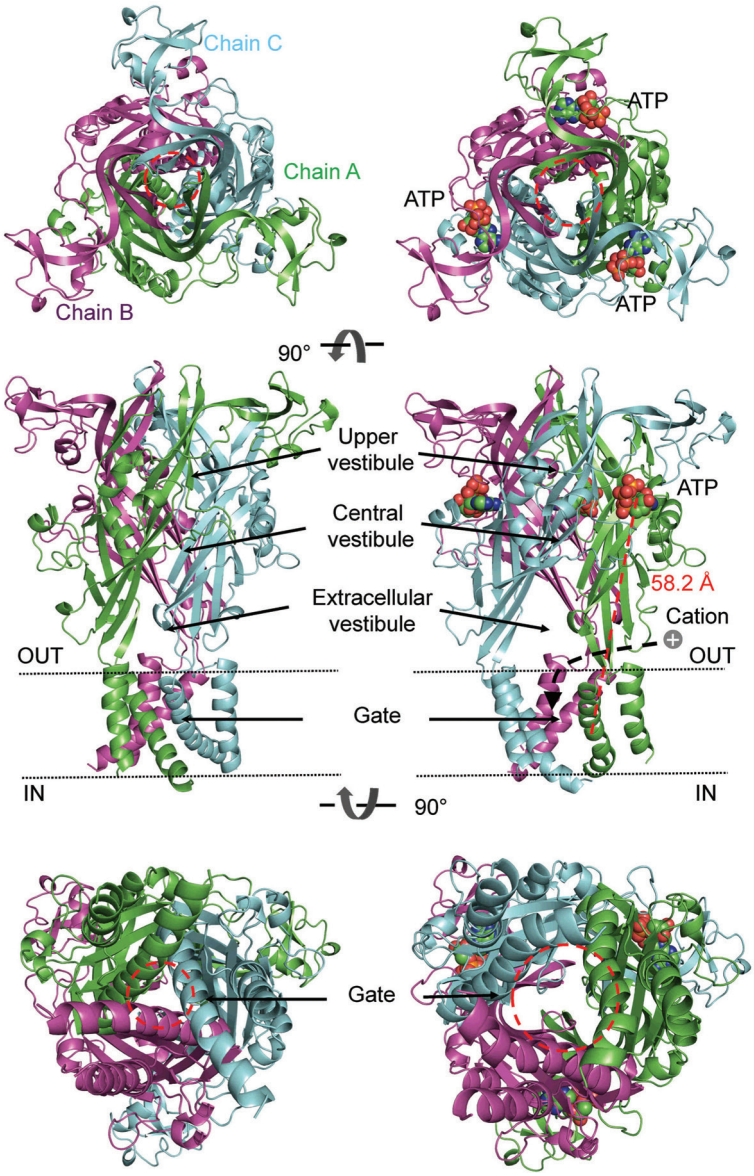

Figure 1. The three-dimensional architectures of the zfP2X4 receptor viewed from the extracellular side (upper), parallel to the membrane (middle) and the intracellular side (lower) at resting (left, PDB ID code: 3H9V) and ATP-bound open (right, PDB ID code: 4DW1) states. The red dashed line indicates the distance between the N9 atom of the purine ring of ATP and the C_α atom of A347. The black dashed arrow indicates the ion influx pathway. Subunits A, B and C are colored by green, magenta and cyan cartoon, respectively. ATP molecules are shown as spheres. Red dashed circles indicate the boundary of the upper vestibule or the gate. All figures were made with PyMol (http://www.pymol.org).

twined with each other[7, 33, 35–37]. The individual subunit of the two families forms different shapes, with ASIC1a resembling a human hand[37] and the P2X4 receptor a dolphin rising from water (Figure 2A and 2B). Different domains of P2X are thus named as head, dorsal fin (DF), left flipper (LF), right flipper (RF), body and fluke (Figure 2A). Benefiting from those crystal structures, progress has been made in the structure-function research on P2X receptors, aiding rational drug design targeting this important ion channel family. Because these channels are ligand-gated ion channels, the gating process of P2X receptors starts with the ligand binding to the channel opening until the ultimate close of the channel, and this involves a series of step-by-step conformational changes. In this review, we focus on the roles of each domain of the P2X receptors and the stepwise domain-domain interactions during channel gating. We also summarize the binding sites of small molecules targeting P2X receptors, which provides insights into the gating mechanism of P2X receptors and the structural basis for future drug design.

Head domain

Located at the extracellular domain, the head domain of P2X subunits is composed of the residues 111–167 (zfP2X4 numbering). Although the sequence of the head domain is not highly

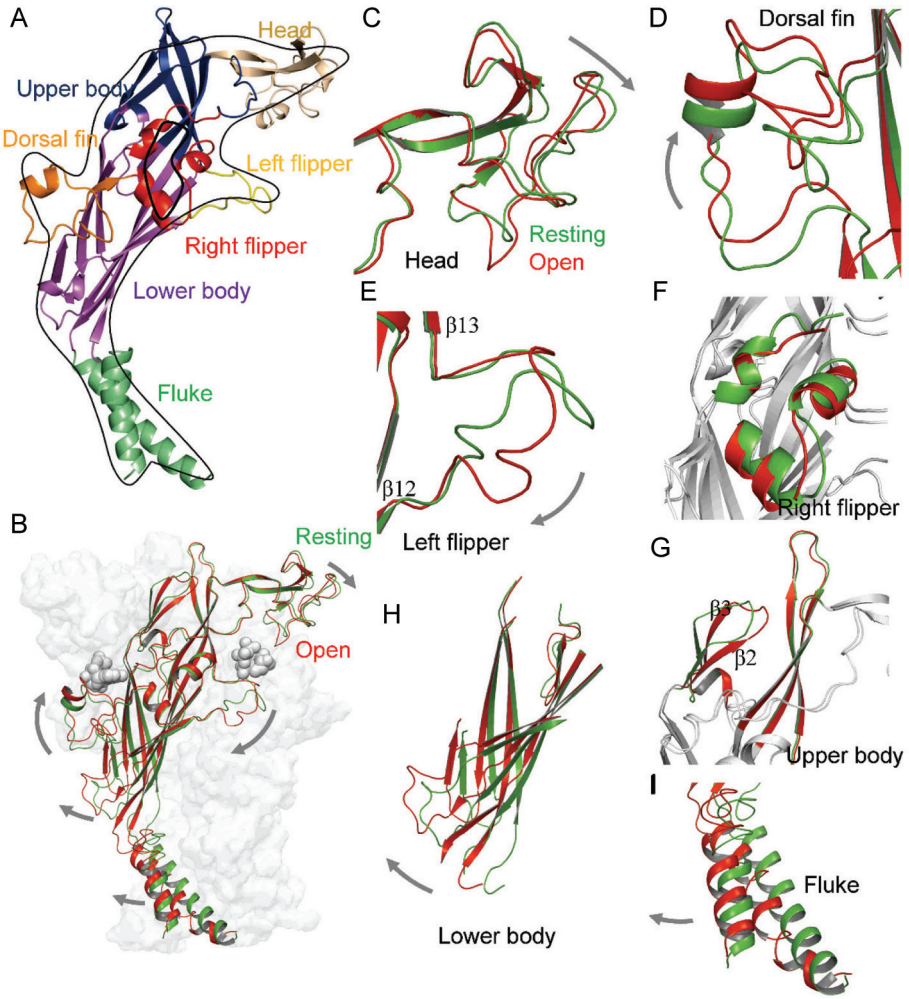

Figure 2. ATP-induced conformational changes of zfP2X4 receptors. (A) The P2X4 subunit has a dolphin-like shape. Distinctive body parts are shown in different colors. (B) Superposition of a single P2X4 subunit at resting (green) and open (red) states. (C–I) Superposition of head (C), dorsal fin (D), left flipper (E), right flipper (F), upper body (G), lower body (H) and fluke (I) domains at resting (green) and open (red) states. The grey arrows indicate the conformational changes after ATP binding.

conserved throughout the P2X family, the architecture of this domain in different subtypes shares certain similarity due to three conserved disulfide bonds that contribute to the folding of P2X receptors[38–42] (Figure 2A and 2C). The architecture of head domain of the zfP2X4 receptor was determined by X-ray diffraction and showed a high similarity in folding pattern with rat P2X4 (rP2X4) resolved by nuclear magnetic resonance, suggesting the conservation of the P2X4 head domain in different species[40]. Deletion of 42 residues in the head domain of P2X1 resulted in the loss of channel function without interfering with membrane trafficking[43], suggesting that the head domain is an integrant domain of channel gating. Using molecular dynamic (MD) simulations and normal mode analysis, previous studies revealed a spontaneous downward motion of the head domain, probably resulting from its inherent dynamics[16, 44, 45] (Figure 2B and 2C). This type of motion coincides with the downward motion of the head domain demonstrated by the ATP-bound open structure and is pivotal for the channel gating of P2X receptors. Labeling L186C (rat P2X2, rP2X2, numbering) using NCS-ATP (a synthesized ATP-derived thiol-reactive compound) impedes subsequent opening of the channel by locking the channel into an ATP binding mode that is incapable of driving the downward motion of head domain[46]. On the contrary, ADP-ribosylation of R125 (mouse P2X7, mP2X7, numbering) (Figure 3) located in the head domain, is sufficient to activate the P2X7 receptor[47], confirming the essential role of the downward motion of head domain in channel gating. It is reasonable to assume that chemicals binding to the head domain interfere with the downward motion, and therefore, alter channel gating[48, 49]. For example, K138 in the head domain is involved in the binding of both suramin and NF449 to the P2X1 receptor (Figure 3)[50, 51]. The data from chimeras and single-point mutations suggest that suramin and NF449 may bind to the site below the head domain of P2X1 and therefore impede the downward motion of this domain. Studies using voltage clamp flurometry and electrophysiology approaches further confirmed the pivotal role of the cysteine-rich head domain in channel acti-

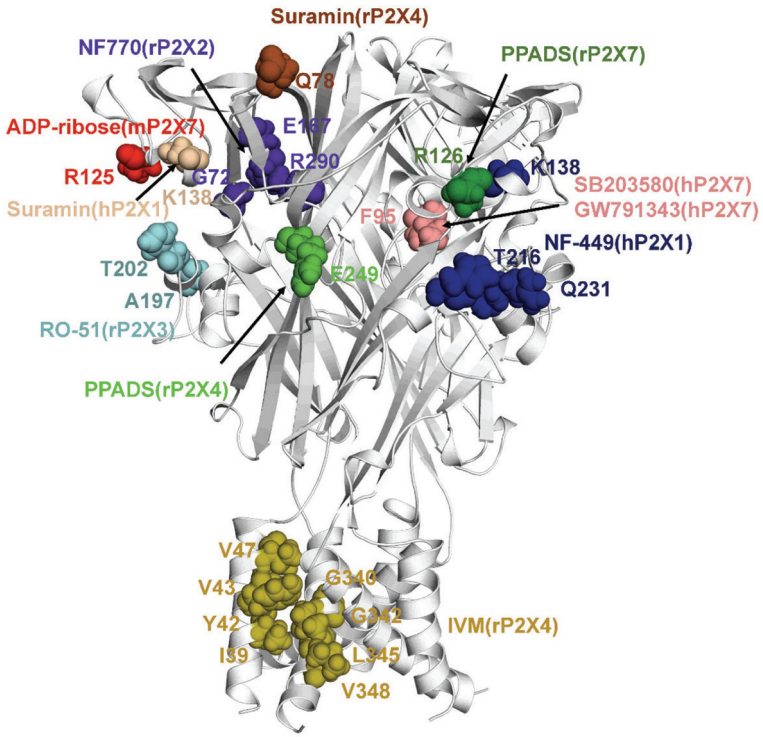

Figure 3. Amino residues involved in small molecule recognition of P2X receptors.

vation and desensitization of the P2X1 receptor. For example, the residues N120 and G123 (rat P2X1, rP2X1, numbering) were associated with channel activation of P2X1 receptor, and P121, E122, I125 (rP2X1 numbering) were correlated with channel desensitization of P2X1 receptor[52].

Dorsal fin domain

The dorsal fin (DF) domain (residues 206–234, zfP2X4 numbering) is a domain structurally coupled with the lower body domain. The upward motion of the DF domain is another allosteric change essential for P2X receptor activation[44, 45] (Figure 2A, 2B and 2D). Similar to the downward motion of the head domain, the upward motion of the DF domain is driven by its inherent dynamics. The bound-ATP directly contacts the DF domain via an interaction between its purine ring and the L217 of the DF domain (zfP2X4 numbering). This interaction induces an upward motion of the DF domain, leading to an expansion of the lower body domain and channel activation (Figures 2H and 4B). Thus, the gating mechanism of P2X receptors mimics a 'lever' system[33], where the head domain and the DF domain function as the two arms of the 'lever'. TNP-ATP, a nonspecific antagonist, impedes the upward motion of the DF with a large steric bulk of trinitrophenyl moieties, and therefore inhibits channel opening[33]. RO-51, a bioavailable P2X3 antagonist, displays a two hundred-fold lower potency on human P2X3 (hP2X3) compared to rat P2X3 (rP2X3), due to two amino acids located in the DF domain, A197 and T202 (Figure 3)[53]. Thus, the upward motion of the DF domain is also essential for the channel gating of P2X receptors, and small molecules interrupting this motion effectively block P2X receptors.

Left flipper domain

The left flipper (LF) domain in P2X4 is a loop structure linking β12 and β13 (Figure 2A and 2E). It is composed of the residues 281–296 (zfP2X4 numbering), with the sequences of the two ends partially conserved. Prior to the structural determination of the zfP2X4 receptor, the contribution of the LF domain to the function of P2X4 was extensively studied. One study revealed that H286 (rP2X4 numbering) is pivotal for the pH sensitivity of the P2X4 receptor in the pathophysiological range[54]. Mutating R278 or D280 (rP2X4 numbering) to alanine could abolish receptor function, potentially from the formation of salt bridge by R278 and D280, which are essential for channel gating the P2X4 receptor[55]. Those studies support an essential role of the LF in channel gating. A comparison between the resting and open structures of zfP2X4 revealed that the LF domain is driven away from the ATP binding site during the ATP bind-to-open process (Figure 2B and 2E)[33], rather than approaching the binding site by the inherent dynamics of the receptors[45]. Alteration in interactions among I208, L217, V291 and K193 (zfP2X4 numbering), induced by ATP binding, correlates well with this movement, indicating that the proper interactions between the DF and LF domains are crucial for channel gating[45], which was further confirmed by other studies[56–58]. The middle region is less conserved among P2X subtypes, which may endow subtype-specific contributions in the channel gating. For instance, an alanine substitution of S275 in the LF domain (rP2X3 numbering) revealed that this position is involved in forming the

binding pocket and correlates with the recovery process of P2X3 from inactivation. In contrast, a corresponding mutation of S289 (S289A) in rP2X4 displays no significant effects on channel activation[57, 59]. Therefore, the downward motion of the LF domain and its movement relative to the DF domain are essential for channel gating in P2X receptors, although the different roles of the LF domain in various subtypes have not been clearly delineated.

Right flipper domain

The right flipper (RF) domain (residues 178–189 and 235–254, zfP2X4 numbering) is formed by three stacked α-helices separated into two segments (Figure 2A and 2F). The superimposition of closed and open structures revealed that the RF domain does not undergo conformational changes during the channel gating of P2X receptors (Figure 2F); however, it does not imply that the RF domain is irrelevant to channel gating. One piece of evidence is that H245, a residue in the RF domain of rP2X2, is involved in zinc/copper potentiation[60]. E249K (rP2X4 numbering) shows sensitivity to PPADS, a chemical that has no effect on the wild-type rP2X4 receptor[61], indicating that RF may act as an anchor when PPADS binds to the channel. Additionally, N187 (zfP2X4 numbering), a conserved glycosylation site located in the RF domain, is important for channel stability and membrane trafficking[38, 62–65]. Due to the limited studies in this region, the contribution of the RF domain to channel gating, membrane trafficking and drug design requires further investigation.

Upper body domain

The upper body domain is composed of residues 75–92, 105–113 and 294–319 (zfP2X4 numbering) (Figure 2A and 2G). Using cysteine scanning mutagenesis along the upper vestibule, Damien *et al* found that MTS reagents had no effect on the currents of mutated P2X receptors[66], indicating that the center pathway is not involved in ion permeation. The superimposition of closed and open structures revealed that the two upper bodies overlap well except for a slight right shift of β2 and β3 (Figure 2G), suggesting that the upper body might not undergo apparent conformational changes in the process of channel opening (Figure 2G), despite the direct contact between ATP and the side chains of R298 and K316 (zfP2X4 numbering). Therefore, Hattori *et al* proposed that the upper body behaved as a rigid body, which acted as a 'brace' in the 'lever' gating mechanism described above[33]. However, fast scanning atomic force microscopy revealed that the long-period application of ATP under Ca^{2+}-free condition caused pore dilation and allowed permeation of large organic molecules, such as NMDG[67–69]. Zhao *et al* also reported the expansion of the upper vestibule of P2X4 during 300-ns MD simulations[45]. Thus, the role of the upper body domain in functional channels requires further clarification.

Lower body domain

The lower body domain is composed of residues 56–74, 93–104, 188–207, 254–281 and 320–330 (zfP2X4 numbering) and is characterized by a β-sandwich motif formed by six β-sheets (Figure 2A and 2H). Alanine/cysteine-scanning studies within the lower body had identified residues involved in ATP binding, supporting the roles of the lower body domain in agonist binding and the conformation transition pathway during channel gating[70–73]. Consistent with those studies, the crystal structure at the open state revealed that ATP contacts side chains and main chains of K70, K72, T189 and K193 in the lower body domain[33]. Thus, the residues of the lower body domain are pivotal for the agonist recognition of P2X receptors.

P2X receptors have a large extracellular domain, with ATP binding sites locating far away (58.2 Å) from channel gates (Figure 1, middle panel). Therefore, a series of conformational changes are required to translate from an extracellular domain into the TM region[74]. ATP binding-induced conformational changes of the LF and DF domains can trigger subsequent outward 'flexing' of the lower body domain and the expansion of the extracellular vestibule, which provides an ion influx pathway[66, 75] and results in an iris-like motion of TM helices to open the channel pore. Double mutations of P62C/H192C, S65C/S190C, and S65C/D315C (rP2X2 numbering) that restrain the expansion of the lower body markedly attenuated ATP-induced maximal currents of mutant receptors, which was rescued by DTT application[76]. Recently, the linker region between the lower body domain and TMs had also been systematically investigated[77–79], mutations of Y54A, Q55A, F198A, W259A, F324A, and G325A (rP2X4 numbering) resulted in a loss of channel function, suggesting that these residues contribute to the conformational transition from the lower body domain to the TM region. These findings confirm the essential role of the lower body domain in conformational transitions during channel gating.

D197 (rat P2X7, rP2X7 numbering), located in the lower body domain, is pivotal for acidic pH-induced channel inhibition[80], suggesting an additional function of the lower body domain that is independent to the movement of the LF and DF domains, and this was confirmed by the importance of the salt bridge E63-R274 (rP2X2 numbering) in this domain for the channel gating[81]. Actually, both the closure of binding site jaw and the movement of the lower body domain are required for the concomitant pore opening of P2X receptors[33]. Zinc is able to elicit inward currents following treatment with AM546 on the mutated (K67C) P2X2 receptor[82], and the zinc-evoked currents are enhanced by lysine substitution at H319, located in the lower body domain[82]. Thus, the lower body domain possesses structural elements that independently affect the channel gating of P2X receptors.

In summary, the lower body domain is not only directly involved in agonist recognition but also able to coordinate the bound-ATP induced conformational changes, conformational transitions, and the final channel pore opening.

Fluke

The TM helices (TM1 and TM2) of a single subunit delineate the 'fluke' of the 'dolphin' (Figure 2A and 2I) and are involved

in many properties of P2X receptors, including unitary conductance and rectification, differential desensitization among subtypes, and voltage-dependence of P2X receptors[83–87]. Functions of the TM domains in channel gating have been deeply studied, and its roles in pore location, ion permeability and structural rearrangements were confirmed by crystal structures[88–97]. Under normal conditions, pore opening of P2X receptors is controlled by ATP binding. However, when cysteine was introduced at position I328 (rP2X2 numbering), the channel pore of the P2X2 receptor was directly opened by propylmethanethiosulfonate (MTSP)[98], suggesting that the rearrangement of the TM domains is sufficient to initiate channel activation. Guided by this idea, azobenzene compounds, namely MEA-TMA and BMA, were linked to P2X2 receptors carrying a cysteine substitution in the TM domain by an electrophilic substitution reaction[99, 100], and the open or closing of the channel pore was controlled by the light-evoked isomerization. This system provides an ideal tool to control the activity of P2X receptors with high spatial and temporal accuracy.

The flukes of the three subunits form the channel pore, with TM2 lining the inner tunnel, and TM1 positioned peripherally to TM2 (Figure 4C). Both TM1 and TM2 are structurally coupled with the lower body. Outward flexing of the lower body induces the TMs to expand in an iris-like motion to open the channel pore. Due to the mismatch occurring in X-ray crystallography caused by rigorous conditions, Heymann *et al* proposed a model in membrane environments that stabilizes intersubunit interactions [101].

N- and C-termini

The N-terminus of P2X receptors is composed of approximately 30 amino acid residues, while the C-terminus com-

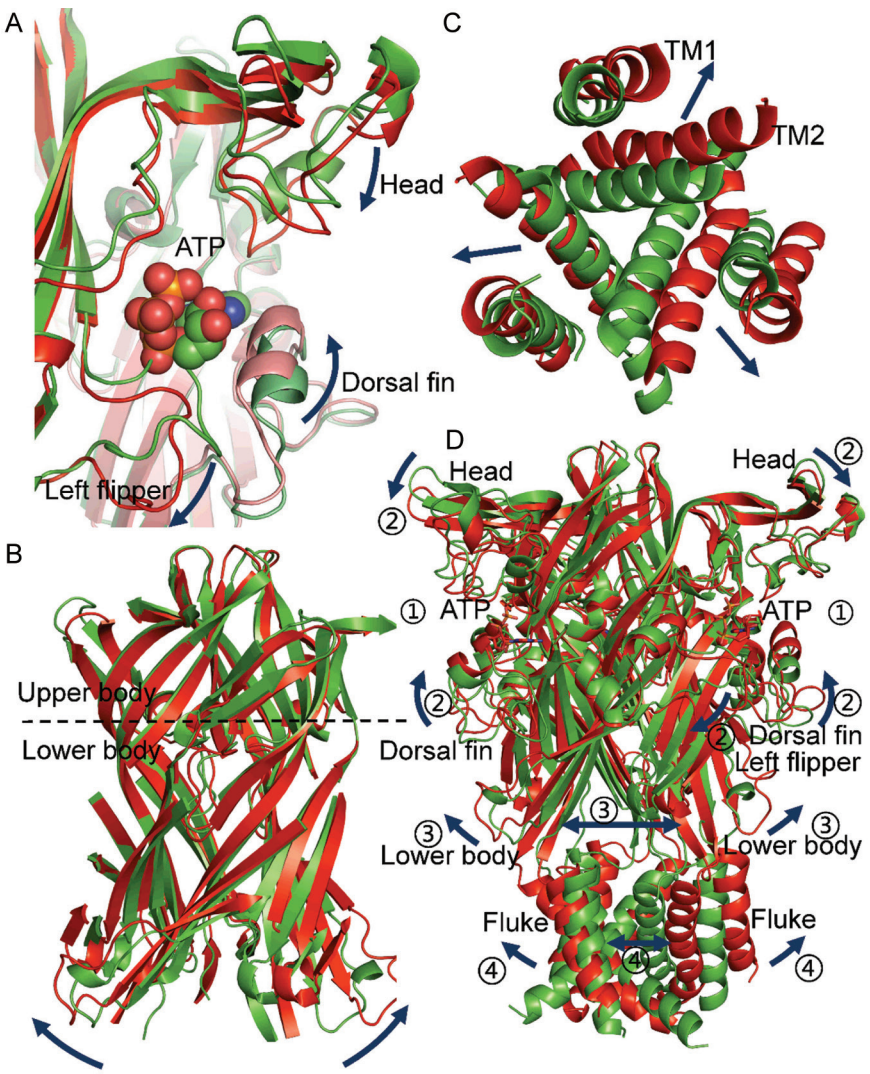

Figure 4. Bound ATP-evoked allosteric changes associated with channel opening in the P2X4 receptor. (A–D) Bound-ATP evoked structural rearrangements at the ATP binding site (A), body domain (B), TM region (C) and overall structure of zfP2X4 receptors (D). The dark-blue arrows indicate the conformational changes after ATP binding. Structures at the resting and open states of zfP2X4 receptors are displayed in green and red, respectively.

prises approximately 30–240 amino acid residues, varying among different subtypes. To obtain a high quality crystal structure, both the N- and C-termini of zfP2X4 were truncated, which might cause the loss of intrinsic property of P2X4 to some extent. For example, the P2X4 receptor shows dynamic ionic selectivity, while the truncated P2X4 produces a constant current in response to long period exposures of ATP[33]. Moreover, the crystal structures made no contribution to research focusing on the intracellular domains. Nevertheless, intracellular domains were found to play important roles in membrane trafficking, channel desensitization, protein-protein interactions, and phospholipids modulation, which was unveiled by various approaches, including Western blot, co-IP, site-directed mutagenesis, and electrophysiology recording[16, 102–106]. The roles of the two termini have been well summarized previously[16, 102–106], and thus, will not be further discussed in this review.

Domain-domain interactions and coordinated motions of multi-domains evoke a final channel opening of P2X receptors

So far, the location and contributions of each domain in P2X functions and channel gating that we have discussed above are at the level of the single subunit. Nevertheless, P2X receptors are intertwined trimeric membrane proteins with inter- and intrasubunit interactions present throughout the entire channel. Based on the 3D structure of the P2X4 receptor, those interactions can be divided into three compartments. (1) The binding sites of ATP are contributed by domains from different subunits, namely, the head and LF domains from the same subunit, and the DF and upper body domains from a neighbor subunit (Figure 4A). Therefore, there are three ATP binding sites in a three-fold symmetric mode, although a recent study showed that ATP binding at only two of the three sites is sufficient for channel opening[107]. (2) The body domains of the three subunits intertwine with each other, forming the fundamental core of the P2X receptors (Figure 4B), which is surrounded by the three ATP binding sites. This 'core' can be further divided into upper and lower sections according to the 'dolphin' body, with the upper part maintaining the stability of the P2X receptors, and the lower part translating conformational changes induced by ATP binding from the extracellular to the TM domain. (3) Flukes of three subunits constitute the TM domain of P2X (Figure 1 and 4C). Three TM2 helices compose the channel pore while TM1 embrace TM2 from outside.

These structural characteristics of P2X determine the gating process of P2X receptors (Figure 4D)[33, 44, 45, 49, 108]. Following ATP binding, the head domain moves downward, the DF domain moves upward and the LF domain is pushed away from the binding site. Because of the coupling between the LF, DF and lower body domains, the relative motions of the LF and DF are capable of driving the outward expansion of the lower body, followed by the movements of TMs and subsequent opening of the ion access route. In conclusion, the gating process consists of a series of complicated and coordinated motions of multiple domains, which leads to the final channel opening of P2X receptors.

Small molecules to change the channel gating of P2X receptors

As discussed above, the gating process of P2X receptors involves a cooperative system composed of multiple domains. Small molecules that interrupt this process by acting on certain sites/domains affect channel gating. According to their effects on channel function, they are classified into antagonists and modulators. Many compounds targeting P2X receptors have been developed. AF-219, a selective P2X3 receptor antagonist, has been used in the treatment of osteoarthritis pain, interstitial cystitis and respiratory disorders[29]. AZD9056 can selectively inhibit the P2X7 receptor and is used for treating rheumatic arthritis[109]. Although both compounds have entered into clinical studies, little is known regarding their binding sites or working mechanisms (Figure 3). In combination with multidisciplinary approaches, including chimera and point mutations, electrophysiology, molecular modeling and molecular docking, Evans' group discovered that NF449, a P2X1 receptor specific inhibitor, could fill up the cleft between the head and the dorsal fin domain, thus preventing the binding of ATP and the downward motion of head domain. It is known that K138 (human P2X1, hP2X1, numbering), located in the head domain, is required for the binding of suramin, a broad spectrum inhibitor of P2X receptors. However, the difference in sensitivity to suramin between human and rat homologue P2X4 was determined by Q78 (rP2X4 numbering) located in the upper body domain[110]. This suggests that suramin may have different binding sites in different subunits and/or have more than one binding site for a certain subunit. Similarly, the mutant E249K of rP2X4 acquires sensitivity to PPADS[61]; however, chimera analysis identified another domain of approximately 100 amino acids (81–183) that accounts for the higher PPADS sensitivity in the human isoform compared to the rat[110]. This domain is in accordance with the spatial location of R126, a residue that is responsible for the species difference in antagonists' effects of the P2X7 receptor[111]. NF770, a suramin derivative that competitively inhibits the P2X2 receptor at nanomolar concentrations, acts on G72, E167 and R290 (rP2X2 numbering), which are also important for ATP binding[112, 113]. Interestingly, nearly all of those identified sites are located in or around the ATP binding pocket except for IVM, a P2X4 positive modulator, which has been identified to act on the TM domains[114–117].

Questions to be answered

Although progress on the channel activation of P2X receptors has been made since the structural determination of P2X receptors, many questions remain to be addressed.

P2X receptors have seven subtypes, exhibiting different affinities for ATP, ranging from nanomolar to millimolar[3], whereas the amino acid residues directly participating in ATP binding are highly conserved among different subtypes[7, 33]. The mechanism underlying those distinct affinities remains to be further investigated.

In light of two crystal structures of P2X4, the process of ATP binding inducing channel open has been deduced. However, the recovery process from open to resting state remains to be a mystery. Furthermore, desensitization kinetics differs among the seven subtypes[3]. Although preliminary research indicated that TMs and intracellular domains play important roles in P2X desensitization, the mechanism requires future studies.

'Pore dilation' is one of the hottest but also toughest questions in P2X research. Two hypothetical mechanisms, namely the gating model and the Pannexin-1 model, have been proposed[3]. In the gating model, pore dilation resulted from long-term ATP action, which leads to additional conformational change[118–124]. While in the Pannexin-1 model, the conformational changes of the P2X receptor resulted in allosterism of an auxiliary protein coupled with the P2X receptor, such as Pannexin-1, permitting molecules to enter cells through those proteins[125, 126]. Unfortunately, the mechanisms underlying the conformational changes from open to the dilated state of both models remain unclear.

Endogenous P2X receptors are assembled in homotrimeric as well as in heterotrimeric forms, such as P2X2/3, P2X4/6 and P2X1/5[8, 11, 127]. Unlike the symmetric gating mechanism of homotrimeric P2X receptors, the gating process of heterotrimeric P2X receptors is more complicated. Limited studies have been performed on the heterotrimeric P2X receptors, mainly on subunit stoichiometry[11, 128, 129]. It remains unexplored in the field of gating mechanisms for heterotrimeric P2X receptors, including the drug-designs targeting heterotrimeric receptors.

P2X receptors and ASICs showed unexpected similarities in their topology, despite their unrelated primary structures. Both contain many vestibules/pockets in their extracellular domains. Multiple pockets/ligand binding sites were identified in ASICs, through which novel toxins and small molecules inhibited or activated ASICs via mechanisms distinct from the acidosis-induced channel activation[130–136]. Therefore, similar to ASICs, finding novel toxins and small molecules to activate or modulate the function of P2X receptors through interactions with those vestibules/pockets in the extracellular domain is possible.

Although the structures of P2X4 at both resting and ATP-bound open states have been determined, structures of other subtypes are required to improve our understanding of the gating process of various P2X receptors. Structures complexed with the allosteric, especially subunit specific molecules, are also in demand to provide the structural basis for rational drug designs. In addition, the structure of the full-length P2X receptor with its intracellular domains has not been developed. With the help of newly improved technology, such as cryo-EM[137, 138], discoveries of more P2X structures are expected.

Concluding remarks

Since ATP was identified as a signal molecule in 1975, seven subtypes of P2X have been cloned and their physiological and pathological functions recognized. As a class of trimeric ion channels, the gating mechanism of P2X receptors differs from previously identified pentameric "cys-loop" ion channel family, tetramer voltage-gated potassium ion channels, TRP channels, or glutamate receptors. The crystal structures of P2X reported in 2009 and 2012 marked the beginning of the post-structure era at the atomic level of P2X research. The gating process of P2X receptors is a complex work by multiple domains. In this work, we highlight the recent achievements in P2X structures and channel gating, aiming to illuminate the correlation between the gating process and the structural elements of P2X receptors. All those studies have paved the way for developing new drugs targeting P2X receptors, which would contribute to novel therapeutic approaches in the future.

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