Review

Moving through the gate in ATP-activated P2X receptors

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P2X receptors are nonselective cation channels gated by extracellular ATP. They represent new therapeutic targets, and they form channels with a unique trimeric architecture. In 2009, the first crystal structure of a P2X receptor was reported, in which the receptor was in an ATP-free, closed channel state. However, our view recently changed when a second crystal structure was reported, in which a P2X receptor was bound to ATP and resolved in an open channel conformation. This remarkable structure not only confirms many key experimental data, including the recent mechanisms of ATP binding and ion permeation, but also reveals unanticipated mechanisms. Certainly, this new information will accelerate our understanding of P2X receptor function and pharmacology at the atomic level.

Molecular architecture of P2X receptors

The concept of purinergic transmission was formulated in 1972, after it was found that ATP acted on nerve and muscle cells as an extracellular signaling molecule [1]. Later, it was shown that ATP binds to two different families of transmembrane proteins, called P2 receptors: ionotropic P2X receptors and metabotropic G-protein-coupled P2Y receptors. P2X receptors carry intrinsic cation-selective pores that switch conformation from closed to open in response to ATP binding, allowing ions to rapidly flow through the membrane. Seven genes (*P2RX1–7*) encode P2X receptor subunits (P2X1–7) that are expressed in virtually all tissues in humans and mice, and also in various eukaryotic organisms. However, to date no evidence supports their presence in *Drosophila*, *Caenorhabditis elegans*, yeast, or bacteria [2], making their evolutionary origins and phylogenicity unclear. Our knowledge of P2X functions at the cellular level (Box 1) has been greatly enhanced with the use of genetically modified mice [3]. It is now firmly established that P2X receptors are involved in many physiological processes including modulation of synaptic transmission, taste, pain sensation, and inflammation, and thus they represent a major class of therapeutic targets.

The molecular architecture of P2X receptors is distinct from those of the 'Cys-loop' and ionotropic glutamate receptors; the other two major classes of ligand-gated ion channels. They are trimeric ion channels and have the simplest fold, in which each subunit crosses the membrane twice in such a way that the two hydrophobic transmembrane segments (TM1 and TM2) are separated by a large ectodomain. This implies that the amino and carboxy termini are intracellular. The extracellular domain carries the ATP binding sites and ten conserved cysteine residues engaged in five disulfide bonds. The transmembrane domain contains the pore for ion permeation; the intracellular domains, of variable size depending on the subtype, are known to regulate function and trafficking of the receptor [4,5]. A functional P2X receptor is composed of three identical or homologous subunits, forming homotrimeric or heterotrimeric channels, respectively. For instance, P2X1 subunits can form heteromeric channels by association with a combination of P2X2, P2X4, or P2X5 subunits, and P2X2 subunits can functionally associate with either P2X3, P2X5, or P2X6 subunits [6,7]. Like other ligand-gated ion channels, P2X receptors have multiple allosteric conformational states, but some of them exhibit a unique pore dilation property (Box 2).

In 2009, the first crystal structure of a P2X receptor (the zebrafish P2X4 receptor) was determined at 3.1 Å resolution [8,9], representing one of the major advances since the initial cloning of P2X receptors in the mid-1990s [9,10]. To help crystallization, the authors had to exclude intracellular domains, leading to a truncated version of the receptor (to be referred to as ΔzfP2X4-B). Nevertheless, the structure definitively confirmed the trimeric stoichiometry previously deduced from biochemical experiments [11,12], and further revealed the unique protein fold of the extracellular domain [13]. It also highlighted that the pore architecture is strikingly similar to that of acid-sensing ion channel (ASIC), another trimeric ion channel activated by protons, for which the X-ray structure has recently been resolved [14]. Finally, the structure, which was resolved in the absence of ATP but in the presence of the inhibitor Gd^{3+}, revealed the conformation of the closed channel state [13]. Overall, this structural advance has provided a firm grounding for further investigations on P2X receptors [11,12,15], and many studies during the past 3 years have contributed to our molecular understanding of receptor activation and desensitization, ion permeation pathways, and allosteric modulation [16–23].

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Box 1. Cellular functions of P2X receptors

Compelling evidence suggests that ATP is released through different mechanisms from living cells, including damaged cells, as well as from dying cells [73,74]. Released ATP opens the central pore in the P2X receptor that is permeable to cations, especially Ca^{2+} [65]. This leads to the depolarization of cells and the generation of downstream calcium signaling. It is now well known that P2X receptors mediate a variety of physiological processes, including fast synaptic transmission, neuromodulation, taste, immunomodulation, inflammation, and sensing pain [75,76]. An important contribution of the P2X1 receptor function has been found in the vas deferens contraction and male infertility with the use of the first P2X knockout mice [77]. Pain-relaying P2X3 and P2X2/3 receptors are expressed in sensory nerve terminals to detect ATP release from peripheral tissue or visceral organs [74]. P2X5 receptors expressed in sensory neurons have been shown to be involved in sensing muscle ischemia [78], whereas P2X4 receptors expressed in microglia have been identified for their critical roles in mediating neuropathic pain [79]. The underlying signaling pathway initiated from chemokine (C-C motif) ligand (CCL)21 to P2X4 and γ-aminobutyric acid type A ($GABA_A$) receptors has been studied in great detail [80,81]. The function of P2X6 subtypes is largely unknown, but it has been shown that they are likely expressed as heteromeric receptors by association with P2X2 and P2X4 subunits in motor neurons in the spinal cord, and relay nociceptive information along the pain pathway [74]. The P2X7 receptor also plays an important role in inflammation and immunity. For instance, activation of microglial P2X7 receptors releases inflammatory cytokines such as interleukin (IL)-1β, allowing microglia to generate full immune responses. This function has been confirmed *in vivo* by P2X7 knockout mice [82]. However, the mechanism underlying P2X7-dependent cytokine release is still under debate [83,84].

Although a picture of the molecular gating of P2X receptors emerged from these studies, the precise mechanism linking ATP binding to channel gating remained unclear until Hattori and Gouaux reported two new crystal structures of the zfP2X4 receptor in 2012. The first one represents another closed conformation of the channel resolved at higher resolution (2.9 Å) than the initial ΔzfP2X4-B structure, whereas the second denotes an open pore state resolved in the presence of ATP at 2.8 Å [8]. The latter was also obtained from a truncated construction (to be referred to as ΔzfP2X4-C), and slowly inactivates in the presence of ATP (a process also known as desensitization). This structure is, to the best of our knowledge, the first example of a vertebrate ligand-gated ion channel trapped by the endogenous agonist in an open-pore conformation. This study illustrated a plausible mechanism of P2X pore opening in response to ATP binding. In this review, we summarize recent functional studies in the context of the newly available structures, and highlight the remaining questions. The rat (r) P2X2 receptor has been extensively studied in terms of structure and function, therefore, we have built two homology models of this receptor based on the two recent crystal structures: one expectedly representing the closed channel state of the receptor, and another representing an ATP-bound, open channel state. Unless stated, residues cited in this review are thus numbered according to the rP2X2 sequence.

Insights from two structures: an overview

To yield a construct that diffracted at 2.8 Å resolution in the presence of ATP, the authors began with the initial truncated version of the ΔzfP2X4-B [13], then further removed several residues from the carboxy terminus and reverted the C51F mutation back to its native residue [8]. Although this optimization represents one of the keys to success, the deletion of intracellular domains raises the question of how close the structure resolved in the crystal is

Box 2. Multiple allosteric conformational states in P2X receptors

Allosteric proteins, including enzymes, transporters, G-protein-coupled receptors, and ion channels, may interconvert among multiple conformational states [85]. In the simplest scheme for P2X receptors, ATP binding drives the receptor from the resting, closed channel state (R state) to an active, open channel state selective to small cations (O_1 state, sometimes denoted by I_1; Figure I). Sustained ATP application drives the receptor to a desensitized, closed channel state (D state), which is refractory to activation. Desensitization kinetics differ among P2X receptors. For example, P2X1 and P2X3 receptors display fast and nearly complete desensitization within 2 s of application of ATP, compared with P2X2, P2X4, and P2X7 receptors that show nearly no desensitization, when expressed in HEK293 cells [75].

A subset of P2X receptors, including P2X2, P2X4, P2X7, P2X2/3, and the recently found heteromeric P2X2/5 [7], displays a second conducting state after prolonged application of ATP. The pore that is initially selective for small cations becomes progressively permeable to larger cations, such as N-methyl-D-glucamine and propidium dyes [86,87]. This state, also known as 'pore dilation' or O_2 (sometimes denoted by I_2), has been characterized by electrophysiological measurements, fluorescent dye uptakes [58,87], and fast-scanning atomic force microscopy [88], but to date, no structure of the dilated pore is known. Furthermore, it is clear that during pore dilation structural rearrangements occur in both the pore and cytosolic regions of the receptor [60]. However, the question of whether the permeability of large molecules results from permeation through the P2X pore or involves a distinct channel still remains controversial [60,84,89–91].

Recent studies have also raised the possibility of the existence of an intermediate closed channel state (F state; Figure I) preceding open states, but following the resting state [92,93]. Such a state has already been proposed for the pentameric channels of the nicotinic receptor family, for which an earlier conformational change called priming or flipping has been identified to take place while the channel is still shut [94,95].

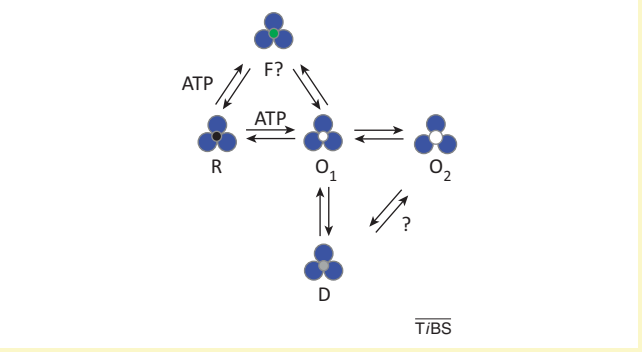

Figure I. Simplified allosteric scheme for P2X receptor function. For clarity, three spheres symbolizing subunits are arranged symmetrically around a central pore to represent the receptor. Following ATP binding, the receptor oscillates between discrete states (indicated by arrows), denoted resting state (R), in which the channel is closed (black pore), open states (O_1 and O_2), in which the pore is open (white pore), and desensitized state (D), in which the channel is closed again (gray pore). For the P2X2 receptor, an intermediate flipped or primed state (F), in which ATP binding primes jaw tightening, but the pore is still shut (green pore), has been proposed [93]. Note that in the O_2 state, the pore further dilates compared with the O_1 state as shown by an increase of the pore size. In this scheme, only O_1 and O_2 states are conducting, and no assumption on ligand binding stoichiometry is made. Question marks indicate putative state or allosteric transition.