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REVIEW

An evolutionary history of P2X receptors

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Abstract Adenosine triphosphate (ATP) is an ancient and fundamentally important biological molecule involved in both intracellular and extracellular activities. P2X ionotropic and P2Y metabotropic receptors have been cloned and characterised in mammals. ATP plays a central physiological role as a transmitter molecule in processes including the sensation of pain, taste, breathing and inflammation via the activation of P2X receptors. P2X receptors are structurally distinct from glutamate and Cys-loop/nicotinic receptors and form the third major class of ligand-gated ion channel. Yet, despite the importance of P2X receptors, both as physiological mediators and therapeutic targets, the evolutionary origins and phylogenicity of ATP signalling via P2X receptors remain unclear.

Keywords ATP · Evolution · P2X · P2Y · Receptor

Abbreviations

LGICs	Ligand-gated ion channels
ESTs	Expressed sequence tags

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Introduction

Ionic movement across biological membranes underpins a multitude of biological processes. Ligand-gated ion channels (LGICs) are responsible for many of the physiological effects of neurotransmitters in both central and peripheral nervous systems. Receptor activation via ligand binding leads to the opening of an ionic pore, or channel, and the movement of ions across biological membranes. The LGIC superfamily can be divided into three major receptor groups based on structural classification: (1) glutamate receptors (kainate, AMPA, NMDA); (2) Cys-loop receptors (nicotinic, 5-HT_3, $GABA_A$, glycine); and (3) P2X receptors. P2X receptors are directly activated by adenosine triphosphate (ATP), leading to opening of a non-selective cation pore, membrane depolarisation and calcium influx [1]. Functional receptors are formed from the assembly of three receptor subunits, in both homo- and heteromeric combinations [2]. This simple ion channel architecture is shared only with ASIC and the recently identified TRIC family of ion channels [3, 4], though no sequence homology exists for either. Each subunit has a double transmembrane topology with a large glycosylated ATP binding domain and relatively short terminal regions. Since the cloning of the first P2X receptors in 1994 [5, 6], our knowledge of this receptor class has been mainly restricted to vertebrates where they can be found on central and peripheral nerves, muscular, glandular and inflammatory cells [7]. The evolutionary origin of P2X receptors remains unclear, though other neurotransmitter-gated ion channels, such as glutamate and nicotinic receptors, have bacterial ancestry. Combined with the fact that purine derivatives, like ATP, have been suggested to have formed primordial transmitter substances [8], it has long been thought that a restriction to vertebrates was unlikely. An enrichment of genomic

information of single-celled and simple multi-celled organisms [9–12] has significantly improved our insight into the phylogenetic distribution of P2X receptors.

Expanding phylogeny

P2X receptors can be found in all vertebrate species (see [13]). Genomic analysis reveals the existence of P2X receptors in many invertebrate marine species including *anemone* (*Nematostella vectensis*), *mollusc* (*Lottia gigantean*) and *urchin* (*Strongylocentrotus pururatus*). Invertebrate P2X receptors are likely to play similar roles as in vertebrate species such as neuronal and muscular functions [14], though this remains to be demonstrated. The first invertebrate P2X receptor was cloned from the parasitic trematode *Schistosoma mansoni* [15]. The existence of P2X receptors in the platyhelminth lineage is further supported by other expressed sequence tags (ESTs) in other species such as *Schmidtea mediterranea* (EG407345). The *Schistosome* receptor displayed many characteristics common to vertebrate receptors such as activation by micromolar ATP and blockade by canonical P2X antagonists [15]. Partial P2X receptor ESTs are also found in members of the nematode lineage, for example *Xiphinema index* and *Haemonchus contortus* (CV509045; CB019691, respectively). It is therefore surprising that P2X receptors are not found in *Caenorhabditis elegans*, despite retaining functional, glutamatergic and GABAergic components of neuromuscular systems [16, 17]. The fruitfly *Drosophila melanogaster*, another neurobiological model organism, also lacks P2X receptors. Furthermore, bioinformatic inquiries fail to recover any insect P2X receptor homologues. Pharmacological evidence does support a role of purinergic signalling in the feeding behaviour of some blood feeding flies and ticks (see [14]). Although P2X receptors participate in communication between taste buds and taste nerves in mammals [18], molecular evidence is required to substantiate any role of P2X receptors in feeding behaviour in insects.

P2X receptors exist in organisms close to the boundary of single and multicellularity, in organisms void of neurones

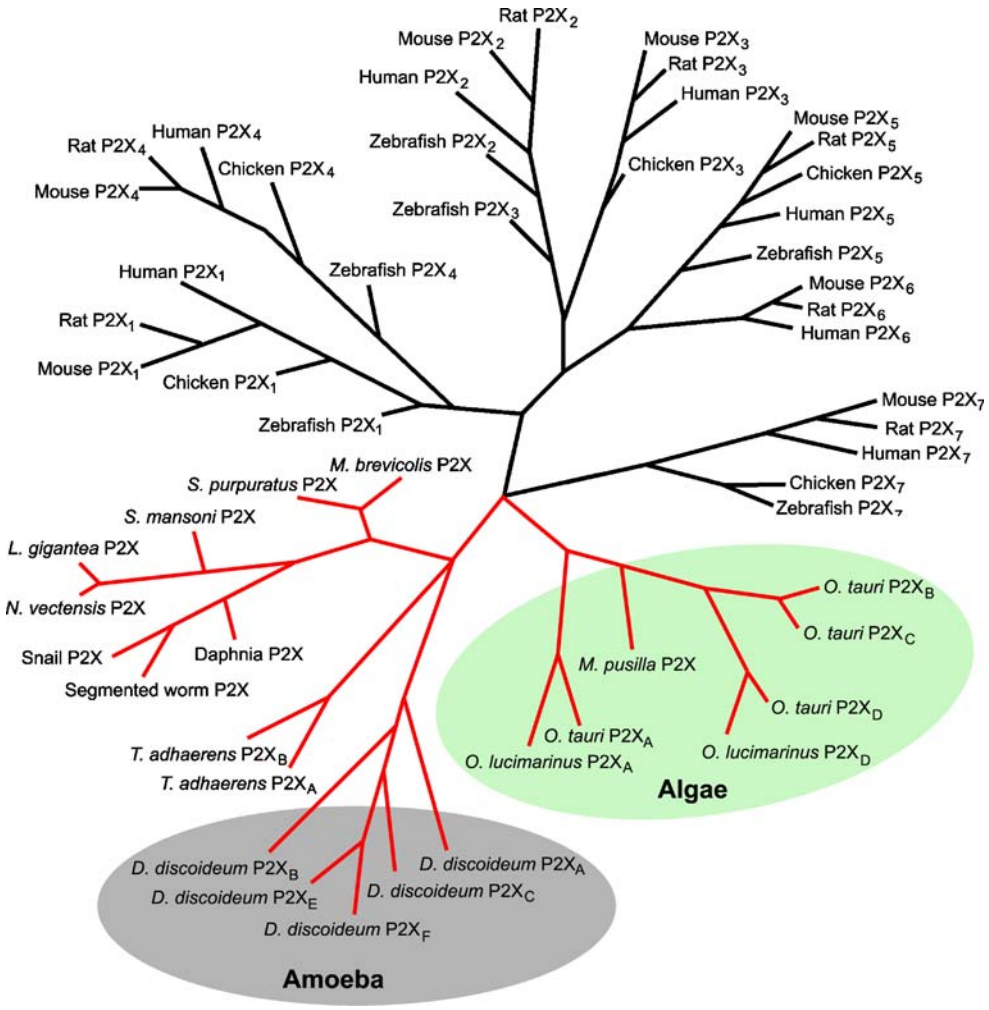

Fig. 1 A radial rooted phylogenetic tree showing the relationship between vertebrate and invertebrate P2X receptors. Vertebrate receptors are shown originated from *black lines* and invertebrate receptors from *red lines*. Clustering of receptor families from algae and amoeba are *highlighted*

and muscle. The genome of one of the earliest multicellular organisms *Trichoplax adhaerens* [12], a simple balloon-like marine animal, encodes two P2X receptor homologues (see Fig. 1). P2X receptors are also present in choanoflagellates (*Monosiga brevicollis*) [19], single-celled organisms considered to be the closest living relatives of animals [11]. The first P2X receptor cloned from a single-celled organism was cloned from the amoeba *Dictyostelium discoideum* [20]. Unlike the invertebrate receptor of *Schistosome*, the *Dictyostelium* receptor was insensitive to canonical antagonists and activated by higher concentrations of ATP (EC_{50}~170 μM). The existence of a receptor for ATP in *Dictyostelium* may not be surprising considering the precedence for extracellular cyclic nucleotide communication during development in this organism. However, the *Dictyostelium* receptor has an exclusively intracellular role, despite the presence of yet unknown cell surface receptors for ATP and ADP [21].

The identification of P2X receptors in *Dictyostelium* amoeba demonstrates the existence of P2X receptors early in the fungi/metazoan lineage. Despite this observation, bioinformatic analysis reveals a lack of P2X receptors in various yeast species, a divergent group of fungi/metazoa. Recently, a functional P2X receptor was cloned from the green algae *Ostreococcus tauri* [19]. This simple organism represents the smallest living eukaryote [10]. Bacterial in size, the cell comprises a single chloroplast and mitochondria and a nucleus with a unitary nuclear pore. It is extremely intriguing to understand what possible role a P2X receptor could play in such an organism. However, exogenous ATP fails to evoke sodium influx in *Ostreococcus* cultures [19], possibly suggesting an intracellular role as for the *Dictyostelium* receptor [20]. Further bioinformatic analysis suggests that P2X receptors may be prevalent within this phylum, with additional homologues identifiable in *Ostreococcus lucimarinus* and *Micromonas pusilla* (Fig. 1). Despite the existence of P2X receptors in simple photosynthetic cells, P2X receptors appear to absent in higher plants such as *Arabidopsis thaliana*. However, studies do suggest a role for ATP signalling in higher plants [22, 23]. Homologues of other neurotransmitter receptors are conserved in higher plants, such as glutamate receptors; however, their physiological roles remain controversial.

In search of prokaryotic P2X

The presence of P2X receptors in green algae tempts speculation about the existence of ancestral P2X receptors in cyanobacteria. Indeed, the precursor gene for mammalian glutamate receptors was identified in a cyanobacterium [24]. However, to date, no prokaryotic P2X receptor has been identified despite a wealth of bacterial genomic information. Such a homology is sorely needed in light of no crystallographic information for P2X receptors and would give great insight into the true evolutionary origins of P2X receptors.

Future directions

The recent years have seen a significant expansion in our knowledge of P2X receptors outside vertebrates. Further effort is required to thoroughly examine P2X receptors in additional species of simple organisms. It is also of critical importance to understand the physiological roles that P2X receptors play in such organisms. Such information is likely to elucidate the biological benefits that have led to the retention of P2X receptors in humans but have resulted in their loss in worms, flies and yeast.

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