Adenosine 5-triphosphate (ATP) is omnipresent in biology. It is therefore no surprise that organisms have evolved multifaceted roles for ATP exploiting its abundance and restriction of passive diffusion across biological membranes. A striking role is the emergence of ATP as a bona fide transmitter molecule, whereby the movement of ATP across membranes serves as a chemical message through a direct ligand-receptor interaction. P2X receptors are ligand-gated ion channels that mediate fast responses to the transmitter ATP in mammalian cells including central and sensory neurons, vascular smooth muscle, endothelium, and leukocytes. Molecular cloning of P2X receptors and our understanding of structure-function relationships has provided sequence information with which to query an exponentially expanding wealth of genome sequence information including protost, early animal and human pathogen genomes. P2X receptors have now been cloned and characterized from a number of simple organisms. Such work has led to surprising new cellular roles for the P2X receptors family and an unusual phylogeny, with organisms such as Drosophila and C. elegans notably lacking P2X receptors despite retaining ionotropic receptors for other common transmitters that are present in mammals. This review will summarize current work on the evolutionary biology of P2X receptors and ATP as a signaling molecule, discuss what can be drawn from such studies when considering the action of ATP in higher animals and plants, and outline how simple organisms may be exploited experimentally to inform P2X receptor function in a wider context.

Keywords: P2X receptor, evolution, molecular, pharmacology, structure-activity relationship, ion channels

INTRODUCTION
Seminal early discoveries identified the role of acetylcholine and norepinephrine in chemical transmission at neuronal synapse. However, the concept of non-adrenergic non-cholinergic (NANC) chemical transmission mediated by adenosine 5-triphosphate (ATP) emerged much later and was met initially with sizable resistance amongst the pharmacology and neuroscience communities (Burnstock, 2012). The development of the hypothesis that ATP can act as a transmitter molecule also came to challenge "Dales Principle" which suggested that a neuron only utilizes one transmitter molecule to communicate. It is now well accepted that neurons of the central and peripheral nervous systems release ATP as a co-transmitter. Indeed ATP is the sole transmitter molecule to act post-syaptically in some instances, for example during sympathetic innervation of submucosal arterioles of the intestine (Evans and Surprenant, 1992). Some molecules that are associated with neurotransmission such as GABA and glutamate are utilized by primitive organisms, which lack nervous systems, for the purpose of cellular signaling (Fountain, 2010).

The human genome encodes a family of cell surface receptors that are activated by extracellular adenine and uridine signaling nucleotides. The metabotropic arm of this family are the G protein-coupled P2Y (mammalian P2Y1,2,4,6,11,12,13,14) receptors that respond to ATP, ADP, UTP, UDP, and UDP-glucose with receptor subtype selectivity, and typically mediate slow responses to nucleotides. P2X receptors (mammalian P2X1-7) are ligand-gated ion channels and mediate fast responses solely to ATP. In mammals, P2X receptor activation is associated with diverse physiological and pathophysiological processes including pain, inflammation, taste and smooth muscle contraction (Khakh and North, 2006). P2X receptors are non-selective cation channels, consequently receptor activation leads to membrane depolarization and cellular calcium influx, both by direct receptor permeation of calcium and voltage-gated calcium channel activation (North, 2002).

Adenosine 5-triphosphate is omnipresent in biology and plays many important roles including energy transfer and as a phosphate donor in enzymatic reactions, though its acceptance as a certified signaling ligand was slow despite early reports of potent physiological action in mammalian systems. The effects of exogenous ATP on insects and invertebrates has been known for some time (for a review see Burnstock and Verkhratsky, 2009), though the identification and cloning of P2X receptors from invertebrates and primitive single-celled organisms has been far less widespread. The identification of genes encoding putative P2X receptors in primitive organisms has been aided by a wealth of structure-function information for mammalian receptors, and an ever-expanding library of curated genomes for single-celled organisms including algae, amoeba and basal fungi. For example, the P2X8 receptor of Dictyostelium discoideum shares only very low primary sequence homology with mammalian P2X receptors (Fountain et al., 2007). A translated BLAST search using the full-length 378 amino acid
sequence of the *Dictyostelium* as a query, provide no homologous mammalian P2X receptor sequences when using an expect value (E values) of 10. However, expression in *Xenopus* oocytes produces a functional ATP-activated ion channel (Schrägmaier et al., 2007) that responds to ATP with an EC50 of 22 μM (Agboh et al., 2004), a partial agonist at mammalian P2X receptors, acts as a full agonist at SCHR-250 receptor homologues with EC50 values in the range 100–500 μM. *Dictyostelium* P2X receptors are insensitive to ATP at concentrations tested (Fountain et al., 2007; Ludlow et al., 2009; Baines et al., 2013). ATP evoked currents can be detected in HEK293 cells expressing *Dd* P2X receptors with EC50 values of 4 and 10 μM, respectively. Permeability studies in HEK293 cells expressing SCHR-250 reveal a high permeability to calcium (PNa/PK = 3.8) that is comparable to mammalian P2X receptors. Cation substitution experiments reveal SCHR-250 and mammalian P2X receptors have conserved ionic pore diameters. Praziquantel is a drug used in the treatment of schistosomiasis. Though the action of praziquantel is dependent upon affecting calcium homeostasis in worms (Kohn et al., 2001), praziquantel does not inhibit SCHR-250 (Agboh et al., 2004).

**Closely non-vértébrale and primitive P2X receptors**

The pharmacological properties of cloned P2X receptors are summarized in Tables 1 and 2.

*Schrägmaier mansoni* (TREMATORIDAE)

The first non-vertebrate P2X receptor was cloned from the human pathogen *S. mansoni*. *Schrägmaier* are parasitic blood fluke and are trematodes belonging to the platyhelminthe genus. *S. mansoni* infection in humans causes schistosomiasis, a chronic illness which can lead to severe damage of multiple organs. *Schistosoma* infection in humans causes schistosomiasis, a chronic illness that can lead to severe damage of multiple organs.

**Table 1** | Agonist sensitivity of cloned P2X receptors.

| ATP         | BaATP | αβγεδATP | βγεδATP |
|-------------|-------|-----------|---------|
| P2X         |       |           |         |
| ATP         |       |           |         |
| DaP2X9      | 97    | 15        | 15      |
| P2X29       | 266   | ND        | 95      |
| P2X4        | 511   | ND        | 86      |
| P2X2        | 44    | 100 μM, 50% | ND      |
| P2X6        | 22    | 4 (75%)  | ND      |
| P2X45       | 6     | 2 (33%)  | 100 μM, 37% |

Values are given as approximate reported EC50 concentrations. EC50 values are given in μM. ND indicates antagonist sensitivity not determined. Maximum responses for partial agonist are given in parenthesis as % maximum ATP response. Where EC50 values have not been determined experimentally, maximum concentrations tested are given in parenthesis with response as % maximum ATP response.
et al., 2007; Ludlow et al., 2009). This makes Dictyostelium P2X receptors very useful tools for understanding antagonist action at P2X receptors as the structural determinants of drug binding in P2X receptors are poorly defined. A common feature shared by both Dictyostelium and mammalian P2X receptors is modulation by divalent metal ions (Virginio et al., 1997; Coddou et al., 2003). \( \text{Cu}^{2+} \) potentially blocks \( \text{DpP2X} \), with a half-maximal inhibitory of 40 nM (Fountain et al., 2007). \( \text{DpP2X}_A, \text{DpP2X}_B \) and \( \text{DpP2X}_E \) are blocked to a varying degree (50-85%) by 100 nM \( \text{Cu}^{2+} \). \( \text{Nd}^{-} \) is less potent at blocking \( \text{DpP2X} \) currents (\( \text{K}_{50} 40 \mu \text{M} \), Fountain et al., 2007).

The most striking feature of Dictyostelium P2X receptor functionality is their exclusive intracellular residence. Although some mammalian P2X receptors exist between intracellular compartments (Qureshi et al., 2007) and the plasma membrane Dictyostelium P2X receptors are targeted inside the cell. Several reports confirm that Dictyostelium P2X receptors reside on the contractile vacuole (Fountain et al., 2007; Ludlow et al., 2009), an osmoregulatory organelle and acidic calcium store (Heuser et al., 1993; Malchow et al., 2006; Sivaramakrishnan and Fountain, 2012). In a study by Fountain et al. (2007) Dictyostelium cells lacking \( \text{DpP2X} \) through genetic disruption were found to swell in response to hypotonic stress but lack any regulatory cell volume decrease, suggesting a severe impairment of contractile vacuole function. The phenotype was reconfirmed in a later study by Baines et al. (2013) who demonstrated that regulatory cell volume decrease could be rescued, or partially rescued, in \( \text{DpP2X}_A \) knockout cells by overexpression of \( \text{DpP2X}_B \), \( \text{DpP2X}_D \) or \( \text{DpP2X}_E \), but not \( \text{DpP2X}_C \) which fail to form functional ion channels when expressed in \( \text{HEK}293 \) or Xenopus oocytes (Ludlow et al., 2009). These data indicate a requirement for ATP activation of P2X receptors for normal contractile vacuole function and osmoregulation. These findings are not in agreement with a study by Ludlow et al. (2009) who demonstrate that Dictyostelium lacking all five P2X receptors still undergo regulatory cell volume decrease, despite a delay in recovery. The differences in phenotype reported can be explained by strain variance. In a recent side-by-side examination (Sivaramakrishnan and Fountain, 2013) of AX2 (used by Ludlow et al., 2009) and AX4 (used by Fountain et al., 2007; Baines et al., 2013) laboratory strains of Dictyostelium, it was found that wild-type AX2 and AX4 vary in the degree of volume recovery following hypotonic swelling and that AX2 but not AX4 can tolerate loss of \( \text{DpP2X}_A \). Within the vacuolar membrane P2X receptors are oriented such that the ATP binding site (ectodomain) faces the vacuole lumen, suggesting that the P2X receptors are positioned to sense changes in luminal ATP. Experiments using purified vacuoles demonstrate that ATP can be translocated into the vacuole lumen, representing a possible mechanism of ATP accumulation (Sivaramakrishnan and Fountain, 2013). Addition of ATP to intact vacuole preparations causes release of stored calcium. The magnitude of calcium release is reduced in \( \text{DpP2X}_A \) knockout vacuoles and ablated in vacuoles lacking all five P2X receptors. These data suggest that vacuoles respond to luminal ATP accumulation by releasing stored calcium via intracellular P2X receptor activation (Sivaramakrishnan and Fountain, 2013). It remains unclear how P2X receptor dependent calcium release contributes to contractile vacuole function though possibilities include facilitation of docking or vacuole fusion. Vesicular P2X receptor activation has been shown recently to facilitate vesicle fusion in mammalian cells in a calcium-dependent fashion (Miklavc et al., 2011; Thompson et al., 2013). Vacuoles isolated from AX2 amoeba release substantially less calcium in response to ATP in comparison to AX4 vacuoles, which may provide some mechanistic insight into the difference in P2X receptor dependency for osmoregulation between the two strains (Sivaramakrishnan and Fountain, 2013).

Extracellular ATP is detectable in suspensions of Dictyostelium (Parish and Weibel, 1980). Early work demonstrated that application of extracellular ATP stimulates \( \text{Ca}^{2+} \) influx in Dictyostelium which was sensitive to the purinergic receptor antagonist suramin (Parish and Weibel, 1980). More recently this has been demonstrated using aequorin expressing strains of Dictyostelium (Ludlow et al., 2008), though in this study the ATP evoked \( \text{Ca}^{2+} \) responses were insensitive to the P2 receptor antagonists suramin and PPADS but did display sensitivity to block by low micromolar \( \text{Ca}^{2+} \) as for the Dictyostelium P2X receptor (Fountain et al., 2007; Ludlow et al., 2009). Despite this, the ATP evoked calcium response remains intact following genetic knockout of all five P2X receptors.

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**Table 2** [Antagonist sensitivity of cloned P2X receptors.]

| Antagonist | PPADS | TNP-ATP | Cu²⁺ | Zn²⁺ |
|------------|-------|---------|------|------|
| DpP2X      | No block (100 μM) | No block (100 μM) | No block (100 μM) | ND |
| DpP2X 50 | No block (100 μM) | No block (100 μM) | No block (100 μM) | 0.04 | ND |
| DpP2X 100 | No block (100 μM) | No block (100 μM) | (100 nM, 85% block) | ND |
| DpP2X 0.04 | ND | ND | (100 nM, 70% block) | ND |
| DpP2X 23 | ND | ND | ND | ND |
| DpP2X 23 5 | Partial > 300 μM | ND | ND | ND |
| DpP2X 23 0.05 | Partial > 100 μM | ND | ND | ND |
| DpP2X 23 0.05 | Partial > 300 μM | 6 | ND | ND |
| DpP2X 23 0.05 | Partial > 300 μM | 6 | ND | ND |

Values are given as approximations of reported \( \text{K}_{50} \) concentrations. \( \text{K}_{50} \) values are given as μM. ND indicates antagonist sensitivity not determined. % block is given where an \( \text{K}_{50} \) value has not been determined.
Ostreococcus tauri (ALGAE)

Ostreococcus are primitive single celled algae and the smallest free-living eukaryotes. They belong to the Prasinophyceae class of unicellular green algae that mainly includes marine planktonic species, and are close to the evolutionary origins of photosynthesis. *O. tauri* encodes a protein of 387 amino acids termed OrIP2X that shares 23% primary sequence identity with the Dictyostelium P2X<sub>7</sub> receptor (Fountain et al., 2007) and around 28% identity with human P2X<sub>2</sub> receptors. Expression of OrIP2X-myc in HEK293 cells produces a 50-kDa protein (Fountain et al., 2008). The receptor contains many of the residues considering important for mammalian P2X receptor function, including conservation of ectodomain lysine residues are positions equivalent to Lys<sup>30</sup> and Lys<sup>290</sup> of rat P2X<sub>2</sub>, though overall the ectodomain is poorly conserved. The N-terminal YXXXXK sequence is retained; however, the C-terminal YXXXK motif shown to promote membrane retention in mammalian receptors (Chaumont et al., 2004) is replaced with a YESVL sequence.

ATP evokes OrIP2X channel opening with a half maximal concentration around 250 μM and activation threshold of around 30 μM. Whole-cell currents display modest desensitization in the presence of ligand. Single channel analysis revealed that OrIP2X open channel properties are flaccid in nature (Fountain et al., 2008). αβ-methylene-ATP evokes very small currents though baATP, γ-imido-ATP or other nucleotides triphosphates elicit no response (Fountain et al., 2008). The antagonist profile of OrIP2X is similar to that of the Dictyostelium P2X<sub>2</sub> receptors with suramin, PPADS and TNP-ATP all failing to cause block up to 100 μM. Unlike the Dictyostelium P2X<sub>2</sub> receptor, OrIP2X receptor currents are unaffected by Cu<sup>2+</sup> up to 100 μM.

In contrast to other P2X<sub>2</sub> receptors, OrIP2X displays poor calcium permeability (PCa/PPNa = 0.4). The poor permeation of calcium is a result of a major structural difference between OrIP2X and mammalian receptors, i.e., the absence of an aspartate residue that is highly conserved amongst other primitive and mammalian P2X receptors in the second transmembrane domain, the conserved aspartate is replaced by an asparagine residue. Asn<sup>293</sup> in OrIP2X is equivalent to Asp<sup>349</sup> in rat P2X<sub>2</sub>. Though a switch from an acidic to a basic moiety can clearly be tolerated at this position in OrIP2X, [N353Δ] mutation renders the receptor non-functional (Fountain et al., 2008). OrIP2X [N353Δ] enhances the calcium permeability (PCa/PPNa = 0.64; Fountain et al., 2008) but not back to the level of mammalian P2X<sub>2</sub> receptors. This suggests other residues in mammalian P2X<sub>2</sub> receptors also contribute to high calcium permeability (Migita et al., 2001; Samways and Egan, 2007). The pore diameter of OrIP2X receptors estimated from the relative permeability of a range of cations suggests a permeability cut-off of 1 nm. This broadly agrees with estimates of mammalian and Dictyostelium P2X receptor pore sizes (Evans et al., 1996; Fountain et al., 2007) and suggests architectural conservation of the selectivity filter between very early P2X receptor proteins and mammalian P2X<sub>2</sub> receptors.

Though OrP2X is clearly expressed at the plasma membrane when overexpressed in HEK293 cells, the subcellular localization of OrP2X in O. tauri has not been confirmed. Such experiments are hampered but the lack of selective antibodies to OrP2X and the size of the organism (around 1 μM in diameter) not being amenable to conventional immunocytochemical studies. However, experiments to determine whether ATP can induce calcium entry in *O. tauri* suspended in artificial sea water provide some indirect data supporting a lack of cell surface expression of P2X receptors. Though ATP does not stimulate calcium influx micromolar capsaicin, the TRPV1 channel agonist, did stimulate influx. A TRPV1 homologue is encoded by this primitive algae.

**TARDIGRADE (Hypsibius dujardini)**

_Hypsibius dujardini_ belongs to the phylum Tardigrade that shares features common to nematodes and arthropods. Around 400 μM in length, these multicellular organisms inhabit moss and freshwater environments, and are capable of lowering their metabolism enough to survive desiccating environments for long periods. Bavan et al. (2009) identified a 330 bp EST from _H. dujardini_ which when translated shared homology with mammalian P2X<sub>1</sub> receptors. The full-length coding region translates to a 480 amino acid protein (Hdp2X<sub>1</sub>) that shares between 36 and 38% sequence identity with human P2X<sub>1</sub>, P2X<sub>3</sub>, and P2X<sub>4</sub>. Phylogenetic analysis suggests Hdp2X<sub>1</sub> is an ancestor of vertebrate P2X receptors and orthologous to other non-vertebrate receptors including _Dictyostelium, S. MAnsori_ and _O. tauri_. Expression of Hdp2X<sub>1</sub> cDNA in Xenopus oocytes produces ATP (EC<sub>50</sub> 45 μM) activated ion channels that mediate transient inward currents that rapidly desensitize in the presence of ligand. Despite this rapid desensitization and in contrast to the human P2X<sub>1</sub> receptor that also displays rapid desensitization during ATP application, Hdp2X<sub>1</sub> currents recover. Current amplitude is fully recovered following 5 min agonist wash-off. Hdp2X<sub>1</sub> can be activated by both BaATP and αβ-methylene-ATP. BaATP acts as a partial agonist at Hdp2X<sub>1</sub> with maximal concentrations producing around 65% of ATP. Though BaATP is less efficacious it acts more potently than ATP with an EC<sub>50</sub> around 12 μM. αβ-methylene-ATP is less efficacious than ATP and evokes current amplitudes that are 50% of ATP responses. BaATP potency and efficacy with regard to ATP at Hdp2X<sub>1</sub> mirror that of BaATP properties at SαP2X<sub>2</sub> receptors (Agboh et al., 2004).

_Hdp2X<sub>1</sub>_ is blocked by the broad-spectrum purinergic receptor antagonists PPADS (IC<sub>50</sub> 15 μM) and Suramin (IC<sub>50</sub> 23 μM), which is in contrast to other primitive P2X receptors that are insensitive to classical antagonists. Micromolar Cu<sup>2+</sup> and Zn<sup>2+</sup> block Hdp2X<sub>1</sub>. Divalent metal ions modulate mammalian P2X receptor function and the _Dictyostelium P2X<sub>2</sub>_ receptor. Cu<sup>2+</sup> and Zn<sup>2+</sup> inhibit ATP-evoked responses with an IC<sub>50</sub> of 20 and

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ATP secreted by neurons or supporting cells of the mollusc CNS. ATP evoked currents mediated by P2X are potentiated by the macrocyclic lactone ivermectin which also potentiates mammalian P2X receptors.

Lymnaea stagnalis (POND SNAIL) The pond snail Lymnaea stagnalis has proven a useful model to study fundamental aspects of the CNS. Its relatively simple CNS contains <22,000 neurons making it amenable to study processes of associative memory and taste. ATP release in molluscan CNS has been studied in real-time (Gruenhagen et al., 2004) highlighting a potential for purinergic receptors in invertebrate CNS function. A full-length P2X receptor orthologue has been cloned from L. stagnalis CNS (Lymp2X). Lymp2X is 435 amino acids in length and shares 31–46% identity with human P2X1–P2X7, sharing most similarity with the human P2X2 receptor. Lymp2X expressed in Xenopus oocytes mediates inward currents that can be activated by ATP, BuATP and α,β-methylene-ATP. ATP is a full agonist that produces half-maximal responses at 6 μm. BuATP is 3-fold more potent than ATP at Lymp2X but acts as a partial agonist, producing a maximal response 66% that of ATP maximally inhibits the receptor by around 8 M. Suramin is less effective with an IC50 of 27 μM and produces incomplete channel block even at a concentration of 300 μM. The suramin resistant component accounts for around 40% of maximum current (Bavan et al., 2012). Lymp2X currents are not potentiated by ivermectin but are potentiated by 100 μM Ca2+ or Zn2+. The level of potentiation is between 45 and 75%. However, the effect of divalent metal ions is biphasic as 1 mM Ca2+ or Zn2+ inhibits the receptor by around 65%. The CNS of Lymnaea has several discernable ganglia including buccal, cerebral, pedal, pleural, left parietal, right parietal, and visceral ganglia. In situ hybridization reveals widespread expression of Lymp2X in neurons of all ganglia, though quantitation of Lymp2X mRNA transcripts reveals highest expression in neurons of pedal ganglia and the lowest levels in pleural neurons (Bavan et al., 2012). Though a physiological role of Lymp2X is yet to be assigned, it is highly likely that the receptor is placed to respond to ATP secreted by neurons or supporting cells of the molluscan CNS.

Boophilus microplus (TICK) The B. microplus tick causes detrimental effects to cattle wellbeing through blood feeding and transmission of disease. The tick P2X receptor homologue BmP2X forms a functional ATP activated ion channel when expressed in Xenopus oocytes (Bavan et al., 2011). The 414 amino acid long receptor shares between 30 and 44% sequence identity with human receptors, sharing the most identity with human P2X4 and least with P2X5. The receptor contains many structural motifs common to mammalian P2X receptors including 18 conserved ectodomain cysteines, positive and aromatic residues implicated in ATP binding and N-terminal putative protein kinase C phosphorylation site. Currents passed by BmP2X exhibit extremely slow kinetics. ATP evoked currents reach peak after almost 5 s, which is in stark contrast to the millisecond activation kinetics of mammalian P2X receptors (North, 2002). Current decay in the presence of agonist are also markedly slow. BmP2X currents decay by around 10% after 20 s exposure to ATP with 50% decay occurring after prolonged (>5 min) agonist application. Despite limited current decay in the presence of agonist, rundown in peak responses is marked. Consecutive ATP applications cause a 12% reduction in peak currents. Bavan et al. (2011) identified sequences positively charged residues in the C-terminus responsible for controlling receptor desensitization. Basic residues in the receptor C-terminus also control the desensitization kinetics of human P2X receptors (Fountain and North, 2006). However, the C-terminus does not contribute to receptor rundown properties. ATP activates BmP2X with an EC50 value of 70 μM, though adenosine, ADP or UTP (all up to 1 mM) do not evoke currents. Suramin antagonizes BmP2X (IC50 = 5 μM) but produces an incomplete block with currents persisting up to 300 μM. Ivermectin potentiates ATP evoked currents at mammalian P2X4 receptors (Priel and Silberberg, 2004), S. mansoni P2X (SmP2X) and H. dujardini (HdP2X) receptors. Despite its broad-spectrum anti-parasitic activity ivermectin does not potentiates BmP2X currents. However, currents are potentiated by amitraz, a triazapentadiene compound used widely in the treatment of tick infestation in cattle. Peak currents are potentiated by 23 and 94% by 1 and 100 μM amitraz, respectively. The identification of BmP2X is of major interest, not only as a target for potential new anti-parasitic drugs, but also as an example on an arthropod P2X receptor. Genomic information reveals that other arthropods including Drosophila melanogaster,Apis mellifera and Anopheles gambiae lack P2X receptors (Fountain and Burnstock, 2009). The existence of P2X receptors in the arthropod phylum suggests selective loss of P2X receptors in some, and likely the majority, of insect species.

GENERAL STRUCTURAL CONSERVATION WITH MAMMALIAN RECEPTORS Functional mammalian P2X receptors assemble as oligomers of three pore-forming subunits (Young et al., 2008; Kawate et al., 2009). This trimeric oligomeric state is unusual amongst other ligand-gated and voltage-gated ion channel families, including ionotropic glutamate and nicotinic acetylcholine receptors (Cys-loop superfamily), but shared by ASIC and intracellular TRIC channels. Our previous work demonstrates that Dicytostelium P2X assemble as trimers, at least when expressed as recombinant receptors in mammalian cells (Fountain et al., 2007), suggesting a conservation of trimer formation by primitive P2X receptors. Expression of Dictyostelium P2X receptors in S. mansoni insect cells also results in strong trimer formation, and the Dictyostelium receptor trimers are of a similar size to that of vertebrate receptor trimers (Valente et al., 2011). The Dictyostelium P2X receptors remain the best structurally characterized primitive P2X receptors. The
A growing wealth of genomic information for single celled and pairing for disulphide bond formation in the human P2X receptors, respectively. Based on the prediction of cysteine–cysteine (cysteine residues, whereas algae (genomic evidence presented for the existence of P2X receptors in osynthetic organisms yet to date the has been no functional or shows no correlation with species phylogeny. Trematode (Ennion and Evans, 2002), this would predict both the O. tauri and M. brevicollis receptor lack a single ectodomain disulphide bond, though at different positions. Strikingly, the Dicyostelium P2X4 receptors lacks cysteines at all equivalent position yet is a functional ATP activated ion channel (Fountain et al., 2007), suggesting a marked difference in ectodomain tertiary structure despite an ability to bind micromolar ATP (Valente et al., 2011).

FUTURE PERSPECTIVES AND EXPERIMENTAL ADVANTAGES
A growing wealth of genomic information for single celled and non-vertebrate species makes it highly likely that our knowledge of P2X receptor phylogeny is set to expand rapidly. Recently several putative P2X receptor sequences were reported from sea sponge (Amphimedon queenslandica), ameboid holozoan (Capsaspora owczarzaki) and nematode (Xiphinema index; Cai, 2012). Interestingly the same report identifies P2X orthologues in three species of basal fungi, namely Amboascus macrosporus, Spicellomyces punctatus, and Barnochytrium dendrobatidis (Cai and Clapham, 2012). Though the function of these newly identified P2X receptors is yet to be demonstrated experimentally, these putative receptors share many of the structural hallmark signs associated with P2X receptor function (Cai, 2012). Their existence suggests some phyla initially thought to lack P2X receptors, such as nematode and fungi (Fountain and Burnstock, 2009), may contain some species that do possess ATP activated ion channels. Identification of P2X receptors in the single celled green algae species Ostreococcus tauri demonstrate that the existence of P2X receptors predates the origins of multicellularity, and that the evolution of these receptor class occurred more than 1 billion years ago. Similar sequences are also present in the genome of Ostreococcus lucimarinus (Palenik et al., 2007). Though OP2X share poor primary structure homology with mammalian P2X receptors the proteins assembly to fully functional ATP activated ion channels. Elucidating the physiological role of P2X receptors in such small organisms will be technically challenging but of immense interest. O. tauri are photosynthetic organisms yet to date the has been no functional or genomic evidence presented for the existence of P2X receptors in higher plants. P2X receptors are notably absent from some species used extensively in neuroscience as model organisms including Caenorhabditis elegans and Drosophila melanogaster (Fountain and Burnstock, 2009). The absence of functional P2X receptors in Drosophila has been used as an experimental advantage for the study of neural circuits and behavior in this genetically amenable model organism. Ecotopic expression of rat P2X5 in Drosophila neurons allows for channel activation by laser-stimulated uncaging of caged ATP injected into specific fly brain areas. This allows pair activation of a specific set of neurons and exposure to a second stimulus such as odor (Zemelman et al., 2003).

SUMMARY
In summary, the phylogenetic distribution of P2X receptors is incomplete but demonstration of functional receptors in simple unicellular organisms suggests evolution of this receptor class occurred over one billion years ago. The fact that many primitive P2X receptors share very low sequence homology with mammalian P2X receptors, including absence of key motifs, yet still retain micromolar sensitivity to ATP and common permeability properties is intriguing. Some low homology receptors which lack sensitivity to common P2X receptors are likely to be useful tools in the future with which to delineate the residues that coordinate drug binding at P2X receptors. Though we have gathered much structural information from cloning and characterization of primitive P2X receptor our understanding of their cell biology and physiology is restricted, but likely to provide fundamental information about why and how the P2X receptor class of ligand-gated ion channels evolved.

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