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Early evolutionary history (from bacteria to hemichordata) of the omnipresent purinergic signalling: A tribute to Geoff Burnstock inquisitive mind

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Abstract

Purines and pyrimidines are indispensable molecules of life; they are fundamental for genetic code and bioenergetics. From the very early life forms purines have acquired the meaning of damage-associated extracellular signaller and purinergic receptors emerged in unicellular organisms. Ancestral purinoceptors are P2X-like ionotropic ligand-gated cationic channels ionotropic P2X receptors showing 20 – 40% of homology with vertebrate P2X receptors; genes encoding ancestral P2X receptors have been detected in Protozoa, Algae, fungi and sponges; they are also present in some invertebrates, but are absent from the genome of insects, nematodes, and higher plants. Plants nevertheless evolved a sophisticated and widespread purinergic signalling system relying on the idiosyncratic purinoceptor P2K1/DORN1 linked to intracellular Ca^{2+} signalling. The advance of metabotropic purinoceptors starts later in evolution with adenosine receptors preceding the emergence of P2Y nucleotide and P0 adenine receptors. In vertebrates and mammals the purinergic signalling system reaches the summit and operates throughout all tissues and systems without anatomical or functional segregation.

Key words: Purinergic signalling, Evolution, ATP, P2X receptors, P2Y receptors, adenosine receptors

"If you can look into the seeds of time,
And say which grain will grow and which will not"
William Shakespeare, *Macbeth*, Act 1, Scene 3.

1. The concept of omnipresent purinergic signalling

The saga of ATP as a neurotransmitter began in early 1960 from experiments carried by Geoffrey Burnstock and his colleagues on teaniae coli [1-3]. These experiments revealed the new type of transmission mediated neither by adrenaline nor by acetylcholine (non-adrenergic, non-cholinergic or NANC transmission); which was quite revolutionary in the days when neurotransmitters were limited to these two substances. By 1970 it become apparent that the transmitter in question is ATP, and hence the concept of purinergic nerves and purinergic neurotransmission has been born [4]; the early history of purinergic research is narrated in [5]. The concept of ATP as chemical neurotransmitter has not been accepted readily; to the contrary, several decades were needed before worldwide acknowledgement [6]. This, however, did not preclude Geoff from contemplating the ATP as ubiquitous neurotransmitter not only in autonomic, but also in the central nervous system; this led him to the next fundamental concept of co-transmission [7]. Again, these were the days when Dale principle (one neurone – one neurotransmitter), supported by all might of John Eccles, stand unassailable; yet co-transmission proved its worth and today the concept of multiple neurotransmitters secreted by an individual neurone or even from individual synaptic terminal is universally accepted.

The concept of purinergic signalling continued to evolve and by early 2000 it become clear that purinoceptors and purinergic mechanisms are operative outside the nervous system; as a matter of fact it turned out that almost every cell type possesses some kind of purinoceptors, and that ATP acts as intercellular signalling molecule literally in all tissues and organs: in blood cells, in bones, in kidney, in the skin, in the reproductive and in the immune system [8]; it is difficult to name a tissue devoid of purinergic signalling [9]. Purinoceptors contribute to regulation of a wide range of processes from oocyte fertilisation to cell proliferation, cell differentiation and cell death [9-11] while malfunction of purinergic signalling accompanies multiple diseases [12-17].

The ubiquity and universality of purinergic signalling led Geoff's inquisitive brain into evolutionary trends behind; in 2005 he presented the view of ATP as the most ancient chemical intercellular transmitter to the public (at the Ciba Foundation symposium). Indeed, the pyrophosphate bonds, phosphorylation of nucleosides and ATP itself appeared on earth in a prebiotic period (there are even speculations for extraterrestrial origin of ATP that arrived to Earth riding the meteorite [18]). Wherever this origin lies, phosphorylated nucleosides define the life as we know it; they are the backbone for RNA/DNA [19], while ATP is an indispensable element of bioenergetics [20]. The choice of ATP as an energy substrate was only possible at low (sub-micromolar) concentrations of ionised Ca^{2+} and hence the cells developed

sophisticated Ca^{2+} homeostatic machinery that is also used as the major intracellular signalling platform [21]. Based on all these considerations, Geoff suggested that ATP was, in early evolution, the first molecule to communicate messages to other cells. He contemplated that primitive cells, which all contained high levels of ATP inside, may release it as an extracellular signaller; and indeed the very first role for ATP could have been a damage signal, because cell damage invariably triggers massive ATP excretion; coincidentally ATP remained the *bona fide* damage-associated molecular pattern (DAMP) throughout most of life forms from protozoa and plants to mammals. This led Geoff to pay substantial attention to evolutionary trends behind purinergic signalling [22-25]. In this essay I shall present the brief overview of the early evolution of purinergic transmission.

2. Evolution of chemical transmission between cells

The intercellular communications are probably as old as life itself, whichever are the life origins – terrestrial, from Haldane-Oparin primordial soup [26; 27], or Panspermic from comets carrying fragments of DNA or viruses or even seeds and fertilised ova [28]. Life on Earth appeared about 3.8 – 4.2 billion years ago [29] with the oldest forms being filamentous microorganisms in seafloor-hydrothermal vent-related precipitates, from the Nuvvuagittuq belt in Quebec, Canada. Already these organisms were in need of perceiving their environment and hence they were likely to possess some sort of receptive systems. Arguably, the very first receptors were associated with ions as indeed most of environmental stresses experienced by primordial organisms were linked to changes in osmotic pressure of their extracellular milieu due to evaporation (under the sun) or dilution (due to the rain). These changes affect the ion concentrations in the water, which inevitably influences intracellular ions too: the antediluvian receptors were most likely ion channels. Indeed bacteria and archea, which probably are the most ancient life forms, contain several types of non-proteinaceous [30; 31] and proteinaceous ion channels (for example single-domain Ca^{2+} channels, Na^+ permeable bacterial Na^+ channels NaChBac, Ca^{2+}-dependent K^+ channels or CLC chloride channels) permeable to Na^+, K^+, Ca^{2+} and Cl^- [32-35]. Environment-related fluctuations in intracellular ions made them the very first intracellular signalling system that operates in all living forms [36; 37]. At the same time intercellular ions must be controlled and evolution selected for a system of pumps and exchangers that may rapidly redress ionic changes associated with cellular responses to stimulation. In essence, cells balance the need for intracellular ionic homeostasis (which, when sabotaged, triggers death) and the need to generate intracellular ion signals to control cell physiology.

Some of ion channels present in ancient unicellular life forms acquired ability to sense extracellular molecules, which are associated with environmental changes and most importantly with danger signals. Those danger signals probably represent the most ancient form of intercellular signalling and are, arguably, mediated by simple molecules, such as, for example, protons or aminoacids released by dying organisms. Thus bacteria appropriated the pentameric ionotropic receptor, which, in its most ancestral form, operated as a H^+-gated channel [38; 39] and the tertrameric K^+-selective glutamate ionotropic receptors in a form of iGluR0 and its analogues [40; 41]. When, around 3.5 billion year ago [42], eukaryotes appear on the scene a seriously diverse set of channels and some receptors have already been in place. Further evolution made three types of ionotropic receptors (Fig. 1), the already

mentioned pentameric and tetrameric and a new class of trimetric receptors. The tetra- and trimeric receptors are, from the earliest times, faithful to their appropriate ligands, respectively to glutamate and ATP; conversely the pentameric receptors are promiscuous and in different species are activated by quite different molecules, including acetylcholine, GABA, glycine and serotonin in mammals, and Zn^{2+}, histamine or H^{+} in invertebrates [43]. All these ionotropic receptors are in operation in unicellular protozoa and algae.

The evolution of the second, and the major class of membrane receptors, the G-protein coupled (GPCR) or metabotropic receptors is associated with eukaryotes [44]. The family of GPCRs is extended and diverse; in the human genome 2.4% of all genes are encoding for ~900 receptors that are responsible for most of neurotransmitters and hormone signalling as well as for special senses such as vision, olfaction and taste [45-47]. All metabotropic receptors are serpentine polypeptides that traverse the plasma membrane seven times (hence they are also known as 7-TM receptors). These peptides are in contact with heterotrimeric G-proteins; the latter act as signal transducers upon GPCRs activation [45]. Quite often, activation of metabotropic receptors translates into intracellular Ca^{2+} signals; this signalling system is conserved throughout evolution [48]. Bacteria and archea are in a possession of distant relatives of GPCRs, known as bacteriorhodopsins, which, however, are not known to act as true receptors [49]; in contrast all eukaryotes from protozoa and fungi to plants and mammals employ metabotropic receptors [50; 51].

The first neurones emerge in hydra (Cnidaria) and comb jellies (Ctenophora) in a form of a diffused nervous system with neuronal net being homogeneously dispersed throughout the animal body. This neuronal network is functionally connected through chemical synapses; the molecular and structural elements of which were likely to evolve earlier, probably already in unicellular organisms [52; 53]. The evolutionary roots of the first neurones are under debate with some evidence favouring the unique evolutionary origin of Ctenophorian nerve cells [54]; while other arguing for the existence of a common secretory cell/neurone precursor [55]. The neurotransmitter landscape in these two earliest forms of nervous system is very different: the hydras use neuropeptides which directly open Na^{+} channels [56] (the Hydra sodium channels, HyNaC 1–4, which are somewhat similar to acid-sensing ion channels, ASICs, and epithelial Na^{+} channels ENaC), whereas comb jellies use glutamate and express 14 types of ionotropic glutamate receptors segregated between different cell types and mediating interneuronal and neuromuscular transmission [54]. Some of these iGluRs are activated by glycine [57], in this distantly resembling the NMDA receptors of mammals. Another interesting peculiarity of intercellular signalling in Cnidaria is associated with wide presence of electrical synapses mediated by innexin-based gap junctions [54]. Genomic analysis of the sea anemone *Nematostella vectensis*, however, revealed a surprising number or ortologues of various receptors, including nicotinic acetylcholine receptors, purinoceptors, adenosine receptors, glutamate receptors as well as many neurotransmitter transporters [58]; which may indicate a more complex neurotransmitter landscape. All in all, the most ancient nervous systems seem to rely chiefly upon ionotropic receptors and ionic signalling, with no proven role for GPCRs.

The next fundamental step in evolution of the nervous system was the centralisation and cephalisaton which led to an appearance of neuronal masses, first in the form of

ganglia, which further evolved into multiganglionic brain in insects, crustaceans and such intelligent molluscs as octopodes [59]. Emergence of vertebrates coincided (or was prompted?) by a revolutionary invention of the radial glia, which underlies the emergence of the new, layers based, architecture of the central nervous system [60; 61]. Further evolution of the nervous system led to significant specialisation and appearance of many types of neurones and glia that utilise numerous neurotransmitters for synaptic and diffuse (volume) intercellular signalling.

3. Evolutionary perspective of purinergic signalling

Direct tracing of the early evolution of chemical transmitters and receptors is of course impossible; fossils do not preserve relevant evidence. To delineate evolutionary trends we have to study living representatives of phylogenetic ladder. The classic purinergic system has three major components: the system for regulated secretion of purinergic signalling molecules, the purinergic receptors and the system for degrading these signalling molecules [43; 62] and here I shall present a concise account of these systems in various phyla from bacteria to early vertebrates.

3.1. Bacteria

Bacteria do not have *bona fide* receptors to purines; excessive search of microbial genomes RefSeq database covering 3595 bacterial species revealed no homologues of any purinoceptors [63]. Despite the absence of identifiable receptors, bacteria are sensitive to environmental purines and pyrimidines. Out of the multitude of evidence on this subject a few examples may be selected. Adenosine, for instance, suppresses growth of unicellular insect parasite *Crithidia fasciculate* [64] and of several pathogenic bacteria such as *Staphylococcus aureus* [65] and *Micrococcus sodonensis* [66]. Extracellular ATP affects proliferation and differentiation of *Streptomyces coelicolor* A3(2) in concentration-dependent manner stimulating at 3 μM and inhibiting at 100 μM [67]; as well, ATP inhibits formation of pigment prodigiosin in gram-negative bacteria *Serratia marcescens* [68]. ATP inhibits growth of many bacteria including *Staphylococcus*, *Pseudomonas* and mycobacteria; it has been postulated that release of ATP from macrophages may exert direct anti-microbial effect [69]. Both purines and pyrimidines instigate sporulation in *Bacillus subtilis* [70; 71], while inhibiting spore germination in *Streptomyces galilaeus* [72]. Regulation of sporulation in *Bacillus subtilis* involves interaction of ATP with SpollAB protein, while ADP stimulates binding of SpollAB protein to SpollAA protein. Both Spoll proteins in turn interact with the transcription factor σ^F [73]. Adenosine has profound effects on *Escherichia coli* growth, gene expression and adherence to host cells [74]. What are the molecular mechanisms of purines action on bacteria remains generally unknown; as mentioned before bacteria do not have true purinoceptors. A high affinity binding site for adenine has been discovered in *Achromobacter xylosoxidans* [75], which may possibly reflect an adenine receptor; yet it is very different from adenine receptors described in mammals [76].

Although being devoid of purinoceptors, bacteria are in possession of two other components of purinergic signalling system. First, many bacterial species (such as *E. coli*, *Salmonella* and *Staphylococcus*) release ATP; the intensity of this release depends on the phase of proliferative cycle, while increase of extracellular ATP supported bacteria survival [77]. Moreover this bacterial release of ATP was reported

to be regulated by an increase in Ca^{2+} concentration in the cytosol of *E. coli* [78]. Thus ATP secreted by bacteria may act as a signalling molecule for bacteria-bacteria or bacteria-host interactions.

Finally, bacteria express the ATP-degrading system. The halophilic bacterium, *Vibrio parahaemolyticus* is in a possession of membrane-bound 5'-nucleotidase [79]; membrane-linked ATPases have been also purified from archaebacteria *Holobacterium salinarium* and *Methanosarcina barkeri* [80]. Classical ATP-degrading enzymes of the nucleoside triphosphate diphosphohydrolase (NTPDases) family were identified in *Legionella pneumophila* [81].

3.2. Protozoa

3.2.1. Social amoeba Dictyostelium discoideum

The social amoeba *D. discoideum* seemingly possesses all components of purinergic signalling system. First, ATP is detected in the media containing suspensions of *D. discoideum* at 0.1 – 0.8 μM, suggesting ATP release pathway(s) [82]; the ATP release is stimulated by changes in cell volume [83]. Second, *D. discoideum* (as well as other amoebae) express ecto-ATPases to degrade and probably protect against excessive extracellular ATP [82; 84]. Finally, D. *discoideum* is endowed with a surprisingly rich repertoire of purinoceptors. First, the genome of social amoeba has five ionotropic ATP receptors which have certain homology with mammalian P2X receptors. These receptors (labelled as P2XA-E) are cationic channels with Ca^{2+} permeability; they are localised at the membrane of intracellular organelles, the vacuoles [85-87]. These intracellular P2X receptors contribute to osmoregulation and may act as intracellular Ca^{2+} release channels [88]. At the same time extracellular administration of ATP to *D. discoideum* triggers cytoplasmic Ca^{2+} signals and cell depolarisation; these reflect plasmalemmal Ca^{2+} influx sensitive to Gd^{3+} [89]. This ATP response is mediated through transient receptor potential channel TRPP homologous to human TRPP1 channel (it is also known as polycystin-2). The TRPP channel in *D. discoideum* is either directly gated by ATP (thus being a purinoceptor) or it is linked to yet unknown plasmalemmal ATP receptor [90]. Finally, amoebae also express plasmalemmal metabotropic receptors activated by cAMP and designated as cAR1 – 4 [89; 91].

3.2.2. Choanoflagellate

The genome of the choanoflagellate *Monosiga brevicollis* contains a gene encoding ionotropic P2X-like receptor; expression of the protein in HEK293 cells led to a formation of ATP-gated cationic channel [92].

3.2.3. Ciliates

The ciliates, *Paramecium* and *Tetrahymena thermophila*, are sensitive to exposure to the nucleotides ATP and GTP, which both trigger avoiding reactions [93] associated with activation of Na^{+} and Mg^{2+} currents, membrane depolarization and Ca^{2+} influx through voltage-gated channels [94-96]. It seems that effects of ATP and GTP on ciliates are mediated through plasmalemmal metabotropic receptors of yet unknown molecular nature [97]. The ATP-degrading system, represented by ectoATPases has been identified in *T. thermophila* and in *Paramecium* [98; 99].

3.2.4. Trypanosoma

Radioligand binding identified two high-affinity plasmalemmal ATP receptors in *Trypanosoma cruzi*; the molecular nature of these receptors remains unknown [97]. Ecto-nucleotidases activity was detected in *Trypanosoma* [100], as well as in another member of the family, *Leishmania* [101].

3.3. Algae

Ostreococcus tauri is primitive green algae, which appeared about 1 billion years ago, and it is related to the evolutionary origin of photosynthetic plants [102]. The genome of *O. tauri* contains a gene for ionotropic P2X-like receptor. This gene, *Ot*P2X encodes a protein of 387 amino-acid residues with a molecular weight ~ 42 KDa. This protein has ~23% homology to P2X receptor of *D. discoideum* and ~28% of homology to human P2X receptors [92]. The OtP2X protein, when expressed in heterologous system, forms a functional ATP-gated cationic channel with ATP EC_{50} ~247 mM and rather low Ca^{2+} permeability (P_{Ca}/P_{Na} ~ 0.39) [92]. Possible functional role for this receptor in the life of *O. tauri* remains unknown [103].

3.4. Fungi

The ortologues of P2X receptors have been identified in the genome of three basal fungi *Allomyces macrogynus*, *Spizellomyces punctatus*, and *Batrachochytrium dendrobatidis*; these receptors have significant sequence similarity with animal P2X receptors [104]. Neither biophysical properties, nor functional significance of these receptors has been studied yet. Some fungi (for instance *Candida albicans*) were reported to release ATP possibly by diffusion through plasmalemmal channels [105].

3.5. Sponges

The ortologue of P2X receptor was found in the genome of sea sponge (*Amphimedon queenslandica*) [104]; whether it exists in other sponges and how it functions remains unknown. Curiously, a tripyridine alkaloid niphatoxin C isolated from the Australian marine sponge *Callyspongia sp* at 10 – 100 μM concentrations inhibited mammalian $P2X_7$ receptors [106]. Similarly several ianterans isolated from the marine sponge Ianthella quadrangulata appeared to be potent (EC_{50} ~ 1 μM) and selective inhibitors of mammalian $P2Y_{11}$ receptors [107].

3.6. Plants

Effects of ATP on plants are many: ATP regulates numerous function including growth, development and regeneration [108; 109]; furthermore ATP triggers cytosolic Ca^{2+} signalling in roots of *Arabidpopsis* plants [110], suggesting the existence of classical receptor system. This system has been identified in recent years as DORN1 (Does not Respond to Nucleotides 1 according to the name of *Aradiptosis* mutant [111]); this receptor has been subsequently named P2K1 to align it with general classification of purinoceptors. Molecularly the P2K1/DORN1 receptor is a plasma membrane-spanning legume-like lectin serine–threonine receptor kinase, which binds ATP with K_D ~ 45 nM and upon binding triggers intracellular Ca^{2+} signals [111; 112].

These Ca^{2+} signals in turn activate numerous downstream signalling pathways (such as for example NO signalling) and induces transcriptional responses [113; 114].

Plant cells release ATP to the apoplast by several pathways. First, ATP was found to be exocytotically secreted at the sites of active growth [115]. Second, ATP can be released by the plasmalemmal transporters such as ABC cassette transporter AtPGP1 or nucleotide transporter PM-ANT1 [116; 117]. Finally, ATP is massively released in response to damage, be this mechanical wounding such as herbivore attack or pathogen-triggered necrosis; such damage-induce ATP release was found to increase extracellular concentration of the latter to 80 nM in the damaged *Arabidopsis* roots [118] and to 80 μM in the plant leaves [119]. Several stressors, such as, for example, osmotic stress, L-glutamate, stress related hormone abscisis acid or pathogen-derived molecules such as yeast extract or mycotoxinbeauvericin, trigger ATP release form plant cells [118-122]. All these facts agree with the classical role of ATP as a DAMP; and indeed in plants ATP acts as a widespread DAMP [122]. Treating plant cells with ATP triggers massive activation of genes associated with wounding [111]. At the same time plant cells secrete ATP in physiological settings: mild mechanical stimulation of plant roots compatible with that experienced during normal growth through the soil evokes release of nanomolar ATP concentrations [123]. This of course indicates that purinergic signalling is utilised in intercellular communications in plants. This signalling seems to be particularly important at the growing roots; these regions of growth shows both the highest degree of physiological ATP release and the highest expression of ectonucleotidases AtAPY1 and AtAPY2 [109].

3.7. Placozoa

Placozoa are the most primitive multicultural animals having only four types of somatic cells and devoid of the nervous system; these phylum is not placed at the very base of the animal kingdom [124]. The homologues of the P2X receptors were identified in the genome and subsequently cloned from *Trichoplax adhaerens* (TaadP2XB receptor); expression of these receptors in HEK293 cells, however, did not result in an assembly of ATP-gated channel [125]. Nonetheless, the role for ATP as a signalling molecule in *Trichoplax adhaerens* has been suggested [126; 127].

3.8. Cnidaria

The genome of cnidarian starlet sea anemone *Nematostella vectensis* contains two orthologues of P2X receptors with 54% residue identity with vertebrate $P2X_4$ receptors; in addition four ortologues of adenosine metabotropic receptors (albeit with rather low ~20% homology to vertebrate ones) have been revealed [58]. The P2X receptors sequence was also identified in the genome of another Cnidarian, in Hydra; this receptor was 48% homologous to the receptor of the sea anemone [58]. The P2X receptor analogue (aepP2X receptor) was identified and cloned from *Hydra vulgaris*; expression of this receptor in HEK293 cells resulted in an appearance of ATP-gated cationic channel [125]. Sensory neurones of the hair bundle of sea anemone were reported to contain ATP-reach storing organelles which possibly may indicate ATP release [128]. Nonetheless functional purinergic transmission is yet to be demonstrated.

3.9. Ecdysozoa

A protostome superphylum *Ecdysozoa* includes nematodes, arthropods, insects, chelicerata, crustaceans, myriapods, tardigrades, and some other smaller phyla.

3.9.1. Nematodes

There is no evidence for expression of purinoceptors in nematodes; the search for the relevant genome sequences fail to identify anything relevant in several nematode species including *Caenorhabditis elegans* and *Caenorhabditis briggsae,* as well as in 37 members of *Ascaridomorpha*, *Spiruromorpha*, *Trihcenillida*, *Dorylaimida*, *Cephalobomorpha*, *Tylenchomorpha*, *Stongyloidea*, *Rhabditoidea*, *Diplogasteromorpha and Panagrolaimomorpha* [129; 130].

3.9.2. Tardigrades

The tardigrades (the "slow walkers") as they were christened by Lazzaro Spallanzani in 1777; although when initially discovered by Johann August Ephraim Goeze in 1773 they received the name of *kleiner Wasserbär* or "little water bears", are the most unique microscopic animals. Their are about 0.2 – 1 mm in length, they have segmented body with eight legs and they live everywhere from hot springs to the deep sea or summits of Himalayas and from African desert to the North pole. These are the most resilient animals, which can even survive the unprotected raid on the outer skin of the cosmic satellite orbiting at ~250 km above Earth for 10 days [131]. There are some conjectures (which are perceived by many as a fantasy) about extraterrestrial origin of these remarkable species [28].

Be this all is it may, the genome of one of the member of the phylum, the tardigrade *Hypsibius dujardini*, contains the sequence encoding the P2X-like ionotropic receptor designated as *Hd*P2X; this sequence is 480 amino acids long and has ~38% homology with P2X receptors of vertebrates. Expression of *Hd*P2X protein in HEK293 cells resulted in a formation of a functional ionotropic receptors sensitive to classical agonists ATP Bz-ATP and α,β-meATP at concentrations ~ 10 - 100 μM as well as to the broad-spectrum purinergic receptor antagonists PPADS and suramin [132].

3.9.3. Arthropoda

The subphylum of *Crustacea* covers a large group of animals represented by crabs, lobsters, crayfish, shrimp, krill and barnacles. Both ionotropic and metabotropic purinoceptors are operational in many representatives of this subphylum. In particular two P2X receptor paralogues have been identified in the genome of the freshwater crustacean *Daphnia pulex*; these receptors were designated *Dpu*P2XA and *Dpu*P2XB. When expressed in HEK293 cells, these proteins assembled into ATP-gated channel, Stimulation of these channels with ATP in mM concentrations evoked non-desensitising, inwardly rectifying cationic current with reversal potential ~ 8 mV; other purinergic agonists ADP, α,β-meATP or β,γ-meATP were ineffective [133].

Crustacea possess quite an elaborated set of metabotropic adenosine and ATP receptors, which act as chemosensors in olfactory and gustatory systems. Several subpopulations of purinoceptors with distinct sensitivity to AMP, ADP and ATP are present in the olfactory system of spiny lobsters *Panulirus argus* and *Panulirus*

interruptus [134; 135]. Lobsters' sensilla, located in the antennae and antennules, are endowed with two types of receptors sensitive to adenosine (resembling adenosine receptors by their agonist profile) and to nucleotides (which, pharmacologically, resemble P2Y receptors); activation of these receptors induces various forms of feeding behaviours [135-137]. The ability to sense ATP in the sea water allows crustaceans (who mostly feed on wounded or recently killed animals) to perceive the "freshness" of the prey – ATP, which is released from dying animal, signals fresh flesh, whereas products of ATP degradation (by nucleotidases of picoplankton [138]) indicate tissue that is dead for a while [139]. The ATP chemosensors of California spiny lobster, *Panulirus interruptus*, show exceptional sensitivity: lobsters can be attracted by ATP in nanomolar concentrations [140]. Olfactory purinoceptors have been detected in the shrimp *Pulaemonetes pugio* and in the blue crab *Callinectes sapidus* [141]. Of note, in decapod crustaceans the organs of olfaction and gustation are anatomically segregated, the former localised on the antennules, the latter on the walking legs, maxillipeds and mouthparts. Sensilla of the walking legs of the spiny lobster, *Panulirus argus,* have special cells sensitive to ATP and AMP [142].

Another arthropod, the Arachnida *Boophilus microplus* (also known as *Rhipicephalus microplus* or Asian blue tick) is also in a possession of the P2X receptor homologue classified as *Bm*P2X [143]. This protein comprises 414 amino acids with 44% of homology with human $P2X_4$ receptor. Expression of *Bm*P2X in Xenopus oocytes resulted in ATP-gated (EC_{50} ~70 μM) channel with slow activation (time to peak ~5 s) and inactivation (50% of decay in 5 min in the continuous presence of ATP) kinetics. The BmP2X currents are potentiated by a drug amitraz used for treating cattle infested by the tick [143].

3.9.4. Insects

Analysis of genome of several insects such as *Drosophila melanogaster*, *Apis mellifera* and *Anopheles gambiae* did not identify any homologues of P2X receptors [25; 144]. Nonetheless insects do have sensitive to purines and pyrimidines and apparently do express some metabotropic purinoceptors.

In particular these receptors are involved in olfactory and gustatory sensations. For example, apical sensilla of the labrum of *Culex pipiens* have functional ATP receptors contributing to the blood feeding behaviour [145]. Some of insect chemoceptors have a remarkable sensitivity: the ED_{50} for ATP for *Glossina palpales palpales* females is 0.5 μM nM, while for males it is 1.5 μM [146]; this means that even tiny amounts of ATP which are much smaller than 1 mM of ATP present in the plasma, can initiate gorging reflex. The gender difference in receptors sensitivity also explains why female mosquitoes are more ferocious than males. The rank of agonist potencies for these chemoceptors is ATP ≥ ADP = 2deoxyADP > AMP-PNP > AMP-PCP >> AMP, which is similar to some P2Y receptors [146]. A similar order of potency for gorging stimulants was found in *Rhodnius prolixus* [147]. The P2Y-like receptors contribute to feeding initiation in mosquitoes *Culex pipiens* and *Culiseta inornata*, which is again suggested the agonist potency order: ADP > ATP = AMP > β,γ-meATP for *C. pipiens* and ADP > ATP > β,γ-meATP > > AMP for *C. inornata* [148]. The molecular nature of insects P2Y-pike receptors are yet to be revealed, although *Drosophila* genome does contain some ortologues of P2Y receptor family [149].

Drosophila gene *CG9753* encodes the adenosine receptor (designated as *DmAdo*R), which shows ~38% of homology to the human A_{2A} receptor [150; 151]. Expression of *DmAdo*R in Chinese hamster ovary cell line results in a functional receptors; stimulation of this receptors with adenosine evokes synthesis of cAMP and triggers cytoplasmic Ca^{2+} signalling [150; 151]. The transcripts of *G9753* were identified in the brain, imaginal discs, ring gland and salivary glands of *Drosophila* larvae, suggesting their functional relevance [150]. Expression of loss of function mutant *DmAdo*R in adult flies causes deficient synaptic transmission and impaired associative learning [152]. In the larvae of the *Calliphora vicina*, the blowfly, adenosine decreases amplitude and frequency of nerve-evoked postsynaptic currents; these effects were simulated by A_2 receptor agonist and suppressed by A_2 receptor antagonist suggesting functional expression of adenosine receptors [153].

3.10. Lophotrochozoa

3.10.1. Platyhelminthes

Ionotropic ATP receptor cloned from the trematode *Schistosoma mansoni* [129; 154] was classified as *SchP2X* [129] or *SmP2X* [154]. This receptor shares 25 – 36% homology with human P2X receptors, being most similar to $P2X_4$ and $P2X_5$ receptors [129; 154]. Expression of recombinant *SchP2X* in *Xenopus* oocytes, resulted in functional ATP-gated channel. Exposure to ATP and Bz-ATP evoked inward currents with EC_{50} of 22 μM and 3.6 μM respectively; AMP-CPP, ADP, UTP, UDP, GTP and ITP were ineffective. Current carried by *SchP2X* channels were blocked by PPADS, suramin, and TNP-ATP, these currents were also potentiated by a modulator of human $P2X_4$ receptors ivermectin [103; 129; 154]. In the presence of ATP *SchP2X* receptors demonstrate pore dilation [154], a phenomenon well known for human $P2X_2$, $P2X_4$ and $P2X_7$ receptors [155].

3.10.2. Planaria

The homologue of P2X receptors was characterised in the freshwater planarian *Dugesia japonica* and designated *Dj*P2X-A [156]. This gene is specifically expressed in planaria stem cells (neoblasts), it encodes a membrane protein and it controls normal proliferation of these neoblasts. The biophysical characterisation of this P2X-like protein is yet to be achieved.

3.10.3. Molluscs

The phylum of Molluscs includes into cephalopods (squid, cuttlefish and octopus) and gastropods (snails and slugs).

The P2X receptor, designated as *Lym*P2X has been cloned from the pond snail *Limnea stagnalis*. This receptor has 435 amino acids and is 31 – 46% identical to the human P2X receptors with maximal homology with $P2X_4$ receptors [157]. Being expressed in *Xenopus* oocytes the *Lym*P2X acts as a ligand-gated channel which can be activated with ATP, BzATP and α,β-methylene-ATP; the EC_{50} for ATP and Bz-ATP are 6 μM and 2 μM, respectively. Currents mediated by *Lym*P2X are inhibited by PPADS and suramin [157]. The *Lym*P2X receptors are expressed in all parts of *L.*

stagnalis CNS. Snail neural cells are capable of secreting ATP [158], which may contribute to excitatory neurotransmission.

Aplysia californica or California sea hare has a single P2X receptor which appears in two isoforms. These receptors, classified as *Ac*P2X, form, when expressed in *Xenopus* oocytes, ligand-gated cationic channels activated by ATP with K_D of 306 μM [159]. The *Ac*P2X receptors are also activated by Bz-ATP and are inhibited by PPADS and suramin. This type of P2X receptors was expressed in chemosensory structures of *Aplysia* and in peripheral organs; in the CNS *Ac*P2X are localised to the insulin-containing neurosecretory cells of the cervical ganglia which are involved in control of growth and reproduction [159].

Molluscs are also in possession of metabotropic purinoceptors. For example adenosine was reported to modulate electrical activity of neurones in the suboesophageal ganglion of the snail, *Helix aspersa*, through A_1 and A_2 adenosine receptors [160]. Adenosine receptor-like proteins and related signalling transduction pathways regulate haemocyte adhesion in abalone, *Haliotis diversicolor* [161].

The AMP receptors are used for chemoception by the common octopus, *Octopus vulgaris*. These receptors are localised in sensory organs in the arms of the animal and AMP appears to be the most potent chemoattractant, which triggers a locomotor response directing the arms towards the meal [162].

3.10.4. Annelida

Main purinergic agonists ATP, ADP, AMP trigger inward cationic current and depolarise noxious and touch cells located in neuronal ganglia of medicinal leech *Hirudo medicinalis* [163], indicating expression of P2X-like receptors. Glial cells of the leech also express metabotropic P2Y-like receptors linked to activation of Na^+ channels and generation of Ca^{2+} signals mediated by $InsP_3$; in addition these glial cells contain adenosine receptors regulating hyperpolarising K^+ channels [164]. Metabotropic P2Y receptors also activate mechanosensitive channels in the growth cones of leech neurones [165] while ATP is the primary activator of microglia in the leech nervous system [166]. Metabotropic purinoceptors were also found to regulate transepithelial Cl^- secretion and Na^+ absorption across the integument of the medicinal leech. It turned out that ATP, applied from either apical or basolateral sides stimulates Na^+ uptake, whereas adenosine stimulated non-Na^+ currents and acted only from the basolateral side [167].

3.11. Echinoderms

The Hemichordata (Acorn worms) and Echinodermata (e.g. sea urchin, starfishes, brittle stars, feather stars, sea cucumber) are currently considered to be a sister phyla of Chordata; it is still unclear whether they represent a parallel evolutionary trait or are related to Chordata. Purines and pyrimidines exert multiple effects on various systems of echinoderms with pharmacology similar to that of adenosine and P2Y purinoceptors of the vertebrates [168-170].

3.12. Recapitulation

The purinergic signalling system emerged at the very dawn of evolution: it is operational in unicellular organisms. Although the purinoceptors have not been detected in Bacteria, there are examples of ATP sensitivity compatible with its broad role as a damage-associated signal. The very first purinoceptors are represented by ionotropic P2X receptors showing 20 – 40% of homology with vertebrate P2X receptors (Fig. 2). Genes encoding these ancestral P2X receptors have been detected in Protozoa, Algae, fungi and sponges; they are also present in some invertebrates, but are absent from the genome of insects, nematodes, and higher plants (for cladograms and detailed descriptions see [104; 159; 171]). Plants have a sophisticated and widespread purinergic signalling system and plant developed the idiosyncratic purinoceptor P2K1/DORN1 linked to intracellular Ca^{2+} signalling (Fig. 2). The advance of metabotropic purinoceptors started later in evolution with adenosine receptors preceding the emergence of P2Y nucleotide and P0 adenine receptors (Fig. 2). In vertebrates and mammals the purinergic signalling system reaches the summit and operates throughout all tissues and systems without anatomical or functional segregation.

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Figure legends

Figure 1. Three classes of ionotropic receptors.

Purinoceptors (trimeric P2X receptors; every subunit is assembled from 2 transmembrane (TM) domains), glutamate receptors (tetrameric AMPA, kainate, KA and NMDA receptors; each subunit is assembled of 3 TM domains), and pentameric receptor channels for acetylcholine (ACh), GABA, glycine and serotonin (each subunit is composed of 4 TM domains). Vertebrate P2X and ionotropic glutamate receptors are non-selective cation channels, whereas pentameric receptors are either non-selective cation channels (nicotinic ACh receptors, serotonin receptors) or chloride channels ($GABA_A$, $glycine_A$ receptors). The existence of Zn^{2+}-gated pentameric cation channels is still a matter of speculation. Invertebrate tissues express a range of pentameric channels with unusual properties.

Modified from [172] with permission.

Figure 2. Evolutionary history of purinoceptors.

Graphical Abstract (for review)

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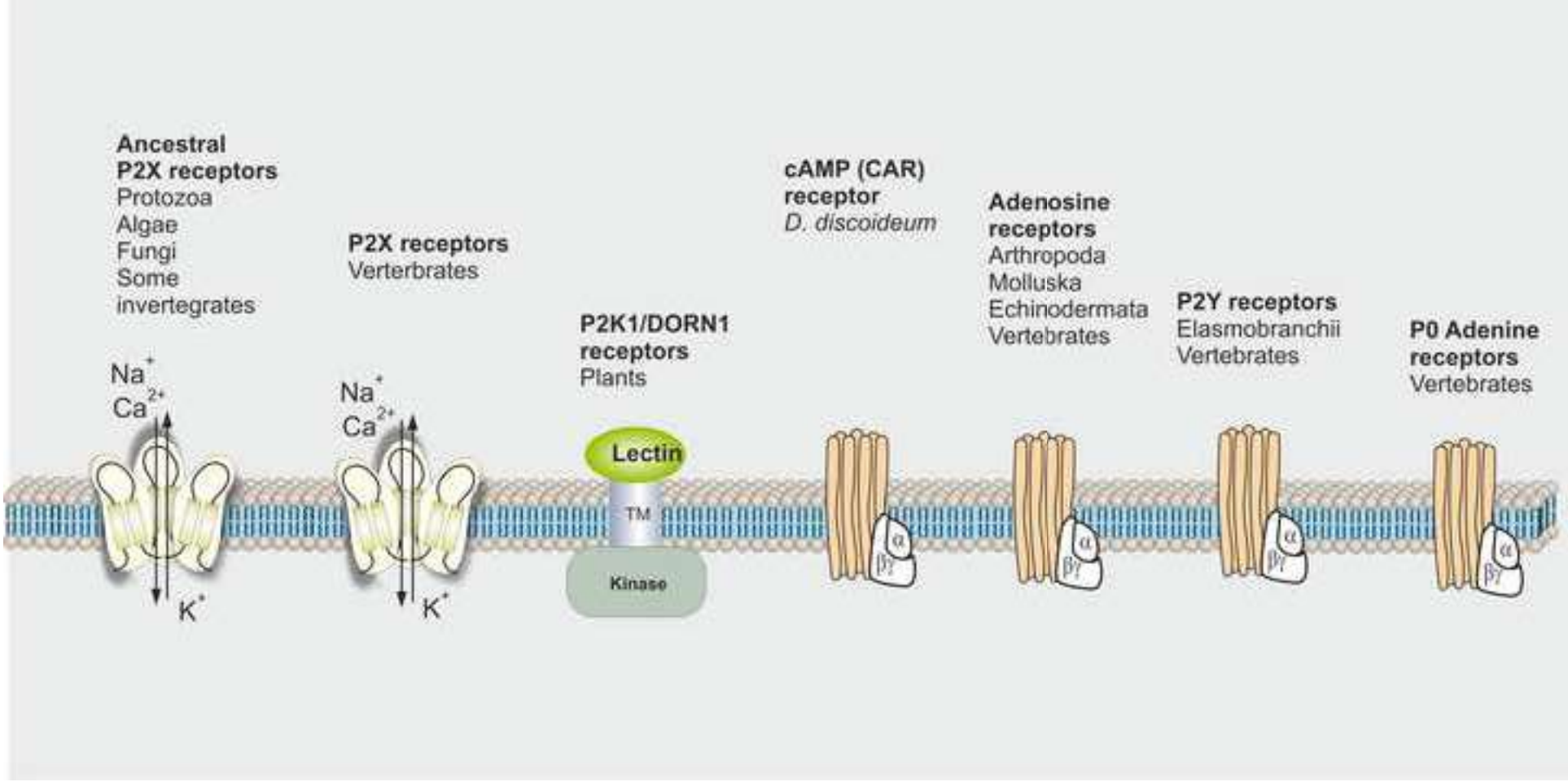

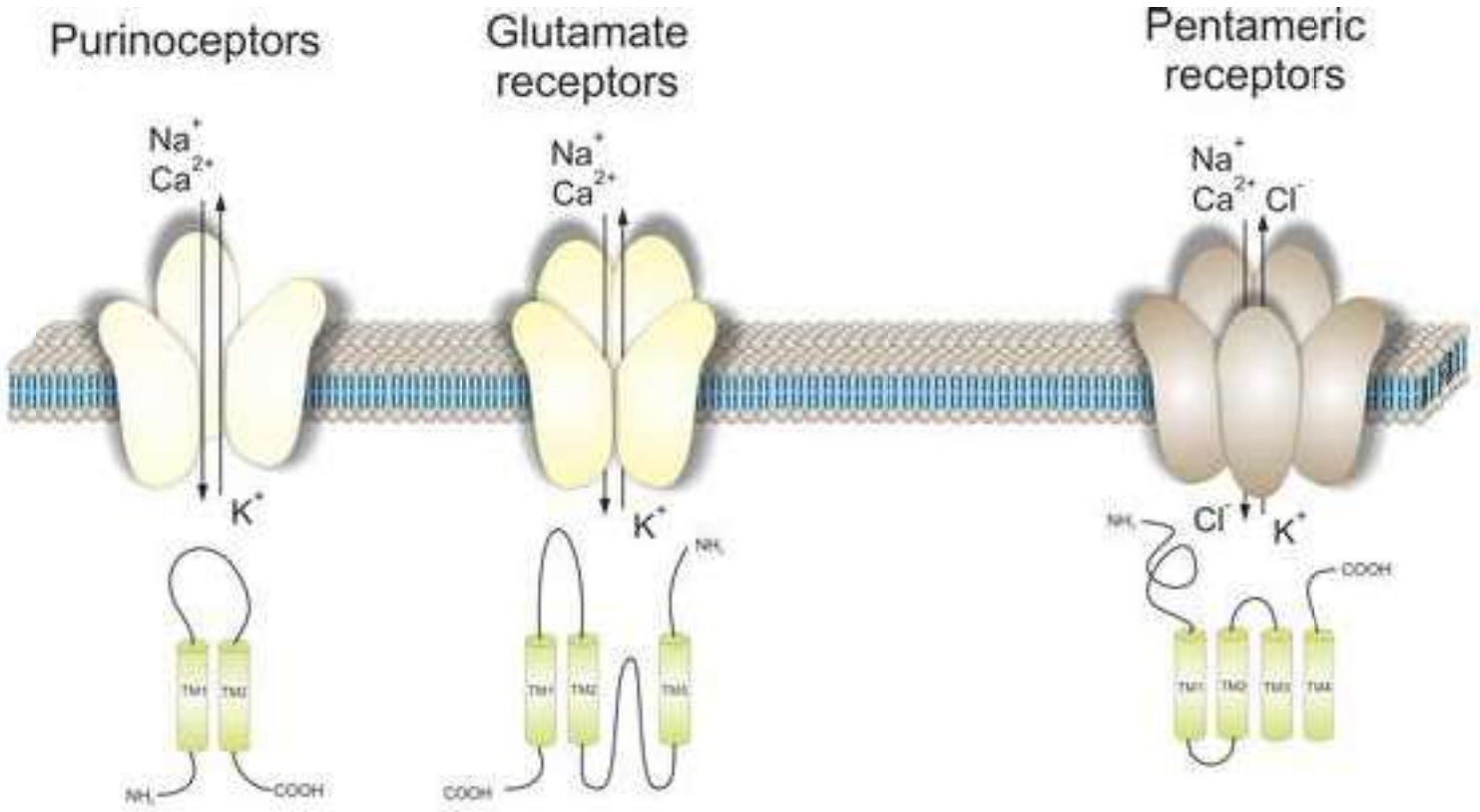

Agonist	ATP	Glutamate	ACh, GABA, Glycine, Serotonin, Histamine
Receptor types	$P2X_{1-7}$	AMPA, KA NMDA	Vertebrates: ACh receptors; Serotoinin receptors; GABA receptors; Glycine receptors; Zn^{2+}-gated cation channels (?) Invertebrates: GABA-gated cationic channels; ACh-gated Cl^- channels; Glutamate-gated Cl^- channels; Histamine-gated Cl^- channels; Serotonin-gated Cl^- channels; pH-gated Cl- channels

Fig. 1.

Figure 2