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REVIEW

The role of G protein-coupled receptors in the early evolution of neurotransmission and the nervous system

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ABSTRACT

The origin and evolution of the nervous system is one of the most intriguing and enigmatic events in biology. The recent sequencing of complete genomes from early metazoan organisms provides a new platform to study the origins of neuronal gene families. This review explores the early metazoan expansion of the largest integral transmembrane protein family, the G protein-coupled receptors (GPCRs), which serve as molecular targets for a large subset of neurotransmitters and neuropeptides in higher animals. GPCR repertories from four pre-bilaterian metazoan genomes were compared. This includes the cnidarian *Nematostella vectensis* and the ctenophore *Mnemiopsis leidyi*, which have primitive nervous systems (nerve nets), the demosponge *Amphimedon queenslandica* and the placozoan *Trichoplax adhaerens*, which lack nerve and muscle cells. Comparative genomics demonstrate that the rhodopsin and glutamate receptor families, known to be involved in neurotransmission in higher animals are also widely found in pre-bilaterian metazoans and possess substantial expansions of rhodopsin-family-like GPCRs. Furthermore, the emerging knowledge on the functions of adhesion GPCRs in the vertebrate nervous system provides a platform to examine possible analogous roles of their closest homologues in pre-bilaterians. Intriguingly, the presence of molecular components required for GPCR-mediated neurotransmission in pre-bilaterians reveals that they exist in both primitive nervous systems and nerve-cell-free environments, providing essential comparative models to better understand the origins of the nervous system and neurotransmission.

KEY WORDS: Neuron, GPCR, Nerve net, Synapse, Evolution

Introduction

G protein-coupled receptors (GPCRs) constitute the largest superfamily among membrane bound proteins that control key physiological functions, such as, neurotransmission, hormone releases, and immune responses among others (Katritch et al., 2013; Lagerström and Schiöth, 2008; Rosenbaum et al., 2009). As such, the large range of integral transmembrane proteins in this family respond to subsequent, diverse array of ligands: including neurotransmitters, hormones, lipids, and odorants (Civelli et al., 2013; Shoichet and Kobilka, 2012). GPCRs in mammals are classified into the five main families according to the GRAFS classification: glutamate, rhodopsin, adhesion, frizzled and secretin (Fredriksson et al., 2003). These five GPCR families are associated with various physiological functions, but the roles of the glutamate and the rhodopsin families are particularly well documented for neurotransmission and regulation of the nervous system (Niswender and Conn, 2010; Schoepp, 2001). Some functions include synaptic transmission, synapse formation, axon guidance and development of neuronal circuits (Collingridge et al., 2004; Hnasko and Edwards, 2012). Of the five GPCR families, the rhodopsin family is the largest, serving as molecular targets for the neurotransmitters serotonin, dopamine, acetylcholine, histamine, adrenaline and norepinephrine. GPCRs in this family also react to several neuropeptides, such as somatostatin, melanocortins, neuropeptide Y and neuropeptide FF. Similarly, glutamate GPCRs are molecular targets for the principal excitatory neurotransmitter of the central nervous system – glutamate. Upon activation, glutamate GPCRs crucially modulate synaptic transmission and neuronal excitability throughout the central nervous system (Cartmell and Schoepp, 2000; Ferraguti and Shigemoto, 2006; Niswender and Conn, 2010; Pinheiro and Mulle, 2008). Adhesion GPCRs are a growing research field, and several of these receptors have shown an important role in the CNS (Araç et al., 2012a; Langenhan et al., 2013; Strokes and Piao, 2010). For example, the brain-specific angiogenesis inhibitor (BAI) subfamily is involved in the control of synaptogenesis (Duman et al., 2013; Stephenson et al., 2014), whereas the latrophilins have been implicated in the control of synaptic transmission (O'Sullivan et al., 2012; O'Sullivan et al., 2014) and GPR56 is associated with brain development (Jeong et al., 2012; Luo et al., 2011; Singer et al., 2013).

GPCRs are well represented in several primitive metazoans, as well as in some pre-metazoan species (de Mendoza et al., 2014; Krishnan et al., 2012; Nordström et al., 2011). Interestingly, several of the pre-bilaterian metazoans, which lack most of the cell types that are commonly found in the bilaterians, still constitute a rich repertoire of GPCRs (Patel, 2012; Technau and Steele, 2011). In this work, we focus on four model organisms that are important for understanding the evolution of the nervous system, which also encode a large number of GPCRs: *Amphimedon queenslandica*, *Trichoplax adhaerens*, *Nematostella vectensis* and the ctenophore *Mnemiopsis leidyi*. *A. queenslandica* is a demosponge belonging to an ancient group of animals that diverged from other metazoans over 600 million years ago. *T. adhaerens* is a placozoan, which represents one of the simplest, free-living animals, whereas *N. vectensis*, a primitive animal that, along with corals, jellyfish and hydras, is contained in the oldest eumetazoan phylum, the cnidaria. The most common feature of these ancient metazoans is their lack of most the cell types found in bilaterians. For example, *A. queenslandica* are simple pore-bearing animals that lack a gut, nervous system and muscles (Degnan et al., 2008; Fahey and Degnan, 2010; Leys and Hill, 2012; Srivastava et al., 2010). *T. adhaerens* is a flat animal that lacks nerves, sensory cells and muscle cells (Ender and Schierwater, 2003; Miller and Ball, 2005; Schierwater, 2005; Srivastava et al., 2008). However, *N. vectensis* is regarded as one of the earliest organisms in animal evolution possessing a nervous system (Putnam et al., 2007; Technau and

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Steele, 2011). *N. vectensis* constitutes an ectodermal and endodermal nerve net making it a simple and diffuse nervous system that runs throughout the animal's body (Marlow et al., 2009; Nakanishi et al., 2012). Also, recently, the ctenophore (*M. leidyi*) genome has been sequenced and the organism also possesses a peripheral subepithelial nerve net, neurons and nerves associated with the tentacles (Jager et al., 2011; Richter and King, 2013; Ryan et al., 2013). Here, we analyse the large expansions of GPCRs, and present the evidence accumulated so far for the presence of neurotransmitters and their roles in early metazoans.

Phylogenetic position of sponges, cnidarians and ctenophores

Understanding the evolutionary origins of the nervous system and neuronal gene families requires an accurate estimation of basal metazoan evolution and precise positioning of pre-bilaterian species (Moroz et al., 2014). The most current phylogenetic analysis based on molecular data places the cnidarians just basal to the bilateral animals (Putnam et al., 2007; Technau and Steele, 2011) (Fig. 1). Likewise, the placozoans are placed basal to cnidarians, whereas the sponges are considered as a sister lineage to all extant animals (Srivastava et al., 2008; Srivastava et al., 2010). Until recently, it was widely accepted that sponges form the earliest diverging and evolving metazoan lineage (a sister group to the rest of Metazoa) and this was supported by their morphological simplicity lacking most cell types. However, the positioning of sponges as basal to all animals is currently being challenged by a recent hypothesis using transcriptomic and genomic data from ctenophore *M. leidyi* (Ryan et al., 2013) (Fig. 1). This is surprising because there is a general understanding that morphological complexity evolved over time and that the sponges are morphologically simpler in comparison with the ctenophore, which possess nerve and muscle cells. Nevertheless, there is additional support from the genomic analysis of another ctenophore *Pleurobrachia bachei*, which places ctenophore as the most basal animal lineage using molecular phylogenetic analysis (Moroz et al., 2014). This topology is also supported by 10 additional transcriptomes from other ctenophores (Moroz et al., 2014). These results provide new concepts for understanding the origins of neural systems and also provide support for theories purporting independent evolution of nerve nets in both the common ancestor of ctenophores and in cnidarian/bilaterian ancestors (Moroz et al., 2014). This scenario is an alternative to another hypothesis, which argues that the first nerve cell originated close the origin of the cnidarians (Holland et al., 2013). Lastly, another hypothesis proposes a single origin of the neural system at the early metazoan ancestor with major losses and/or gains of sophisticated cell types, including nerve and muscle cells, during the course of the metazoan evolution. Additional genomes and a coherent phylogenetic approach using several molecular markers are likely to provide more data to test the robustness of these hypotheses (Nosenko et al., 2013).

Remarkably rich GPCR repertoires in early metazoans

Recent studies based on large-scale genomic analyses have shown that these primitive metazoans possess complex GPCR-mediated signalling systems (Fig. 1) (de Mendoza et al., 2014; Nordström et al., 2011). One of the most notable events is a vast expansion of the rhodopsin family at the origin of metazoans. The ctenophore *M. leidyi* contains around 600 genes coding for rhodopsin-like GPCRs, whereas the sponges and placozoans have ~150 and 400 genes, respectively (Fig. 1). The cnidarians, which are placed basal to the bilaterians and are known to have a nervous system, have the largest repertoire of GPCRs in pre-bilaterians, with a remarkable expansion of over 1000 genes coding for rhodopsin-like GPCRs. In addition to the rhodopsins, adhesion GPCRs expanded with highly diverse N-terminal functional regions, ranging from short N-termini with no domains, to very large N-termini containing multiple cadherin, calx-β, scavenger receptor cysteine-rich (SRCR) domain, hormone receptor (HRM) domain and immunoglobulin domain repeats. Furthermore, these early metazoans contain expansions of the glutamate family of GPCRs, which possess conserved N-terminal functional domains, including a ligand-binding domain (the so called Venus flytrap domain) and a cysteine-rich domain (CRD or NCD3G).

Rhodopsin GPCRs in pre-bilaterians and the evolution of neurotransmission

Genome-wide analysis and similarity-search approaches suggest that *M. leidyi* contains rhodopsin family receptors that have their top five closest hits as serotonin, dopamine and adrenergic receptors from bilaterians (Fig. 2). However, immunohistochemical and pharmacological data suggest that ctenophores do not use serotonin, acetylcholine, dopamine, noradrenaline, adrenaline, octopamine, histamine or glycine as neurotransmitters. Indeed, ctenophores lack any of the enzymes necessary to synthesise these conventional low molecular mass neurotransmitters (Moroz et al., 2014; Ryan et al., 2013). This is surprising because sponges have neurotransmitter-synthesising enzymes, although they lack the nerve-net-like

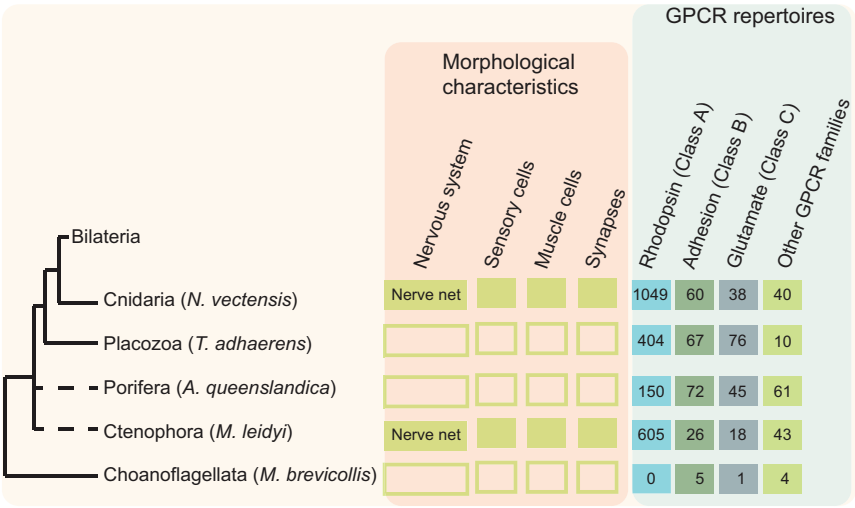

Fig. 1. Summary of GPCR repertoires in pre-bilaterian metazoans. The most current phylogenetic position of early metazoan lineages is shown on the left. Dotted lines represent the uncertainty over the positioning of sponges and ctenophores, although ctenophore is currently suggested as the sister lineage to all other animals (Ryan et al., 2013). The number of receptors in each family of GPCRs was obtained by performing a search in each proteome against the Pfam database. It must be noted here that the number of putative GPCRs predicted by automated Pfam search engine may slightly vary from a more refined or manually curated dataset excluding false positives and fragmentary gene sequences. Other GPCR families include Frizzled, Dicty_CAR, Lung_7-TM_R, GpcrRhopsn4 and Ocular_alb-like GPCRs belonging to the GPCR_A Pfam clan (for more information see GPCR_A clan in the Pfam database, ID: CL0192).

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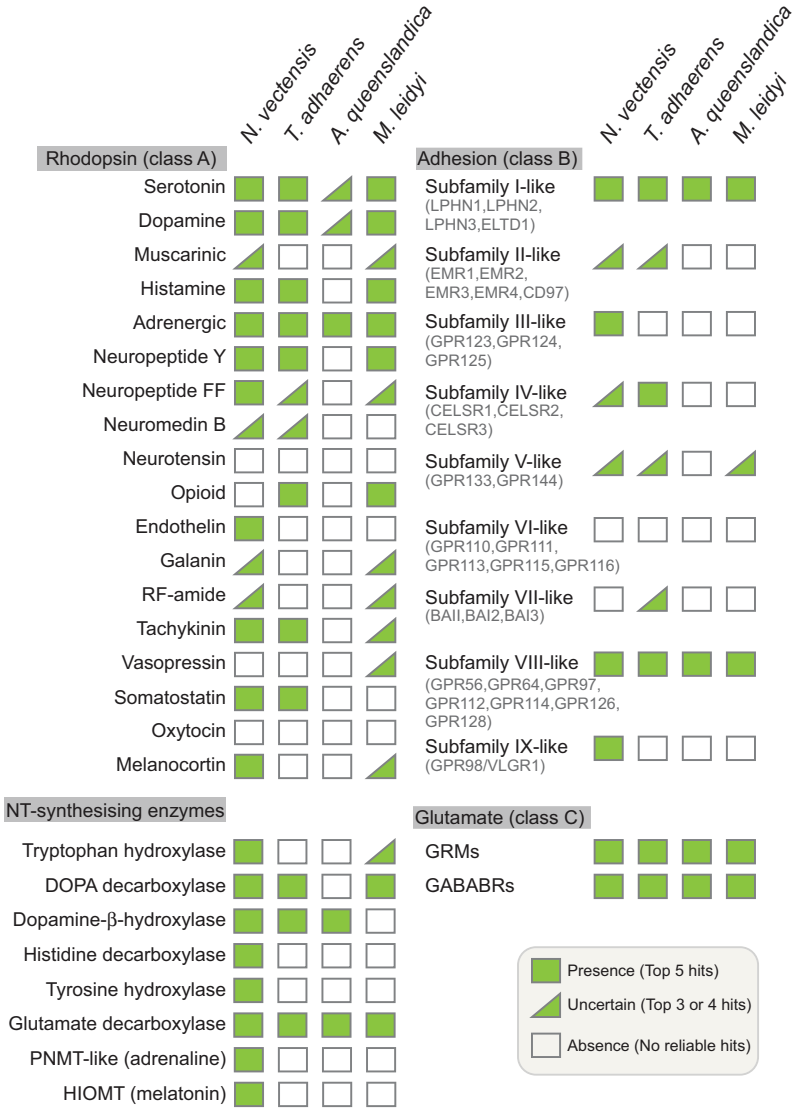

Fig. 2. Summary of putative GPCR homologues and neurotransmitter-synthesising enzymes found in pre-bilaterian metazoans. Green boxes indicate presence, white boxes, absence and half-filled squares the uncertainty over the hits obtained. The list of receptors shown in the illustration was estimated by a BLAST search using the putative GPCR sequences from all four proteomes as queries against the GPCR database obtained from the Swiss-Prot database. This GPCR database contained the manually annotated and reviewed list of the GPCRs from well-characterised vertebrates as well as from several well-known invertebrate model organisms. Also, the list was cross verified with whole-genome analysis and comparative genomic studies (Anctil, 2009; Moroz et al., 2014; Nikitin, 2014; Putnam et al., 2007; Riesgo et al., 2014; Ryan et al., 2013; Srivastava et al., 2008; Srivastava et al., 2010).

environment found in ctenophores. A recent study, which attempted an extensive analysis on eight transcriptomes from all sponge classes, found that dopamine-β-hydroxylase, tryptophan hydroxylase, DOPA decarboxylase and phenylalanine hydroxylase are all present in sponges, and may be found across all sponge transcriptomes (Riesgo et al., 2014) (Fig. 2). Interestingly, *Tethya wilhelma*, a demosponge, uses chemical messengers, including serotonin, to induce rhythmic body contractions (Ellwanger and Nickel, 2006). Another study also reported serotonin-derived alkaloids in sponges (Salmoun et al., 2002). Furthermore, sponges have non-motile cilia that line the inner epithelium of the osculum (an excretory structure that regulates the water flow) and these cilia function as a sensory organ to coordinate whole-animal responses (Ludeman et al., 2014). Although sponges may harbour the molecular machinery required for synthesis of neurotransmitters, it is rather complicated to provide evidence for neurotransmitter signalling in sponges, because they lack a nervous system, nerve net or nerve cells. This may explain the differential roles for some of the conventional neurotransmitters in a nerve-cell-free environment.

A closer inspection of *T. adhaerens* revealed that the primitive metazoan also contains a few neurotransmitter-synthesising enzymes (Fig. 2). Components of *T. adhaerens* neurotransmitter biosynthesis include DOPA decarboxylase and dopamine-β-hydroxylase (Srivastava et al., 2008). Also, enzymes for proneuropeptide (pNP) processing, maturation and secretion have been identified in *T. adhaerens* (Jékely, 2013). A similarity search approach suggests that *T. adhaerens* contains receptors that have their top hits as bilaterian serotonin, dopamine, histamine, adrenergic, neuropeptide Y and neuropeptide FF receptors (Fig. 2). This is consistent with the whole genome report, which suggested the presence of putative neurotransmitter- and neuropeptide-binding receptors (Srivastava et al., 2008). Furthermore, it was recently discovered that *T. adhaerens* has four insulin genes, five neuropeptide precursors and most of the enzymes required for peptide processing, including the prohormone convertase, carboxypeptidase and amidating monooxygenase (Nikitin, 2014). Additionally, two leucine-rich repeat-containing GPCRs (LGRs) were found in *T. adhaerens*. However, glycoprotein hormones, the known ligands for LGRs, were absent (Nikitin, 2014). Although *T. adhaerens* contains several GPCRs, including the peptide and amine binding receptors, attempts to build phylogenetic trees and resolve their relationships with cnidarian and bilaterian GPCRs suggest that the *T. adhaerens* rhodopsins have diverged considerably and seem to have undergone lineage-specific diversification (Nikitin, 2014).

There is ample evidence that acetylcholine, glutamate, γ-aminobutyric acid (GABA), glycine, epinephrine, norepinephrine,

dopamine and serotonin transmitters, as well as neuropeptides, are involved in cnidarian neurotransmission (Kass-Simon and Pierobon, 2007; Watanabe et al., 2009) (Fig. 2). Among these, the neuropeptide RFamide family of GPCRs is the most studied among the cnidarians. RFamide-positive neurons were found in *N. vectensis*, *Hydra*, *Hydractinia* and jellyfish, and they are found in all classes of cnidarians (Watanabe et al., 2009). Moreover, the components for neurotransmitter biosynthesis in cnidarians are more complete when compared with the other pre-bilaterians. Choline acetyltransferase, acetylcholinesterase, glutamate decarboxylase, tryptophan hydroxylase, DOPA decarboxylase, dopamine-β-hydroxylase are all present in *N. vectensis* and other cnidarians (Anctil, 2009) (Fig. 2). Apart from the experimental verification for some of the neurotransmitters and neuropeptides, bioinformatic analyses found neuropeptide receptors similar to NPY, NPFF, galanin, tachykinin, GnRH/vasopressin-like, melanocortin, insulin-like and glycoprotein hormone-like receptors (Anctil, 2009). Furthermore, chordate-like olfactory receptors (ORs), a family of chemosensory receptors belonging to the large rhodopsin-like GPCRs, were discovered in *N. vectensis*. The presence of epithelial sensory cells supports the idea that the *N. vectensis* OR-like receptors may play a crucial role in sensing the environment (Churcher and Taylor, 2011; Marlow et al., 2009; Nakanishi et al., 2012; Niimura, 2009). These findings provide insights that the pre-bilaterians exhibit a GPCR-mediated sensory system and suggested that the genetic complexity commonly assumed to have arisen much later in animal evolution is actually an ancestral feature.

Functional roles of some of these neurotransmitters and neuropeptides are also demonstrated. Neuropeptides Hym-53, Hym-355 and CGLW amide trigger the oocyte maturation and spawning in hydrozoan jellyfish (Takeda et al., 2013). The GLW amide was shown earlier to have a role in muscle contraction and metamorphosis in *Hydra* (Takahashi et al., 2003). Similarly, another neuropeptide, Hym-176, induces ectodermal muscle contraction in *Hydra* (Yum et al., 1998). Also, FRamides in *Hydra magnipapillata* induce elongation as well as contraction of the body (Hayakawa et al., 2007). Thus, these neuropeptides are probably involved in regulating muscle contraction in cnidarians (Fujisawa, 2008; Grimmelikhuijzen et al., 1996; Takahashi, 2013). Furthermore, the roles of non-peptidergic neurotransmitters have been characterised in cnidarians. Serotonin is involved in metamorphosis of the hydrozoans *Phialidium gregarium* and *Eudendrium racemosum* (McCauley, 1997; Zega et al., 2007). And, the neurotransmitter glycine affects the endodermal pacemakers and thereby regulates elongation and contraction of the hydra body column (Ruggieri et al., 2004). Similarly, electrophysiological experiments show that glutamate and GABA and their receptors are involved in pacemaker activity in *Hydra* (Kass-Simon et al., 2003). Lastly, the amino acid transmitters, GABA, glycine and glutamate are implicated in the coordination of feeding response in *Hydra*.

Although studies suggest roles for neurotransmitters in cnidarians, the molecular functions of the pre-bilaterian rhodopsin GPCRs are still obscure. A current challenge is to understand the functional implications of the expansion of rhodopsin GPCRs in sponges and placozoans. Studying this topic is, however, difficult because sponges and placozoans are not readily suitable for laboratory purposes. Similarly, the functions of the most ancient rhodopsin GPCRs in ctenophores have not yet been characterised. Systematic cross-genome comparative analysis and robust phylogenetic analysis can serve as an initial step to explore the relationships between cnidarians and other bilaterian GPCRs. Based on the current evolutionary hypothesis, which argues for independent origins of the nerve cells in ctenophores and in a cnidarian/bilaterian ancestor, it could be possible that the rhodopsin family expanded independently in these pre-bilaterians and may have diverse functions based on the morphological characteristics of the organism. Also, considering the fact that rhodopsin shares a common ancestor before the divergence of metazoans, it is possible that these large expansions may have evolved to perform neuronal functions in ctenophores and cnidarians, and that this ability is secondarily lost in sponges and placozoans during the course of metazoan evolution. Further comparative genomics, as well as developmental or neurobiological studies would lead to a better understanding and reconcile the conjectures over the origins and evolution of metazoan neurotransmission. For this purpose, expansion of the rhodopsin family GPCRs could serve as one of the essential datasets to further understand and study the origins of the nervous system and neurotransmission from a GPCR perspective.

Emerging neurobiological roles of adhesion GPCRs: comparison with pre-bilaterians

The functions of adhesion GPCRs in pre-bilaterian organisms are mostly unknown. Therefore, we approached the question of the roles of pre-bilaterian adhesion GPCRs by considering adhesion GPCRs in vertebrate model organisms and searching for putative adhesion orthologues or adhesion genes with similar domain compositions in the genomes of pre-bilaterian metazoans. This is a conventional approach, and has been used in practice to provide insights into the possible roles of genes in distant organisms. For example, the composition of the ursynapse and protosynapse (Emes and Grant, 2012; Emes et al., 2008; Ryan and Grant, 2009), as well as genetic components at the early origins of multicellularity (Brooke and Holland, 2003; Ruiz-Trillo et al., 2007), were previously inferred by comparing the gene repertoires of the distant metazoans with vertebrates and invertebrates (Brooke and Holland, 2003; Ruiz-Trillo et al., 2007). We primarily address the emerging roles of the adhesion GPCRs in vertebrate model organisms and compare the pre-bilaterian adhesion genes with a focus on the characteristics of N-terminal domains.

Cell–cell adhesion plays an integral role in synapse formation and development, synaptogenesis and synaptic plasticity (Bukalo and Dityatev, 2012; Dalva et al., 2007; Li and Sheng, 2003; Washbourne et al., 2004; Yamagata et al., 2003). The most extensively described cell adhesion molecules in synaptic transmission and formation are neurexins, neuroligins, cadherins and members of the immunoglobulin (Ig) superfamily (Craig and Kang, 2007; Dean and Dresbach, 2006; Lisé and El-Husseini, 2006; Scott and Palmer, 1993; Shapiro et al., 2007; Takeichi, 2007). Unlike the classical cell–cell adhesion molecules, the adhesion GPCRs are not characterised by their roles in regulation and/or development of the nervous system. However, in the past ten years, progress has been made in exploring the neurobiological roles of the adhesion GPCRs in mammals and other vertebrates. For example, among the nine adhesion GPCR subfamilies (see Fig. 2), subfamily I latrophilins are well known for their roles in synapse development and triggering neurotransmitter release. The presynaptic latrophilin 1 (LPHN1) interacts with the postsynaptic teneurin-2 (also known as Lasso, an endogenous ligand of latrophilin 1), forming a trans-synaptic complex and thus affecting synaptic development (Boucard et al., 2014; Silva et al., 2011). And, latrophilins interact with the postsynaptic fibronectin leucine-rich transmembrane (FLRT) family members. The ectodomains of the latrophilin 3 (LPHN3) and FLRT3 interact with high affinity in a ‘trans’ configuration and

regulate development of excitatory synapses (O'Sullivan et al., 2012). In addition, latrophilin-1interacts with the postsynaptic neurexin I-α and -β, forming a trans-synaptic complex and thus modulating synaptic functions (Boucard et al., 2012; O'Sullivan et al., 2014; Südhof, 2001).

The brain-specific angiogenesis inhibitor family (BAIs; subfamily VII adhesion GPCRs) members are well characterised for their roles in synaptogenesis, involving regulation of synapses and synaptic density (for a review, see Stephenson et al., 2014). Recently, Duman and colleagues provided evidence that BAI1 can regulate synaptogenesis by coupling with Rac1, mediated by a specific interaction and recruitment of the Par3/Tiam1 polarity proteins to postsynaptic sites (Duman et al., 2013). Also, the C-terminal region of BAI1 interacts with PDZ-domain-containing proteins, including MAGI-2 and MAGI-3 (Stephenson et al., 2013). PDZ domains are protein-interaction domains that are concentrated in the post-synaptic density and regulate synaptic transmission (Kim and Sheng, 2004). Furthermore, the N-termini of BAIs encodes thrombospondin type 1 repeats (TSRs), which regulate synaptogenesis and neural development (Adams and Tucker, 2000; Risher and Eroglu, 2012). These TRS repeats act as major interacting regions for BAI interaction partners, including the C1q-like proteins (Bolliger et al., 2011), which are highly expressed in the CNS and aid in synapse formation (Yuzaki, 2010). Gene knockout studies also suggest a role in synaptogenesis for other BAI family members (Jeong et al., 2006; Lanoue et al., 2013; Okajima et al., 2011).

Cadherin EGF LAG seven-pass G-type receptors (CELSRs), or alternatively, 7TM-cadherins/flamingo, constitute the subfamily III adhesion GPCRs and comprise large N-termini (~2500–3500 amino acids) containing cadherin, EGF and laminin G (LAG) domain repeats. CELSRs play a crucial role in nervous system development and planar cell polarity (Boutin et al., 2012; Feng et al., 2012; Tissir and Goffinet, 2006), and are also involved in axonal development and dendritic patterning (Berger-Müller and Suzuki, 2011; Tissir et al., 2005). The CELSR homologue in *Drosophila* was demonstrated to play a crucial role in synaptogenesis, as well as for the survival of axons and synapses (Bao et al., 2007). In humans, mutations in *CELSR1* lead to one of the most severe neural tube defects called craniorachischisis (Tissir and Goffinet, 2006). Moreover, knockout of CELSR2 and CELSR3 in mice leads to hydrocephalus (a lethal condition that accumulates excessive cerebrospinal fluid in the brain), suggesting that CELSR2 and CELSR3 are required for the development and function of ependymal cilia (Tissir et al., 2010). In addition, GPR56, which belongs to subfamily VIII is another adhesion GPCR that is involved in brain development (Singer et al., 2013). In humans, a mutation in *GPR56* causes bilateral frontoparietal polymicrogyria (BFPP) syndrome, a condition where the cerebral cortex is malformed (Piao et al., 2004). Also, very large G-protein-coupled receptor 1 (VLGR1, subfamily IX) is highly expressed during embryonal neurogenesis in mouse (McMillan et al., 2002).

Expansions of adhesion GPCRs in pre-bilaterian lineages

In comparison with their closest unicellular metazoan relatives, such as choanoflagellates and filastereans, the adhesion GPCRs expanded significantly in early metazoan lineages. Among all the pre-bilaterians, sponges, with a remarkable number of 72 genes, encode the largest repertoire of adhesion GPCRs. Similarly, *N. vectensis* and *T. adhaerens* harbour about 60 and 67 genes, respectively, whereas the ctenophore *M. leidyi* contains 26 genes coding for adhesion GPCRs (Fig. 1). It seems that adhesion GPCRs expanded significantly after the origin of metazoans (de Mendoza et al., 2014). One possible explanation is that expansion of adhesion GPCRs soon after the origin of metazoans was driven by the evolution of multicellularity, because cell–cell adhesion is one of the major factors involved in driving multicellularity (Abedin and King, 2010; Brooke and Holland, 2003; Müller, 2003; Ruiz-Trillo et al., 2007).

A BLAST search against the Swiss-Prot database using pre-bilaterian adhesion GPCRs as queries revealed that the ancient adhesion GPCRs are more similar to subfamilies I and VIII (Fig. 2.), which are associated with neurobiological roles in vertebrates (see above). Moreover, the VLGR1-like receptor (subfamily IX) is present in *N. vectensis* (Nordström et al., 2009) and a few receptors from *T. adhaerens* had their top hits as CELSRs (subfamily III) in the BLAST search. In an unpublished study, we attempted to classify these ancient adhesion GPCRs by performing a comparative phylogenetic analysis with the classified adhesion GPCRs from vertebrates. The unrooted topology obtained, based on the alignment of conserved seven transmembrane (7TM) regions, suggested that the pre-bilaterian adhesion GPCRs lack one-to-one orthologous relationships with known vertebrate homologues. However, we observed that a few sponge adhesion GPCRs are placed basal to human (used as a vertebrate representative) subfamily VIII adhesion GPCRs, and that a few adhesion GPCRs from *T. adhaerens*, *N. vectensis* and *A. queenslandica* are placed on the same node that clustered human subfamilies I and II. Although this phylogenetic clustering does not necessarily imply that these adhesion GPCRs have roles analogous to the mammalian homologues, it could be interesting to explore whether the ancestral Group I-, II- and VIII-like adhesion GPCRs in the pre-bilaterians are associated with neurobiological functions.

N-terminal domains of pre-bilaterian adhesions

One of the most notable features of the adhesion GPCRs is that each subfamily has a unique pattern of N-terminal domain architecture (Langenhan et al., 2013). This molecular signature is often used as a marker to distinguish between the subfamilies as well as to hypothesise the potential functional roles of the receptors (Schiöth et al., 2010). For instance, *N. vectensis* encodes an adhesion GPCR containing cadherin domains, EGF and laminin G (LAG) domain repeats similar to the bilaterian CELSRs (Fig. 3). However, *T. adhaerens*, which lacks nerve or sensory cells, contains a CELSR-like homologue, with its signature cadherin and LAG domain repeats, implying that these ancient CELSR-like adhesions possess multiple roles, depending upon the physiology and behaviour of the organism. *N. vectensis* also has a VLGR1-like homologue, with multiple calx-β repeats, EPTP and laminin_G_3 repeats in their N-termini, sharing high sequence similarity with the vertebrate VLGR1 receptors. Interestingly, among the pre-bilaterians analysed in this study, the sponge adhesion GPCRs encode the most varied domain architecture: at least 11 of these contain a hormone receptor domain (HRM), although no hormones have been found in sponges (Fig. 3). Furthermore, seven sponge adhesion GPCRs have immunoglobulin domain repeats, and another two contain multiple repeats of the SRCR domain (Fig. 3). To the best of our knowledge, SRCR repeats are not found in other GPCRs, whereas SRCR repeats are often found in proteins associated with immune system functions (Martínez et al., 2011; Sarrias et al., 2004). In contrast to the sponges and other pre-bilaterians, *M. leidyi* adhesion GPCRs are relatively limited in the number of N-terminal domains and the majority of receptors contain only the 7TM domain and the GPS site. However, domains such as pentaxin, epimerase and GCC2 are present, to name a few.

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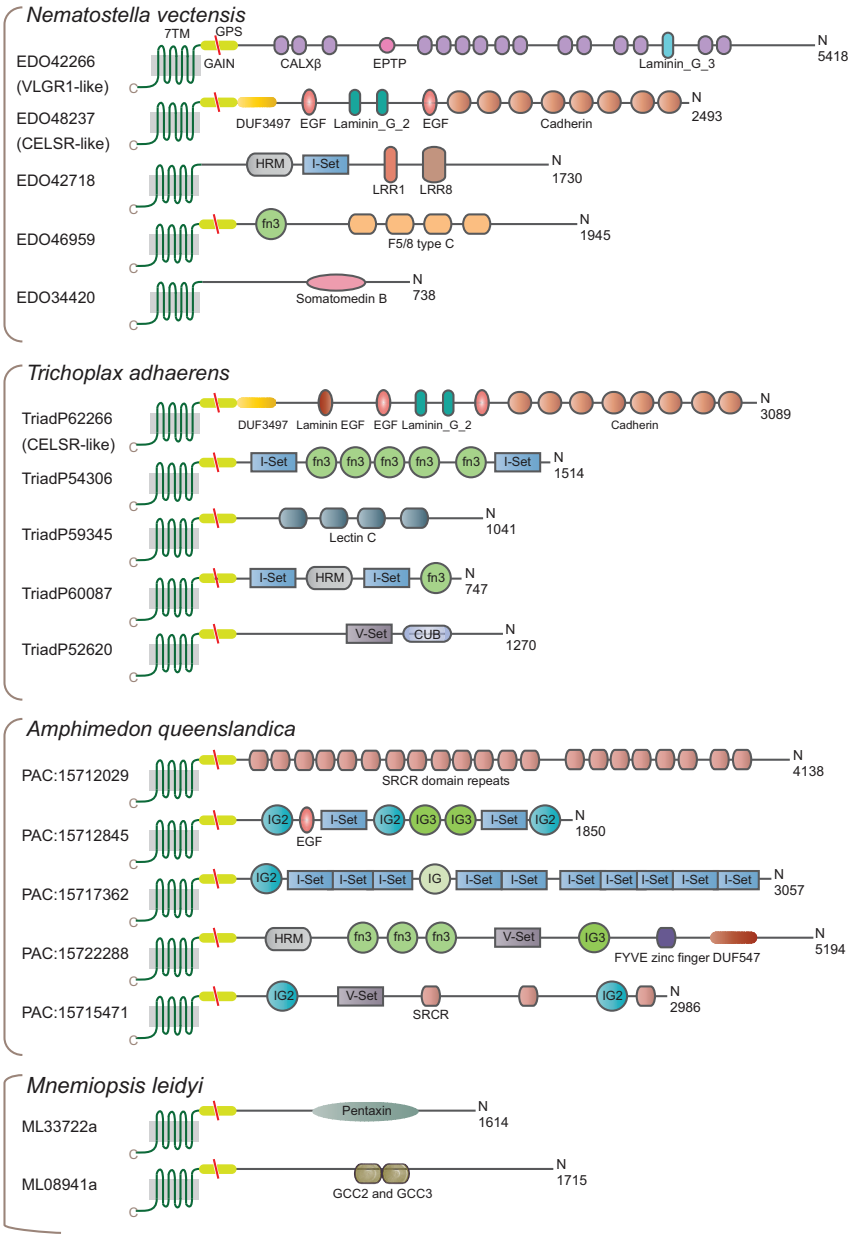

Fig. 3. Schematic drawing of domain organisation in representative adhesion GPCRs encoded in early metazoans. The illustration includes a selection of domains structures of adhesion GPCRs in pre-bilaterians. Each panel shows the intracellular C-terminal end, seven integral transmembrane helices embedded in the membrane and the long N-terminus with multiple functional domains. For display purposes, the length of the N-terminus of each panel does not correspond to a measured scale of amino acids and instead the overall length of the receptors was shown at the right corner of each panel. It must be noted that the presence of a GPCR autoproteolysis-inducing (GAIN) domain containing the GPCR-proteolytic site (GPS) in few receptors may not necessarily imply that the receptor undergoes autoproteolysis leading to a bipartite segmentation of adhesion GPCRs, into N-terminal and C-terminal fragments. The N-terminal domain organisations that are most similar to the known vertebrate adhesion GPCRs such as VLGR1 and CELSRs are found in both *N. vectensis* and *T. adhaerens* genomes. The domains shown in the figure include: 7TM, heptahelical transmembrane; cadherin domain; CALXβ, calx-beta domain; CUB domain; DUF3497, domain of unknown function 3479; DUF547; domain of unknown function 547; EGF, epidermal growth factor; EPTP, epitempin/epilepsy-associated repeat; F5/8 type C domain; fn3, fibronectin type III domain; FYVE zinc finger domain; GAIN, GPCR autoproteolysis-inducing domain; GCC2 and GCC3 domain; GPS, GPCR-proteolytic site; HRM, hormone receptor motif (HBD); IG_2/IG_3, immunoglobulin domains; I-Set; immunoglobulin I-set domain; laminin EGF domain; laminin_G_2 domain; Laminin_G_3 domain; Lectin C domain; LRR, leucine-rich repeat; pentraxin domain; somatomedin B domain; SRCR, scavenger receptor cysteine-rich domain; V-Set, immunoglobulin V-set domain.

Another important characteristic of adhesion GPCRs is the presence of a GPCR autoproteolysis-inducing (GAIN) domain located proximal to the first transmembrane helix (Fig. 3). The GAIN domain encompasses about 320 amino acids and contains the GPS (GPCR-proteolytic site) motif, a stretch of about 40 residues embedded within the large domain (Araç et al., 2012b). The GAIN domain mediates autoproteolytic events that trigger cleavage-based bipartite segmentation of adhesion GPCRs, which leads to the dissociation of the N-terminal fragment from the C-terminal fragment (Araç et al., 2012b). This intra-molecular processing at the GPS site in the GAIN domain is attributed to several factors, including signalling and membrane trafficking, as well as for the formation of heterodimeric GPCR complexes. The GAIN domain is evolutionarily ancient and is found in the amoebozoa *Dictyostelium discoideum*, the ciliate *Tetrahymena thermophila* and the choanoflagellate *Monosiga brevicollis* (Araç et al., 2012b). In pre-bilaterians, the GAIN domain seems to be present in about 50% of adhesion GPCRs, whereas the others lack a GAIN domain region, including the long N-terminus.

Single-pass membrane proteins – a possible interaction partner to adhesion GPCRs in pre-bilaterians?

Recent studies suggest that vertebrate family I and II adhesion GPCRs interact with post-synaptic single-pass transmembrane proteins. Therefore, we wanted to explore whether pre-bilaterians comprise homologues of single transmembrane helix post-synaptic proteins. Interestingly, a recent evolutionary analysis on teneurins, an endogenous ligand of latrophilins (see above), suggested that these single-pass transmembrane proteins, which are involved in neurogenesis, were absent in the genomes of sponges, cnidarians, placozoans and ctenophores (Tucker et al., 2012). This implies that the pre-bilaterian adhesion GPCRs that are similar to family I do not have ligands or interactions partners analogous to those in vertebrates. Surprisingly, the pre-synaptic neurexins that are well renowned for their role in synaptogenesis, are found in pre-bilaterians, including the cnidarians (Putnam et al., 2007; Riesgo et al., 2014), placozoans (Srivastava et al., 2008), sponges (Riesgo et al., 2014) and ctenophores (Moroz et al., 2014; Ryan et al., 2013).

Furthermore, to investigate whether putative homologues of fibronectin leucine-rich transmenbrane (FLRT) family are present in early metazoans, we performed a comprehensive search in the genomes of eumetazoans, placozoans, sponges and ctenophores. We identified several proteins with LRR repeats and a single transmembrane helix at the C-terminal end, but they are divergent from FLRT family members, indicating that the FLRT family is likely to be specific to bilaterians. Given that these early metazoans are rich in single-pass membrane proteins that constitute a substantial proportion of their membrane proteome, it would be interesting to explore whether these proteins interact with the adhesion GPCRs to aid cell-cell adhesion and communication affecting synapse formation and development.

Glutamate family of GPCRs in pre-bilaterians

GPCR-mediated glutamate signalling in early metazoans

In vertebrates, the glutamate family of receptors predominantly includes the metabotropic glutamate receptors (GRMs) and GABABRs, which are best characterised for their roles in modulation of synaptic transmission and neuronal excitability throughout the central nervous system (Niswender and Conn, 2010). Homologues of these neuromodulatory receptors are found in all metazoans, several pre-metazoan lineages, and also in the last eukaryotic common ancestor (LECA), suggesting that glutamate GPCRs have roles beyond their functions in a nervous system (de Mendoza et al., 2014; Krishnan et al., 2012). Although glutamate is largely known to be present in neurons and acts as the principal excitatory neurotransmitter, several lines of evidence suggest that glutamate and its receptors (including the GRMs) are present in a variety of non-excitable cells (for a review, see Nedergaard et al., 2002). For example, glutamate and GABA are both found in sponges that are often known to lack epithelial or sensory cells, or general tissue-level organisation. Early evidence for the presence of glutamate receptors in sponges was provided a decade ago when a metabotropic glutamate/GABA-like receptor was cloned from *Geodia cydonium* and was demonstrated to affect the concentrations of intracellular calcium upon activation by L-glutamate (Perovic et al., 1999). Recently, Elliott and Leys showed that glutamate triggers the contraction of the sponge canal system, whereas GABA inhibits glutamate-induced contraction, forming the synchronised behaviour of the incurrent and excurrent canal system of the demosponge *Ephydatia muelleri* (Elliott and Leys, 2010). Similarly, *Tethya wilhelma*, another demosponge, displayed contractions of its body column when exposed to GABA and L-glutamate (Ellwanger et al., 2007; Ellwanger and Nickel, 2006; Nickel, 2004). Sequencing of the *T. adhaerens* genome showed that this primitive metazoan encodes about 85 genes (76 were found in the latest Ensembl release) coding for glutamate family GPCRs, including a large expansion of GRMs (Srivastava et al., 2008), but the functions of these putative glutamate family receptors are mostly unknown. Several key enzymes, including glutamate dehydrogenase and glutaminase, which are involved in the synthesis of glutamate, as well as the glutamate decarboxylase, which synthesises GABA, are present in *T. adhaerens*. Thus it is conceivable that GRM-like receptors and GABAB-like receptors could be functioning as glutamate and GABA receptors in *T. adhaerens*, modulating the physiology and behaviour of the organism.

Several studies have provided biochemical evidence for the presence of glutamate and GABA, and their receptors in cnidarians (for a review, see Kass-Simon and Pierobon, 2007). More recently, immunohistochemistry studies have demonstrated that glutamate and GABA exist in the nerve plexus within the tentacles of a sea anemone *Phymactis papillosa*, suggesting that glutamate and GABA function as neuronal signalling molecules in the primitive nervous system of cnidarians (Delgado et al., 2010). Furthermore, GABA receptors modulate the discharge of nematocysts in hydra (Scappaticci et al., 2010; Scappaticci and Kass-Simon, 2008). Nematocysts, which are also called cnidae, are sophisticated stinging cells found in all cnidarians and are required for prey capture and defence mechanisms (Beckmann and Özbek, 2012). This suggests that the nematocyst discharge, induced by GABA- mediated neurotransmission in the hydra nervous system, could be required for prey capture and that this mechanism could also exist in other cnidarians. In contrast to the cnidarians, the role of glutamate GPCRs in ctenophores remains obscure. However, recent whole-genome analysis of the ctenophore *P. bachei* provided evidence that the comb jelly has eight genes coding for glutaminases and that L-glutamate acts as a neurotransmitter to induce muscle contractions (Moroz et al., 2014). It must be mentioned that L-glutamate has a role in inducing contractions of the *E. muelleri* canal system, and that it also affects the concentrations of intracellular calcium in *G. cydonium* (as discussed above). This suggests that glutamate and its receptors in pre-bilaterians are capable of inducing similar effects in both neuron-rich and neuron-free environments. Also noteworthy is that receptors such as kainate receptors (kainateGluR), AMPA receptors (AMPAGluR) and NMDA receptors, which belong to ionotropic glutamate family receptors (iGluRs), are present in early metazoans (Burkhardt et al., 2014). Specifically, kainate and AMPA receptors both have early metazoan origins and are present in *T. adhaerens* and *N. vectensis*, whereas NMDA receptors probably emerged in an early eumetazoan ancestor (Burkhardt et al., 2014). However, ctenophores contain a diversified set of iGluRs, because they seem to have undergone lineage-specific adaptations with unique exon and intron organisation (Moroz et al., 2014). Taken together, it seems evident that glutamate acts as a signalling molecule in early metazoans, and that it may induce diverse intracellular responses through a variety of receptors, depending on the morphological characteristics and environmental adaptations of the organism.

Conclusions and perspectives

In this review, we highlight the large expansion of GPCR gene families before the divergence of bilateria. Comparative genomics reveals that the molecular components required for GPCR-mediated neurotransmission exist in organisms with a primitive nervous system, as well as in organisms that lack nerve cells or a nervous system. This includes some of the important enzymes required for the synthesis of neurotransmitters and neuropeptides, although these species do not comprise all the genes required for complete pathways as they appear in mammals. Genomic comparisons also demonstrate that adhesion GPCRs first expanded at the early origins of metazoans. Until recently, the roles of adhesion GPCRs were unclear; however, an emerging view suggests that they play a role as cell–cell adhesion molecules in the developing nervous system, which guide synaptic formation, neuronal patterning, and plasticity in vertebrates. Thus, an interesting question is whether pre-bilaterian adhesion GPCRs mediate cell–cell adhesion and have a possible role in the origins of synapses and synapse formation. Taken together, it is conceivable that some of the basic molecular components required for GPCR-mediated neurotransmission are an ancestral feature and during the course of metazoan evolution these genetic components have facilitated the evolution of a nervous system along with several other neuronal gene families. However, because the most recent hypothesis supports multiple origins of the neural systems, it is

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important to understand the functions of GPCRs and other neuronal gene families in *T. adhaerens* and *A. queenslandica*, which lack a nervous system. Progress on this front will require expanded comparisons of genomes with robust phylogenetic studies to analyse these GPCR families, as well as several other crucial elements, including all presynaptic and post-synaptic proteins. Important questions remain. For example, do GPCRs in sponges and placozoans share orthologous relationships with the GPCRs found in cnidarians and ctenophores? Are GPCRs in sponges and placozoans capable of inducing intracellular responses in non-excitable cells similar to those in excitable cells? What are the functional implications of the expansions of GPCRs in sponges, placozoans, cnidarians and ctenophores? Overall, the genomic machinery required for neuronal functioning in pre-bilaterians provides a great platform for further comparative genomics and functional studies, which aim to delineate the key evolutionary events that shaped the first nervous system in early metazoans.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

A.K. wrote the initial and complete drafts of the manuscript. A.K. performed all required revisions of the manuscript. H.B.S. contributed in editing and finalising the manuscript. Both authors read and approved the final manuscript.

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References

Abedin, M. and King, N. (2010). Diverse evolutionary paths to cell adhesion. *Trends Cell Biol.* **20**, 734-742.

Adams, J. C. and Tucker, R. P. (2000). The thrombospondin type 1 repeat (TSR) superfamily: diverse proteins with related roles in neuronal development. *Dev. Dyn.* **218**, 280-299.

Anctil, M. (2009). Chemical transmission in the sea anemone *Nematostella vectensis*: A genomic perspective. *Comp. Biochem. Physiol.* **4D**, 268-289.

Araç, D., Aust, G., Calebiro, D., Engel, F. B., Formstone, C., Goffinet, A., Hamann, J., Kittel, R. J., Liebscher, I., Lin, H. H. et al. (2012a). Dissecting signaling and functions of adhesion G protein-coupled receptors. *Ann. N. Y. Acad. Sci.* **1276**, 1-25.

Araç, D., Boucard, A. A., Bolliger, M. F., Nguyen, J., Soltis, S. M., Südhof, T. C. and Brunger, A. T. (2012b). A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *EMBO J.* **31**, 1364-1378.

Bao, H., Berlanga, M. L., Xue, M., Hapip, S. M., Daniels, R. W., Mendenhall, J. M., Alcantara, A. A. and Zhang, B. (2007). The atypical cadherin flamingo regulates synaptogenesis and helps prevent axonal and synaptic degeneration in *Drosophila*. *Mol. Cell. Neurosci.* **34**, 662-678.

Beckmann, A. and Özbek, S. (2012). The nematocyst: a molecular map of the cnidarian stinging organelle. *Int. J. Dev. Biol.* **56**, 577-582.

Berger-Müller, S. and Suzuki, T. (2011). Seven-pass transmembrane cadherins: roles and emerging mechanisms in axonal and dendritic patterning. *Mol. Neurobiol.* **44**, 313-320.

Bolliger, M. F., Martinelli, D. C. and Südhof, T. C. (2011). The cell-adhesion G protein-coupled receptor BAI3 is a high-affinity receptor for C1q-like proteins. *Proc. Natl. Acad. Sci. USA* **108**, 2534-2539.

Boucard, A. A., Ko, J. and Südhof, T. C. (2012). High affinity neurexin binding to cell adhesion G-protein-coupled receptor CIRL1/latrophilin-1 produces an intercellular adhesion complex. *J. Biol. Chem.* **287**, 9399-9413.

Boucard, A. A., Maxeiner, S. and Südhof, T. C. (2014). Latrophilins function as heterophilic cell-adhesion molecules by binding to teneurins: regulation by alternative splicing. *J. Biol. Chem.* **289**, 387-402.

Boutin, C., Goffinet, A. M. and Tissir, F. (2012). Celsr1-3 cadherins in PCP and brain development. *Curr. Top. Dev. Biol.* **101**, 161-183.

Brooke, N. M. and Holland, P. W. (2003). The evolution of multicellularity and early animal genomes. *Curr. Opin. Genet. Dev.* **13**, 599-603.

Bukalo, O. and Dityatev, A. (2012). Synaptic cell adhesion molecules. *Adv. Exp. Med. Biol.* **970**, 97-128.

Burkhardt, P., Grønborg, M., McDonald, K., Sulur, T., Wang, Q. and King, N. (2014). Evolutionary insights into premetazoan functions of the neuronal protein homer. *Mol. Biol. Evol.* **31**, 2342-2355.

Cartmell, J. and Schoepp, D. D. (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *J. Neurochem.* **75**, 889-907.

Churcher, A. M. and Taylor, J. S. (2011). The antiquity of chordate odorant receptors is revealed by the discovery of orthologs in the cnidarian *Nematostella vectensis*. *Genome Biol. Evol.* **3**, 36-43.

Civelli, O., Reinscheid, R. K., Zhang, Y., Wang, Z., Fredriksson, R. and Schiöth, H. B. (2013). G protein-coupled receptor deorphanizations. *Annu. Rev. Pharmacol. Toxicol.* **53**, 127-146.

Collingridge, G. L., Isaac, J. T. and Wang, Y. T. (2004). Receptor trafficking and synaptic plasticity. *Nat. Rev. Neurosci.* **5**, 952-962.

Craig, A. M. and Kang, Y. (2007). Neurexin-neuroligin signaling in synapse development. *Curr. Opin. Neurobiol.* **17**, 43-52.

Dalva, M. B., McClelland, A. C. and Kayser, M. S. (2007). Cell adhesion molecules: signalling functions at the synapse. *Nat. Rev. Neurosci.* **8**, 206-220.

de Mendoza, A., Sebé-Pedrós, A. and Ruiz-Trillo, I. (2014). The evolution of the GPCR signaling system in eukaryotes: modularity, conservation, and the transition to metazoan multicellularity. *Genome Biol. Evol.* **6**, 606-619.

Dean, C. and Dresbach, T. (2006). Neuroligins and neurexins: linking cell adhesion, synapse formation and cognitive function. *Trends Neurosci.* **29**, 21-29.

Degnan, B. M., Adamska, M., Craigie, A., Degnan, S. M., Fahey, B., Gauthier, M., Hooper, J. N., Larroux, C., Leys, S. P., Lovas, E. et al. (2008). The Demosponge *Amphimedon queenslandica*: reconstructing the Ancestral Metazoan Genome and deciphering the origin of animal multicellularity. *Cold Spring Harb. Protoc.* **2008**, pdb emo108.

Delgado, L. M., Couve, E. and Schmachtenberg, O. (2010). GABA and glutamate immunoreactivity in tentacles of the sea anemone *Phymactis papillosa* (LESSON 1830). *J. Morphol.* **271**, 845-852.

Duman, J. G., Tzeng, C. P., Tu, Y. K., Munjal, T., Schwechter, B., Ho, T. S. and Tolias, K. F. (2013). The adhesion-GPCR BAI1 regulates synaptogenesis by controlling the recruitment of the Par3/Tiam1 polarity complex to synaptic sites. *J. Neurosci.* **33**, 6964-6978.

Elliott, G. R. and Leys, S. P. (2010). Evidence for glutamate, GABA and NO in coordinating behaviour in the sponge, *Ephydatia muelleri* (Demospongiae, Spongillidae). *J. Exp. Biol.* **213**, 2310-2321.

Ellwanger, K. and Nickel, M. (2006). Neuroactive substances specifically modulate rhythmic body contractions in the nerveless metazoon *Tethya wilhelma* (Demospongiae, Porifera). *Front. Zool.* **3**, 7.

Ellwanger, K., Eich, A. and Nickel, M. (2007). GABA and glutamate specifically induce contractions in the sponge *Tethya wilhelma*. *J. Comp. Physiol. A* **193**, 1-11.

Emes, R. D. and Grant, S. G. (2012). Evolution of synapse complexity and diversity. *Annu. Rev. Neurosci.* **35**, 111-131.

Emes, R. D., Pocklington, A. J., Anderson, C. N., Bayes, A., Collins, M. O., Vickers, C. A., Croning, M. D., Malik, B. R., Choudhary, J. S., Armstrong, J. D. et al. (2008). Evolutionary expansion and anatomical specialization of synapse proteome complexity. *Nat. Neurosci.* **11**, 799-806.

Ender, A. and Schierwater, B. (2003). Placozoa are not derived cnidarians: evidence from molecular morphology. *Mol. Biol. Evol.* **20**, 130-134.

Fahey, B. and Degnan, B. M. (2010). Origin of animal epithelia: insights from the sponge genome. *Evol. Dev.* **12**, 601-617.

Feng, J., Han, Q. and Zhou, L. (2012). Planar cell polarity genes, Celsr1-3, in neural development. *Neurosci. Bull.* **28**, 309-315.

Ferraguti, F. and Shigemoto, R. (2006). Metabotropic glutamate receptors. *Cell Tissue Res.* **326**, 483-504.

Fredriksson, R., Lagerström, M. C., Lundin, L. G. and Schiöth, H. B. (2003). The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol. Pharmacol.* **63**, 1256-1272.

Fujisawa, T. (2008). Hydra peptide project 1993-2007. *Dev. Growth Differ.* **50 Suppl. 1**, S257-S268.

Grimmelikhuijzen, C. J., Leviev, I. and Carstensen, K. (1996). Peptides in the nervous systems of cnidarians: structure, function, and biosynthesis. *Int. Rev. Cytol.* **167**, 37-89.

Hayakawa, E., Takahashi, T., Nishimiya-Fujisawa, C. and Fujisawa, T. (2007). A novel neuropeptide (FRamide) family identified by a peptidomic approach in *Hydra magnipapillata*. *FEBS J.* **274**, 5438-5448.

Hnasko, T. S. and Edwards, R. H. (2012). Neurotransmitter corelease: mechanism and physiological role. *Annu. Rev. Physiol.* **74**, 225-243.

Holland, L. Z., Carvalho, J. E., Escriva, H., Laudet, V., Schubert, M., Shimeld, S. M. and Yu, J. K. (2013). Evolution of bilaterian central nervous systems: a single origin? *Evodevo* **4**, 27.

Jager, M., Chiori, R., Alié, A., Dayraud, C., Quéinnec, E. and Manuel, M. (2011). New insights on ctenophore neural anatomy: immunofluorescence study in *Pleurobrachia pileus* (Müller, 1776). *J. Exp. Zool. B* **316**, 171-187.

Jékely, G. (2013). Global view of the evolution and diversity of metazoan neuropeptide signaling. *Proc. Natl. Acad. Sci. USA* **110**, 8702-8707.

Jeong, B. C., Kim, M. Y., Lee, J. H., Kee, H. J., Kho, D. H., Han, K. E., Qian, Y. R., Kim, J. K. and Kim, K. K. (2006). Brain-specific angiogenesis inhibitor 2 regulates VEGF through GABP that acts as a transcriptional repressor. *FEBS Lett.* **580**, 669-676.

Jeong, S. J., Luo, R., Li, S., Strokes, N. and Piao, X. (2012). Characterization of G protein-coupled receptor 56 protein expression in the mouse developing neocortex. *J. Comp. Neurol.* **520**, 2930-2940.

Kass-Simon, G. and Pierobon, P. (2007). Cnidarian chemical neurotransmission, an updated overview. *Comp. Biochem. Physiol.* **146A**, 9-25.
Kass-Simon, G., Pannaccione, A. and Pierobon, P. (2003). GABA and glutamate receptors are involved in modulating pacemaker activity in hydra. *Comp. Biochem. Physiol.* **136A**, 329-342.
Katritch, V., Cherezov, V. and Stevens, R. C. (2013). Structure-function of the G protein-coupled receptor superfamily. *Annu. Rev. Pharmacol. Toxicol.* **53**, 531-556.
Kim, E. and Sheng, M. (2004). PDZ domain proteins of synapses. *Nat. Rev. Neurosci.* **5**, 771-781.
Krishnan, A., Almén, M. S., Fredriksson, R. and Schiöth, H. B. (2012). The origin of GPCRs: identification of mammalian like Rhodopsin, Adhesion, Glutamate and Frizzled GPCRs in fungi. *PLoS ONE* **7**, e29817.
Lagerström, M. C. and Schiöth, H. B. (2008). Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discov.* **7**, 339-357.
Langenhan, T., Aust, G. and Hamann, J. (2013). Sticky signaling–adhesion class G protein-coupled receptors take the stage. *Sci. Signal.* **6**, re3.
Lanoue, V., Usardi, A., Sigoillot, S. M., Talleur, M., Iyer, K., Mariani, J., Isope, P., Vodjdani, G., Heintz, N. and Selimi, F. (2013). The adhesion-GPCR BAI3, a gene linked to psychiatric disorders, regulates dendrite morphogenesis in neurons. *Mol. Psychiatry* **18**, 943-950.
Leys, S. P. and Hill, A. (2012). The physiology and molecular biology of sponge tissues. *Adv. Mar. Biol.* **62**, 1-56.
Li, Z. and Sheng, M. (2003). Some assembly required: the development of neuronal synapses. *Nat. Rev. Mol. Cell Biol.* **4**, 833-841.
Lisé, M. F. and El-Husseini, A. (2006). The neuroligin and neurexin families: from structure to function at the synapse. *Cell. Mol. Life Sci.* **63**, 1833-1849.
Ludeman, D. A., Farrar, N., Riesgo, A., Paps, J. and Leys, S. P. (2014). Evolutionary origins of sensation in metazoans: functional evidence for a new sensory organ in sponges. *BMC Evol. Biol.* **14**, 3.
Luo, R., Jeong, S. J., Jin, Z., Strokes, N., Li, S. and Piao, X. (2011). G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. *Proc. Natl. Acad. Sci. USA* **108**, 12925-12930.
Marlow, H. Q., Srivastava, M., Matus, D. Q., Rokhsar, D. and Martindale, M. Q. (2009). Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev. Neurobiol.* **69**, 235-254.
Martínez, V. G., Moestrup, S. K., Holmskov, U., Mollenhauer, J. and Lozano, F. (2011). The conserved scavenger receptor cysteine-rich superfamily in therapy and diagnosis. *Pharmacol. Rev.* **63**, 967-1000.
McCauley, D. W. (1997). Serotonin plays an early role in the metamorphosis of the hydrozoan *Phialidium gregarium*. *Dev. Biol.* **190**, 229-240.
McMillan, D. R., Kayes-Wandover, K. M., Richardson, J. A. and White, P. C. (2002). Very large G protein-coupled receptor-1, the largest known cell surface protein, is highly expressed in the developing central nervous system. *J. Biol. Chem.* **277**, 785-792.
Miller, D. J. and Ball, E. E. (2005). Animal evolution: the enigmatic phylum placozoa revisited. *Curr. Biol.* **15**, R26-R28.
Moroz, L. L., Kocot, K. M., Citarella, M. R., Dosung, S., Norekian, T. P., Povolotskaya, I. S., Grigorenko, A. P., Dailey, C., Berezikov, E., Buckley, K. M. et al. (2014). The ctenophore genome and the evolutionary origins of neural systems. *Nature* **510**, 109-114.
Müller, W. E. (2003). The origin of metazoan complexity: porifera as integrated animals. *Integr. Comp. Biol.* **43**, 3-10.
Nakanishi, N., Renfer, E., Technau, U. and Rentzsch, F. (2012). Nervous systems of the sea anemone *Nematostella vectensis* are generated by ectoderm and endoderm and shaped by distinct mechanisms. *Development* **139**, 347-357.
Nedergaard, M., Takano, T. and Hansen, A. J. (2002). Beyond the role of glutamate as a neurotransmitter. *Nat. Rev. Neurosci.* **3**, 748-755.
Nickel, M. (2004). Kinetics and rhythm of body contractions in the sponge *Tethya wilhelma* (Porifera: Demospongiae). *J. Exp. Biol.* **207**, 4515-4524.
Niimura, Y. (2009). On the origin and evolution of vertebrate olfactory receptor genes: comparative genome analysis among 23 chordate species. *Genome Biol. Evol.* **1**, 34-44.
Nikitin, M. (2014). Bioinformatic prediction of *Trichoplax adhaerens* regulatory peptides. *Gen. Comp. Endocrinol.* doi:10.1016/j.ygcen.2014.03.049.
Niswender, C. M. and Conn, P. J. (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* **50**, 295-322.
Nordström, K. J., Lagerström, M. C., Wallér, L. M., Fredriksson, R. and Schiöth, H. B. (2009). The Secretin GPCRs descended from the family of Adhesion GPCRs. *Mol. Biol. Evol.* **26**, 71-84.
Nordström, K. J., Sällman Almén, M., Edstam, M. M., Fredriksson, R. and Schiöth, H. B. (2011). Independent HHsearch, Needleman–Wunsch-based, and motif analyses reveal the overall hierarchy for most of the G protein-coupled receptor families. *Mol. Biol. Evol.* **28**, 2471-2480.
Nosenko, T., Schreiber, F., Adamska, M., Adamski, M., Eitel, M., Hammel, J., Maldonado, M., Müller, W. E., Nickel, M., Schierwater, B. et al. (2013). Deep metazoan phylogeny: when different genes tell different stories. *Mol. Phylogenet. Evol.* **67**, 223-233.
O'Sullivan, M. L., de Wit, J., Savas, J. N., Comoletti, D., Otto-Hitt, S., Yates, J. R., III and Ghosh, A. (2012). FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development. *Neuron* **73**, 903-910.
O'Sullivan, M. L., Martini, F., von Daake, S., Comoletti, D. and Ghosh, A. (2014). LPHN3, a presynaptic adhesion-GPCR implicated in ADHD, regulates the strength of neocortical layer 2/3 synaptic input to layer 5. *Neural Dev.* **9**, 7.
Okajima, D., Kudo, G. and Yokota, H. (2011). Antidepressant-like behavior in brain-specific angiogenesis inhibitor 2-deficient mice. *J. Physiol. Sci.* **61**, 47-54.
Patel, N. H. (2012). Evolutionary crossroads in developmental biology. *Development* **139**, 2637-2638.
Perovic, S., Krasko, A., Prokic, I., Müller, I. M. and Müller, W. E. (1999). Origin of neuronal-like receptors in Metazoa: cloning of a metabotropic glutamate/GABA-like receptor from the marine sponge *Geodia cydonium*. *Cell Tissue Res.* **296**, 395-404.
Piao, X., Hill, R. S., Bodell, A., Chang, B. S., Basel-Vanagaite, L., Straussberg, R., Dobyns, W. B., Qasrawi, B., Winter, R. M., Innes, A. M. et al. (2004). G protein-coupled receptor-dependent development of human frontal cortex. *Science* **303**, 2033-2036.
Pinheiro, P. S. and Mulle, C. (2008). Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nat. Rev. Neurosci.* **9**, 423-436.
Putnam, N. H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V. V. et al. (2007). Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317**, 86-94.
Richter, D. J. and King, N. (2013). The genomic and cellular foundations of animal origins. *Annu. Rev. Genet.* **47**, 509-537.
Riesgo, A., Farrar, N., Windsor, P. J., Giribet, G. and Leys, S. P. (2014). The analysis of eight transcriptomes from all poriferan classes reveals surprising genetic complexity in sponges. *Mol. Biol. Evol.* **31**, 1102-1120.
Risher, W. C. and Eroglu, C. (2012). Thrombospondins as key regulators of synaptogenesis in the central nervous system. *Matrix Biol.* **31**, 170-177.
Rosenbaum, D. M., Rasmussen, S. G. and Kobilka, B. K. (2009). The structure and function of G-protein-coupled receptors. *Nature* **459**, 356-363.
Ruggieri, R. D., Pierobon, P. and Kass-Simon, G. (2004). Pacemaker activity in hydra is modulated by glycine receptor ligands. *Comp. Biochem. Physiol.* **138A**, 193-202.
Ruiz-Trillo, I., Burger, G., Holland, P. W., King, N., Lang, B. F., Roger, A. J. and Gray, M. W. (2007). The origins of multicellularity: a multi-taxon genome initiative. *Trends Genet.* **23**, 113-118.
Ryan, T. J. and Grant, S. G. (2009). The origin and evolution of synapses. *Nat. Rev. Neurosci.* **10**, 701-712.
Ryan, J. F., Pang, K., Schnitzler, C. E., Nguyen, A. D., Moreland, R. T., Simmons, D. K., Koch, B. J., Francis, W. R., Havlak, P., Smith, S. A. et al.; NISC Comparative Sequencing Program (2013). The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* **342**, 1242592.
Salmoun, M., Devijver, C., Daloze, D., Braekman, J. C. and van Soest, R. W. (2002). 5-hydroxytryptamine-derived alkaloids from two marine sponges of the genus *Hyrtios*. *J. Nat. Prod.* **65**, 1173-1176.
Sarrias, M. R., Grønlund, J., Padilla, O., Madsen, J., Holmskov, U. and Lozano, F. (2004). The Scavenger Receptor Cysteine-Rich (SRCR) domain: an ancient and highly conserved protein module of the innate immune system. *Crit. Rev. Immunol.* **24**, 1-38.
Scappaticci, A. A. and Kass-Simon, G. (2008). NMDA and GABA B receptors are involved in controlling nematocyst discharge in hydra. *Comp. Biochem. Physiol.* **150A**, 415-422.
Scappaticci, A. A., Jr, Kahn, F. and Kass-Simon, G. (2010). Nematocyst discharge in *Hydra vulgaris*: Differential responses of desmonemes and stenoteles to mechanical and chemical stimulation. *Comp. Biochem. Physiol.* **157A**, 184-191.
Schierwater, B. (2005). My favorite animal, *Trichoplax adhaerens*. *BioEssays* **27**, 1294-1302.
Schiöth, H. B., Nordström, K. J. and Fredriksson, R. (2010). The adhesion GPCRs; gene repertoire, phylogeny and evolution. *Adv. Exp. Med. Biol.* **706**, 1-13.
Schoepp, D. D. (2001). Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther.* **299**, 12-20.
Scott, J. A. and Palmer, E. L. (1993). Neural network analysis of ventilation-perfusion lung scans. *Radiology* **186**, 661-664.
Shapiro, L., Love, J. and Colman, D. R. (2007). Adhesion molecules in the nervous system: structural insights into function and diversity. *Annu. Rev. Neurosci.* **30**, 451-474.
Shoichet, B. K. and Kobilka, B. K. (2012). Structure-based drug screening for G-protein-coupled receptors. *Trends Pharmacol. Sci.* **33**, 268-272.
Silva, J. P., Lelianova, V. G., Ermolyuk, Y. S., Vysokov, N., Hitchen, P. G., Berninghausen, O., Rahman, M. A., Zangrandi, A., Fidalgo, S., Tonevitsky, A. G. et al. (2011). Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities. *Proc. Natl. Acad. Sci. USA* **108**, 12113-12118.
Singer, K., Luo, R., Jeong, S. J. and Piao, X. (2013). GPR56 and the developing cerebral cortex: cells, matrix, and neuronal migration. *Mol. Neurobiol.* **47**, 186-196.
Srivastava, M., Begovic, E., Chapman, J., Putnam, N. H., Hellsten, U., Kawashima, T., Kuo, A., Mitros, T., Salamov, A., Carpenter, M. L. et al. (2008). The *Trichoplax* genome and the nature of placozoans. *Nature* **454**, 955-960.
Srivastava, M., Simakov, O., Chapman, J., Fahey, B., Gauthier, M. E., Mitros, T., Richards, G. S., Conaco, C., Dacre, M., Hellsten, U. et al. (2010). The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature* **466**, 720-726.
Stephenson, J. R., Paavola, K. J., Schaefer, S. A., Kaur, B., Van Meir, E. G. and Hall, R. A. (2013). Brain-specific angiogenesis inhibitor-1 signaling, regulation, and enrichment in the postsynaptic density. *J. Biol. Chem.* **288**, 22248-22256.
Stephenson, J. R., Purcell, R. H. and Hall, R. A. (2014). The BAI subfamily of adhesion GPCRs: synaptic regulation and beyond. *Trends Pharmacol. Sci.* **35**, 208-215.

Strokes, N. and Piao, X. (2010). Adhesion-GPCRs in the CNS. *Adv. Exp. Med. Biol.* **706**, 87-97.
Südhof, T. C. (2001). alpha-Latrotoxin and its receptors: neurexins and CIRL/latrophilins. *Annu. Rev. Neurosci.* **24**, 933-962.
Takahashi, T. (2013). Neuropeptides and epitheliopeptides: structural and functional diversity in an ancestral metazoan Hydra. *Protein Pept. Lett.* **20**, 671-680.
Takahashi, T., Kobayakawa, Y., Muneoka, Y., Fujisawa, Y., Mohri, S., Hatta, M., Shimizu, H., Fujisawa, T., Sugiyama, T., Takahara, M. et al. (2003). Identification of a new member of the GLWamide peptide family: physiological activity and cellular localization in cnidarian polyps. *Comp. Biochem. Physiol.* **135B**, 309-324.
Takeda, N., Nakajima, Y., Koizumi, O., Fujisawa, T., Takahashi, T., Matsumoto, M. and Deguchi, R. (2013). Neuropeptides trigger oocyte maturation and subsequent spawning in the hydrozoan jellyfish *Cytaeis uchidae*. *Mol. Reprod. Dev.* **80**, 223-232.
Takeichi, M. (2007). The cadherin superfamily in neuronal connections and interactions. *Nat. Rev. Neurosci.* **8**, 11-20.
Technau, U. and Steele, R. E. (2011). Evolutionary crossroads in developmental biology: Cnidaria. *Development* **138**, 1447-1458.
Tissir, F. and Goffinet, A. M. (2006). Expression of planar cell polarity genes during development of the mouse CNS. *Eur. J. Neurosci.* **23**, 597-607.
Tissir, F., Bar, I., Jossin, Y., De Backer, O. and Goffinet, A. M. (2005). Protocadherin Celsr3 is crucial in axonal tract development. *Nat. Neurosci.* **8**, 451-457.
Tissir, F., Qu, Y., Montcouquiol, M., Zhou, L., Komatsu, K., Shi, D., Fujimori, T., Labeau, J., Tyteca, D., Courtoy, P. et al. (2010). Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. *Nat. Neurosci.* **13**, 700-707.
Tucker, R. P., Beckmann, J., Leachman, N. T., Schöler, J. and Chiquet-Ehrismann, R. (2012). Phylogenetic analysis of the teneurins: conserved features and premetazoan ancestry. *Mol. Biol. Evol.* **29**, 1019-1029.
Washbourne, P., Dityatev, A., Scheiffele, P., Biederer, T., Weiner, J. A., Christopherson, K. S. and El-Husseini, A. (2004). Cell adhesion molecules in synapse formation. *J. Neurosci.* **24**, 9244-9249.
Watanabe, H., Fujisawa, T. and Holstein, T. W. (2009). Cnidarians and the evolutionary origin of the nervous system. *Dev. Growth Differ.* **51**, 167-183.
Yamagata, M., Sanes, J. R. and Weiner, J. A. (2003). Synaptic adhesion molecules. *Curr. Opin. Cell Biol.* **15**, 621-632.
Yum, S., Takahashi, T., Koizumi, O., Ariura, Y., Kobayakawa, Y., Mohri, S. and Fujisawa, T. (1998). A novel neuropeptide, Hym-176, induces contraction of the ectodermal muscle in Hydra. *Biochem. Biophys. Res. Commun.* **248**, 584-590.
Yuzaki, M. (2010). Synapse formation and maintenance by C1q family proteins: a new class of secreted synapse organizers. *Eur. J. Neurosci.* **32**, 191-197.
Zega, G., Pennati, R., Fanzago, A. and De Bernardi, F. (2007). Serotonin involvement in the metamorphosis of the hydroid *Eudendrium racemosum*. *Int. J. Dev. Biol.* **51**, 307-313.