# Evolutionary origin of synapses and neurons – Bridging the gap

*Pawel Burkhardt*[1)*] *and Simon G. Sprecher*[2)*]

**The evolutionary origin of synapses and neurons is an enigmatic subject that inspires much debate. Non-bilaterian metazoans, both with and without neurons and their closest relatives already contain many components of the molecular toolkits for synapse functions. The origin of these components and their assembly into ancient synaptic signaling machineries are particularly important in light of recent findings on the phylogeny of non-bilaterian metazoans. The evolution of synapses and neurons are often discussed only from a metazoan perspective leaving a considerable gap in our understanding. By taking an integrative approach we highlight the need to consider different, but extremely relevant phyla and to include the closest unicellular relatives of metazoans, the ichthyosporeans, filastereans and choanoflagellates, to fully understand the evolutionary origin of synapses and neurons. This approach allows for a detailed understanding of when and how the first pre- and postsynaptic signaling machineries evolved.**

**Keywords:**
evolution; neuron; origin; protein–protein interactions; synapse

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**Abbreviations:**
**CamKII,** calcium/calmodulin-dependent protein kinase II; **CASK,** calcium/calmodulin dependent serine protein kinase; **Dlg,** discs large; **Erc/Cast,** ELKS/RAB6-interacting/CAST family member; **FMRFamide,** Phe-Met-Arg-Phe amide; **GKAP,** guanylate kinase-associated protein; **Munc13,** mammalian uncoordinated-13; **Munc18,** mammalian uncoordinated-18; **PSD-95,** post synaptic density protein 95; **Shank,** SH3 and multiple ankyrin repeat domains protein; **SNAREs,** soluble N-ethylmaleimide-sensitive-factor attachment receptors.

## Exciting times for the debate about the evolutionary origin of neurons

> "Ideas about invertebrate phylogeny are often presented as though they were widely agreed-upon theories or, worse yet, as though alternative ideas did not even exist"
>
> Brusca & Brusca [1]

Nervous systems within the metazoan kingdom are surprisingly diverse both in cell number and functional complexity. The nervous system of the nematode *Caenorhabditis elegans* consists of only 302 neurons while the brains of mammals, including humans, are comprised of multiple billions of neural cells. But also the diversity of neuron types within the nervous system is striking making "neuron" likely the most diverse cell type existing [2–3]. Distinct neuron types are defined for instance by the neurotransmitter or neuropeptide they use, their morphological and anatomical properties, whether they receive sensory input or control motor output but also by their physiological and membrane properties. However, defining what makes all neurons distinct from other cell types at a molecular basis remains challenging, since many features that are essential for a neuron to function can also be found in other somatic cells. One key characteristic that almost all neurons have in common is that they are able to communicate to each other (or to non-neuronal cells) via specialized synaptic connections [3–4]. Thus, the emergence of intercellular communication via pre- and postsynaptic molecular machineries may be considered a turning point in evolution allowing cells to transmit and integrate information.

Yet, neurons are not absolutely essential for all metazoan life since entire lineages of non-bilaterian metazoans appear to completely lack neurons. Conversely, many molecular components of neurons, such as synaptic proteins, evolved before neurons were present [5]. This raises fundamental questions regarding the evolutionary origin of the nervous

systems as well as neurons, as the basic cellular unit. Intuitively, one might assume that neurons evolved only once. However, recent studies challenge this view and suggest that neurons might have evolved multiple times independently [6–8]. The monophyletic origin of neurons is therefore strongly debated based on arguments supporting either homology by a secondary loss in certain clades or alternatively convergent evolution with multiple origins. Currently, most of our understanding and recent discussions on the origin of neurons is biased toward a metazoan perspective. This review summarizes and clarifies recent uncertainties about the evolutionary origin of synapses and neurons by uniting the latest findings from very different, but extremely relevant phyla. We here first depict current views regarding the phylogeny of non-bilaterian metazoans and their implications on nervous system evolution. We propose to include close relatives of metazoans (e.g. ichthyosporeans, filastereans, and choanoflagellates) to bridge this apparent gap and to answer some of the key remaining questions. We discuss the emergence and co-regulation of complex synaptic signaling machineries put into context of the seemingly "neuron-less" sponge and placozoan phyla and discuss the appearance of the first synapses and neurons in metazoans.

## Current and opposing views on the phylogeny of non-bilaterian metazoans and their implications for the origin of neurons

While the presence of complex nervous systems is a unifying feature of all bilaterians, the origin of neurons and nervous systems during early evolution of metazoans remains highly debated. Reasons are the unresolved phylogeny of non-bilaterian metazoans, meaning that there is no broad agreement on relationships at the base of the metazoan tree and the fact that not all non-bilaterian metazoan phyla have neurons. Among the four non-bilaterian phyla poriferans (sponges) and placozoans (Trichoplax) do not have recognizable neurons or a nervous system, while ctenophores (comb jellies) and cnidarians (sea anemones, corals, jellyfish, and hydroids) both have clearly recognizable neurons and in some cases even comparably complex nervous systems (Fig. 1). The identity of the metazoan lineages that diverged first is an intense matter of debate. All four non-bilaterian metazoan lineages have at least one species with a sequenced genome. Two of these – sponges and ctenophores – are currently the most frequently discussed candidate lineages to have first diverged from other metazoans.

Until very recently, it was widely agreed that sponges were the sister-group to the rest of metazoans with great support from phylogenetic analyses, comparative embryology and paleontology (Fig. 1A) [9–13]. The phylogenetic position of placozoans within the metazoan tree is somewhat unclear as well. When the complete mitochondrial genome of the placozoan *Trichoplax adhaerens* [14] was analyzed, it was concluded that placozoans are the sister-group to the rest of metazoans, although recent phylogenomic analyses of whole genome sequences revealed placozoans as the sister group to cnidarians and bilaterians [15]. However, increasing evidence now instead supports ctenophores as the sister-group to the rest of metazoans [6, 7, 16] (Fig. 1B). While some data support the hypothesis that ctenophores branched first, other data argue against it [17]. For example, several phylogenetic studies clearly support ctenophores as the sister-group to the remaining metazoans [6, 7, 18–22], while other phylogenetic studies support sponges as the sister-group ([10, 23, 24], see also [25, 26]). Remarkably, these phylogenetic studies show that the placement of sponges or ctenophores as the sister-group to the remaining metazoans might depend on which model is used to reconstruct phylogenetic trees, as well as on the quality and quantity of data analyzed [6, 7, 21, 23, 24]. Strong support for ctenophores being the sister group to the remaining metazoans comes from a recent careful analysis using a maximum likelihood framework which examined the incorporation of gene-wise and site-wise phylogenetic signal into their analysis [22]. In contrast, other recent phylogenetic analyses suggest that long-branch attraction might be the cause for the basal position of ctenophores [23, 24]. The authors show that taxon sampling and the choice of model type (site-homogeneous versus site-heterogeneous model type) has a drastic effect on the placement of long branches and correcting for these places sponges as the sister group to the rest of metazoans [24].

If ctenophores represent the sister-group to the rest of metazoans, this radically challenges the view on the early evolution of these cell types. It would mean that neurons might have either evolved twice independently or, alternatively, were lost from both sponges and placozoans [26, 27]. The absence of some key synaptic proteins (Synaptotagmin1, CASK, and Neuroligin) and some neuronal patterning genes in ctenophores was used as argument for an independent origin of synapses and neurons [7, 28]. Interestingly, also many canonical neurotransmitters known from cnidarians and bilaterians are absent in ctenophores (e.g. serotonin, dopamine, noradrenaline) [7] suggesting that the ctenophore nervous system may largely use different neurotransmitters. However, for instance the use of glutamate and glycine for neuronal communication is shared between ctenophores, cnidarians, and bilaterians [7, 28–30]. Moreover, many components critical for synaptic transmission are actually present in ctenophores and are very similar to the ones found in cnidarians and bilaterians [6, 7, 28], thus questioning an independent origin. In addition, many developmental genes used for determining a neural cell fate or genes patterning the nervous system are present in ctenophores [6, 7, 28].

Phylogenetic analyses might not resolve early metazoan phylogeny any time soon. Thus, genetic studies in ctenophores dedicated to the understanding of nervous system development and function in ctenophores will be key to clarify the current dispute on commonalities or differences of neurons in ctenophores and other metazoans. For instance, it will be relevant to address if neuronal gene homologs in ctenophores are involved in ctenophore neural cell types or not. While it seems intuitive that these genes provide the same


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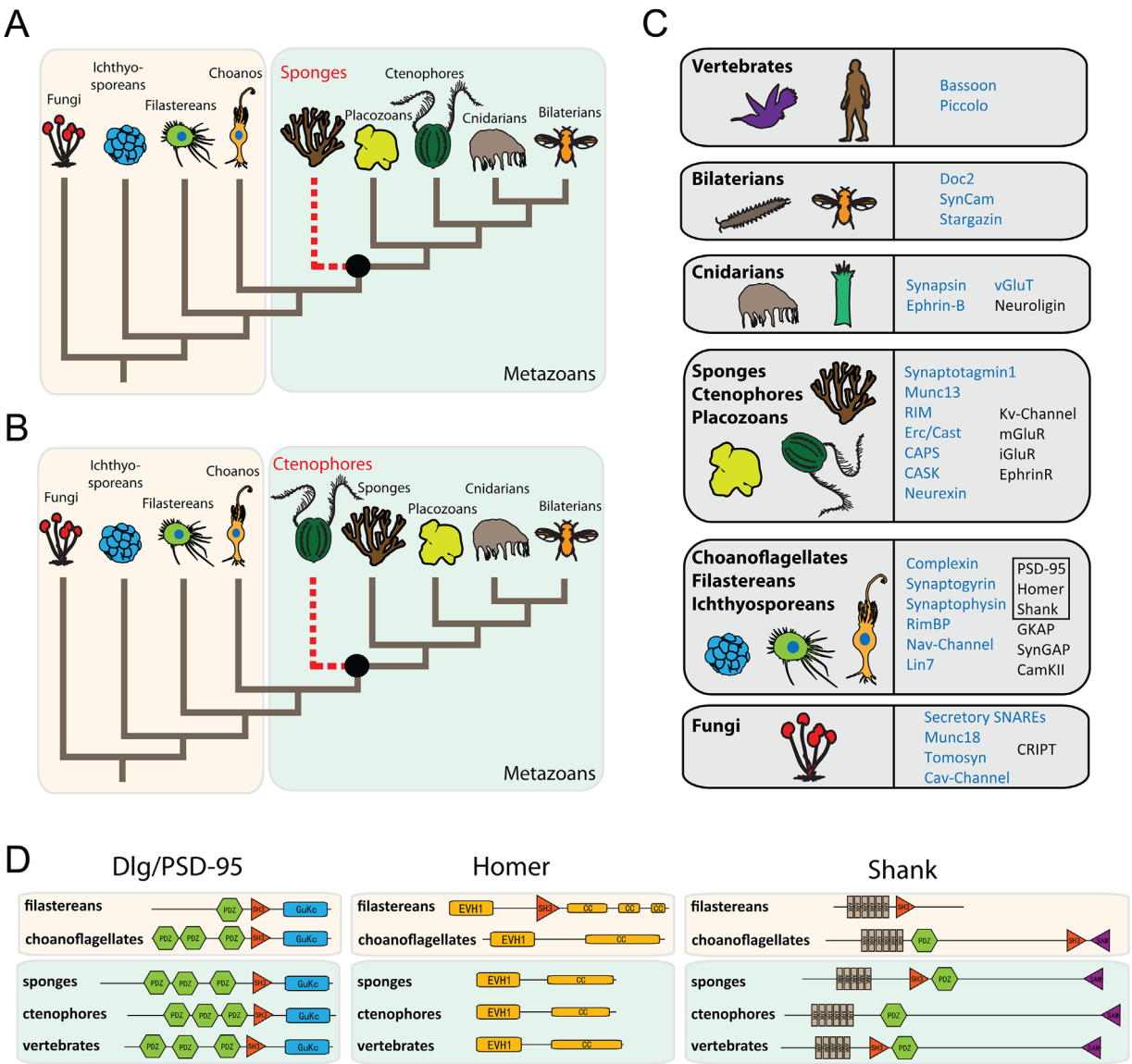


**Figure 1.** Current views on the phylogeny of non-bilaterian metazoans and rich repertoire of proto-synaptic proteins in protists closely related to metazoans. **A:** Phylogenetic tree showing sponges as the sister lineage to all other metazoans. Black circle: Urmetazoan. **B:** Phylogenetic tree showing ctenophores as the sister lineage to all other metazoans. Black circle: Urmetazoan. All illustrations were reused with modifications from phylopic.org. **C:** Proto-synaptic and synaptic proteins in different lineages. Note the prominent expansion of proto-synaptic and synaptic proteins in ichthyosporeans/filastereans/ choanoflagellates and sponges/ctenophores/placozoans. **D:** The protein domain organization of Dlg/PSD-95, Homer and Shank from the filasterean *Capsaspora owczarzaki*, the choanoflagellate *Salpingoeca rosetta*, the sponge *Oscarela carmela*, the ctenophore *Pleurobrachia bachei* and the vertebrate *Homo sapiens* are shown.

cellular function in ctenophores and cnidarians or bilaterians, it cannot be excluded that neurons in ctenophores use different strategies to achieve a similar function. Similarly, the genetic pathways of neurogenesis in ctenophores and neural patterning genes remain currently largely unknown. Understanding similarities and differences in nervous system formation between ctenophores and cnidarians or bilaterians will provide a second relevant line of research. If the function of neuronal genes is conserved between ctenophores and cnidarians/bilaterians it would support a single origin of neurons and would establish the homology of neural cell types in ctenophores and cnidarians or bilaterians.

## Rich repertoire of proto-synaptic proteins in protists closely related to metazoans

Many molecular and cellular features, which are essential for nervous system function and considered typical neuronal properties are in fact neither specific to synapses and neurons nor to metazoans. A prominent example is the presence of voltage-gated channels in viruses and bacteria [31, 32], although their roles in these organisms remain largely unknown. Even rapid sodium based action potentials can

occur in unicellular protists [33]. For example, the marine diatom *Odontella sinensis*, a unicellular, non-motile organism, is able to generate fast action potentials that show similar biophysical properties to metazoan action potentials [34]. Moreover, ionotropic glutamate receptors (iGluRs) have been identified in plants, where they function in development of roots,transport of ions, chemotaxis, and reproduction [35, 36], thus highlighting the challenges to identify molecular, physiological, and genetic properties that define neurons and make them distinct from other somatic cells.

Studies of protists that are close relatives of metazoans, like the ichthyosporean *Creolimax fragrantissima*, the filasterean *Capsaspora owczarzaki* and the two choanoflagellate species *Monosiga brevicollis* and *Salpingoeca rosetta* (Fig. 1A and B) are gaining increased attention when it comes to elucidate the origin of synaptic proteins. These organisms possess synaptic protein homologs although they never developed synapses and neurons. We refer to these proteins as proto-synaptic proteins, as they are clearly homologues to proteins which function at metazoan synapses and may interact with other proto-synaptic proteins in organisms with no synapses and neurons, in a very similar manner as observed in neurons. For example, the genomes of close relatives of metazoans, ichthyosporeans, filastereans and choanoflagellates, encode for Dlg/PSD-95, Homer and Shank (Figs. 1C,D and 2) [37–39]. In addition, many vesicle membrane proteins (e.g. Synaptophysin and Synaptogyrin), proteins involved in exocytosis (e.g. Complexin), and signaling (e.g. CaMKII) are present in the genomes *C. owczarzaki* and choanoflagellates [5, 37, 40–42] (Fig. 1C). Moreover, voltage-gated sodium and calcium channels [43–45] were identified in the genomes of choanoflagellates (Fig. 1C).

Proto-synaptic protein number was further expanded during the rise of metazoans. For example, important synaptic adhesion protein homologs like Neurexin and Ephrin receptors are present in sponges, ctenophores and placozoans (Fig. 1C). In addition, many active zone proteins (e.g. RIM, Erc/Cast, CASK) (Fig. 1C) are present in sponges [41, 46, 47], ctenophores [6, 7, 48], and placozoans [15]. Thus, many proto-synaptic proteins already existed in close relatives of metazoans and the majority of synaptic protein homologs were present when the first metazoans evolved (Fig. 1C) [37, 38, 40, 49].

## Co-regulation of proto-synaptic genes in close relatives of metazoans

The presence of proto-synaptic proteins in close relatives of metazoans (Fig. 1C) suggests that some molecular machineries critical for synaptic transmission evolved prior to the origin of synapses. Thus, studies of proto-synaptic proteins in these clades provide insight into putative ancestral functions of these cellular specializations and evolutionary precursors of synaptic signaling complexes [50, 51]. In the metazoan nervous system, the interplay between specialized presynaptic and postsynaptic molecular machineries allows the translation of electrical membrane currents into chemical signals in the presynaptic cell, which in turn elicit electrical currents (or intracellular signaling pathways) in the postsynaptic cell. It is worth mentioning, that even in metazoans with synapses and neurons synaptic proteins are functionally diverse and fulfil different roles in other cell types (Fig. 2) [52]. This seems to be the case for nearly every synaptic protein found for example in vertebrates (Fig. 2). For instance, Dlg/PSD-95 functions as a scaffolding protein and clusters iGluRs to the plasma membrane of postsynapses, whereas the same protein is an important component of septate junctions in epithelial cells [53] (Fig. 2). Currently, very little is known about the ancestral function of synaptic proteins (Fig. 2). One example is the protein Homer, which is expressed in the nucleus and binds to Flotillins in choanoflagellates and vertebrate astrocytes [41] and highlights that many proto-synaptic genes may be pleiotropic.

The ichthyosporean *C. fragrantissima* comprises amoeboid and colonial (multinucleate) life stages (Fig. 3A) [54, 55]. Interestingly, the transition to the colonial stage is associated with significant upregulation of secretory SNAREs, Homer, and Shank (Fig. 3A) [51]. The filasterean *C. owczarzaki* can switch between filopodial, cystic and aggregative life stages (Fig. 3B). Remarkably, the proto-synaptic genes Dlg/PSD-95, CaMKII and GKAP are upregulated in the aggregative life stage (perhaps functioning in a complex like the Dlg/CaMKII/GKAP complex in vertebrate neurons [56, 57] to cluster cation channels on particular plasma membrane areas important for the aggregation of *C. owczarzaki* cells), but other proto-synaptic genes like Homer and Shank and many presynaptic genes (secretory SNAREs) are upregulated in the cystic life stage (Fig. 3B) [58]. A recent analysis of the regulatory genome of the filasterean *C. owczarzaki* indicates that while the regulation of transcriptional activity by distal enhancers is likely a metazoan innovation, many transcription factor networks that are important for metazoan development and multicellularity are conserved in *C. owczarzaki* [59]. Notably, transitions between different life stages in *C. owczarzaki* are additionally linked to proteome and phosphoproteome changes and they alter key proteins, for instance transcription factors involved in metazoan multicellularity [59, 60]. The choanoflagellate *S. rosetta* comprises of single (attached, swimmers) and colonial life stages [61, 62] (Fig. 3C) and many proto-synaptic genes (secretory SNAREs, voltage gated sodium channel [Nav-channel]) are upregulated in colonies [63]. Strikingly, neighboring cells in *S. rosetta* colonies are connected by fine cytoplasmic bridges [61], which might mediate cell–cell signaling using presynaptic protein homologs. Surprisingly, while the protein Homer is upregulated in colonies as well, Shank, Dlg/PSD-95, and CamKII are upregulated in attached cells. In vertebrate neurons Dlg/PSD-95 and Shank can also induce filopodia [64], thus the choanoflagellate proteins could function in a similar complex inducing long cellular protrusions that resemble filopodia (a hallmark of choanoflagellate attached cells [65]). In addition, CamKII has previously been reported to interact with PSD-95 in mouse CNS postsynapses [66] and these two proteins might interact in choanoflagellates as well. These data indicate that already in close relatives of metazoans some of the proto-synaptic proteins might be co-regulated and provide first insights into the evolution of synaptic signaling machineries. Given the relative limited number of different conditions (e.g. life cycle stages) that were tested for the ichthyosporean


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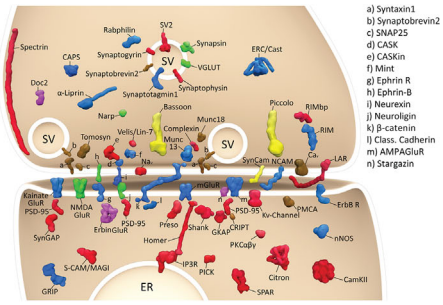


| Proteins | 'Canonical' synaptic function | Non-synaptic function | Ancestral function |
|---|---|---|---|
| **Transmitter/peptide Secretion** | | | |
| SNARE proteins: Syntaxin 1/2, SNAP-25, Synaptobrevin 1/2 | synaptic vesicle fusion | vesicle fusion in pancreatic cells [88], acrosome vesicle fusion [89] | polarized vesicle fusion in many eukaryotes, transcriptionally co-regulated in close relatives of metazoans [51] |
| Sec1/Munc18 | template for SNARE assembly | controls SNARE protein assembly during sperm acrosomal exocytosis [90] | controls SNARE protein assembly [50] |
| Complexin | stimulatory & inhibitory role in vesicle fusion | facilitates exocytotic release in mast cells [91] | unknown, localizes in gland cells of placozoans [73] |
| Synaptotagmin 1/2 | calcium sensor of neuronal exocytosis | controls mast/pancreatic beta-cell exocytosis [93, 94, 95] | unknown |
| Synapsin | modulates neurotransmitter release | unkown | unknown, colocalizes with secretroy SNAREs in gland cells of placozoans [72] |
| **Active Zone** | | | |
| Munc13 | synaptic vesicle priming | sustained insulin secretion in pancreatic beta-cells [96] | unknown |
| CAPS | calcium dependent secretion activator | insulin granule priming, exocytosis, and stability [97] | unknown |
| RIM | active zone protein | insulin secretion in pancreatic beta-cells [98] | unknown |
| Erc/Cast (ELKS) | RIM binding proteins, organization of active zone | activation of transcription factor NF-kB [99] | unknown |
| **Adhesion & Signalling** | | | |
| Neurexin | Synapse formation & adhesion, binds to Neuroligin | insulin secretion in pancreatic beta-cells [100] | component of septate junctions in cnidarians [110] |
| Neuroligin | Synapse formation & adhesion, Neurexin binding partner | vascular remodeling/angiogenesis in arteries [101] | unknown |
| CamKII | Calmodulin-dependent kinase critical for learning & memory | cardiac signaling [92] | unkown, evolutionary conserved molecular mechanim of subunit exchange [67] |
| **Transmembrane receptors** | | | |
| iGluRs | glutamate binding ion channels | mediate cardiac electrophysiology [102] | unknown |
| mGluRs | G-protein coupled glutamate binding receptors | glutamate uptake in astrocytes [103], hormone production in adrenal glands & pancreas [104] | unknown |
| **Postsynaptic scaffolding** | | | |
| Dlg/PSD-95 | iGluRs and Neuroligin localization, critical scaffolding protein | regulator of T cell activation [105]; septate junction structure [53] | unknown, present at cell-cell contacts in placozoans [72] |
| Shank | Homer and actin cytoskeleton binding, critical scaffolding protein | actin co-ordination in liver epithelial cells [106] | unkown, transciptionally co-regulated with Homer in close relatives of metazoans [51] |
| Homer | Shank and mGluRs localization, critical scaffolding protein | NFAT activation in T cells [107], calcium signaling in muscle cells [108] | unknown, binds Flotillins in the nucleus of choanoflagellates [41] |
| GKAP | Dlg and Shank crosslinking protein | aassociation with microtubules, centrosome positioning, and cell polarity [109] | unknown, transciptionally co-regulated with Shank in close relatives of metazoans [51] |

**Figure 2.** Many synaptic proteins have functions outside the nervous system. **Top:** Graphic representation of an excitatory vertebrate synapse indicating the subcellular distribution of key pre- and postsynaptic proteins, modified from [41]. **Bottom:** Highlighted are key synaptic proteins with their well described function in synapses (canonical function) and their function outside of synapses (non-synaptic function). First insights into ancestral functions of synaptic proteins have been gained from studies of close relatives of metazoans and non-bilaterians metazoans. Illustrations of organisms were reused with modifications from phylopic.org.

*C. fragrantissima* (2 conditions), the filasterean *C. owczarzaki* (3 conditions), and the choanoflagellate *S. rosetta* (4 conditions), there is a small likelihood that some of the proto-synaptic genes analyzed might be co-regulated just by chance. Thus, it will be key to validate these findings with other techniques to fully understand the proto-synaptic signaling machinery in close relatives of metazoans.

Moreover, a recent understanding of how the enzyme CaMKII functions at a molecular level emerged from studies on a CaMKII homolog from choanoflagellates [67]. At synapses CaMKII, which is composed of several subunits forming a ring (Fig. 3C box) and can exchange subunits with each other, has an important role in long-term memory formation [68]. Biochemical and structural studies on choanoflagellate CaMKII provided direct evidence into how subunits of CaMKII can interchange and thus spread information [67] (Fig. 3C box) and is another exciting example for how close relatives of metazoans can reveal important, previously unknown insights into the molecular mechanism of metazoan synaptic protein function.

It is worth mentioning, that (obviously) not all proto-synaptic proteins are co-regulated in close relatives of metazoans, suggesting an extensive rewiring of regulatory networks over time that allowed proto-synaptic proteins to be expressed in the same cell and to function together.

## Neuronal components in non-bilaterian metazoans and the first appearance of neurons

While close relatives of metazoans clearly have no synapses, a study in sponges provides some insights into the assembly of a synapse [69]. This study shows that in the sponge *Amphimedon queenslandica* a global co-regulation of postsynaptic genes is lacking although some postsynaptic signaling complexes are transcriptionally co-regulated [69]. Thus, synapses may have evolved by expanding preexisting protein complexes and ancient postsynaptic protein complexes may continue to function in synapses of present metazoans [69]. Another explanation would be that sponges lost synapses and neurons and that these modules are remnants of neurons. Obviously, the existence of synaptic proteins alone is not sufficient to make up a neuron. Thus, further molecular features such as the expression of dedicated ion channels to propagate voltage changes, the intracellular machinery allowing the formation of directed "neurite"-like membrane protrusions or the biosynthesis of neurotransmitters have to be taken into account when aiming to resolve the origin of first neurons.

First insights into potential evolutionary precursors of neurons have recently been gained from studies in sponges

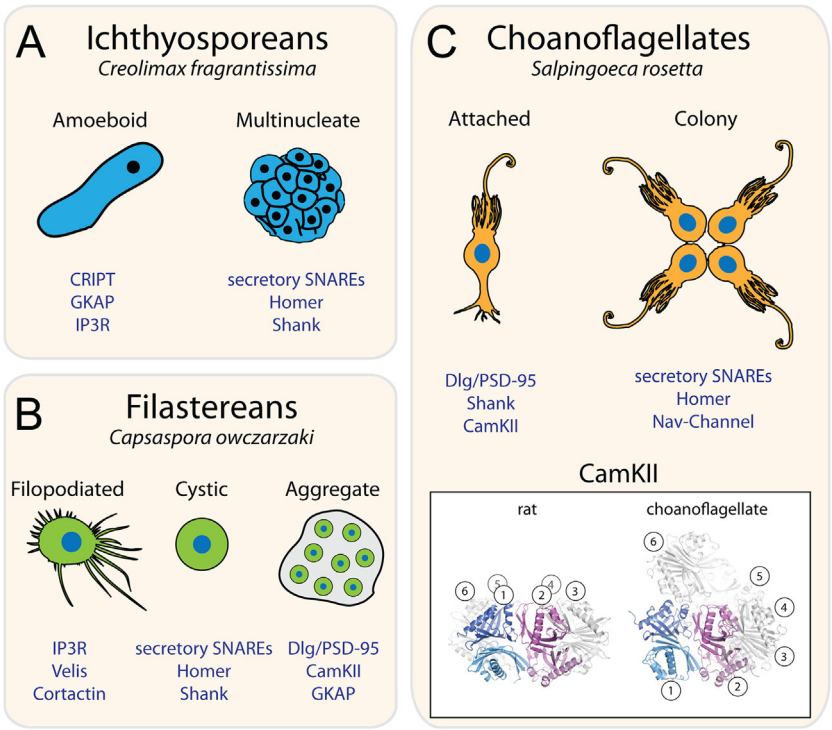


**Figure 3.** Co-regulation of proto-synaptic genes in close relatives of metazoans. **A:** Illustrations of amoeboid and colonial (multinucleate) life stages of the ichthyosporean *Creolimax fragrantissima*. Secretory SNAREs and Homer/Shank are upregulated in the colonial life stage [51]. **B:** The filasterean *Capsaspora owczarzaki* can switch between filopodial, cystic and aggregative life stages. Dlg/PSD-95, CaMKII, and GKAP are upregulated in the aggregative life stage, but Homer/Shank) and secretory SNAREs are upregulated in the cystic life stage [58]. **C:** Illustrations of single and colonial life stages of the choanoflagellate *Salpingoeca rosetta*. Secretory SNAREs, Nav-channel, Homer are upregulated in colonies [63]. The known binding partners of Homer, the proteins Shank, GKAP and Dlg/PSD-95 are upregulated in attached cells. **Box:** Structures of rat and choanoflagellate hub assemblies of CaMKII. Adapted with permission. [67] Copyright 2016, eLife Sciences Publications, Ltd. Choanoflagellate CaMKII forms a ring-opened spiral assembly and provides direct evidence into how subunits of CamKII can interchange and thus spread information. Adapted with permission. [67] Copyright 2016, eLife Sciences Publications, Ltd. Illustrations of close relatives of metazoans were reused with modifications from phylopic.org.

and placozoans. Larvae from the sponge *A. queenslandica* possess a cell type in their epithelia called globular cell (Fig. 4A and B) [38, 70]. These globular cells express postsynaptic scaffolding protein homologs like Dlg/PSD-95, Homer and GKAP suggesting an assembly into a protein-protein complex [38]. On the other hand, extensive expression analyses and immunolocalization studies of synaptic protein homologs in adult sponges are still missing. Numerous studies on different sponges using electron microscopy failed to recognize obvious synaptic structures with a postsynaptic density. Many cells in the gelatinous matrix within a sponge (the so-called mesohyl) are in steady motion with little time for "direct contact" [71]. In contrast, pinacocytes (cells at the surface of sponges) are motionless and keep contact with neighboring cells (Fig. 4C and D) and numerous vesicles can be observed at contact sites of neighboring cells (Fig. 4E). In addition, Smith and colleagues have characterized the different cell types in the placozoan *T. adhaerens* in more detail and found that so called gland cells display neuron-like properties [72, 73] (Fig. 4F and G), as they express secretory SNARE proteins, complexin and synapsin (abundant protein of synaptic vesicles in bilaterians) and contain potential secretory vesicles, features of metazoan pre-synaptic specializations (Fig. 4G). Moreover, an antibody against FMRFamide stains these cells, indicating that placozoan gland cells may secrete an FMRFamide-like peptide. The findings that sponges and placozoans possess specialized cells that display neuron-like properties offer some exciting hypotheses. It is possible, that the first neurons evolved before sponges and placozoans diverged, and in sponges neurons transformed into globular cells and in placozoans into gland cells [74]. On the other hand, neurons may have evolved after sponges and placozoans branched off from the metazoan tree [74]. Under this scenario, sponge globular cells, placozoan gland cells, and neurons in all other metazoans have evolved from a primordial secretory or sensory cell [74, 75].

When looking at the first appearance of neurons in metazoans investigations on ctenophore neurons are particularly informative due to the debate on their phylogenetic position. The majority of neurons in ctenophores form a subepidermal nerve net on the surface of the body (Fig. 4H) [7, 27, 76, 77]. So far, most observed synaptic connections display an organization, which is referred to as the "pre-synaptic triad," an odd presynaptic organization by a string of vesicles docked at the plasma membrane, followed by one or several mitochondria as well as an ER sac (Fig. 4I) [78]. However, the organization of synapses as pre-synaptic triads is not restricted to ctenophores as it can be found in neurons of the nerve net of many cnidarians (Fig. 4J and K) [3, 75, 79, 80]. In the nerve net of the cnidarian *Cyanea capillata* it was shown that that these synapses are bidirectional, excitatory chemical synapses [81]. It will be interesting to study if ctenophore synapses also are bidirectional, excitatory chemical synapses and to compare presynaptic (e.g. active zone molecules) and postsynaptic proteins have similar distributions/localization patterns in triad synapses between ctenophores and cnidarians. The observation that ctenophore synapses display similarities with cnidarian synapses at the ultrastructural level may provide an argument for a common structural organization and common origin [4, 75].

The currently proposed different scenarios of nervous system evolution critically depend on the phylogenetic tree of the metazoan kingdom. Resolving the phylogeny of early branching metazoans will thus be a key step toward a better


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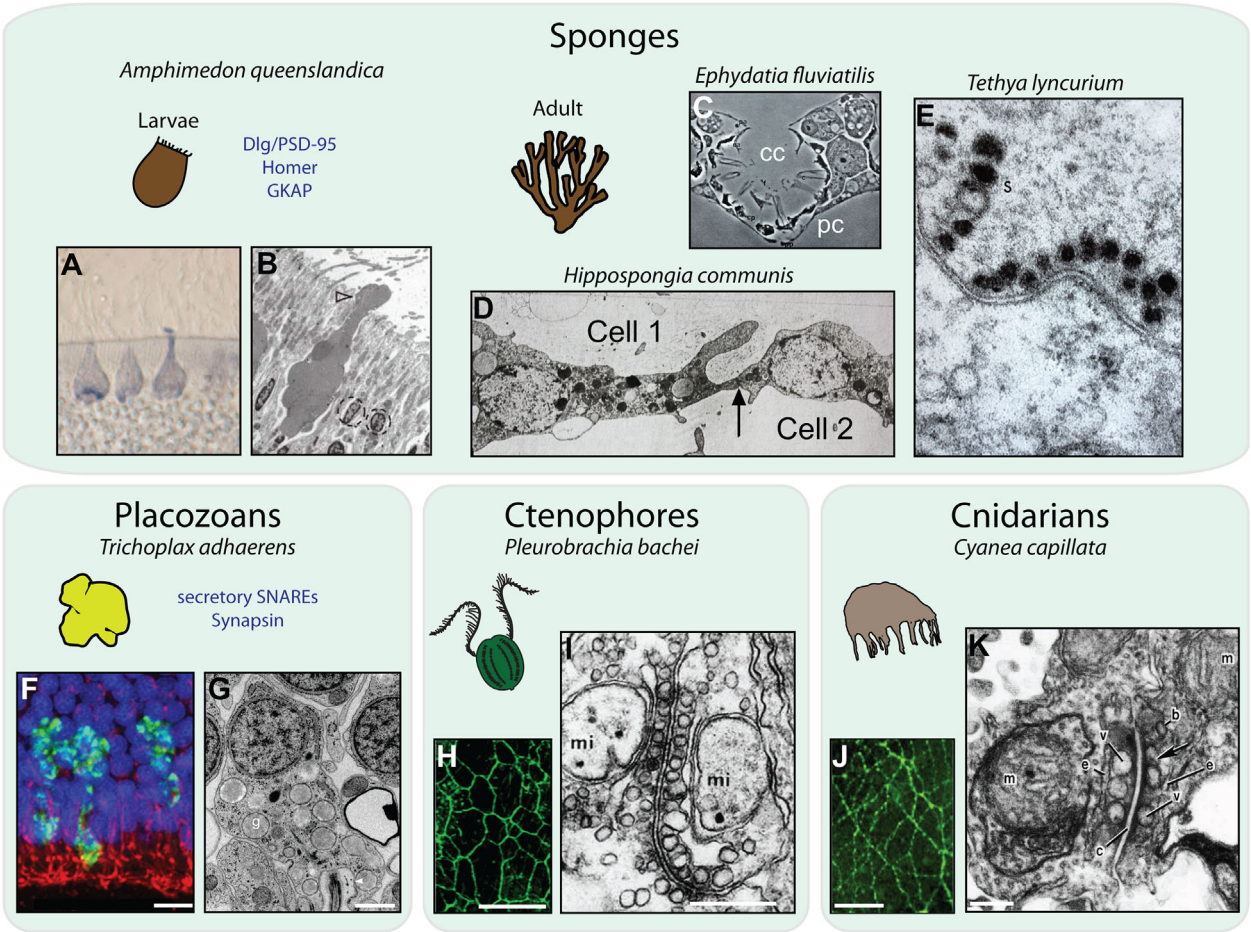


**Figure 4.** Insights into evolutionary precursors of neurons and the first appearance of neurons. **A:** Section of whole mount in situ hybridized larvae showing expression of the post-synaptic protein GKAP in *Amphimedon queenslandica* (modified from [38]). **B:** Electron micrograph of a globular cell from *A. queenslandica* larvae. Globular cells (arrowhead) are filled with large electron dense vesicles (modified from [70]). **C:** Choanocyte chamber (cc) and surrounding pinacocytes (pc) in the sponge *Ephydatia fluviatilis* (modified from [83]). **D:** Pinacocyte cell-cell contact (black arrow) in the sponge *Hippospongia communis* (modified from [84]). **E:** Numerous vesicles (s) can be observed at some contact sites of neighbouring cells in the sponge *Tethya lyncurium* (modified from [85]). **F:** Secretory SNAREs (in this case Synaptobrevin) are detected in gland cells of *Trichoplax adhereans* (modified from [72]). **G:** Electron micrograph of a ciliated gland cell reveals membrane-enclosed vesicles comparable in size with the stained vesicles shown in (F), (modified from [72]). **H:** The nerve net of the ctenophore *Pleurobrachia bachei* visualized by staining with tyrosinated alpha-tubulin antibodies (modified from [7]). **I:** The ctenophores pre-synaptic triad: a string of vesicles docked at the plasma membrane, followed by a sac of endoplasmic reticulum and one or several mitochondria (mi) (modified from [86]). **J:** The nerve net of the cnidarian *Cyanea sp.* labeled with antibody to FMRFamide (modified from [87]). **K:** Electron micrograph of a bidirectional, excitatory chemical synapse in the cnidarian *Cyanea capillata*. m, mitochondria; v, synaptic vesicles; e, elongated cisternae; c, synaptic cleft; b, bulbous cisternae (modified from [79]). Illustrations of metazoans were reused with modifications from phylopic.org. Scale bars: 20 μm in F, 0.5 μm in G; 60 μm in H, 100 nm in I; 25 μM in J, 200 nm in K.

understanding about the origin of neurons. The presence of neuron-like cells in all non-bilaterian metazoans may be used as an argument for a common origin of all neurons or may be regarded as distinct types of proto-neurons. It will be key to learn more about the developmental origin, physiology and molecular features of these enigmatic cells in order to further unveil how similar or distinct they are from various types of neurons found in cnidarians or bilaterians. Independent of whether ctenophores are the sister-group to the rest of metazoans or not the striking differences in nervous system organization [82] raises numerous intriguing questions. While the presence of certain neuronal development genes in the ctenophore genome suggests that they may provide similar functions in ctenophores and cnidarians or bilaterians functional developmental studies remain sparse. Moreover, the lack of some critical synaptic proteins in ctenophores should not be used as a criterion for independent origin of neurons, as similar examples can be found in other organisms with neurons. For example, the genome of the cnidarian *Hydra magnipapillata* does not encode for the key synaptic adhesion protein neuroligin [37] and the genome of *C. elegans* does not encode for voltage-gated Na-channels or the postsynaptic scaffolding protein Homer, despite the presence of clearly homologous nerve cells and nervous systems in these organisms. Thus, further insights on how similar or distinct ctenophore neurons function from

their cnidarian or vertebrate counter parts, on a cellular or a network level and how a seemingly different set of neurotransmitters and neuromodulator are employed will shed light into current controversies about nervous system evolution.

## Concluding remarks

Recent sequencing of genomes from non-bilaterian metazoans and their closest relatives has greatly enhanced our understanding on the origin of synapses, a central characteristic of neurons. It now becomes clear that many proto-synaptic genes were already present when the first metazoans appeared. More recent work shows, that even in close relatives of metazoans some proto-synaptic genes seem to be co-regulated at the transcriptional level and suggests that parts of the synaptic signaling machinery might have been co-opted from ancestral roles that may still be observable today in their close relatives. Choanoflagellate cells for example have evolved ways to connect to each other. The specific upregulation of voltage gated Na−channels and secretory SNAREs in *S. rosetta* colonies makes it tempting to speculate that choanoflagellate cells can communicate with each other by electrical and/or chemical signaling using proto-synaptic proteins, a hypothesis that can be tested in the future with cell labeling, electrophysiological experiments, calcium imaging, and even proteomic interaction studies. A picture is emerging where an ancestral secretion apparatus consisting of secretory SNAREs, Munc18, Complexin, and a Munc13-like protein is already present in close relatives of metazoans and thus probably originated before the first metazoans emerged (Fig. 2). On the other hand, a postsynaptic-like scaffold (a scaffold comprising of Dlg/PSD-95, Homer, Shank, and GKAP which in metazoans with synapses clusters receptors at plasma membranes) likely evolved in metazoans only, despite the fact that these proteins are expressed in close relatives of metazoans and key residues for protein-protein interactions are present. Together, these findings show that close relatives of metazoans possess different precursors of synaptic activity. Therefore, by just viewing at the origin of synapses and neurons from a metazoan perspective the view of synapse and neuron evolution is incomplete. Including the closest unicellular relatives of metazoans into the question relating the origin and evolution of synapses and neurons is therefore of great importance.

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