Getting nervous: an evolutionary overhaul for communication

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Abstract

The evolution of a nervous system as a control system of the body's functions is a key innovation of animals. Its fundamental units are neurons, highly specialized cells dedicated to fast cell-cell communication. Neurons pass signals to other neurons, muscle cells, or gland cells at specialized junctions, the synapses, where transmitters are released from vesicles in a Ca^{2+}-dependent fashion to activate receptors in the membrane of the target cell. Reconstructing the origins of neuronal communication out of a more simple process remains a central challenge in biology. Recent genomic comparisons have revealed that all animals, including the nerveless poriferans and placozoans, share a basic set of genes for neuronal communication. This suggests that the first animal, the Urmetazoan, was already endowed with neurosecretory cells that probably started to connect into neuronal networks soon afterwards. Here, we discuss scenarios for this pivotal transition in animal evolution.

Key-words

regulated secretion, vesicle trafficking, endosome, lysosome, SNARE proteins, Rab proteins, CATCHR complex

Introduction

The nervous system is responsible for (i) sensing and responding to the environment and (ii) coordinating all of the body's activities. It computes external and internal cues and signals these to muscles, organs, and glands (the endocrine system). Its basic building blocks are nerve cells (neurons), highly specialized polarized cells dedicated to fast intercellular communication. Three basic types of nerve cells can be distinguished: sensory neurons, activated through various types of sensory input; interneurons, which connect to other neurons; and motor neurons, which transmit signals to muscles. Their stimulation triggers an electrical impulse (the action potential, a sub-threshold depolarization of the plasma membrane) which propagates along the membrane up to an area of the cell where it is transduced chemically across the membrane: In this subcellular compartment, voltage-gated Ca^{2+} channels open up, and the influx of Ca^{2+} prompts the rapid secretion of chemical neurotransmitters/neuropeptides from vesicles, which activate cognate receptors on the membrane of the target cell. Accordingly, although neurons exist in many different types and shapes (102), the area devoted to chemical signal transfer where two neurons "join together", the synapse (from the Greek "*syn* + *haptein*"), is highly conserved. Both the pre-synaptic (sending) and post-synaptic (receiving) parts require a highly specific environment and dedicated molecular machines to carry out directional chemical transmission in a highly regulated manner (reviewed in (3, 67, 73, 102, 119, 129, 134)).

The question of how these outstanding communication cells have evolved out of a simple system has fascinated neuroscientists for more than a century (for historical overviews of different evolutionary scenarios, see e.g. (10, 84, 87, 98)). As virtually all animals with nervous systems have muscles and as the communication between neurons and muscles coordinates animal behavior, it has been proposed that neurons and muscles cells have co-evolved from a primordial epithelium of electrically coupled cells, in parallel or sequentially. Another concept suggests that secretory cells may have evolved in the epithelium to coordinate surrounding cells in a paracrine fashion; next, these secretory cells evolved into neurosecretory cells and neurons by developing a conducting segment with electrical properties between its receptive and secretory poles, while muscle cells developed in close relationships to these cells.

So humble a beginning

The control of the body by a nervous system only evolved in animals, but not in other multicellular organisms such as plants or fungi, and must have provided them with a tremendous advantage. But what makes animals distinctive? What exactly are animals? Our commonsense understanding is that they can move independently and that they have senses to react quickly to the environment. Nervous systems and muscles can be found in three (of the five) basic groups of animals: cnidarians, ctenophores (commonly known as comb jellies), and bilaterians (Fig. 1) (17, 99, 100). Bilaterians, animals with bilateral symmetry, forming the majority of animal phyla, mostly have a centralized nervous system. Most of our knowledge about nerve cells comes from the study of bilaterian animals, particularly vertebrates; much less is known about the other animals. The more simple cnidarians and ctenophores have a decentralized nerve net. How exactly diffuse nerve nets evolved into centralized nervous systems is an unresolved question (14, 23, 116). Remarkably, cnidarian synapses can be bidirectional and their nerve cells do not exhibit a clear separation between dendrites and axons (23, 116) as established for typical neurons in "higher" animals. Very little is known about the nerve cells of ctenophores (66).

Intriguingly, no true nerve or muscle cells have been reported in the two remaining basic groups of animals: poriferans (sponges) and placozoans. For this reason, they are thought to have branched off early in animal history and, until recently, have not been paid much attention by those addressing the origins of neuronal communication. However, as we will discuss in the following, their coordination systems can shed more light onto the origins of neuronal communication and provide novel insights into its fundamental functional principles. Both lineages have a simple body plan of epithelial-cell-like layers interspersed with a few differentiated types of cells. Poriferans are passive filter-feeders that are also able to slowly contract their body (4, 42). Placozoans, represented by only one species with cryptic diversity (*Trichoplax adhaerens*) are millimeter-sized free-living marine animals that glide on cilia and graze on algae (44, 131). Actually, no single criterion can serve to distinguish all animals from all other eukaryotes. Nervous system and muscles, as they did not evolve at the onset of animal evolution, cannot be regarded as true defining features of animals. Instead, animals are usually defined as multicellular eukaryotic heterotrophs that typically reproduce sexually.

According to molecular clock studies, the first animals appeared around 750 million years ago (Mya) during the Cryogenian period, whereas the first fossil evidence of animals comes from Ediacaran period (635–542 Mya). In the early phase of the Cambrian period (541–485 Mya), by around 520 Mya, nearly all animal phyla are represented in the fossil record. This burst of evolutionary changes is known as the "Cambrian Explosion" and signifies an increase in animals with calcified body parts, whereas the soft-bodied and probably smaller forms of earlier times left only a few fossil traces (36, 41, 46, 147). It is speculated that the ocean was filled with microorganisms which filter-feeding porifera-like animals could live on. The sea floor might have been covered by a layer of algae and other microbes onto which placozoan-like organisms could graze (12).

Animals probably arose from heterotrophic protists closely related to extant choanoflagellates (Fig. 1) (80, 117). Choanoflagellates live as single-celled and/or colony-forming organisms. They capture bacteria using an apical flagellum/cilium surrounded by a collar of actin-filled microvilli held together at their base by a fine porous extracellular proteinaceous mesh (38). Such tentacle-like microvilli are also found in other related single-celled organisms (holozoans) and in some animals. The water current produced by flagellar movement drives prey onto the sticky surface of the microvilli, where it is trapped and engulfed by phagocytosis at the base of the collar (38). This mode of uptake resembles that of the choanocytes of sponges.

Choanoflagellates, like other unicellular organisms, can swim toward nutrients and away from noxious substances, thanks to a variety of membrane receptors and signal transduction modules, including various types of ion channels. The flagellum, which has many specific sensory receptors concentrated at its base, also plays an important role in sensing and relaying signals from the environment (146). A colonial protometazoan, composed of similarly non-differentiated yet multifunctional epithelial cells would have been able to sense a rich spectrum of environmental cues via numerous flagella facing the extracellular space. In a multicellular organism, however, any signals received needed to be integrated to achieve coherent behavior of the entire organism. The multicellular green algae *Volvox*, which forms spherical colonies that may resemble that of the hypothetical ancestor of metazoans, exemplifies how such tasks can be mastered by a uniform population of epithelial cells: in *Volvox,* the beats of the flagella are mainly synchronized through

hydrodynamic coupling; movement towards light is achieved through differences in beat frequency, which is controlled by the photoreceptors in the eyespot of each cell (142).

It is conceivable, however, that it was soon no longer sufficient for a colonial bacterivore to leisurely float in the primordial ocean. Organisms with the ability to actively move had an advantage. In order to make the leap to true multicellularity with specialized cell types, evolutionary innovations were required. At minimum, in adhesive interactions between cells that maintain a spherical form, a mean of transferring nutrients to cells that have taken up other tasks and of communication between cells in order to coordinate the behavior of the entire organism was needed. Can the transition to multicellularity or even the advent of neuronal communication be traced by comparative genomics?

The genomic perspective

For about a century after the publication of Darwin's *Origin of Species* in 1859, biologists had to rely solely on morphological and developmental characteristics to establish the branching order of the animal tree of life. The advances in sequencing and computing technologies achieved over the past two decades enabled us to start looking into the evolution of animals from a genomic perspective and to bring the analysis of the branching order to a new level. Though the first animal genome projects focused on bilaterians, including our own species, genomes of early-branching animals and of our unicellular relatives were added gradually so that a wide repertoire of genomes representing all major animal phyla can now be utilized (43, 136). It was a major surprise when the genome of a cnidarian, the sea anemone *Nematostella vectensis* (114), turned out to have a gene repertoire and organization that was more similar to our own genome than to that of the fruitfly *Drosophila melanogaster* or the nematode *Caenorhabditis elegans*, suggesting that the genome of the eumetazoan ancestor was already complex, and that the genomes of the fruit fly and nematode worm underwent major rearrangements. Analyses of additional genomes further entrenched this notion and revealed that not only the genome of *N. vectensis,* but also these of the placozoan *T. adhaerens* (132) and the demosponge *Amphimedon queenslandica* (133) – animals without nervous system contain the “gene repertoire for neuronal communication”. In other words, these genomes contain an array of genes coding for proteins considered as landmarks of synaptic transmission involved in

vesicle exocytosis, synapse building (active zone and post-synaptic scaffolds, and cell adhesion), and signaling (various type of receptors). This suggests that there may not be such a thing as a clear marker for the onset of synaptic communication. It also suggests that a closer inspection of poriferans and *T. adhaerens* might shed light on the origins of neurons and how these became the organism's control center.

Comparisons of animal genomes with that of the choanoflagellate *Monosiga brevicollis* (117, 121) have revealed the increased complexity of many gene families involved in different aspects of multicellularity, suggesting a major genomic overhaul during the rise of animals. Unicellular organisms already expressed proteins that, broadly speaking, can be considered as involved in neuronal function (e.g. various ion channel families; reviewed in (25, 29, 85) and in a special edition of *The Journal of Experimental Biology* (6)). Many of the molecular innovations of animals actually built on already existing modules, which did not necessarily display the same functionality in the unicellular ancestors. Indeed, while true innovations are rare, it is commonly agreed upon that certain molecules multiplied and were co-opted for a novel function by evolutionary tinkering (65). Subtle changes in the binding surfaces of a protein or more drastic changes such as protein domains shuffling suffice to make this happen, and factors that are no longer needed are often discarded. Hence biophysical, structural, morphological, and (cellular) biological studies, albeit time-consuming, are essential to give insights into the functional changes accompanying gene multiplication, which would, in turn, help to clarify genome homology searches. In the following sections, we outline some key cellular changes that we find particularly fascinating. The epithelium emerged at the onset of animal evolution as the basic building block of the metazoan body plan (31, 83, 139). All cell types, including the highly polarized neurons, eventually evolved from epithelial cells by modulating existing cellular features (13). Thus, in order to understand how neurons evolved, one needs to take a closer look at epithelial cells.

Epithelial cell adhesion

In animal epithelia, several types of junctions link cells laterally to each other (adherens and occluding junctions) or basally to the extracellular matrix (ECM) via integrins, providing them with (i) a mechanical link, hence tissue stability; (ii) a seal, inducing a differential

environment at their apical vs. basal side; and (iii) a hub for communication and paracellular exchange. Most of the proteins comprising these junctions (e.g. integrins, ECM proteins, and cadherin) were shaped at the onset of animal evolution from the precursor forms present in the single-celled ancestor (117, 121). It often seems that in early-branching animals, junctional epithelial features may not have yet adopted a similar structure and function to that of their bilateral counterparts, rendering them sometimes difficult to locate or identify as such.

Adherens junctions are found in sponge epithelia in rudimentary form (47, 83) and in *T. adhaerens* (130). They are notably made of cadherins, cell surface receptors that function in a Ca^{2+}-dependent manner (91, 95, 97). By binding to actin filaments, they form a circumferential contractile belt near the apical surface of epithelial cells. Integrins, a broad family of transmembrane proteins expressed at the basal side of the cells, bind to the ECM (35, 48, 63, 106). Occluding junctions, which seal and control paracellular transport across the epithelial layer, also evolved early (51) and are categorized into septate junctions and tight junctions (153). Generally, occluding junctions show a higher degree of variation than adherens juntions. Strictly speaking, septate junctions are reported only in invertebrates (64), although some of their building elements are found in vertebrate paranodes (61). Interestingly, in *C. elegans,* and possibly other animals as well*,* septate and adherens junctions merge. Tight junctions seem to have emerged first in chordates and are located apical to adherens junctions, whereas septate junctions are found on the basal side. The point in evolution at which septate junctions developed true barrier properties is unclear. While the *T. adhaerens* genome encodes for their key components, septate junctions have not been clearly observed morphologically (130).

Overall, junctional proteins are linked to the actin cytoskeleton and can translate actomyosin-generated forces throughout the epithelial tissue, triggering coordinated internal movement of the organism, independently from the outer ciliary layer. Integrins, cadherins/adherens junction proteins, and occluding junction proteins serve as a hub for intracellular signaling molecules, helping the cell to sense its immediate environment and providing the foundation for the complex developmental programs that evolved in the animal kingdom (30, 57, 62).

Interestingly, several proteins that are members of key protein families composing the occluding junctions are also present at synaptic junctions where they link pre- and

postsynaptic membranes (54, 61), act as scaffold proteins for receptors, and act as intracellular signaling molecules. Among these are neurexins, immunoglobulin cell adhesion molecules, Na/K-ATPase transporters, and membrane guanylate kinases (MAGUKs) such as Dlg/PSD95 and ZO-1. This suggests that the intercellular connection between neurons may be based on molecular foundations that were established for epithelial function at the onset of animal evolution. A rigorous phylogenetic analysis is required to (in)validate this hypothesis; it would also be interesting to find out at which point neuroligins, a family of non-catalytic cholinesterase-like molecules that interact with neurexins, were recruited as adhesion proteins to synaptic junctions. The emergence of the postsynaptic repertoire in animal evolution has been discussed elsewhere (5, 27, 39, 45). Again, if simple synapses emerged from cellular junctions, it might be a formidable task to tell them apart morphologically in early animals.

Epithelial cell polarity

The plasma membrane of epithelial cells is segregated by the circumferential belt of adherens junctions into apical and basolateral domains with different lipid and protein compositions. Embedded in the apical domain is the ciliary pocket, a membrane patch with special vesicular trafficking requirements (146). The epithelial polarity is, in part, established and maintained via mutually antagonistic interactions among three protein complexes, mostly comprising cytosolic proteins recruited to the plasma membrane: the Par and Crumbs complexes at the apical domains, and the Scribble complex at the basolateral domain (120, 138). Only some components of these polarity complexes seem to have been present in unicellular holozoans (117). For example, only one of the three key subunits of the Scribble complex, the MAGUK protein Discs large (Dlg), has a unicellular precursor, whereas the two others, Scribble and lethal giant larvae (Lgl), originated in the metazoan lineage. Lgl can be linked directly to the underlying membrane trafficking machinery, as it interacts with syntaxin 4, a SNARE protein (Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor) involved in basolateral secretion in vertebrates (145).

Specific membrane-trafficking pathways (Fig. 2a) are essential for shaping the composition of the specific surface subdomains of the epithelial cell. By constantly recycling adhesion molecules, receptors, and transporters between the plasma membrane and

endosomes, the cell can adapt quickly to signals received. The ability to deliver cargo to specific regions of the plasma membrane is shared between all eukaryotic cells. The trafficking pathways in the budding yeast *Saccharomyces cerevisiae* are particularly well-studied: Secretory vesicles are delivered to a specific plasma membrane site, the daughter bud, during growth (118). The delivery of any transport vesicle in the eukaryotic cell depends on a molecular machine involving SNARE proteins, Sec1/Munc18 proteins, Rab GTPases, vesicle tethering complexes, and other regulatory proteins (Fig. 2b) (11, 18, 59). This is also the case of the Ca^{2+}-driven release of neurotransmitters or peptide hormones at the synapse (67, 119, 134). However, yeast cells, like most other eukaryotic cells, do not release transmitters in a Ca^{2+}-dependent manner. Noticeably, something must have changed to allow animal cells to secrete transmitters in a spatially and temporally manner. For example, the ability to sort material to different regions on the plasma membrane seems to have been improved significantly in epithelial cells, where different populations of apical and basolateral early endosomes and common recycling endosomes exist, which permits the transport of cargo across the entire cell, a process referred to as transcytosis (9, 21, 122). In view of their pivotal function, we have started to look into the evolution of the factors responsible for vesicular docking and fusion in animals. Here we discuss their significant expansion.

An expansion of trafficking factors

SNARE proteins constitute the engine of the molecular machine involved in vesicular fusion (Fig. 2b). SNARE proteins are small cytoplasmically oriented membrane-associated proteins with a relatively simple domain architecture. Heterologous sets of SNARE proteins assemble into tight, membrane-bridging complexes that pull together the membranes of two compartments. If one disregards lineage-specific expansions, the set of SNARE proteins of all five basic animal groups is surprisingly uniform. Interestingly, the key changes in the SNARE repertoire of basal animals were found mostly in the SNARE proteins involved in the endosomal/lysosomal trafficking steps (Fig. 2b) (74, 75). Likewise, only the Sec1/Munc18 protein involved in lysosomal trafficking, Vps33, has been duplicated. It thus seems that the transition to multicellularity in animals saw an increased capacity to sort cargo between endosomes and the plasma membrane, possibly reflecting the evolution of specific

trafficking routes to different populations of endosomes (Fig. 2a). Of note, duplicates of some of these factors were also found in choanoflagellates (Fig. 2b). Although our phylogenetic analysis was not able to find out whether these were the same duplications (75), this suggests that the cell's sorting capacities had already expanded before animals evolved. Later, major expansions of several trafficking factors occurred in vertebrates, caused by the two rounds of whole-genome duplications that occurred in this lineage. This expanded set certainly allowed for more finely tuned regulation and tissue-specific specialization in vertebrates.

An expansion of regulatory factors

Among the several factors regulating the core vesicle fusion machinery are CATCHR proteins (Complex Associated with Tethering Containing Helical Rods) (59, 150). These form elongated arrays of stacked α-helical bundles with flexible hinge regions, which tether transport vesicles to the site of fusion. Two types of CATCHR proteins play a role in secretion: the exocyst complex (90, 148) and MUN domain-containing protein (68). In animal cells, the exocyst complex controls housekeeping, “constitutive” secretion steps, whereas the MUN-domain proteins control secretion steps that are regulated by Ca^{2+} influx, such as neurotransmitter release in neurons (Fig. 3). Originally discovered in yeast, the exocyst complex was probably already present in the last common eukaryotic ancestor (LECA) and has not expanded much since. It is composed of eight subunits, two subunits of which, Sec3 and Exo70, directly bind to $PI(4,5)P_2$ in the inner leaflet of the plasma membrane. It controls numerous cellular processes, such as morphogenesis, ciliogenesis, cell migration, and, in neurons, axon and dendrite outgrowth. It is also important for cell polarization, as it guides the delivery of cadherin to the plasma membrane. MUN-domain proteins are reported in many eukaryotes, such as in fungi and in plants, where their role is elusive (77, 109). The MUN domain is structurally strikingly similar to other CATCHR family members. In animals, MUN-domain proteins (comprising (M)unc13s, BAP3/Baiap3, and CAPS/Unc31) regulate the release of classical neurotransmitters from synaptic vesicles and peptides from dense-core vesicles in neurons and endocrine cells, and also participate in regulated granule exocytosis in hematopoietic cells (33, 40, 72, 79). In animals, MUN-domain proteins are flanked by C2 domains, Ca^{2+}-binding modules that have been initially described as the second

conserved region of protein kinase C (PKC) and whose general function is to mediate the Ca^{2+}-triggered binding, often supported by $PI(4,5)P_2$-binding, of a protein to a membrane (82). Munc13s further contain a C1-domain, a diacylglycerol binding domain, which is the other conserved region of PKC. CAPSs have yet another different membrane attachment domain, a pleckstrin homology domain, which binds to phosphatidylinositol lipids. Altogether, MUN-domain proteins in animals can steer a protein onto a membrane upon an increase of the intracellular Ca^{2+} level thanks to Ca^{2+}-binding/membrane-binding modules. Although their precise function is not entirely clear, all protein subtypes are thought to tether vesicles in a primed state to the site of fusion. The priming step might reflect the fact that Ca^{2+} influx through voltage-gated channels is needed to make the fusion machinery ready. Note that Ca^{2+}-dependent priming has not been reported for the exocyst complex. Although relatively limited, the available phylogenetic data suggest that in contrast to the exocyst complex, which is evenly represented in eukaryotes, the subfamilies of MUN-domain proteins have clearly expanded, in combination with domain rearrangements. It would be fascinating to find out when exactly this evolutionary change took place. Most eukaryotes seem to have only one MUN-domain protein that does not have flanking C2 domains; Munc13-like proteins (with C1 and C2 domains) are present in unicellular holozoans, and all animals are equipped with members of two or three of the MUN-domain protein subfamilies. Rigorous phylogenetic analysis would be necessary to clarify whether the holozoan MUN-domain proteins had already split into the three subfamilies found in animals (Fig. 2b). In any case, the presence of MUN-domain proteins in unicellular holozoans suggests that these must have already possessed a Ca^{2+}-regulated tethering machine for secretion; whether it was used for cell-cell communication and whether the release was triggered by extracellular cues is unknown.

Additional regulatory factors of the vesicular fusion process comprise other Ca^{2+}-sensing proteins (111, 119, 134) which have appeared in the animal lineage (151). Among these, synaptotagmins and synaptotagmin-like proteins, containing tandem C2 domains, play an important role in controlling Ca^{2+}-dependent secretion in various processes, including the release of neurotransmitters and peptide hormones. Synaptotagmins are usually anchored in the membrane of the secretory vesicle via a transmembrane region, whereas synaptotagmin-like factors are soluble proteins. The *N*-terminal domain of synaptotagmin-like factors such as Rabphilin, as its name suggests, is composed of a binding

domain for secretory Rab GTPases. Rim/Unc10 is another multidomain protein with tandem C2 domains, which is present only in animals. Its *N*-terminal domain can form a tripartite complex with a secretory Rab protein and the *N*-terminal C2 domain of Munc13. Rim is considered to be a central organizers of the active zone, a region of the synapse dedicated to the release of neurotransmitters (119, 134). In essence, in animal cells, Ca^{2+}-regulated secretion steps have been brought to the fore, thanks to multiplications and appendage of C2 domains, while constitutive secretion proceeds.

Another intriguing expansion of vesicle trafficking factors in animals occurred among Rab proteins, and in particularly in Rab subfamilies involved in trafficking towards the plasma membrane (76). Rabs are considered as signposts that regulate the movement and arrival of vesicles (152). They are small G-proteins that function as molecular switches cycling between a GTP-bound 'on' form and a GDP-bound 'off ' form. The conversion occurs through GTP hydrolysis and involves a major conformational change. In their GTP-bound form, Rabs are anchored to the vesicle membrane and specifically recruit various effector proteins involved in the corresponding trafficking event. In their GDP-bound form, they are extracted from the bilayer. Among the novel Rab proteins are Rab3 and Rab27, which are key to the release from small synaptic vesicles (usually loaded with small classical neurotransmitters) or large dense core vesicles (containing neuropeptides) (50), respectively. Of note, many neurons co-release both types of transmitter.

Together, this suggests that in animals, several regulating factors were added to the Ca^{2+}-dependent tethering machinery, which fine-tuned the process, and also reflects the emergence of specialized cells. These features, shared by all animal groups, were probably already established in the Urmetazoan.

Lysosomes, transporters, and transmitters

Choanoflagellates, like many other eukaryotes, feed by phagocyting bacteria, which are then degraded in the lysosomal compartment. In the colonial bacterivorous protometazoan, improved sorting capacities between plasma membrane domains and endosomal/lysosomal compartments must have come in handy for providing nutrients to cells in the interior: nutrients could be delivered at the basal side using secretory vesicles derived directly from lysosomes and not only via membrane transporters.

Recent studies have revealed that the lysosomal compartment is not simply a degradative compartment but has a master role in cellular nutrient sensing and distribution (34, 86, 107, 110, 149), as reflected by the large variety of lysosomal storage disorders (24). The conserved mechanistic Target Of Rapamycin Complexes (mTORC), Rag GTPases, and vacuolar ATPase (v-ATPase) are involved in sensing and regulating the level of intracellular amino acids together with the arginine transporter SLC38A9, a membrane transporter of the solute carrier protein (SLC) superfamily (115, 143). Interestingly, this arginine-sensing mechanism might have evolved in early-branching animals (124). The large SLC superfamily includes various different types of active and facilitative transporters that are found in all domains of life (32, 58, 125). By and large, animals made use of a rich repertoire of transporters that were already present in single-celled eukaryotes. Of the 400 different human SLCs, about 30 can be found on lysosomes (20). Interestingly, the transporters that load synaptic vesicles with classical neurotransmitters (usually amino acids or their derivatives) are driven by the proton gradient generated by v-ATPase (8, 103) and are related to some lysosomal SLCs (20).

It should also be pointed out that classical neurotransmitters are loaded into synaptic vesicle-specific transporters that belong to entirely different SLC families (8, 103). In animals, glutamate (and other anionic neurotransmitters) are taken up by SLC17 transporters, and monoamines and acetylcholine are taken up by SLC18 transporters, whereas GABA and glycine accumulate through a SLC32 transporter. One SLC17 transporter with unknown properties is present in choanoflagellates, and some SLC17 transporters are expressed in early-branching animals; the aforementioned SLC18 and SLC32 transporters are also present in the calcareous sponge *Sycon ciliatum*. This implies that different types of chemicals were potentially available as neurotransmitters very early in animal evolution. Interestingly, a putative glutamate transporter (i.e. an SLC17-type), together with several other enzymes involved in the production of different types of classical fast neurotransmitters, was found to be expressed only in the endoderm, mostly around the pharynx of *N. vectensis* (104). Thus it is conceivable that the first neurotransmitter-loaded vesicles were not destined primarily for chemical transmission inside the body but contained nutrients (55, 71). It is conceivable that, in the beginning, classical transmitters/nutrients were loaded fortuitously into vesicles already containing peptide hormones and released in a Ca^{2+}-dependent manner. Functional investigations could shed

light on the time in evolution at which amino acids started to be used for chemical transmission. In this context, it should be noted that peptide hormones and their respective receptors appeared in early metazoans as well. In fact, neurotransmission in cnidarians, and possibly other early-branching animals, is dominated by peptide hormones (70, 78, 92). Interestingly, peptide hormones are derived from larger precursor molecules by lysosomal proteases (60), suggesting that they could also have arisen as a by-product of lysosomal degradation. These molecules, secreted in response to specific nutrients or hazardous bacteria, could signal or be sensed by other cells and thus change the other cells' behavior. Since peptide hormones are not released exclusively from neuronal cells but also from epithelial cells (16), one can suggest that this communication mechanism evolved before the appearance of different cell types in the metazoan body.

Last, it should be noted that most of the peptides released by neurons act by binding to G protein-coupled receptors and are therefore considered to evoke a slower response than small molecule transmitters (140), which open ligand-gated ion channels. Interestingly, however, some neuropeptides act via peptide-gated Na channels of the degenerin/epithelial Na channel (DEG/ENaC) family (53, 126) or the insect chemoreceptor family (19), and may mediate fast transmission in early-branching animals.

Emergence of true multicellularity

It is conceivable that with time, certain cells of the colonial premetazoan tended to specialize in sensing some environmental cues rather than taking up food particles – possibly because they were located at spots where bacteria capture was less likely or because they were better positioned than other cells to sense a given cue. Novel cell types gradually refined and reshaped the existing cellular modules for sensing, eating, moving, and transducing environmental cues. Division of labor truly gained momentum when it increased the fitness of the entire organism (2, 13), as possibly did the emergence of novel cells types in the epithelium, yielding a new feeding mode in early-branching animals. Though choanoflagellates and porifera feed by taking up entire bacteria via phagocytosis, some early-branching animals, such as placozoans, cnidarians and ctenophores, have developed mucoid-ciliary particle feeding (12, 135). This new feeding mode allowed them to make use of a new food source that could not be swallowed entirely by phagocytosis:

eukaryotic cells, such as algae for the placozoan *T. adhaerens* or even entire animals for cnidaria and ctenophora. They evolved novel epithelial cell types that produce digestive enzymes to predigest material on the outside, enabling small nutrients to be taken up as catabolites through selective transporters and pinocytosis. However, large particles may still be taken up by phagocytosis. Maybe other novel cell types released mucus on the apical side, whereas cells that took up nutrients had to invest much less into producing enzymes to break up larger particles within lysosomes.

Later in evolution, it is likely that epithelial cells, such as the endocytes of our intestine, no longer took up nutrients via phagocytosis but solely via membrane transporters in the microvilli on the apical surface, whereas the export of substances into the extracellular space at the basolateral side was carried out by other transporters or via secretory vesicles. Cells of the epithelium may also have transmitted information to the basolateral ECM by a Ca^{2+}-dependent release mechanism. For example, by propagating along the lateral membrane a change in potential induced by the activation of ion channels on the apical side, yielding an influx of Ca^{2+}-ions at a remote (basal) release site or by triggering a signaling cascade, leading to vesicular release and the opening of Ca^{2+} channels at the basal side (25). Gap junctions, which are narrow pores between adjacent cells that allow small molecules and ions to move from one cell to another, providing the means for direct metabolic and electrical coupling between cells, are not present in sponges or placozoans and thus might have evolved later (1, 47).

To capture their prey, cnidarians and ctenophores have developed sophisticated control mechanisms that require muscle-like cells, sensory cells, and neurons, forming a nerve net. Although less striking, *T. adhaerens* has also developed a complex control program, as it moves towards its algae food source, stops over it, activates enzyme-releasing secretory cells, and takes up nutrient while performing remarkable movements before resuming crawling (130). Such control over different other cell types, such as those involved in motility and digestion, could easily be conveyed by endocrine cells. Two types of “gland cells” have been described in *T. adhaerens* which bear resemblance to neuroendocrine cells, as they contain large dense-core vesicles, several neuronal proteins (131) (including a T-type Ca^{2+} channel homolog (127)), and have a cilium surrounded by microvilli. A subpopulation interspersed in the feeding epithelium may be appropriately positioned to sense nutrients and communicate with neighboring cells (possibly enzyme-secreting cells) via paracrine

secretion. Another subpopulation of cells is evenly spread at the rim close to the edge of the animal and hence is also in a favorable control position. Such cells may be compared to the enteroendocrine cells (EECs) of our intestine. EECs can sense nutrients as well as bacteria and can relay these sensory signals onto the enteric nerves. EECs are thought to signal in a paracrine fashion by releasing peptide hormones, but recently they were shown to have a cellular process, referred to as a neuropod, that is physically connected to the neurons, underlining the resemblance of EECs to neurons (52). Similar extensions, referred to as neurites, have been observed in neurosecretory cells in the digestive epithelium of cnidarians (37, 96, 135). The fine morphology and connectivity of gland cells in *T. adhaerens* have yet to be investigated. This might provide a glimpse into an early stage of the nervous system.

Interestingly, chemosensory cells, such as EECs, sense amino acids on their apical side, and use some of them as neurotransmitters on their basolateral side. It is thus conceivable that an initial connection between two neuroendocrine cells may have been achieved simply by the shift of a chemosensory cell out of the epithelium in such a way that the apical side of this cell lay close to the basolateral side of another chemosensory cell that remained in the epithelium (Fig. 4). Such a connection could have been fostered using existing junctions as hubs for signaling molecules (5, 27, 39, 45). Their evolution into morphological synapses would have increased the speed and efficiency of communication and they could have given rise to a more complex circuit resembling a nervous system. In fact, it is an old idea that an ancestral polarized secretory cell gave rise to neurons and endocrine cells (2, 55, 84, 98, 108, 128). In bilaterians, this idea has been in conflict with the fact that neurons essentially originate from the ectoderm, whereas chemosensory cells such as EECs derive from the endoderm. However, in the present context, it is important to underline that in non-bilaterians some neurons are essentially derived from the endoderm (26, 56, 85, 96, 104), making it possible that originally, the entire epithelium gave rise to sensory cells with a paracrine signaling function, from which endocrine cells and neurons then evolved. In this connection, it should also be noted that close evolutionary links exist between neuroendocrine and immune cells (22, 88, 105), as both draw on similar molecular machines that are involved in Ca^{2+}-regulated secretion (33, 40, 72, 79).

In epithelial cells, released transmitters could have influenced the ciliary beat and/or triggered actomyosin-based local contractions, which are readily sensed by neighboring cells

thanks to cell-cell junctions. Evolution sustained this changeover, as actomyosin-induced contractions allowed for larger movements and deformations of the organism. True muscles possibly formed when the actomyosin network became anchored to the foundation provided by a solid ECM, enabling motile animals to become larger. The tight interplay between cellular movements and neuroendocrine cells was probably the starting point for the evolution of more complex motile animals. Diffuse nerve nets soon started to be centralized around sensory organs and innervated muscle tissue. These animals were able to search actively for food (93). The first macropredators and protective mineralized skeletons appeared, changing ocean life during the Cambrian explosion (12, 36, 41, 46, 147).

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Figure legends

Fig. 1 - Schematic morphology-based phylogenetic tree, showing the major groups of animals

Nervous systems and muscles can be found in three basic groups of animals: cnidarians, ctenophores, and bilaterians (17, 99, 100). Bilaterians are animals with bilateral symmetry and comprise the majority of animal phyla, most of which have centralized nervous systems, whereas the more simple cnidarians and ctenophores (commonly known as comb jellies) have a decentralized nerve net. Cnidarians and ctenophores possess two primary germ layers: the ectoderm and endoderm. Bilaterians have a third tissue layer, the mesoderm, between the endoderm and the ectoderm. The two other basic groups (poriferans (sponges) and placozoans) do not have nerve and muscle cells. The body plan of both lineages is simple. Poriferans have two epithelial-like cell layers: the choanoderm, a layer of flagellated collar cells (choanocytes) in the interior, whereas the body surface is covered by the pinacoderm. The flat body of the placozoan *Trichoplax adhaerens* consists of two epithelial layers around a more loosely packed interior. Comparisons between the genomes from animals and their close unicellular relatives (as representatives of unicellular holozoans, choanoflagellates are shown) have uncovered a major genomic overhaul at the base of the animal kingdom. Many novel genes play a role in key aspects of multicellularity, including factors involved in tyrosine signaling, transcription regulation, cell adhesion, cell polarity, and factors with a putative neuronal role. This suggests that the last common animal ancestor, the Urmetazoan, had various different cell types, including cells that were able to communicate via Ca^{2+}-dependent secretion, although probably none of the early-branching animals still resembles the Urmetazoan. The phylogenetic position of ctenophores is indicated by a dashed line, since a lively debate has been sparked recently as to whether sponges or ctenophores are the most basal animals (94, 123, 144). As the evidence for the scenario of ctenophores as the most basal animal is currently not strong and as our assessment is not greatly affected by this, we decided against discussing it here in detail, but the interested reader may refer to the discussions in (69, 89, 112, 137). Animal silhouettes are from http://phylopic.org; credits to Robbi Bishop-Taylor, T. Michael Keesey, Noah Schlottman, Michelle Site, M. Garfield, K. Anderson, Mali'o Kodis, and Thomas Hegna.

Fig. 2 - Expansion of vesicle docking and fusion proteins involved in endosomal sorting and in secretion during the rise of animals

(a) Features of polarized epithelial cells

It is commonly assumed that the metazoan ancestor was organized into a hollow colony of ciliated collar cells, which are responsible for food uptake. In early-branching animals, the epithelium usually lines the outer surface of the organism as a protective and nutritive layer. Epithelial cells are connected via adherens junctions that partition the plasma membrane into distinct apical and basolateral domains, which have distinct lipid compositions and specific sets of proteins. Typically, the apical surface of cells in the nutritive epithelium contains various microvilli and can also have a central flagellum or cilium. The flagellum is a microtubule-based extension of the cell covered by the plasma membrane, allowing locomotion via a dynein-motor – a molecular machine already established in the last common eukaryotic ancestor (LECA). Various types of receptors are localized on the apical surface, often concentrated around the ciliary pit. The basolateral domain contains integrin receptors that anchor the cell onto the extracellular matrix. The different surface proteins reach their destination via vesicle trafficking. After synthesis, membrane proteins are transported through the biosynthetic pathway from the endoplasmic reticulum (ER) to the Golgi apparatus and the trans-Golgi network (TGN) via sequential trafficking steps. In the TGN, they are sorted into distinct carriers to bring them to the appropriate plasma membrane domain or towards endosomal/lysosomal compartments. Some cargo proteins can also traffic through endosomal compartments before reaching the final surface domain. In epithelial cells, one can distinguish apical early endosomes (AEE), basolateral early endosomes (BEE), and common recycling endosomes (CRE). In addition, a slower apical recycling route through the apical recycling endosomes (ARE) has been described (9, 21, 122).

(b) The molecular machines involved in the principal aspects of vesicular trafficking are highly conserved among all eukaryotes, not only among different species but also among different trafficking steps within the cell. At each trafficking step, the core of each vesicle fusion machine consists of SNARE proteins, which assemble into a tight complex between the membranes. Their activity is orchestrated by various other conserved factors including Sec1/Munc18 (SM), tethering, Rab, and other regulatory proteins that are recruited during different phases of the reaction (shown as a stacked venn diagram) (11, 18, 59) (67, 119,

134). The color scheme indicates the different layers of the vesicle fusion machine. The molecular machines involved in different basic trafficking steps inside of the cell (a) are indicated using the same color code.

The evolutionary transition to animals saw several gene expansions of the factors involved in secretion and in endosomal sorting, whereas the machines of the first biosynthetic steps were less affected. The evolutionary history of factors with an established endosomal role are shown on the left side; the history of secretory factors is shown on the right side. The evolutionary time points of the major changes are shown on the top. The prototypical repertoires of selected factors of the LECA, early-branching metazoans, and vertebrates are shown, disregarding some lineage-specific duplications (75, 76). As a representative of unicellular holozoans, the repertoire of the choanoflagellate *Monosiga brevicollis* was used (27). Structural investigations of *M. brevicollis* proteins have shown that their mode of interaction has been largely maintained in animals (28). These specific expansions suggest that animals possess more finely tuned regulation mechanisms and cell-specific specialization for secretion and endosomal sorting. Interestingly, some factors that diversified in animals are present as duplicated already in *Monosiga*. This comprises the factors involved in endosomal trafficking but also in Ca^{2+}-dependent vesicle docking. In addition, novel factors such as complexin seem to have been added onto the fusion machinery before the rise of animals (27). Note that the rise and expansion of C2 domain proteins such as synaptotagmins, synaptotagmin-likes, and Rim that occurred in the animal lineage is not shown, since their phylogeny is not entirely resolved yet. Also note that the Scribble complex subunit Lgl arose through a duplication of the regulatory SNARE protein tomosyn during the rise of animals (49, 74). In contrast to Lgl, the *C*-terminal tail region of tomosyn has preserved a synaptobrevin-like SNARE domain, which enables the protein to form stable SNARE complexes with syntaxin 1 and SNAP-25 (113). Consequently, tomosyn is thought to act as an inhibitor of transmitter release, because it lacks a transmembrane anchor and can thus only form non-fusogenic SNARE complexes (15). Although the *C*-terminal SNARE domain has deteriorated in Lgl, it seems plausible that Lgl has maintained a function comparable to that of tomosyn.

Fig. 3 - Comparison of molecular machines driving the release from secretory vesicles

The docking and fusion of secretory vesicles with the plasma membrane is driven by a complex protein machinery. The key factors involved belong to structurally conserved protein families. Although the core fusion factors are often shared by different types of secretory vesicles, they can use different sets of regulatory proteins. Among these are Rab proteins and are tethering complexes that belongs to the CATCHR (Complex Associated with Tethering Containing Helical Rods) family and coordinate SNARE complex assembly (59, 150).

(a) During polarized secretion, the vesicle is tethered to the plasma membrane by the octameric exocyst complex (90, 148). The exocyst complex also interacts with Rho GTPases and the vesicle motor myosin V (not shown).

(b) During Ca^{2+}-dependent secretion, such as the release of neurotransmitters from synaptic vesicles, the vesicle is tethered to the plasma membrane by MUN-domain protein such as Munc13. MUN-domain proteins also belongs to the CATCHR family but have additional flanking C2 domains that allow the protein to bind to the membrane in a Ca^{2+}-dependent manner (68). Various other regulatory proteins such as complexin, synaptotagmin, and Rim are known to participate in regulated secretion. The latter two emerged during animal evolution, whereas complexins emerged earlier (27). The release of synaptic vesicles is triggered by the influx of Ca^{2+} through voltage-gated Ca^{2+} channels. Note that tandem C2 domain proteins have been found to also control lysosomal secretion, a process that might have originally been used to discard waste material or to repair the plasma membrane in all eukaryotes (7, 25). This process can also make use of dysferlins, a more ancient protein family with multiple C2 domains (81). Interestingly, neurotransmitter release from ribbon synapses of cochlear inner hair cells is controlled by otoferlin, a dysferlin family member. This process appears not to require “neuronal” SNARE proteins (101) and was found not to depend on MUN-domain tethering proteins (141) and currently remains difficult to categorize.

Fig. 4 - Hypothetical evolutionary scenario for the transition from paracrine to synaptic communication.

(a) The primordial epithelium of uniform multifunctional polarized epithelial cells. In the apical domain, around the cilium, various receptors are localized, which can respond to chemical or physical environmental stimuli (red). Signals are passed electrically and chemical transmitters are released into the interior of the colonial organism in a Ca^{2+}-dependent fashion by vesicles at the basal membrane (blue). From there, they reach neighboring cells in a paracrine fashion, coordinating their behavior. Food particles are taken up by phagocytosis at the apical side and digested in lysosomes, and metabolites are released at the basolateral side (gray) to feed cells in the interior of the colony. During evolution, certain epithelial cells started to specialize in sensing environmental cues, whereas other cells remained concentrated on food uptake but probably kept the ability to transmit signals in a paracrine fashion. Other, well-positioned epithelial cells might have strengthened their actin cytoskeleton to drive the movement of larger epithelial areas. These movements started to be coordinated not only through cellular junctions but also via paracrine signals.

(b) It is conceivable that, in the next evolutionary step, sensory cells started to sink into the interior to sense the Ca^{2+}-dependent release from sensory cells that had remained in the epithelium. The establishment of specialized junctions between the sensory cells, the synapse, allowed them to communicate more efficiently. In a similar fashion, muscle-like cells moved to the interior but needed to anchor to a strong basement membrane to exert force. Note that comparable scenarios have been proposed by (2, 55, 98).

References

1. Abedin M, King N. 2010. Diverse evolutionary paths to cell adhesion. *Trends Cell Biol*. 20(12):734–42
2. Achim K, Arendt D. 2014. Structural evolution of cell types by step-wise assembly of cellular modules. *Curr Opin Genet Dev*. 27:102–8
3. Ackermann F, Waites CL, Garner CC. 2015. Presynaptic active zones in invertebrates and vertebrates. *EMBO Rep.* 16(8):923–38
4. Adamska M. 2016. Sponges as models to study emergence of complex animals. *Curr Opin Genet Dev*. 39:21–28
5. Alié A, Manuel M. 2010. The backbone of the post-synaptic density originated in a unicellular ancestor of choanoflagellates and metazoans. *BMC Evol Biol*. 10(1):34
6. Anderson PAV. 2015. The Journal of Experimental Biology: 218 (4). *Journal of Experimental Biology*. 218(4):501–3
7. Andrews NW, Almeida PE, Corrotte M. 2014. Damage control: cellular mechanisms of plasma membrane repair. *Trends in Cell Biology*. 24(12):734–42
8. Anne C, Gasnier B. 2014. Vesicular neurotransmitter transporters: mechanistic aspects. *Curr Top Membr*. 73:149–74
9. Apodaca G, Gallo LI, Bryant DM. 2012. Role of membrane traffic in the generation of epithelial cell asymmetry. *Nat Cell Biol*. 14(12):1235–43
10. Arbas EA, Meinertzhagen IA, Shaw SR. 1991. Evolution in nervous systems. *Annual Review of Neuroscience*. 14:9–38
11. Archbold JK, Whitten AE, Hu S-H, Collins BM, Martin JL. 2014. SNARE-ing the structures of Sec1/Munc18 proteins. *Current Opinion in Structural Biology*. 29:44–51
12. Arendt D, Benito-Gutierrez E, Brunet T, Marlow H. 2015. Gastric pouches and the mucociliary sole: setting the stage for nervous system evolution. *Philos Trans R Soc Lond B Biol Sci*. 370(1684):
13. Arendt D, Musser JM, Baker CVH, Bergman A, Cepko C, et al. 2016. The origin and evolution of cell types. *Nat Rev Genet*. 17(12):744–57
14. Arendt D, Tosches MA, Marlow H. 2016. From nerve net to nerve ring, nerve cord and brain--evolution of the nervous system. *Nat Rev Neurosci*. 17(1):61–72
15. Ashery U, Bielopolski N, Barak B, Yizhar O. 2009. Friends and foes in synaptic transmission: the role of tomosyn in vesicle priming. *Trends Neurosci*. 32(5):275–82
16. Attenborough RMF, Hayward DC, Kitahara MV, Miller DJ, Ball EE. 2012. A “neural” enzyme in nonbilaterian animals and algae: preneural origins for peptidylglycine α-amidating monooxygenase. *Mol Biol Evol*. 29(10):3095–3109
17. Ax P. 1996. *Multicellular Animals*. Berlin, Heidelberg: Springer Berlin Heidelberg
18. Baker RW, Hughson FM. 2016. Chaperoning SNARE assembly and disassembly. *Nat Rev Mol Cell Biol*. 17(8):465–79
19. Benton R. 2015. Multigene Family Evolution: Perspectives from Insect Chemoreceptors. *Trends Ecol Evol (Amst)*. 30(10):590–600
20. Bissa B, Beedle AM, Govindarajan R. 2016. Lysosomal solute carrier transporters gain momentum in research. *Clin. Pharmacol. Ther.* 100(5):431–36
21. Blasky AJ, Mangan A, Prekeris R. 2015. Polarized protein transport and lumen formation during epithelial tissue morphogenesis. *Annu Rev Cell Dev Biol*. 31:575–91
22. Bosch TCG. 2013. Cnidarian-microbe interactions and the origin of innate immunity in metazoans. *Annu Rev Microbiol*. 67:499–518

23. Bosch TCG, Klimovich A, Domazet-Lošo T, Gründer S, Holstein TW, et al. 2017. Back to the Basics: Cnidarians Start to Fire. *Trends Neurosci*. 40(2):92–105
24. Boustany R-MN. 2013. Lysosomal storage diseases--the horizon expands. *Nat Rev Neurol*. 9(10):583–98
25. Brunet T, Arendt D. 2016. From damage response to action potentials: early evolution of neural and contractile modules in stem eukaryotes. *Philos Trans R Soc Lond B Biol Sci*. 371(1685):20150043
26. Burke RD, Moller DJ, Krupke OA, Taylor VJ. 2014. Sea urchin neural development and the metazoan paradigm of neurogenesis. *Genesis*. 52(3):208–21
27. Burkhardt P. 2015. The origin and evolution of synaptic proteins - choanoflagellates lead the way. *J Exp Biol*. 218(Pt 4):506–14
28. Burkhardt P, Stegmann CM, Cooper B, Kloepper TH, Imig C, et al. 2011. Primordial neurosecretory apparatus identified in the choanoflagellate Monosiga brevicollis. *Proc Natl Acad Sci USA*. 108(37):15264–69
29. Cai X, Wang X, Patel S, Clapham DE. 2015. Insights into the early evolution of animal calcium signaling machinery: a unicellular point of view. *Cell Calcium*. 57(3):166–73
30. Case LB, Waterman CM. 2015. Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. *Nat Cell Biol*. 17(8):955–63
31. Cereijido M, Contreras RG, Shoshani L. 2004. Cell adhesion, polarity, and epithelia in the dawn of metazoans. *Physiol Rev*. 84(4):1229–62
32. César-Razquin A, Snijder B, Frappier-Brinton T, Isserlin R, Gyimesi G, et al. 2015. A Call for Systematic Research on Solute Carriers. *Cell*. 162(3):478–87
33. Chang HF, Bzeih H, Chitirala P, Ravichandran K, Sleiman M, et al. 2017. Preparing the lethal hit: interplay between exo- and endocytic pathways in cytotoxic T lymphocytes. *Cellular and molecular life sciences : CMLS*. 74(3):399–408
34. Chantranupong L, Wolfson RL, Sabatini DM. 2015. Nutrient-sensing mechanisms across evolution. *Cell*. 161(1):67–83
35. Cromar G, Wong K-C, Loughran N, On T, Song H, et al. 2014. New tricks for “old” domains: how novel architectures and promiscuous hubs contributed to the organization and evolution of the ECM. *Genome Biol Evol*. 6(10):2897–2917
36. Cunningham JA, Liu AG, Bengtson S, Donoghue PCJ. 2017. The origin of animals: Can molecular clocks and the fossil record be reconciled? *Bioessays*. 39(1):1–12
37. Davis LE. 1974. Ultrastructural Studies of the Development of Nerves in Hydra. 14(2):551–73
38. Dayel MJ, King N. 2014. Prey Capture and Phagocytosis in the Choanoflagellate Salpingoeca rosetta. *PLoS ONE*. 9(5):e95577
39. de Mendoza A, Suga H, Ruiz-Trillo I. 2010. Evolution of the MAGUK protein gene family in premetazoan lineages. *BMC Evol Biol*. 10(1):93
40. de Saint Basile G, Ménasché G, Fischer A. 2010. Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. *Nat Rev Immunol*. 10(8):568–79
41. Droser ML, Gehling JG. 2015. The advent of animals: The view from the Ediacaran. *Proc Natl Acad Sci USA*. 112(16):4865–70
42. Dunn CW, Leys SP, Haddock SHD. 2015. The hidden biology of sponges and ctenophores. *Trends Ecol Evol (Amst)*. 30(5):282–91
43. Dunn CW, Ryan JF. 2015. The evolution of animal genomes. *Curr Opin Genet Dev*. 35:25–32
44. Eitel M, Osigus H-J, DeSalle R, Schierwater B. 2013. Global diversity of the placozoa.

PLoS ONE. 8(4):e57131
45. Emes RD, Grant SGN. 2012. Evolution of synapse complexity and diversity. *Annual Review of Neuroscience*. 35:111–31
46. Erwin DH. 2015. Early metazoan life: divergence, environment and ecology. *Philos Trans R Soc Lond B Biol Sci*. 370(1684):20150036–36
47. Fahey B, Degnan BM. 2010. Origin of animal epithelia: insights from the sponge genome. *Evol Dev*. 12(6):601–17
48. Fahey B, Degnan BM. 2012. Origin and evolution of laminin gene family diversity. *Mol Biol Evol*. 29(7):1823–36
49. Fasshauer D, Jahn R. 2007. Budding insights on cell polarity. *Nat Struct Mol Biol*, May, pp. 360–62
50. Fukuda M. 2013. Rab27 effectors, pleiotropic regulators in secretory pathways. *Traffic*. 14(9):949–63
51. Ganot P, Zoccola D, Tambutté E, Voolstra CR, Aranda M, et al. 2015. Structural molecular components of septate junctions in cnidarians point to the origin of epithelial junctions in eukaryotes. *Mol Biol Evol*. 32(1):44–62
52. Gribble FM, Reimann F. 2016. Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium. *Annu Rev Physiol*. 78:277–99
53. Gründer S, Assmann M. 2015. Peptide-gated ion channels and the simple nervous system of Hydra. *J Exp Biol*. 218(Pt 4):551–61
54. Harden N, Wang SJH, Krieger C. 2016. Making the connection - shared molecular machinery and evolutionary links underlie the formation and plasticity of occluding junctions and synapses. *J Cell Sci*. 129(16):3067–76
55. Hartenstein V. 2006. The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. *J Endocrinol*. 190(3):555–70
56. Hartenstein V, Stollewerk A. 2015. The evolution of early neurogenesis. *Dev Cell*. 32(4):390–407
57. Heller E, Fuchs E. 2015. Tissue patterning and cellular mechanics. *Journal of Cell Biology*. 211(2):219–31
58. Hoglund PJ, Nordstrom KJV, Schioth HB, Fredriksson R. 2011. The Solute Carrier Families Have a Remarkably Long Evolutionary History with the Majority of the Human Families Present before Divergence of Bilaterian Species. *Mol Biol Evol*. 28(4):1531–41
59. Hong W, Lev S. 2014. Tethering the assembly of SNARE complexes. *Trends Cell Biol*. 24(1):35–43
60. Hook V, Funkelstein L, Lu D, Bark S, Wegrzyn J, Hwang S-R. 2008. Proteases for processing proneuropeptides into peptide neurotransmitters and hormones. *Annu. Rev. Pharmacol. Toxicol.* 48:393–423
61. Hortsch M, Margolis B. 2003. Septate and paranodal junctions: kissing cousins. *Trends in Cell Biology*. 13(11):557–61
62. Humphrey JD, Dufresne ER, Schwartz MA. 2014. Mechanotransduction and extracellular matrix homeostasis. *Nat Rev Mol Cell Biol*. 15(12):802–12
63. Hynes RO. 2012. The evolution of metazoan extracellular matrix. *J Cell Biol*. 196(6):671–79
64. Izumi Y, Furuse M. 2014. Molecular organization and function of invertebrate occluding junctions. *Semin Cell Dev Biol*. 36:186–93
65. Jacob F. 1977. Evolution and tinkering. *Science*. 196(4295):1161–66

66. Jager M, Manuel M. 2016. Ctenophores: an evolutionary-developmental perspective. *Curr Opin Genet Dev*. 39:85–92
67. Jahn R, Fasshauer D. 2012. Molecular machines governing exocytosis of synaptic vesicles. *Nature*. 490(7419):201–7
68. James DJ, Martin TFJ. 2013. CAPS and Munc13: CATCHRs that SNARE Vesicles. *Front Endocrinol (Lausanne)*. 4:187
69. Jekely G, Paps J, Nielsen C. 2015. The phylogenetic position of ctenophores and the origin(s) of nervous systems. *Evodevo*. 6(1):1
70. Jékely G. 2013. Global view of the evolution and diversity of metazoan neuropeptide signaling. *Proc Natl Acad Sci USA*. 110(21):8702–7
71. Jorgensen EM. 2014. Animal evolution: looking for the first nervous system. *Current Biology*. 24(14):R655–58
72. Joshi S, Whiteheart SW. 2016. The nuts and bolts of the platelet release reaction. *Platelets*, pp. 1–9
73. Kavalali ET, Jorgensen EM. 2014. Visualizing presynaptic function. *Nat Neurosci*. 17(1):10–16
74. Kloepper TH, Kienle CN, Fasshauer D. 2007. An elaborate classification of SNARE proteins sheds light on the conservation of the eukaryotic endomembrane system. *Mol. Biol. Cell*. 18(9):3463–71
75. Kloepper TH, Kienle CN, Fasshauer D. 2008. SNAREing the basis of multicellularity: consequences of protein family expansion during evolution. *Mol Biol Evol*. 25(9):2055–68
76. Klöpper TH, Kienle N, Fasshauer D, Munro S. 2012. Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis. *BMC Biol*. 10:71
77. Koch H, Hofmann K, Brose N. 2000. Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. *Biochem J*. 349(Pt 1):247–53
78. Krishnan A, Schiöth HB. 2015. The role of G protein-coupled receptors in the early evolution of neurotransmission and the nervous system. *J Exp Biol*. 218(Pt 4):562–71
79. la Roche de M, Asano Y, Griffiths GM. 2016. Origins of the cytolytic synapse. *Nat Rev Immunol*. 16(7):421–32
80. Leadbeater BSC. 2015. *The choanoflagellates : evolution, biology, and ecology*. Cambridge: Cambridge University Press
81. Lek A, Evesson FJ, Sutton RB, North KN, Cooper ST. 2012. Ferlins: regulators of vesicle fusion for auditory neurotransmission, receptor trafficking and membrane repair. *Traffic*. 13(2):185–94
82. Lemmon MA. 2008. Membrane recognition by phospholipid-binding domains. *Nat Rev Mol Cell Biol*. 9(2):99–111
83. Leys SP, Nichols SA, Adams EDM. 2009. Epithelia and integration in sponges. *Integr Comp Biol*. 49(2):167–77
84. Lichtneckert R, Reichert H. 2007. Origin and Evolution of the First Nervous System. In *Evolution of Nervous Systems*, pp. 289–315. Elsevier. 27 p.
85. Liebeskind BJ, Hillis DM, Zakon HH, Hofmann HA. 2016. Complex Homology and the Evolution of Nervous Systems. *Trends Ecol Evol (Amst)*. 31(2):127–35
86. Lim C-Y, Zoncu R. 2016. The lysosome as a command-and-control center for cellular metabolism. *J Cell Biol*. 214(6):653–64

87. Mackie GO. 1990. The Elementary Nervous-System Revisited. *Am. Zool.* 30(4):907–20
88. Margolis KG, Gershon MD, Bogunovic M. 2016. Cellular Organization of Neuroimmune Interactions in the Gastrointestinal Tract. *Trends Immunol*. 37(7):487–501
89. Marlow H, Arendt D. 2014. Evolution: Ctenophore Genomes and the Origin of Neurons. *Current Biology*. 24(16):R757–61
90. Martin-Urdiroz M, Deeks MJ, Horton CG, Dawe HR, Jourdain I. 2016. The Exocyst Complex in Health and Disease. *Front. Cell Dev. Biol.* 4(400):24
91. Miller PW, Clarke DN, Weis WI, Lowe CJ, Nelson WJ. 2013. The evolutionary origin of epithelial cell-cell adhesion mechanisms. *Curr Top Membr*. 72:267–311
92. Mirabeau O, Joly J-S. 2013. Molecular evolution of peptidergic signaling systems in bilaterians. *Proc Natl Acad Sci USA*. 110(22):E2028–37
93. Monk T, Paulin MG. 2014. Predation and the origin of neurones. *Brain Behav. Evol.* 84(4):246–61
94. Moroz LL, Kocot KM, Citarella MR, Dosung S, Norekian TP, et al. 2014. The ctenophore genome and the evolutionary origins of neural systems. *Nature*. 510(7503):109–14
95. Murray PS, Zaidel-Bar R. 2014. Pre-metazoan origins and evolution of the cadherin adhesome. *Biology Open*. 3(12):1183–95
96. Nakanishi N, Renfer E, Technau U, Rentzsch F. 2012. Nervous systems of the sea anemone Nematostella vectensis are generated by ectoderm and endoderm and shaped by distinct mechanisms. *Development*. 139(2):347–57
97. Nichols SA, Roberts BW, Richter DJ, Fairclough SR, King N. 2012. Origin of metazoan cadherin diversity and the antiquity of the classical cadherin/β-catenin complex. *Proc Natl Acad Sci USA*. 109(32):13046–51
98. Nickel M. 2010. Evolutionary emergence of synaptic nervous systems: what can we learn from the non-synaptic, nerveless Porifera? *Invertebrate Biology*. 129(1):1–16
99. Nielsen C. 2008. Six major steps in animal evolution: are we derived sponge larvae? *Evol Dev*. 10(2):241–57
100. Nielsen C. 2012. *Animal evolution : interrelationships of the living phyla*. Oxford ; New York : Oxford University Press
101. Nouvian R, Neef J, Bulankina AV, Reisinger E, Pangršič T, et al. 2011. Exocytosis at the hair cell ribbon synapse apparently operates without neuronal SNARE proteins. *Nat Neurosci*. 14(4):411–13
102. O'Rourke NA, Weiler NC, Micheva KD, Smith SJ. 2012. Deep molecular diversity of mammalian synapses: why it matters and how to measure it. *Nat Rev Neurosci*. 13(6):365–79
103. Omote H, Miyaji T, Hiasa M, Juge N, Moriyama Y. 2016. Structure, Function, and Drug Interactions of Neurotransmitter Transporters in the Postgenomic Era. *Annu. Rev. Pharmacol. Toxicol.* 56:385–402
104. Oren M, Brickner I, Brikner I, Appelbaum L, Levy O. 2014. Fast neurotransmission related genes are expressed in non nervous endoderm in the sea anemone Nematostella vectensis. *PLoS ONE*. 9(4):e93832
105. Ottaviani E, Malagoli D, Franceschi C. 2007. Common evolutionary origin of the immune and neuroendocrine systems: from morphological and functional evidence to in silico approaches. *Trends Immunol*. 28(11):497–502

106. Ozbek S, Balasubramanian PG, Chiquet-Ehrismann R, Tucker RP, Adams JC. 2010. The evolution of extracellular matrix. *Mol. Biol. Cell*. 21(24):4300–4305
107. Palm W, Park Y, Wright K, Pavlova NN, Tuveson DA, Thompson CB. 2015. The Utilization of Extracellular Proteins as Nutrients Is Suppressed by mTORC1. *Cell*. 162(2):259–70
108. Pearse AGE. 1968. Common Cytochemical and Ultrastructural Characteristics of Cells Producing Polypeptide Hormones (the APUD Series) and their Relevance to Thyroid and Ultimobranchial C Cells and Calcitonin. *Proceedings of the Royal Society of London B: Biological Sciences*. 170(1018):71–80
109. Pei J, Ma C, Rizo J, Grishin NV. 2009. Remote homology between Munc13 MUN domain and vesicle tethering complexes. *J Mol Biol*. 391(3):509–17
110. Perera RM, Zoncu R. 2016. The Lysosome as a Regulatory Hub. *Annu Rev Cell Dev Biol*. 32:223–53
111. Pinheiro PS, Houy S, Sørensen JB. 2016. C2-domain containing calcium sensors in neuroendocrine secretion. *J Neurochem*. 139(6):943–58
112. Pisani D, Pett W, Dohrmann M, Feuda R, Rota-Stabelli O, et al. 2015. Genomic data do not support comb jellies as the sister group to all other animals. *Proc Natl Acad Sci U S A*. 112(50):15402–7
113. Pobbati AV, Razeto A, Böddener M, Becker S, Fasshauer D. 2004. Structural basis for the inhibitory role of tomosyn in exocytosis. *J Biol Chem*. 279(45):47192–200
114. Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, et al. 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science*. 317(5834):86–94
115. Rebsamen M, Pochini L, Stasyk T, de Araújo MEG, Galluccio M, et al. 2015. SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. *Nature*. 519(7544):477–81
116. Rentzsch F, Layden M, Manuel M. 2017. The cellular and molecular basis of cnidarian neurogenesis. *Wiley Interdiscip Rev Dev Biol*. 6(1):e257
117. Richter DJ, King N. 2013. The genomic and cellular foundations of animal origins. *Annu Rev Genet*. 47(1):509–37
118. Riquelme M. 2013. Tip growth in filamentous fungi: a road trip to the apex. *Annu Rev Microbiol*. 67:587–609
119. Rizo J, Xu J. 2015. The Synaptic Vesicle Release Machinery. *Annu Rev Biophys*. 44(1):339–67
120. Rodriguez-Boulan E, Macara IG. 2014. Organization and execution of the epithelial polarity programme. *Nat Rev Mol Cell Biol*. 15(4):225–42
121. Rokas A. 2008. The origins of multicellularity and the early history of the genetic toolkit for animal development. *Annu Rev Genet*. 42:235–51
122. Román-Fernández A, Bryant DM. 2016. Complex Polarity: Building Multicellular Tissues Through Apical Membrane Traffic. *Traffic*. 17(12):1244–61
123. Ryan JF, Pang K, Schnitzler CE, Nguyen AD, Moreland RT, et al. 2013. The Genome of the Ctenophore Mnemiopsis leidyi and Its Implications for Cell Type Evolution. *Science*. 342(6164):1242592–92
124. Saxton RA, Chantranupong L, Knockenhauer KE, Schwartz TU, Sabatini DM. 2016. Mechanism of arginine sensing by CASTOR1 upstream of mTORC1. *Nature*. 536(7615):229–33
125. Schlessinger A, Yee SW, Sali A, Giacomini KM. 2013. SLC classification: an update.

Clin. Pharmacol. Ther. 94(1):19–23
126. Schüler A, Schmitz G, Reft A, Ozbek S, Thurm U, Bornberg-Bauer E. 2015. The Rise and Fall of TRP-N, an Ancient Family of Mechanogated Ion Channels, in Metazoa. *Genome Biol Evol*. 7(6):1713–27
127. Senatore A, Raiss H, Le P. 2016. Physiology and Evolution of Voltage-Gated Calcium Channels in Early Diverging Animal Phyla: Cnidaria, Placozoa, Porifera and Ctenophora. *Front Physiol*. 7:481
128. Shaham S. 2010. Chemosensory organs as models of neuronal synapses. *Nat Rev Neurosci*. 11(3):212–17
129. Sheng M, Hoogenraad CC. 2007. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu Rev Biochem*. 76:823–47
130. Smith CL, Reese TS. 2016. Adherens Junctions Modulate Diffusion between Epithelial Cells in Trichoplax adhaerens. *Biol Bull*. 231(3):216–24
131. Smith CL, Varoqueaux F, Kittelmann M, Azzam RN, Cooper B, et al. 2014. Novel Cell Types, Neurosecretory Cells, and Body Plan of the Early-Diverging Metazoan Trichoplax adhaerens. *Curr Biol*. 24(14):1565–72
132. Srivastava M, Begovic E, Chapman J, Putnam NH, Hellsten U, et al. 2008. The Trichoplax genome and the nature of placozoans. *Nature*. 454(7207):955–60
133. Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier MEA, et al. 2010. The Amphimedon queenslandica genome and the evolution of animal complexity. *Nature*. 466(7307):720–26
134. Südhof TC. 2012. The presynaptic active zone. *Neuron*. 75(1):11–25
135. Takashima S, Gold D, Hartenstein V. 2013. Stem cells and lineages of the intestine: a developmental and evolutionary perspective. *Dev Genes Evol*. 223(1-2):85–102
136. Telford MJ, Budd GE, Philippe H. 2015. Phylogenomic Insights into Animal Evolution. *Curr Biol*. 25(19):R876–87
137. Telford MJ, Moroz LL, Halanych KM. 2016. Evolution: A sisterly dispute. *Nature*. 529(7586):286–87
138. Tepass U. 2012. The apical polarity protein network in Drosophila epithelial cells: regulation of polarity, junctions, morphogenesis, cell growth, and survival. *Annu Rev Cell Dev Biol*. 28:655–85
139. Tyler S. 2003. Epithelium--the primary building block for metazoan complexity. *Integr Comp Biol*. 43(1):55–63
140. van den Pol AN. 2012. Neuropeptide transmission in brain circuits. *Neuron*. 76(1):98–115
141. Vogl C, Cooper BH, Neef J, Wojcik SM, Reim K, et al. 2015. Unconventional molecular regulation of synaptic vesicle replenishment in cochlear inner hair cells. *J Cell Sci*. 128(4):638–44
142. Wan KY, Goldstein RE. 2016. Coordinated beating of algal flagella is mediated by basal coupling. *Proc Natl Acad Sci USA*. 113(20):E2784–93
143. Wang S, Tsun Z-Y, Wolfson RL, Shen K, Wyant GA, et al. 2015. Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. *Science*. 347(6218):188–94
144. Whelan NV, Kocot KM, Moroz LL, Halanych KM. 2015. Error, signal, and the placement of Ctenophora sister to all other animals. *Proc Natl Acad Sci USA*. 112(18):5773–78
145. Wirtz-Peitz F, Knoblich JA. 2006. Lethal giant larvae take on a life of their own.

Trends in Cell Biology. 16(5):234–41
146. Wood CR, Rosenbaum JL. 2015. Ciliary ectosomes: transmissions from the cell's antenna. *Trends Cell Biol*. 25(5):276–85
147. Wray GA. 2015. Molecular clocks and the early evolution of metazoan nervous systems. *Philos Trans R Soc Lond B Biol Sci*. 370(1684):20150046–46
148. Wu B, Guo W. 2015. The Exocyst at a Glance. *J Cell Sci*. 128(16):2957–64
149. Xu H, Ren D. 2015. Lysosomal physiology. *Annu Rev Physiol*. 77:57–80
150. Yu I-M, Hughson FM. 2010. Tethering factors as organizers of intracellular vesicular traffic. *Annu Rev Cell Dev Biol*. 26:137–56
151. Zhang D, Aravind L. 2010. Identification of novel families and classification of the C2 domain superfamily elucidate the origin and evolution of membrane targeting activities in eukaryotes. *Gene*. 469(1-2):18–30
152. Zhen Y, Stenmark H. 2015. Cellular functions of Rab GTPases at a glance. *J Cell Sci*. 128(17):3171–76
153. Zihni C, Mills C, Matter K, Balda MS. 2016. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat Rev Mol Cell Biol*. 17(9):564–80

Fig. 1 - Varoqueaux & Fasshauer

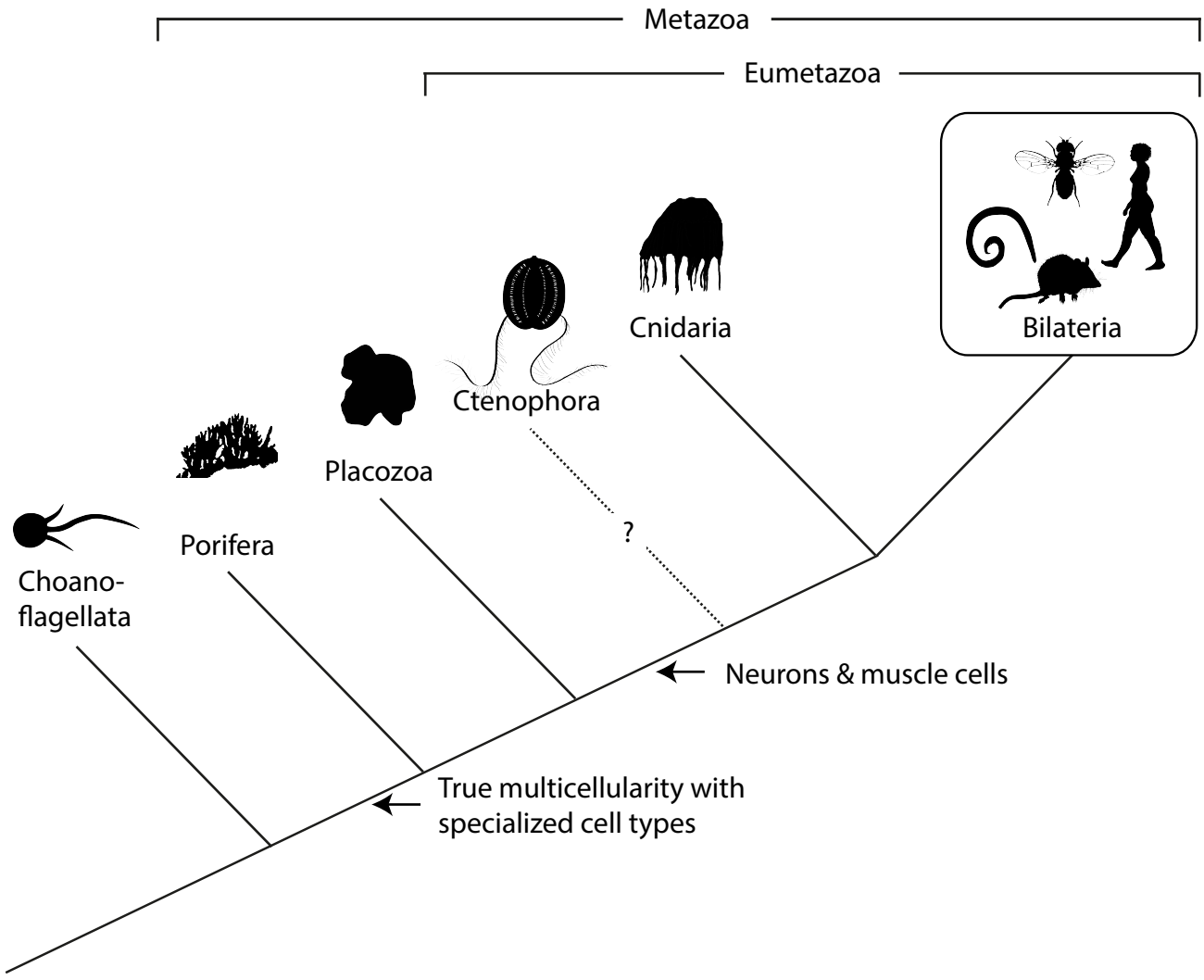
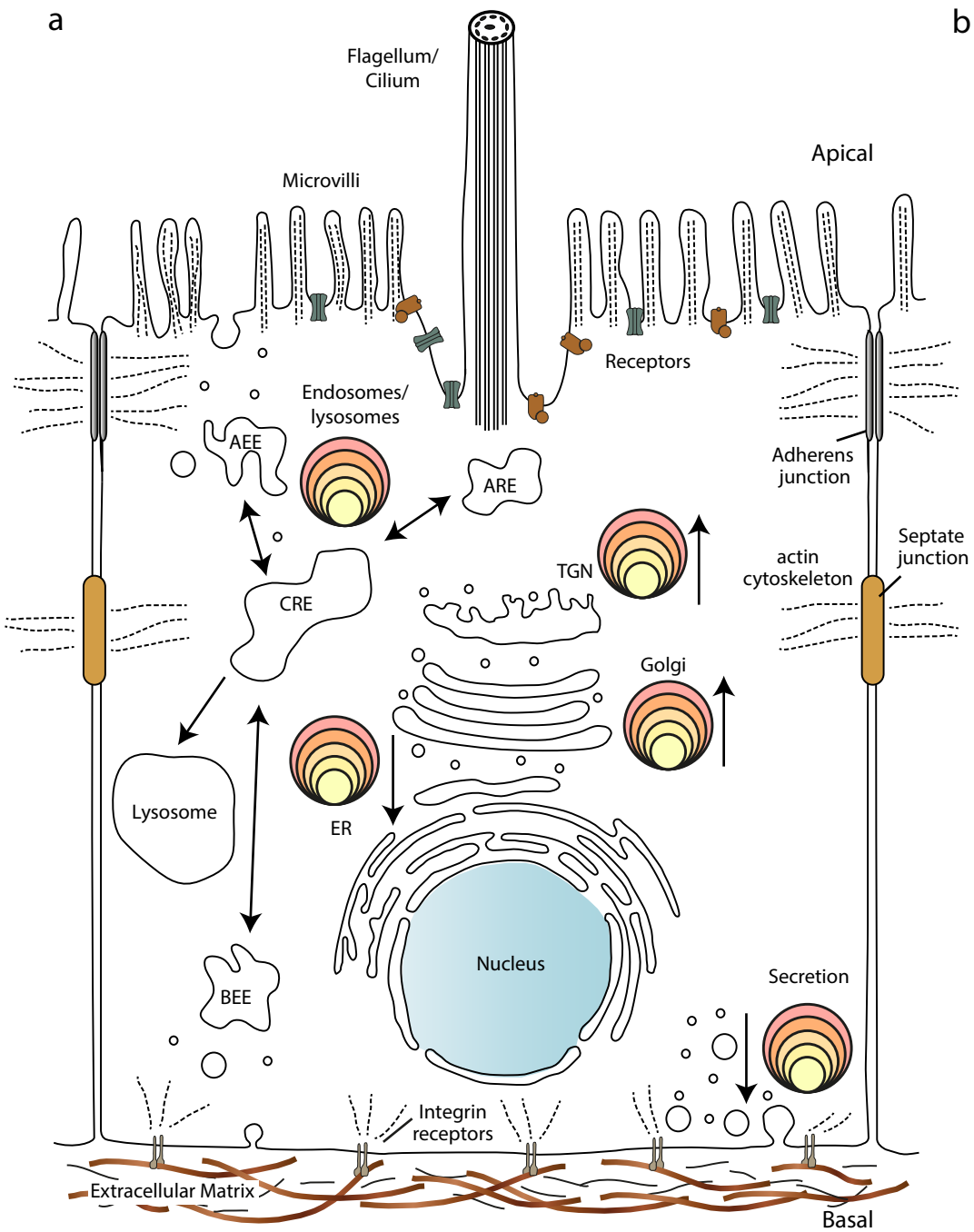
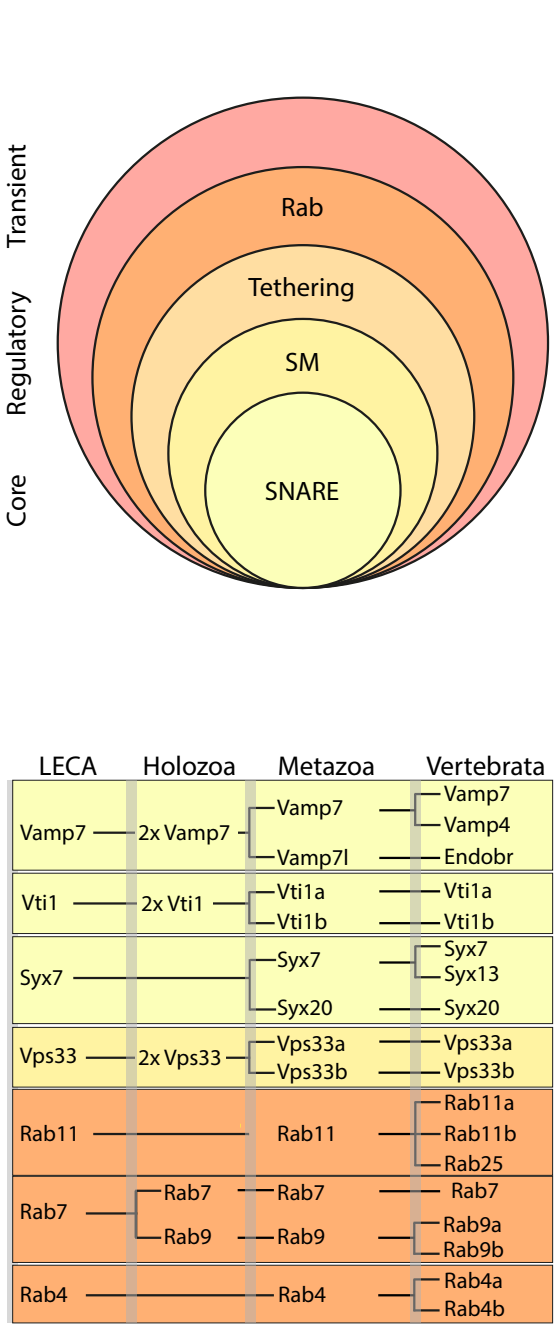


Fig. 2 - Varoqueaux & Fasshauer





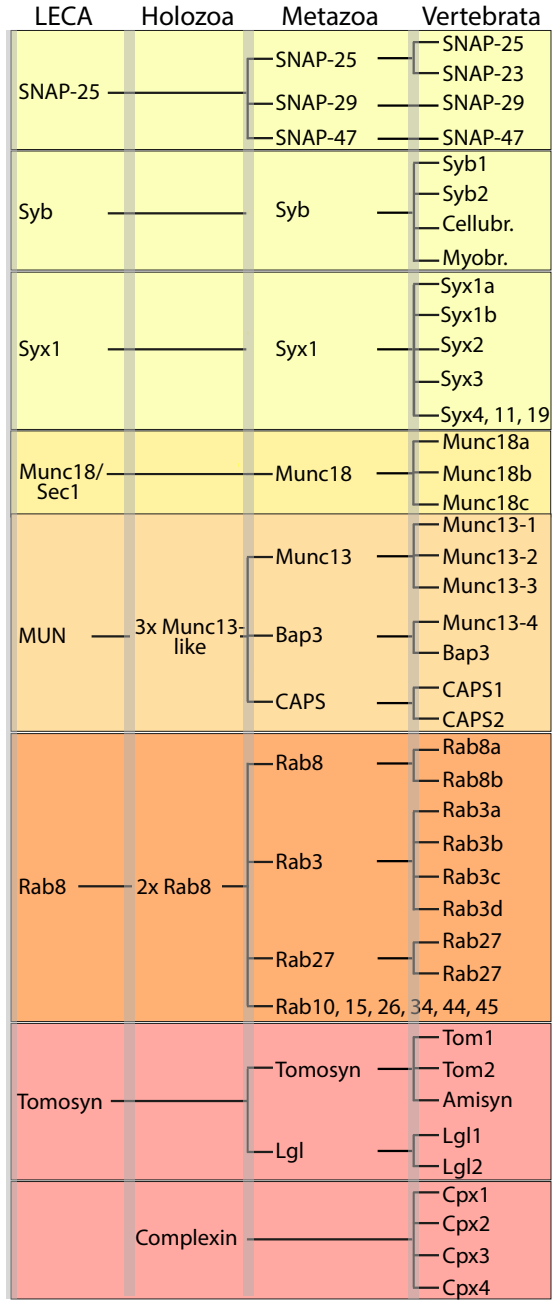


Fig. 3 - Varoqueaux & Fasshauer

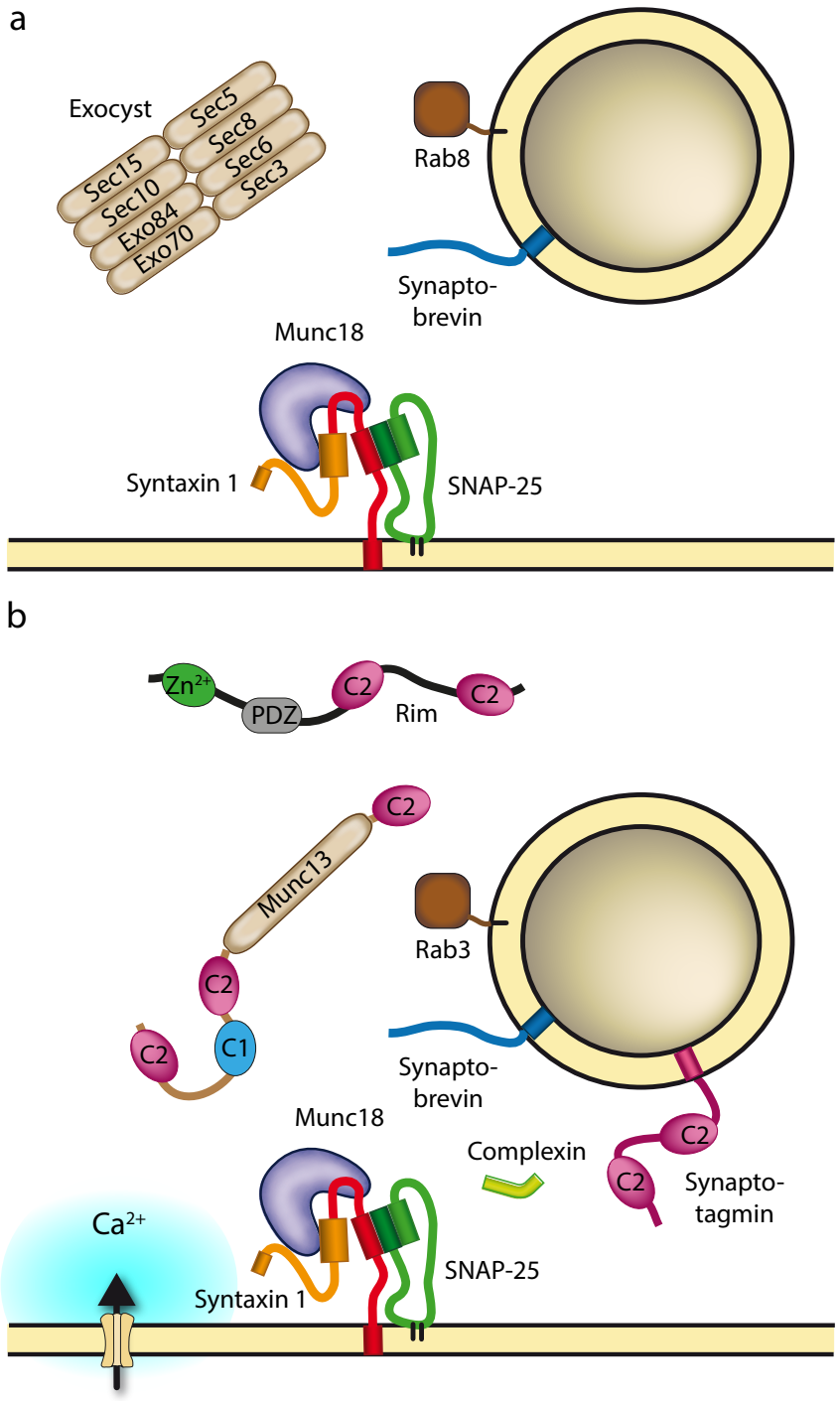


Fig. 4 - Varoqueaux & Fasshauer