Abstract

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Reference


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Origins of neurogenesis, a cnidarian view

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ABSTRACT

New perspectives on the origin of neurogenesis emerged with the identification of genes encoding post-synaptic proteins as well as many “neurogenic” regulators as the NK, Six, Pax, bHLH proteins in the Demospomg sponge genome, a species that might differentiate sensory cells but no neurons. However, poriferans seem to miss some key regulators of the neurogenic circuitry as the Hox/paraHox and Otx-like gene families. Moreover as a general feature, many genes families encoding evolutionarily-conserved signaling proteins and transcription factors were submitted to a wave of gene duplication in the last common eumetazoan ancestor, after Porifera divergence. In contrast gene duplications in the last common bilaterian ancestor, Urbilateria, are limited, except for the bHLH Atonal-class. Hence Cnidaria share with Bilateria a large number of genetic tools. The expression and functional analyses currently available suggest a neurogenic function for numerous orthologs in developing or adult cnidarians where neurogenesis takes place continuously. As an example, in the Hydra polyp, the Clytia medusa and the Acropora coral, the Gsx/cnox2 ParHox gene likely supports neurogenesis. Also neurons and nematocytes (mechano-sensory cells) share in hydrozoans a common stem cell and several regulatory genes indicating that they can be considered as sister cells. Performed in anthozoan and medusozoa species, these studies should tell us more about the way(s) evolution hazards achieved the transition from epithelial to neuronal cell fate, and about the robustness of the genetic circuitry that allowed neuromuscular transmission to arise and be maintained across evolution.

INTRODUCTION

Urbilateria and its older sisters Cnidaria and Ctenophora

In 1978, Ed Lewis in his seminal Nature paper (Lewis, 1978) predicted the evolutionary conservation of DNA-binding regulatory proteins that would control patterning along the anterior-posterior axis through cis-regulatory elements. Since then, the accumulation of molecular and genetic data indeed proved the wide conservation of the genetic networks regulating shared developmental processes among bilaterians, not only for the specification of the anterior to posterior axis but also the dorso-ventral axis, the head patterning and the eye specification (De Robertis, 2008). As anticipated, the main cellular differentiation processes in bilaterians also make use of evolutionarily-conserved genetic circuitries as those used for myogenesis (Yun and Wold, 1996), neurogenesis (Bertrand et al., 2002; Acampora et al., 2005; Denes et al., 2007; Tessmar-Raible et al., 2007), gametogenesis (Cox et al., 1998). Since 1991, orthologs of these bilaterian regulatory genes were identified not only in cnidarians (see below) but also in poriferae (Larroux et al., 2006; Larroux et al., 2008) and some could even be traced in choanoflagellates (King et al., 2008).

The zootype hypothesis proposed first that a same set of regulatory genes, namely homeobox genes, define the anterior to posterior (AP) axis in all animal species at an early and transient developmental stage (Slack et al., 1993). Subsequently the Urbilateria hypothesis proposed that, beside the AP axis, deuterostomes and protostomes also received from a common putative ancestor, named Urbilateria a genetic toolkit that specifies their dorso-ventral axis, including their neural tube (De Robertis, 2008). In the absence of extant Urbilatarian species, the Ctenophora and Cnidaria that diverged earlier in animal evolution but display anatomical polarities and differentiate a nervous system, are obvious candidates to test these hypotheses (Figure 1). In fact the initial expression analyses performed at the cellular level supported the hypothesis of a common origin for neurogenesis and also for the specification of the apical nervous system in cnidarians and anterior nervous system in bilaterians (Gauchat et al., 1998; Galliot and Miller, 2000). However this simple rule of the universal conservation of developmental genetic toolkits between animal phyla received some assault when it appeared that the zootype hypothesis could not be verified in cnidarians (Gauchat et al., 2000; Schierwater and Desalle, 2001; Chourrout et al., 2006; Kamm et al., 2006; Lee et al., 2006; Ryan et al., 2007; Chiori et al., 2009; Quiquand et al., 2009), and it is nowadays admitted that the specification of the embryonic AP axis by the Hox gene families only arose after Cnidaria divergence.

However what is true for the AP axis might not be true for the specification of the nervous systems. Alain Ghysen wrote about the Origin and Evolution of the Nervous System: “The extreme variability of behaviors and survival strategies among triploblasts would be subordinate on the previous attainment by the urbilaterners of a high level of developmental stability in the building of elementary functional circuits. According to this view, the initial triploblast radiation may have been contingent upon reaching this highly evolved stage of neural development” (Ghysen, 2003). In other words, the neurogenic circuitry was already established in a very stable way in Urbilateria (Arendt et al., 2008), suggesting that it might be possible to trace back some features of this ancestral nervous system in cnidarians that differentiate a rather sophisticated nervous system with numerous cellular and functional similarities to bilaterian ones. In bilaterians, homologous tasks such as differentiating nerve cells (Simionato et al., 2008) and mechanosensory organs (Ghysen, 2003), developing eyes (Pichaud and Desplan, 2002; Gehring, 2004), regionalizing the neural tube along the dorso-ventral axis (Denes et al., 2007; Mieko Mizutani and Bier, 2008) or patterning the tripartite brain (Lichtneckert and Reichert, 2005) rely on a shared set of transcription factors. We propose here to review the current knowledge about the molecular mechanisms that support neurogenesis in cnidarians and discuss some scenario that lead to this unique evolutionary transition.
The complex life cycle of cnidarians

Cnidaria is supposed to have diverged about 650 million years ago, preceding the Cambrian explosion, the period when ancestors to most extant bilaterian phyla arose from a common hypothetical ancestor named Urbilateria (Figure 1). Cnidarians are most often marine animals that commonly display a radial symmetry and are made up of two cell layers, the ectoderm and the endoderm, separated by an extracellular matrix named mesoglea (Bouillon, 1994b). However this “diploblastic” criterion is disputed as numerous cnidarian species actually differentiate “mesodermal” derivatives as striated muscle at one or the other stage of their life cycle (Seipel and Schmid, 2006). Cnidarian species cluster in two distinct classes (Bridge et al., 1995; Collins et al., 2006): the anthozoans that live exclusively as polyps (sea pens as Renilla, stony corals as Acropora, sea anemones as Aiptasia, Anthopleura, Nematostella) and the medusozoans that display a complex life cycle with a parental medusa stage and a sessile polyp stage. Among those, the cubozoans (Tripedalia cystophora) and scyphozoans (Aurelia aurita, Cassiopea xamachana) predominantly live as medusae, whereas the hydrozoans (Podocoryne, Clytiya, Cladonema, Eleutheria) usually follow a life cycle where they alternate between these two forms. However some hydrozoan species have lost the medusa stage as the marine Hydra polyps (Galliot and Schmid, 2002). Similarly the staurozoans that were only recently characterized as a group (Collins et al., 2006), live exclusively as polyps. Cnidarian polyps are basically a tube with a single opening circled by a ring of tentacles, which has a mouth-anus function. Cnidarians together with ctenophores (combjellies) are the first phyla where movements are governed by a neuromuscular system, as exemplified by their active feeding behavior that requires coordinated movements of their tentacles (Westfall and Kinnamon, 1984; Westfall, 1996). Therefore, cnidarians and ctenophores provide appropriate model systems to trace back the first-evolved nervous systems (Anderson and Spencer, 1989). In contrast, poriferans (sponges), which diverged earlier during evolution and are capable of chemical conduction (Leys et al., 1999), do not display any cell types exhibiting synaptic conduction and usually feed by passive filtration.

Anatomy of the cnidarian nervous systems

The cnidarian neurons form nerve nets and nerve rings

In textbooks the organization of the cnidarian nervous system is described as a “diffuse nerve net” homogenously distributed along the polyps, which can be visualized by neuron-specific immunostaining. However in adults, this nerve net is certainly not homogenous as the distribution of neurons is not uniform, neither at the qualitative nor at the quantitative levels. For example, in Hydra, distinct subsets of neurons with specific spatial distribution could be identified (Grimmelikhuijzen et al.,

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**FIGURE 1:** Origin of neurogenesis and progressive acquisition of a central nervous system along animal evolution. The differentiation of cells with synaptic transmission can be traced back to the last common ancestor of eumetazoans, whereas the differentiation of sensory cells possibly emerged in the last common ancestor of choanoflagellates and metazoans; in Porifera choanocytes are proposed to correspond to sensory cells. Both Ctenophora and Cnidaria differentiate a nervous system; they diverged prior to Bilateria but their respective positions are controversial. Similarly the position of Placozoa in Metazoa is debated. * indicate species with sequenced genome; † species that differentiate eyes,” species that have lost the medusa stage.
1989; Koizumi et al., 1990) and the nerve cell density is at least six fold higher in the head region than in the body column (Figure 2). Similarly in medusa the RFamide neurons are clearly more abundant in the manubrium and the tentacle bulbs (Grimmelikhuijzen and Spencer, 1984) (Figure 5). In addition to the nerve net, a dense anatomical structure, named the nerve ring was identified at the base of tentacles in some Hydra species (Koizumi et al., 1992), following the bell margin in jellyfish (Mackie, 2004), around the oral opening in Nematostella (Marlow et al., 2009). Nerve rings are considered as annular forms of central nervous system, involved in the coordination of behaviors (Grimmelikhuijzen and Westfall, 1995; Mackie, 2004; Garm et al., 2007; Koizumi, 2007).

Although the sensory systems and the behavioral repertoire are more elaborate in medusae than in polyps, the analysis of the differentiation of the nervous system is so far the most achieved in the Hydra polyp (Koizumi, 2002). Nonetheless the current emergence of new experimental cnidian model systems (e.g. Acropora, Nematostella, Clytia, Cladonema, Hydataclina, Cassiopea, Aurelia, Tripedalia,) should soon complete the picture. In Hydra, neurons, which represent about 3% of the total cell number (David, 1973) are either sensory cells or ganglion neurons. Cell bodies of most sensory neurons are located within the ectodermal layer, their processes reaching the surface (Figure 4F), whereas the bipolar and multipolar ganglion neurons (Figure 4G-I), which are the most common type of neuronal cells, are spread in both cell layers, along the mesoglea and function as interneurons. In jellyfish sensory neurons can actually function as sensory-motoneurons, establishing bilateral synaptic connections with their target cells, namely myoepithelial cells and nematocytes (Anderson, 1985; Garm et al., 2006). In sea anemones, sensory neurons are associated with smooth muscle fibers, suggesting that they also behave as sensory-motoneurons (Grimmelikhuijzen et al., 1989). Synaptic transmission in cnidarians relies on fast neurotransmitters (glutamate, GABA, glycine) as well as slow ones (catecholamines, serotoneine) and neuropeptides (see in Table 1). For a recent update about neurotransmission in cnidian nervous systems, see (Kass-Simon and Pierobon, 2007).

### The nematocytes (or cnidocytes) are phylum-specific mechanoreceptor cells

Beside neurons, cnidarians differentiate highly specialized mechanoreceptor cells that play a key role in the capture of preys and defense – see in (Bouillon, 1994a; Tardent, 1995). These phylum-specific stinging cells, named nematocytes (or cnidocytes, giving their name to the phylum), are abundant, representing 35% of the cells in Hydra (David, 1973). They display variable morphologies and functions; in anthozoans, spirocytes (Figure 3) are mechanosensory cells involved in adhesion to prey and non-prey (Kass-Simon and Scapaticci, 2002). Mature nematocytes are stimulated by chemicals or preys that contact their cnidocil, then respond in nanoseconds by discharging the toxic content of a thick-wall capsule named nematocyst (Figure 4) (Nuchter et al., 2006). The nematocyst discharge immobilizes the prey by releasing large droplets of venom through an evertting tube (Tardent, 1995). The prey then releases the peptide glutathione, which induces the feeding response, i.e. tentacle bending and mouth opening (Loomis, 1955; Lenhoff et al., 1982; Shimizu, 2002).

Although electrical activity could be recorded in nematocytes (Anderson and McKay, 1987; Brinkmann et al., 1996), it is not clear how the information sensed by the cnidocil apparatus is transduced to target the discharge function. In fact, nematocyst discharge can occur in the absence of neuronal control indicating that nematocytes can behave as autonomous mechanoreceptor-effector units (Aerne et al., 1991). However ultrastructural studies showed the presence of two-cell as well as three-cell synaptic pathways in the tentacle epidermis of a sea anemone, including synaptic connections between nematocytes and surrounding neurons (Holtmann and Thuem, 2001; Westfall et al., 2002). This neuronal control is supposed to pace down the spontaneous firing activity of nematocytes.

### Neurogenesis and nematogenesis in cnidarians

#### Neurogenesis and nematogenesis in the planula (swimming larva)

In developing hydrozoans, scyphozoans and anthozoans, neurogenesis and nematogenesis are initiated in late gastrula, as soon as the ectodermal and endodermal cell layers are established (Figure 2A). In hydrozoans and anthozoans, cells located in the endoderm, named interstitial stem cells in hydrozoans, give rise to nematoblasts and neuroblasts, which migrate towards the ectodermal layer (Figure 3). In Podocoryne larva, the nematocytes appear in the endoderm at 24 hours post-fertilization (hpf), homogenously distributed before migrating to the ectodermal layer, while a subset of larger nematocytes accumulates at the posterior end, the future oral pole (Groger and Schmid, 2001). At 24 hpf the first RFamide sensory neurons are detected in the mid-body region with neurites oriented along the anterior posterior axis. Few hours later, tyrosin-tubulin nerve cells are detected in the anterior region, with lateral neurites forming rings. Progressively novel tyrosin-tubulin neurons with lateral neurites differentiate towards the posterior pole forming repetitive units along the anterior posterior axis, while anterior and posterior connections also appear. This anterior to posterior development of the nervous system with repetitive units is highly reminiscent of the formation of the central nervous system in bilaterians (Groger and Schmid, 2001).

In the scyphozoan Aurelia planula where all neurons are ectodermal, the RFamide neurons differentiate first in the vicinity of the aboral pole and progressively form a dense graded plexus along the aboral half (Nakanishi et al., 2008). Similarly the Acropora planula develops asymmetrically, with the sensory nerve cells expressing RFamide, Pax-C or Emx that appear denser at the aboral pole but rare or absent from the oral pole (de Jong et al., 2006; Miller et al., 2000), and the ganglion and sensory neurons expressing cnox-2am (Gsx ortholog) restricted to the ectoderm of the mid-body region (Hayward et al., 2001). Therefore the diffuse larval nerve net is already highly regionalized. In addition, some anthozoan planula as Nematostella develop at the anterior/aboral pole a transient sensory organ, named the apical tuft, which senses the signals that will induce settlement of the larva and its subsequent metamorphosis. The high density of sensory neurons at the aboral pole of the hydrozoan and scyphozoan planulae are supposed to play a role similar to the apical tuft. At the time of metamorphosis, part of the larval nervous system degenerates as observed in the hydrozoan and scyphozoan larvae where the aboral RFamide neurons disappear to reappear at the oral pole.
of the polyp. Thus a complex reorganization of the nervous system is linked to the metamorphosis process, with complex migration patterns (Kroither et al., 1990; Martin, 2000; Nakashishi et al., 2008). A similar process also probably occurs in metamorphosing anthozoans (de Jong et al., 2006).

**Neurogenesis and nematogenesis in the adult polyp**

The cnidarian polyps display an oral-aboral polarity, with differentiated tissues at the extremities but no sensory organs as recognized in medusae. In *Hydra* three distinct stem cell populations provide all cell types, the ectodermal epithelial cells, the endodermal epithelial cells and the interstitial cells, which are multipotent stem cells restricted to the ectoderm of the body column, continuously providing neurons, mechanoreceptor cells (nematocytes), gland cells and gametes when the animals follow the sexual cycle (Bode, 1996; Bosch, 2009). The epithelial stem cells divide every three to four days when the interstitial stem cells divide faster, once a day. Surprisingly enough, interstitial stem cells seem to be lacking in non-hydrozoan species, where it was proposed that neurons differentiate directly from epithelial cells. However cell lineage tracing analyses are required in non-hydrozoan model systems to clarify this question.

In *Hydra*, the nematocyte and neuronal differentiation pathways appear to share a common bipotent progenitor (Holstein and David, 1990; Mljikovic-Licina et al., 2007) before following distinct regulations: interstitial cells committed to the nematocyte lineage that are located in the ectodermal layer of the body column, undergo up to five synchronous cell cycle divisions, forming clusters of syncitial nematoblasts (Figure 4). Once they stop proliferating, the nematoblasts start differentiating their nematocyst vacuole, which can be of four distinct types (Holstein and Emschermann, 1995). Differentiated nematocytes then migrate to their definitive location, namely the tentacles, according to a process that relies on contact guidance from surrounding tentacles (Campbell and Marcum, 1980). In the tentacles, nematocytes are embedded within large epithelial cells named battery cells, each battery cell containing several nematocytes, themselves connected to sensory neurons by synapses. After discharge of their capsule, nematocytes are eliminated and replaced by new ones.

**FIGURE 2:** Schematic views of neurogenesis and nematogenesis during the cnidarian life cycle.

A) Neurogenesis in the developing Podocoryne hydrozoan. As for all medusozoan species with a medusa stage, the mature jellyfish release the gametes. At mid-gastrula stage (b) the precursors of nerve cells and nematocytes (pr) arise in the endoderm, rapidly differentiate and migrate to the ectoderm, forming a diffuse network throughout the swimming planula larva (c). At this stage the nerve cells (nv), detected here with an anti-tyrosine tubulin antibody, show laterally oriented neurites that form a ladder (Groger and Schmid, 2001). The anterior pole contains RFamide+ neurons (nv) and the posterior pole large mature nematocytes (ne). Upon metamorphosis, the larval anterior pole becomes the aboral region of the polyp (also named foot) and the larval posterior pole provides the oral region (also named head). B) In polyps the nerve net is much denser in oral and aboral regions than in the body column. In intact *Hydra* (a), neurogenesis takes place in the body column where interstitial stem cells provide neuronal progenitors that migrate and differentiate in the upper and lower regions of the body column. In head-regenerating *Hydra* (b), de novo neurogenesis takes place at the tip to reform in two days the apical nerve net. Progenitors are detected in the tip at 24 hpa and neurons after 32 hpa. C) In the adult medusa (a) neurogenesis takes place in three regions: the manubrium (b), the tentacle bulb (c) and the sensory organs, which may contain eyes (d) and statocysts. b) Closer view of a *Cladonema* manubrium with the mouth opening directed to the bottom and the nerve net detected with the anti-RFamide antibody; cell bodies (c.b) and neuronal projections (n.p). c) Staggered nematogenesis in tentacle bulbs: stem cells located in the most proximal position (α) initiate nematocyte differentiation less proximally (β), until nematocytes migrate distally in the maturation area (γ) and finally reach the tentacle when mature (δ) as shown by (Denker et al., 2008c). Tentacle bulbs are also the site of intense neurogenesis, as depicted on the right with RFamide nerve cells that project from the bulb to the tentacle. Neuronal precursors can also be found in the α zone as suggested by the Gsx expression in *Cladina* (see Figure 5). d) Drawing of a *Cladonema* eye after (Weber, 1981) with the tripartite lens, the ciliated photoreceptor cells (ph.c) and the pigment cells (pi.c).
In contrast, the differentiation of nerve cells appears more direct: interstitial cells committed to this pathway are found predominantly along the body column, possibly in the head region but neither in the tentacles nor in the foot region. These progenitors go through S phase, get arrested in G2 until a signal will let them divide and terminally differentiate as a sensory or ganglion neuron (Schaller et al., 1989; Bode, 1996). Neuronal differentiation is more intense in the upper body column and peduncle region than in the central body column and mature neurons receive signals from the head and foot regions to migrate, explaining the higher neuronal densities recorded at the extremities. One striking finding was the high level of neuronal plasticity observed in adult Hydra polyps (Bode, 1992) with changes in neuropeptide phenotype according to the position of the neurons along the body column (Koizumi and Bode, 1986), but also transdifferentiation from ganglion to sensory neurons (Koizumi et al., 1988). This plasticity was also observed in the nematocyte lineage (Fujisawa et al., 1986).

Beside the highly dynamic adult homeostatic context, the regulation of neurogenesis can also be investigated in developmental contexts in Hydra as regeneration of the head and foot regions after bisection, asexual reproduction through budding when animals are well fed, reaggregation after tissue dissociation. After bisection, nematocytes and neurons disappear from the head and foot regions to migrate, explaining the higher neuronal densities recorded at the extremities. One striking finding was the high level of neuronal plasticity observed in adult Hydra polyps (Bode, 1992) with changes in neuropeptide phenotype according to the position of the neurons along the body column (Koizumi and Bode, 1986), but also transdifferentiation from ganglion to sensory neurons (Koizumi et al., 1988). This plasticity was also observed in the nematocyte lineage (Fujisawa et al., 1986).

The nerve-free Hydra paradigm

In Hydra, neurogenesis can be disconnected from patterning by producing « nerve-free » polyps, which lack the interstitial lineage derivatives, namely nematocytes, sensory and ganglion neurons, and are thus named "nerve-free" or "epithelial" hydra. Such animals can be obtained by different means: either chemically, upon colcemid, colchicine (Campbell, 1976) and hydroxyurea treatments (Yaross and Bode, 1978b), or genetically as in the nf-1 Hydra magnipapillata mutant that completely lacks the interstitial lineage (Sugiyama and Fujisawa, 1978), or in the temperature sensitive sf-1 mutant (Terada et al., 1988). It is also possible to maintain "pseudo-epithelial" hydra, which are depleted of all somatic interstitial lineage derivatives, but still contain stem cells restricted to the germ cell lineages (Nishimiya-Fujisawa and Sugiyama, 1995). As anticipated nerve-free animals completely lose their autonomous feeding behavior and can only be maintained by force-feeding (manual introduction of the food through the mouth opening with a pipette and subsequently washing of the gastric cavity). Nevertheless, epithelial hydra exhibit developmental patterning processes, like budding and regeneration (Marcum and Campbell, 1978a) although head regeneration in such hydra is significantly slower and less efficient (Mijovic-Licina et al., 2007).

The manipulation of such animals turned out to be very informative, showing that the differentiation of interstitial cells into nematocytes was not position dependent, whereas that of nerve cells was indeed position dependent, i.e. enhanced in the upper and lower parts of the body column (Yaross and Bode, 1978b). This position-dependent regulation of neurogenesis seems to be largely under the control of epithelial cells (Koizumi et al., 1990; Minobe et al., 1995). Together with experiments performed on chimeras formed between morphologically-distinct strains (Marcum and Campbell, 1978b), these data suggested that the interstitial lineage, and more specifically the neurons do not play a significant role in hydra morphogenesis (Fujisawa, 2003). However the situation is probably more complex as in the absence of nerve cells, the genetic circuitry is likely reprogrammed in the epithelial cells as already reported (Hornberger and Hassel, 1997). Moreover interstitial cells and their derivatives appear involved in the fine tuning of the morphogenetic processes driven by the epithelial cells, as for instance in the reg-16 mutant where head regeneration that is strongly deficient, can be reestablished upon depletion of the interstitial lineage (Sugiyama and Waneik, 1993). Similarly the dramatic apoptosis of the neuronal and nematocyte lineages in head-regenerating tips immediately after mid-gastric amputation leads to the activation of the head regeneration program (Chera et al., 2009).
intermingled with pigment cells, or more complex as A. aurelia (Martin, 2002) the jellyfish can differentiate photoreception organs photosensitive structures and the polyp can sense light in non-ancestor innovation that took place in the Cnidaria organs was a major innovation in animal evolution, an but the clustering of photoreceptor cells to form sensory Light sensing is wi...ing of the head-regenerating Hydra. Following mid-gastric section, the tip of the head-regenerating half is immediately depleted of neurons (J, outline), progressively repopulated with neuronal progenitors (K) and mature neurons (L, M). However the apical nervous system at 40 hours post-amputation (hpa) is still less dense than in adult polyps (N, topview). mo: mouth opening; te: tentacles. Scale bars: 2 μm (A,B,D), 5 μm (C,F-I), 50 μm (J-N).

**Neurogenesis and nematogenesis in the adult medusa**

The manubrium and the tentacle bulbs

In the mature medusa, the manubrium and the tentacle bulbs are the sites of intense production of neurons and nematocytes as observed in the hydrozoan jellyfish (Figures 2C and 5). In contrast to Hydra polyps where all stages of nematogenesis and neurogenesis overlap along the body column, the expression analysis of neuronal and nematocyte markers coupled to in vivo cell labeling and morphological analyses revealed that the differentiation stages follow a proximo-distal gradient along the tentacle bulbs (Denker et al., 2008b) as depicted in Figure 2Cc. Moreover the tentacle bulb isolated from the medusa has the capacity to survive for several days in culture, opening the possibility for manipulations and functional studies.

The medusa-specific sensory organs: the ocellus, the camera eye and the rhopalia

Light sensing is widely spread in non-metazoan species but the clustering of photoreceptor cells to form sensory organs was a major innovation in animal evolution, an innovation that took place in the Cnidaria-Bilateria ancestor (Gehring, 2004). In cnidarians, both the medusa and the polyp can sense light in non-visible photosensitive structures (Santillo et al., 2006) but only the jellyfish can differentiate photoreception organs (Martin, 2002). These can be either simple ocelli as in Aurelia that are composed of photosensitive cells intermingled with pigment cells, or more complex as camera eyes with a lens as observed in Cladonema (Figure 2Cd) and Tripedalia. The most complex eyes with a cornea, lens and ciliated photoreceptor cells forming retina are found in cubomedusae. In scyphozoan and cubozoan medusae, eyes associate with pressure sensing organs named statocytes to form complex sensory organs named rhopalia (Figure 5F-I), connected to the nerve ring (Garm et al., 2006). Therefore rhopalia were proposed to be part of the central nervous system. Behaviors can in fact be regulated through visual input, as the observed modulations of the swim pacemaker according to the light intensity in Tripedalia (Garm and Bielecki, 2008). Non-visible photosensitive structures also regulate animal behaviors (see in (Santillo et al., 2006)) as the pacemakers that regulate the periodic contractions of the Hydra body (Passano and Mccullough, 1962; Passano and Mccullough, 1963), the locomotion of eyeless pelagic species (Plickert and Schneider, 2004) or the triggering of spawning supported by the expression of opsins in gonads (Suga et al., 2008).

Beside adult eyes, pigmented photoreceptor cells were identified in the Tripedalia larva, which does not contain any nervous system (Nordstrom et al., 2003). These single cell ocelli appear quite original since they most probably function completely autonomously, sensing the light through their photoreceptors and regulating the animal behavior thanks to the motor-cilium they differentiate. Moreover these photoreceptors are rhodometic (microvilli) as observed in most invertebrates and not ciliated as in adult cnidarian eyes and vertebrates. It would be of interest to identify other cases of cnidarian larval eyes. In some species as...
Cladonema, adult eyes can fully regenerate (Stierwald et al., 2004). Given the great variety of eye morphology, the question of a unique origin for all animal eyes or a repeatedly convergent evolution is a long-standing one (Arendt, 2003; Nilsson, 2004), still debated after the discovery of shared regulators of eye differentiation as the Pax and Six genes (Kozmik et al., 2003; Stierwald et al., 2004) and shared effectors as opsins (Suga et al., 2008; Kozmik et al., 2008).

Elements of the cnidarian neurogenic circuitry

1.- Growth factor signaling pathways in cnidarian neurogenesis

The deep conservation of the signaling machinery that support developmental processes in bilaterians came as a surprise in cnidarians, when components of the insulin-like (Steele, 2002), Wnts (Hobmayer et al., 2000; Wikramanayake et al., 2003; Kusserow et al., 2005; Teo et al., 2006; Momose and Houliston, 2007), Notch (Kasbauer et al., 2007), VEGF (Seipel et al., 2004a), FGF (Matus et al., 2007b; Sudhop et al., 2004; Rentzsch et al., 2008) and Hedgehog (Matus et al., 2008) pathways were uncovered (Figure 6). Not only the ligands, receptors and intra-cellular components were identified but also the antagonists as the Dickkopf3 and Dickkopf1/2/4 Wnt-antagonists (Fedders et al., 2004; Guder et al., 2006b) and the Gremlin, Noggin and Follistatin BMP-antagonists (Matus et al., 2006; Rentzsch et al., 2006). This amazing conservation was actually confirmed by the even more surprising presence of these pathways in sponges (Nichols et al., 2006; Adamska et al., 2007) and partally in choanoflagellates (King et al., 2008).

However the experimental evidences concerning the contribution of these pathways to neurogenesis in cnidarians are still limited, although four of them are likely involved in neurogenesis (Table 1). The FGF pathway supports the differentiation of the apical sensory organ in Nematostella planula as demonstrated by loss-of-function assays (Rentzsch et al., 2008), but a similar role at the aboral pole of medusozoan planulae remains to be shown. The canonical Wnt pathway appears to

FIGURE 5: Neurogenesis in hydrozoan and scyphozoan medusae.
A-C) Bottom view of the Clytia medusa nervous system with the manubrium (m) and the tentacle bulbs (tb) containing numerous RFamide sensory neurons (purple-pink). Note the delicate nerve net in the velum (B) and the endogeneous bioluminescence in the tentacle bulbs (green, A,C). D) The proliferative (arrow) and differentiating (arrowheads) zones of the tentacle bulbs express the ParaHox gene Gsx (red; blue: DAPI staining). E) In the Podocoryne medusa, the differentiating neurons in the tentacle bulbs strongly express the CREB transcription factor detected with the anti-hydra CREB antibody (red). Scale bars: 500 µm (A), 50 µm (B,C) 10 µm (D,E). F-I) Rhopalia in the immature Aurelia medusa (ephyra). Most of the adult features can be already observed: the mouth (m), the developing stomach (s), three types of radial canals: adradial, perradial and interradial (ic) and the rhopalia (r; arrows), each of them guarded by a pair of tappets that contain a diffuse nerve net (F, G). At the base of the rhopalia, a stratified epithelium includes columnar ciliated cells with basal axons and cells with intra-epithelial flagella stained with α-tubulin (G, H, arrowhead). In the center of the rhopaliun the elongated ovoidal lithostyle (I) contains the photoreception organs named ocelli that contain pigmented cells, and a terminal statocyst (st) that senses gravitation.
**Table: Signaling and Homeoproteins**

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<tr>
<th>Antizoonas</th>
<th>Medusozoonas</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FGF (FGF1, FGF2, FGF2a)</strong></td>
<td>Nv:pl, apical tuft (lof)</td>
<td>?</td>
</tr>
<tr>
<td>Wnt3</td>
<td>Nv: Hm (Wnt3): apical organizer, terminal differentiation of nematoblasts</td>
<td>(Kussrew et al., 2005) (Hodmayer et al., 2010) (Grimmelikhuijzen et al., 2002) (Katsukura et al., 2003) (Katsukura et al., 2007) (Muller et al., 2007) (Tao et al., 2006)</td>
</tr>
<tr>
<td>Dkk1/2/4</td>
<td>Hm: expressed in body column, gland cells, putative neurogenic</td>
<td>(Udder et al., 2006b)</td>
</tr>
<tr>
<td>Dkk3</td>
<td>Hm: differentiating nematocytes</td>
<td>(Feidler et al., 2004)</td>
</tr>
<tr>
<td>BMP2/4</td>
<td>Am larva: oral region</td>
<td>?</td>
</tr>
<tr>
<td>BMP5/8</td>
<td>Nv-pl: pan-endodermal, apical tuft</td>
<td>(Matus et al., 2006)</td>
</tr>
<tr>
<td>chordin</td>
<td>Nv:ga: blastopore, asymmetric ectodermal oral-aboral expression</td>
<td>(Matus et al., 2006, Rentzsch et al., 2007)</td>
</tr>
<tr>
<td>follistatin</td>
<td>Nv-pl: circumanal, neurogenesis</td>
<td>(Matus et al., 2006)</td>
</tr>
<tr>
<td>gremlin, GDF5</td>
<td>Nv:ga: asymmetric endo, oral-aboral, Nv-pl: apical tuft</td>
<td>?</td>
</tr>
<tr>
<td>noggin</td>
<td>Nv-pl (Noggin1): endo, facing the apical tuft; pharyngeal asymmetrical</td>
<td>(Matus et al., 2006)</td>
</tr>
<tr>
<td>Jun kinase</td>
<td>Hm: differentiating nematocytes</td>
<td>(Phipps et al., 2005)</td>
</tr>
<tr>
<td>RSK 2</td>
<td>Hm: cells, neurons, nematoblasts, epithelial cells apical organizer (lof)</td>
<td>(Kotluss et al., 2004, Chen et al., 2006) (Cheru et al., 2007)</td>
</tr>
<tr>
<td>Notch</td>
<td>Nv-pl: similar to Musashi</td>
<td>(Martin et al., 2009) (Kashbauer et al., 2007) (Kashbauer et al., 2007)</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>?</td>
<td>(Ishii et al., 2003)</td>
</tr>
</tbody>
</table>

**Table: Peptidases**

| GL/Wamides (NP) | Ae: inhibits muscle contraction | (Grimmelikhuijzen et al., 2002) |
| Kxamides (NP) | ? | (Trum et al., 1989) (Hansen et al., 2002) |
| PWamide (EP) | ? | (Takahashi et al., 1997, Takahashi et al., 2009) (Lenti, 1965) |
| RFamide (NP) | (I, II, III) Ae: endo. neurons | (Grimmelikhuijzen et al., 2002) (Hansen et al., 2002) (Pernet et al., 2004) (Katsukura et al., 2003) (Katsukura et al., 2004) |
| RGamide (NP) | ? | (Takahashi et al., 2000, Hansell et al., 2002) |
| Rlamide (NP) | (II, III) Ae: inhibit muscle contraction | (Grimmelikhuijzen et al., 2002) |
| RNamide (NP) | (II, III) Ae: antagonist action on longitudinal and circular muscles | (Grimmelikhuijzen et al., 2002) |
| RFamide (NP) | (V) Ae: tentacle contractions | (Grimmelikhuijzen et al., 2002) |
| RWamide (NP) | (II, III) Ae: slow contraction of endo. muscles; Cpl: sphinder contraction | (Grimmelikhuijzen et al., 2002) |

**Table: Antp-class Homeoproteins**

| Edx | Ant: aboral larval sensory neurons | Hm: neurogenic | ? |
| Nat | Ant: oral larval ectoderm (neurons ?) | Hm: body column neurons | ? |
| Gsa / Anthox2 / cnox-2 (Parahox) | Am-pl (cnox-2): ecto. bipolar and multipolar neurons except aboral | Hm: apical neurogenesis (lof), nematogenesis aboral, body column | (de Jong et al., 2006) (Mikosk-Litosa et al., 2004) (Galle et al., 2005) |
| Pdx/Xio (Parahox) | Nv-pl, Nv-po (Xio/Cdx): endo. ventral midline stripes | Hm: ecto. progenitors | (Ryan et al., 2007) (Quin Goddard et al., 2009) |
| Cdx (Parahox) | ? | Hm: ecto. oral/aboral | (Ryan et al., 2007) (Quin Goddard et al., 2009) |

**Table: PG-1 (Hox)**

| Nv-pl (2x) (Ax1a): endo. pharyngeal ring | Hm: mechanano-sensory cells | (Finnerty et al., 2004) (Ryan et al., 2007) |
| Nv-po (Ax1a): oral, mouth opening; endo. base and tips of tentacles | Hm: oral sensory cells in statocysts | (Kamm et al., 2006) (Jakob and Schierwater, 2007) (Yanze et al., 2001) (Chiori et al., 2009) |

**Table: PG-2 (Hox)**

<p>| Nv-pl (Ax1): body-wall endoderm | no ortholog ? | (Finnerty et al., 2004) (Matus et al., 2006, Ryan et al., 2007) |
| Nv-po (Ax2): pair of mesenteries | no ortholog ? | (Finnerty et al., 2004) (Ryan et al., 2007) |
| Nv-pl (Ax2b): pharyngeal endo. | no ortholog ? | (Finnerty et al., 2004) (Ryan et al., 2007) |
| Nv-pl (Ax2b): ventral midline | no ortholog ? | (Finnerty et al., 2004) (Ryan et al., 2007) |
| Nv-po (Ax2b-ax3b): ventral pair mesenteries, endo. tentacle base | no ortholog ? | (Finnerty et al., 2004) (Ryan et al., 2007) |
| Nv-pl (Ax2a): asymmetric body wall | no ortholog ? | (Finnerty et al., 2004) (Ryan et al., 2007) |
| Nv-po (Ax2a): ventral mesenteries, endo. tentacle base | no ortholog ? | (Finnerty et al., 2004) (Ryan et al., 2007) |
| orphan Hox-like | no ortholog ? | (Masuda Nakagawa et al., 2000) |</p>
<table>
<thead>
<tr>
<th>PRD-class Homeoproteins</th>
<th>?</th>
<th>Hc: apical neurons and precursors, organizer during head formation</th>
<th>(Gauchat et al., 1998; Mijovic-Licina et al., 2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>prdl-6</td>
<td>?</td>
<td>Hv: body neural cells, proliferating nematoblasts</td>
<td>(Gauchat et al., 2004; Mijovic-Licina et al., 2004)</td>
</tr>
<tr>
<td>homeobrain</td>
<td>?</td>
<td>Hv: neural plate, restricted to tentacles</td>
<td>(Marlow et al., 2009)</td>
</tr>
<tr>
<td>Gsc</td>
<td>?</td>
<td>Hv: neural plate, pharyngeal, apical tuff, asymmetric directive axis</td>
<td>(Marlow et al., 2009)</td>
</tr>
<tr>
<td>Gtp</td>
<td>?</td>
<td>Hv: neural plate, oral nerve ring</td>
<td>(Marlow et al., 2009)</td>
</tr>
<tr>
<td>Rx</td>
<td>?</td>
<td>Hv: neural subsets</td>
<td>(Matus et al., 2007a)</td>
</tr>
<tr>
<td>Repo</td>
<td>?</td>
<td>Hv: nerve ring</td>
<td>(Marlow et al., 2009)</td>
</tr>
<tr>
<td>Gta</td>
<td>?</td>
<td>Hv: neural plate, pharyngeal, apical tuff, asymmetric directive axis</td>
<td>(Marlow et al., 2009)</td>
</tr>
<tr>
<td>Pax-A/C (pox neuro)</td>
<td>?</td>
<td>Hv: putative neural, sprinocectodermal precursors and neural cell types</td>
<td>(Matus et al., 2007a)</td>
</tr>
<tr>
<td>Pax-B (Pax25/56)</td>
<td>?</td>
<td>Hv: patterning of the nerve ring</td>
<td>(Matus et al., 2007a)</td>
</tr>
<tr>
<td>Pax-D (Pax37)</td>
<td>?</td>
<td>Hv: embryonic stripes neurogenesis</td>
<td>(Matus et al., 2007a)</td>
</tr>
<tr>
<td>NF-κB</td>
<td>?</td>
<td>Hv: late nematogenesis, sensory neurons</td>
<td>(Uéno et al., 1995; Hayakawa et al., 2004; Lindgens et al., 2004; Muller et al., 2003; Seipel et al., 2004)</td>
</tr>
</tbody>
</table>

**Table 1: Putative regulators of cnidarian neurogenesis identified in analyses performed at the cellular expression level and/or functional level. Abbreviations:** Ac: anthox (anthozoan Hox/ParaHox gene); Cx: cnxox (Hox/ParaHox gene); ecto.: ectodermal, endo.: endodermal; EP: epitheliopeptide; ga: gastrula; gof: gain of function assay; i-cells: intestinal cells; lof: loss of function assay; med: medusae; NP: neuropeptide; pl: planula; po: polyp; TeBu: tentacle bulb; x: copy number for a given gene family. **Species code:** Ab: Anthopleura Bailey; Ae: A. elegantissima; Af: A. fuscoviridis; Am: Acropora millipora; Ch: Clyta hemispherica; Cp: Caliactis parasitica; Cr: Cladonema radiata; Hy-AEP: Hydra sexual strain; He: Hydractinia equinata; Hm: Hydra magnipapillata; Ha: Hydractinia symbiolongicarpus; Hv: Hydra vulgaris; Nv: Nematostella vectensis; P: Podocoryne carnea; Ps: Protanthea simplex; Rk: Renilla coelenter; Tc: Tripedalia cystophora.

| Abbreviations: | Hv: Hydractinia equinata; Nv: Nematostella vectensis; P: Podocoryne carnea; Ps: Protanthea simplex; Rk: Renilla coelenter; Tc: Tripedalia cystophora. |
|-----------------|-------------------------------------------------------------|-----------------------------------------------|

**Abbreviations:** Hv: Hydractinia equinata; Nv: Nematostella vectensis; P: Podocoryne carnea; Ps: Protanthea simplex; Rk: Renilla coelenter; Tc: Tripedalia cystophora.
2. The transcription factors in cnidarian neurogenesis

In bilaterians, homeoproteins in combination with the bHLH proteins bring a major contribution to neurogenesis during development and adulthood (Guillemaut, 2007). According to the sequence of their homeodomain, homeogenes fall into classes, which do have cnidarian representatives (Galliot et al., 1999; Holland and Takahashi, 2005; Ryan et al., 2006). Genes from the ANTP, PRD, SIN, POU and LIM classes perform neurogenic tasks in bilaterians, but in cnidarians, expression and in few cases functional data are only available for the ANTP, PRD and SIN gene families. We will review here what is currently known about the neurogenic function of those gene families in Cnidaria.

The neurogenic function of the non-@HoX (NK-like) ANTP-class homeogenes

The ANTP-class of homeogenes contains numerous gene families that distribute into two sub-classes: the non-Hox (also named NK-type) and the Hox/paraHox families (Gauchat et al., 2000; Holland, 2001). The non-Hox families are highly conserved from cnidarians to bilaterians and thus form well defined sister groups (Gauchat et al., 2000; Schierwater and Desalle, 2001; Chourrout et al., 2006; Kamm et al., 2006; Ququand et al., 2009). We will consider here only those that are putative regulators of the cnidarian nervous systems.

Divergent roles for the empty spiracle / emx gene family in hydrozoans and anthozoans: The emx genes are involved in forebrain formation in vertebrates with a special emphasis on the cytoarchitecture of the cerebral cortex (Cecchi et al., 2000), whereas mutation of the Drosophila homologue, Emx, eliminates the deutero- and tritocerebrum (Hirth et al., 1995). In the hydrozoan Hydra, the Emx homologue is expressed in endodermal epithelial cells of the hypostome (head region) and up-regulated in posterior regions of the planula larva experimentally converted to anterior fate (Mokady et al., 1998). However in the coral Acropora, Emx is expressed in sensory neurons of the aboral half of the larva until their density drastically decreases at the time of metamorphosis (de Jong et al., 2006). Therefore Emx might belong to the ancestral neurogenic genetic families that distribute into two sub-families that conserve the ParaHox gene families, which are highly conserved among bilaterians, exhibiting a dual function, during segmentation and neurogenesis, the latter one being considered as ancestral (Gibert, 2002). However, a cnidarian engrailed ortholog was not identified so far, suggesting that this gene family arose later during evolution or was lost in cnidarians, implying thus that it was not essential at the origins of neurogenesis.

The @Hox Not ortholog, a marker for apical sensory neurons: Not homeobox genes are involved in neurogenesis in bilaterians: in Drosophila, the Not-like 90Bbre gene participates in the differentiation of the neuroblasts of the posterior brain (Dessain and McGinnis, 1993), in Xenopus Noto-2 promotes notochord formation (Gont et al., 1996), in chicken Cnot1 and Cnot2 are expressed in the early neurectoderm (Stein et al., 1996), in zebrafish the Not-related floating head gene is required for neurogenesis of the epiphysis (Masai et al., 1997). In Hydra, the cnot gene is expressed in sensory neurons at the root of the tentacles (Figure 7A). During tentacle formation, budding or regrowth, cnot transcripts start to be expressed in a limited number of neuronal cells at the place where tentacle rudiments will emerge. Hence the Hydra cnot gene appears to be restricted to the differentiation of a limited subset of neurons.

The msh/msx gene, a candidate regulator of neurogenesis: The msh/msx homeogene family is highly conserved from Porifera to bilaterians (Larroux et al., 2007). Beside the homeodomain, msx genes also encode some Groucho-interacting domains that are conserved in Nemataostella but not in hydrozoans (Takahashi et al., 2008). In Drosophila, the msh gene is involved in both dorsoventral patterning and neurogenesis, specifying neuroblasts in the dorsal neuroectoderm. In the leech, Le-msx transcripts are present in embryonic stem cells, and subsequently restricted to the neural tissue. In amphioxus, msx is expressed in dorsal cells of the neural tube, similarly to the msx3 expression pattern observed in mice embryos. From these results, it was proposed that the msh/msx genes specify the differentiation of the dorsal/lateral neural tube in an evolutionarily conserved manner (Cornell and Ohlen, 2000). In the coral Acropora, msx3-Am is expressed in the ectoderm of the oral region but no cell-type specificity was noted (de Jong et al., 2006). In Hydra Msx is expressed exclusively in neurons along the body column (Figure 7A), forming a nerve net in the central region of the polyp (Mijlkovic-Licina et al., 2004). In contrast in the jellyfish Podocoryne, msx appears involved in the maintenance of progenitors during medusa budding and transdifferentialization (Galle et al., 2005). As above more studies are required to conclude about a conserved neurogenic fonction for msx in cnidarians.

The @EHG gene family appears missing in cnidarians: The evolutionarily-conserved neurogenic function of homeogenes was first reported with the engrailed homeoprotein in arthropods, annelids and chordates (Patel et al., 1989). In vertebrates, two engrailed-related genes (en-1 and en-2) specify the cerebellar territory (Wassef and Joyner, 1997), whereas in Drosophila, engrailed exhibits a dual function, during segmentation and neurogenesis, the latter one being considered as ancestral (Gibert, 2002). However, a cnidarian engrailed ortholog was not identified so far, suggesting that this gene family arose later during evolution or was lost in cnidarians, implying thus that it was not essential at the origins of neurogenesis.

The ParaHox and Hox-like cnidarian genes

The Hox/ParaHox gene families, which are highly conserved among bilaterians, exhibit a much lower level of conservation from cnidarians to bilaterians that the ANTP non-Hox families (Gauchat et al., 2000; Schierwater and Desalle, 2001; Chourrout et al., 2006; Kamm et al., 2006; Ququand et al., 2009). Recent analyses showed that the three ParaHox families (cnox2/Anthox2/Gsx, Pdx/Xlox, Cdx/Cnox4) and the three Hox families (CBA). However, the expression of these Hox/ParaHox genes appears tightly regulated during developmental processes in hydrozoans and anthozoans, suggesting that they act as developmental genes. Hox genes likely participate in the development or the maintenance of the cnidarian nervous system as the Clytia Hox1 (PG1) in stacocysts, the Nemataostella Anthox1 (PG9-like) in the apical tuft, or the Eleutheria Cnox-3 (PG9-like) in the oral ring (see in Table 1). However cellular and functional
analyses are required to confirm this statement. Evidences for a neurogenic function were only obtained for the cnox2/Anthox2/Gsx paraHox gene. Gsx, a regulator of nematogenic and neurogenic precursors: Gsx genes belong to the ParaHox gene cluster (Brooke et al., 1998) and in phylogenetic analyses group together with the Pdx/PG2/PG3 gene families (Quiquand et al., 2009). In Drosophila embryos, the Gsx ortholog named ind is expressed as a longitudinal band in the intermediate neuroectoderm where it promotes activation of proneural genes in the specific set of neuroblasts (Weiss et al., 1998). The cnox-2/Gsx gene family is currently the most widely studied in cnidarians (Schierwater et al., 2002; Finnerty et al., 2003) and its regulation has been documented in Hydra (Schummer et al., 1992; Gauchat et al., 2000; Miljkovic-Licina et al., 2007), Hydractinia (Cartwright et al., 1999), Podocoryne (Yanze et al., 2001), Clytia (Chiori et al., 2009; Quiquand et al., 2009), Acropora (Hayward et al., 2001; de Jong et al., 2006) and Nematostella (Finnerty et al., 2003). In anthozoans, cnox-2 Am expressing cells display a neuronal morphology and are restricted to the oral pole of the larva; in the developing Nematostella planula (swimming larva), Gsx is expressed in the future head region. In the Podocoryne and Clytia larvae, early zygotic Gsx transcripts are initially localised in the anterior endoderm before extending towards the posterior pole, i.e. the future head region. In the Hydra adult polyp, cnox-2 is expressed in the head region and along the body column in a subset of...
neuronal cells in the apical region (Figure 7A) and in dividing interstitial cells and clusters of nematoblasts in the body column (Figure 7B). During head regeneration, these two types of cnox-2 expression are submitted to opposite regulations: in head-regenerating tips induction of cnox-2 expression is observed from 24 hours post-amputation (hpa), first in proliferating neuronal cells then in de novo differentiated neurons, whereas cnox2 expression in nematoblasts vanishes soon after amputation (Mlijkovic-Licina et al., 2007). Hence the two cnox-2 expressing cell populations respond differently to the signals propagated during head regeneration. Internally, the regulation is detected in the neural plate, the apical cells during head formation in Hydra correlates well with that observed during larval development in Podocoryne, Clytia, Acropora and Nematostella. Moreover in cnox-2(RNAi) knocked-down Hydra, the apical nerve net is not maintained in adult polyps and head regeneration is significantly delayed (Figure 7C), suggesting a contribution of cnox-2 progenitors and/or neurons in the head patterning process (Mlijkovic-Licina et al., 2007). In the Clytia medusa, Gsx is expressed in the tentacle bulbs, proximally in clustered interstitial cells and more distally in neurons (Figure 5D) as reported by (Chiori et al., 2009). The same group also reported about a Cdx ortholog expressed in differentiating nematoblasts in the tentacle bulb. These data definitely support a role for the cnidarian ParaHox genes in the regulation of the nervous system.

The PRD-class genes as regulators of nematogenesis, neurogenesis and eye differentiation

The PAIRED-class (PRD-class) gene families distribute into three main sub-classes: the paired-like genes, the Otx-related genes and the Pax genes (Galliot et al., 1999). Most PRD-class gene families carry out neurogenic functions in bilaterians. Twenty bilaterian PRD-class gene families do have representatives in cnidarians (Galliot et al., 1999; Ryan et al., 2006) whereas the sponge Amphimedon genome contains eight paired-like genes and a single Pax gene but no Otx-related genes (Larroux et al., 2008). In developing and adult cnidarians, most paired-like and Pax gene families exhibit regulations that suggest a specific role during neurogenesis.

The aristaeless-like paired-like gene, prdl-a is expressed as a regulator of neurogenesis. In intact Hydra polyps, Prdl-a is predominantly expressed in neuronal precursors and sensory neurons located in the most apical region (Figure 7A), being overexpressed in multihedated mutants (Gauchat et al., 1998). However, during the early stages of head regeneration and budding, prdl-a is transiently expressed in a distinct cell lineage, the endodermal myoepithelial cells located in the presumptive head region. This transient wave of endodermal expression occurs concomitantly with the raise in organizer activity detected in the regenerating stump by transplantation experiments (MacWilliams, 1983). Subsequently prdl-a is reexpressed in the differentiating neurons of the presumptive head region (Gauchat et al., 1998; Mlijkovic-Licina et al., 2004). This biphasic mode of expression observed during patterning processes is highly reminiscent of that displayed by the vertebrate paired-like genes Hesx1/Rox, Otx2 and Gsc during early mouse development (Thomas and Beddington, 1996; Rhinn et al., 1998); these genes that support early head patterning in the embryo, are expressed as two successive waves, a first one in the anterior visceral endoderm / hypoblast that induces a second one in the sus-jacent neuroectoderm of the rostral region (Foley and Stern, 2001). This similarity suggested an ancient commitment of « neurogenic » paired-like genes in apical/anterior nervous system patterning (Galliot and Miller, 2000).

The aristaeless-like paired-like gene prdl-b: In contrast to prdl-a, prdl-b is expressed in proliferating nematoblasts and in a subset of neurons in the gastric region but is not expressed during patterning processes (Gauchat et al., 2004; Mlijkovic-Licina et al., 2004). These data suggest that some cnidarian paired-like genes like prdl-a already exhibit two separate functions with distinct regulations, one during maintenance of apical neurogenesis in homeostatic conditions and another during patterning processes, whereas others as prdl-b would be restricted to neuronal differentiation in homeostatic conditions.

Goosecid (Gsc) in Hydra apical sensory neurons: The Hydra goosecid homolog, CnGsc, is expressed in the adult polyp in sensory neurons of the hypostome but also in endodermal epithelial cells at the base of tentacles and along the body column (Broun et al., 1999). During patterning processes, CnGsc is first repressed in the regenerating stump or growing bud, and reexpressed at later stages in the presumptive head region, suggesting that it is not involved in the organizer activity that drives head regeneration. However, when expressed in Xenopus embryos, CnGsc exhibits organizer activity (Broun et al., 1999). A single Gsc ortholog is present in Nematostella (Ryan et al., 2006) but its regulation and function are currently unknown.

The Rx and repo-related genes: In Nematostella Nvrx1 the ortholog of the Retinal homeobox gene Rx that is expressed upstream of Pax6 during eye development in vertebrates, is expressed in scattered ectodermal neuronal-like cells in planula and polyps, suggesting a role in the specification of a neuronal subset (Matus et al., 2007a). Similarly, NvRepo the ortholog of the glial-specific paired-like gene Repo is expressed at the oral nerve ring in planula and polyps (Marlow et al., 2009).

The cnidarian Otx-related genes are expressed as putative regulators of morphogenetic movements and of the nerve ring. Among PRD-class genes, Otx/Otd orthologs were identified in Podocoryne, Hydra and Nematostella however their neurogenic function is currently doubtful. In Podocoryne and Hydra Otx genes are likely involved in cell migration, like that observed during the budding process (Muller et al., 1999; Smith et al., 1999). However in the Hydra polyp Otx is also expressed in the neurogenic zones, i.e. in the tentacle zone and along the body column, but was not detected in neurons (Smith et al., 1999). In Podocoryne, Otx expression is actually initiated during the budding process and maintained in the striated muscle cells of the medusa but was not detected during planula development (Muller et al., 1999).

In anthozoans two Otx genes were identified in the coral Acropora and three clustered ones in the Nematostella although with unclear phylogenetic relationships between these paralogs (de Jong et al., 2006; Mazza et al., 2007). The three Nematostella Otx genes show a very similar biphasic expression pattern, with a first wave during gastrulation, restricted to the earliest involving endoderm that rapidly occupies the aboral region, and a later wave at the oral pole, detected as an endodermal pharyngeal
ring surrounding the presumptive mouth and in the first developing tentacles (Mazza et al., 2007). In Acropora, the two Otx genes exhibit distinct regulations, with OtxA predominantly ectodermal, also detected as a ring at the oral pole and along the body column in scattered cells, and OtxB, endodermal throughout development (de Jong et al., 2006). Thus a common trait for the Otx anthozoan genes would be their expression at a place and at a time when the nerve ring forms. If confirmed, this would suggest that the Otx function in apical/anterior neuronal patterning emerged in the CBA.

The Pax genes as regulators of neurogenesis and eye differentiation in cnidarians: In bilaterians the Pax gene families that likely derive from five uberrubarian ancestors, play a critical role in neurogenesis, eye development as well as myogenesis, segmentation and organogenesis. The identification of a single Pax gene in Porifera and of three Pax families in Cnidaria proved that Pax genes were submitted to an early wave of gene duplications likely after the divergence of Porifera (Hoshiyama et al., 2007; Matus et al., 2007a; Larroux et al., 2008). The anthozoans express four Pax gene families that represent three ancestral families: Pax-A and Pax-C for Pax neuro, Pax-B for Pax2/5/8, and Pax-D for Pax3/7 (Catmull et al., 1998; Miller et al., 2000). By contrast hydrozoans have lost the Pax3/7 ortholog and only express Pax-A as Pax neuro ortholog and Pax-B related to Pax2/5/8. Therefore a definitive Pax4/6 ortholog has not been found in cnidarians. However these cnidian Pax proteins bind the consensus Paired-response elements with broader specificity than the mammalian ones, likely allowing more flexibility (Miller et al., 2000; Sun et al., 2001; Plaza et al., 2003).

The expression data suggests that Pax genes are already involved in neurogenesis in cnidarians as in Nematostella, the PaxA/C genes (pox-neuro related) are expressed in putative neuronal/spirocyte precursors and neural cell types (Matus et al., 2007a) similarly to the Acropora PaxC gene (Miller et al., 2000). The Nematostella PaxB is expressed in scattered ectodermal cells and around the oral region suggesting a role in the patterning of the nerve ring (Matus et al., 2007a). The PaxD genes are present as a single copy in Acropora but four in Nematostella where expression of only two could be detected. In both species the PaxD genes bind the consensus Paired-response elements with broader specificity than the mammalian ones, likely allowing more flexibility (Miller et al., 2000; Sun et al., 2001; Plaza et al., 2003).

The coral PaxB and PaxD genes cannot induce by themselves eye formation when expressed in Drosophila imaginal discs but can achieve such task when chimeric (Plaza et al., 2003). In the jellyfish Podocoryne that does not differentiate eyes, Pax-B is expressed in the early steps of neuronal cell differentiation (Groger et al., 2000). These data strongly speak for a neurogenic function for Pax genes that arose in the CBA, and further studies in Trichoplax (Hadrys et al., 2005) and Porifera (Larroux et al., 2006) should trace the ancestral function of Pax genes in cell specification. However the expression of Pax genes is clearly not restricted to the nervous system in cnidarians as NvPaxC appears to be expressed also in gland cells, NvPaxB at the endodermal/ectodermal boundary of the pharynx, and Pc Pax-B in the entodocoton during medusa formation.

The Six genes and the eye differentiation in jellyfish: In contrast to the Pax genes, the Six gene families were already established in the CBA and remained highly conserved along cnidian and bilaterian evolutions. A single Six gene was identified in Porifera, related to the Six1/2-so family (Hoshiyama et al., 2007) whereas three distinct families were identified in cnidarians: Six1/2, Six3/6 and Six4/5, suggesting a wave of gene duplication that took place between Porifera and eumetazoans. In two hydrozoan medusae, Podocoryne (no eyes) and Cladonema (no eyes) these three families are likely involved in neurogenesis being expressed along the manubrium, in the nerve ring and/or the tentacle bulbs (Stierwald et al., 2004). Moreover the Six1/2 and Six3/6 genes are expressed in the eye cup, at low and high levels respectively (Stierwald et al., 2004). During eye regeneration Six1/2 and Six3/6 but not Six4/5 are up-regulated very early, Six1/2 preceding Six3/6 suggesting that Six1/2 is acting rather upstream in the cascade directing eye formation but is probably not required for eye maintenance. In the scyphozoan jellyfish Aurelia the Six1/2 ortholog is also expressed in the rhopalia (Bebenek et al., 2004). Finally given the genetic interactions that occur between the Pax and Six genes during eye specification (but also for muscle specification and kidney differentiation) in bilaterians, one can speculate that this interaction was already at work in cnidarians (Hoshiyama et al., 2007). Hence several key components of the genetic circuitry driving eye specification in bilaterians are already available and properly regulated in cnidarians. However the same question remains disputed (Fernald, 2004; Kozmik et al., 2008): How to explain that the same gene regulatory network supports eye differentiation in a large variety of phyla? Does it reflect a common origin for all the eyes across the phyla or rather a reiterated recruitment of the same regulatory network in distinct contexts?

The bHLH genes as candidate regulators of neurogenesis and myogenesis in hydrozoans: As for many gene classes involved in developmental processes, the complement of basic Helix-loop-Helix (bHLH) genes was already established when Cnidaria arose and remained strikingly stable over the evolution (Simionato et al., 2007). The bHLH genes were initially characterized in genetic analyses as proneural genes, i.e. directing the ectodermal cells towards a neuronal fate: In Drosophila, the achaete and scute genes exhibit a proneural function in sensory organ formation (Jan and Jan, 1994), and the vertebrate orthologs play a similar proneural function during development (Benowitz, 2002). Surprisingly a sponge bHLH gene was recently shown to display proneural properties when expressed...
in Xenopus or Drosophila (Richards et al., 2008). Hence pieces of the metazoan neurogenic circuitry predated the emergence of a nervous system.

The type A achaete-scute ortholog appears restricted to the nervous system in hydrozoans. The Achaete-Scute (ASH) genes distibute in two distinct classes, A and B. The A class is represented by four genes in Drosophila and C. elegans, two in mouse whereas the class B is absent in Drosophila but present in mouse, C. elegans and Podocoryne indicating an ancient duplication event (Ledent et al., 2002; Seipel et al., 2004c). In Hydra the type A ortholog, CnASH, is expressed in clusters of differentiating nematoblasts (Grens et al., 1995; Lindgens et al., 2004) and in sensory neurons at the base of tentacles (Hayakawa et al., 2004). When ectopically expressed in Drosophila instar larvae, CnASH led to the formation of ectopic sensory organs similarly to the Drosophila cognate genes when ectopically expressed; moreover a partial rescue was noted when CnASH was expressed in achaete/scute double mutants (Grens et al., 1995). In the jellyfish Podocoryne carnea, two Achaete/Scute genes were analyzed: the first one, Ash1, also related to class A, consistently showed an expression in differentiating nematocytes (Muller et al., 2003) whereas the second, Ash2, related to the class B, is likely involved in the differentiation of secretory cells (Seipel et al., 2004c). These data suggest that the neurogenic function of type A ASH genes is ancestral and conserved from cnidarians to bilaterians.

The Atonal-like gene Atl1 is a candidate proneural gene in the jellyfish Podocoryne. In the developing jellyfish Podocoryne, an Atonal-like gene (Atl1) was found expressed in endodermal neuronal precursors, and in adulthood, in mechanosensory cells and neuronal precursors located in the tentacle bulbs and the manubrium. Moreover, when in vitro transdifferentiation is induced, Atl1 is upregulated in proliferating neuronal precursors arising from adult striated muscle cells (Seipel et al., 2004c). Interestingly this study also mentions that during medusa budding, the striated muscle precursors in the entocodon expressed Atl1, highlighting the fact that neurogenesis and myogenesis that are supposed to share a common origin, indeed make use of common regulators.

The putative neurogenic function of the nuclear receptors RXR and COUP-TF
Nuclear receptors (NRs) are ligand-dependent transcription factors activated by steroid hormones and non-steroid molecules such as retinoic acid, thyroid hormone and vitamin D (Moras and Gronemeyer, 1998). However, some of the NRs are considered as “orphans”, i.e. lack a well-identified ligand (Benet et al., 2006). In cnidarians, a variety of nuclear receptors were characterized including a unique COUP-TF gene in Hydra but six in Acropora, a FTZ-F1 gene in Nematostella and a RXR gene in Nematostella and Tripedalia (Kostrouch et al., 1998). Therefore, the NRs gene families diversified very early during evolution, before divergence of Cnidaria (Figure 6).

A putative function in eye differentiation for the nuclear receptor RXR: As in vertebrates, the Tripedalia RXR ortholog might also regulate the expression of the crystallin genes as it is predominantly expressed at the medusa stages and it specifically recognizes in vitro direct repeats identified in the crystallin gene promoter of this cubozoan jellyfish (Kostrouch et al., 1998).

Moreover, similarly to its vertebrate cognates, the Tripedalia RXR transcription factor is potentially regulated by retinoic acid as it binds the 9-cis retinoic acid as a ligand. Further studies in hydrozoan and scyphozoan jellyfish should establish whether the RXR function is a common trait in cnidarian eye differentiation.

The neurogenic and nematogenic function of the nuclear receptor COUP-TF: In all bilaterian species the orphan COUP-TF genes were clearly associated with neurogenesis (Park et al., 2003). In mice, COUP-TF1 disruption results in multiple defects of the central nervous system (Qi et al., 1997) and together with Pax6 and Emx2, it acts as an early intrinsic factor for early regionalisation of the neocortex (Zhou et al., 2001). Hence COUP-TF genes, which in most contexts behave as potent negative transcriptional regulators (Achatz et al., 1997), bring a major contribution to both neurogenesis and the CNS patterning during the embryonic life, as well as in neurophysiology of the adult nervous system (Pereira et al., 2000; Cooney et al., 2001). According to these data, neurogenesis is considered as the ancestral developmental function of COUP-TF genes whereas the vertebrate COUP-TFI1 gene seems to be devoted to mesenchymo-epithelial interactions during organogenesis (Pereira et al., 1999). In Hydra, hyCOUP-TF is expressed in few interstitial cells, in proliferating and differentiating nematoblasts, as well as in neurons of the body column (Gauchat et al., 2004). In mice, the COUP-TF1 actually seems to be turned on later than prdf-b, at a time when nematoblasts enter the differentiation phase (Fig. 4). In the neuronal cell lineage, hyCOUP-TF expressing cells correspond to a subset of small bipolar neurons. When animals were rendered “nervle-free”, hyCOUP-TF expressing cells disappeared in few days. During budding and regeneration, hyCOUP-TF expression vanished in regions where either apical or basal differentiation occurred (Gauchat et al., 2004). Moreover the Hydra hyCOUP-TF expressed in mammalian cell cultures can repress the transactivation induced by the RAR-RXR nuclear receptors. In summary, the Hydra hyCOUP-TF is supposed to promote the differentiation of both nematocytes and neurons, reflecting hence an ancestral neurogenic function for the COUP-TF NR family.

Various transcription factor families involved in neurogenesis

The neurogenic function of the C2H2 zinc-finger transcription factors, Zic, Gli: The Zic and Gli transcription factors form two highly related evolutionarily-conserved families that interact with each other and bind their target sequences thanks to their C2H2 zinc finger domains (Aruga et al., 2006). In vertebrates, ascidians and nematode, Zic genes exert multiple functions during neuronal development including early neural patterning in vertebrates as evidenced by the region-specific morphogenetic alterations of the central nervous system induced upon inactivation of the different Zic genes in mice (Aruga, 2004; Merzdorf, 2007). However, they also specify mesodermal derivatives as in amphioxus and ascidians (Gostling and Shimeld, 2003) and the Drosophila Zic ortholog Odd-paired is a segmentation gene not involved in neuronal differentiation. In Hydra a HyZic gene is expressed in the nematocyte lineage where it is turned on during the first synchronous divisions of nematoblasts and off at the final
runs of division, before differentiation of mature nematocytes occurs (Lindgrens et al., 2004). In nerve-free animals Hyzic expression is rapidly turned off supporting the hypothesis that Hyzic function is restricted to the early stages of nematocyte differentiation; moreover in cnox-2(RNAi) hydra Hyzic expression is abolished (Figure 7D), suggesting that Hyzic is directly or indirectly regulated by the Gsx ortholog (Miljkovic-Licina et al., 2007). Further studies in anthozoan and medusozoan species should confirm and refine the neurogenic function of Zic genes, i.e. restricted or not to nematocyte differentiation, and involved or not in patterning of the cnidarian nervous system.

The Fox and MADS box transcription factors in cnidarians: The Sox genes encode High Mobility Group (HMG) transcription factors that are already present in choanoflagellates and sponges (King et al., 2008; Larroux et al., 2008) and diversified early in metazoan evolution with representatives of the groups B, C and F in sponges, B, C, E and F in cnidarians and ctenophores (Magie et al., 2005; Jager et al., 2006; Jager et al., 2008; Shinzato et al., 2008). In bilaterians Sox genes are involved in germ cell specification, mesendodermal patterning, neural induction, development of the central and peripheral nervous systems and organogenesis (Guth and Wegner, 2008). In Nematostella, Acropora and Clytia, 14, 6 and 10 Sox genes respectively were identified (Figure 6); among those, the expression patterns of the Acropora and Nematostella SoxC orthologs suggest some role in the specification of the ectodermal sensory neurons during development (Table 1). Nematostella and SoxB2 genes in ectodermal neuronal-like cells in developing Nematostella suggest some ancestral neurogenic function. Similarly the expression of the SoxB and SoxE genes in the neurosensory structures of the adult cnidophore comb jelly Pleurobrachia evoke some role in the maintenance of the nervous system (Jager et al., 2008). Consequently some Sox genes might have been recruited at the time of the emergence of the nervous system.

The Fox and MADS box transcription factors: The Fox genes encode transcription factors that bind DNA thanks to their winged-helix domain and are involved in the development of the nervous system in deuterostomes (Mazet and Shimeld, 2002; Mazet et al., 2005). Among the 20 families identified in bilaterians, at least 6 families already diversified in sponges whereas 9 new families appeared in cnidarians and only 3 in urbilaterians (Magie et al., 2005; Larroux et al., 2006; Chevalier et al., 2006; Larroux et al., 2008). In Clytia two of these gene families already diversified, the FoxB gene being expressed in numerous places where neurogenesis takes place including in the sensory organs named statocysts that develop along the bell rim (Chevalier et al., 2006). As FoxB genes are implicated in neurogenesis in bilaterians, these data suggest an evolutionarily-conserved function in neurogenesis for some of the Fox families. In Hydra, Budhead, a fork head/HNF3 ortholog rather appears involved in apical specification (Martinez et al., 1997). Concerning the MADS box transcription factors, the expression of the Nematostella MeF2 gene is consistent with a role in the differentiation of ectodermal cell types including nematocytes and neurons (Martinale et al., 2004) whereas the Hydra SRF ortholog possibly plays a similar function in the interstitial cell precursors and nematoblasts (Hoffmann and Krogh, 2001).

The basic leucin zipper (b-ZIP) CREB transcription factor. The bZIP transcription factors, defined by the presence of a basic domain followed by a leucine zipper domain involved in DNA-binding and dimerization respectively, form a large class of transcription factors that can be traced in fungi, plants and animals. In bilaterians, this class is formed of 19 families, 13 of them being already expressed in cnidarians (Figure 4). Phylogenetic analyses including cnidarian sequences indeed concluded that an early wave of gene duplications took place in the last common-CBA (Amoutzias et al., 2007). In bilaterians, the CREB transcription factor that is targeted by a wide variety of stimulus, regulates multiple developmental and physiological processes including neuron survival and neuron degeneration (Manatsiotis et al., 2002), nervous development, learning and memory (Lonze and Ginty, 2002). In Drosophila, Aplysia, rats and mice, CREB-dependent transcription is required for synaptic plasticity and learning and memory processes, more specifically for the transition from short-term to long-term memory, suggesting that CREB is an universal modulator of memory in bilaterians (Barco et al., 2006). In Hydra, the CREB transcription factor was initially identified as a key regulator of the early stage of head regeneration (Galliott et al., 1995; Kaloulis et al., 2004). However, the CREB protein was also detected at strong levels in proliferating progenitors, including progenitors for the nematocyte and neuronal cell lineages, as well as mature nematocytes, ganglion and bipolar sensory neurons (Chera et al., 2007). In hydrozoan medusae CREB might play a similar role, being strongly expressed in differentiating neurons in the tentacle bulbs (Figure 5E, here Podocoryne). Future work should tell us more about the various functions of CREB in cnidarian nervous systems.

The Runx and CBPβ genes in Nematostella neurogenesis: The DNA-binding of the Runx transcription factors is enhanced upon heterodimerization, specially with CBPβ when co-expressed. These two gene families appears to form a rather stable old couple, already present in Porifera and Cnidaria, which did not diversify prior to the emergence of bilaterians (Sullivan et al., 2008). In the adult Nematostella, Runx and CBPβ are expressed in putative neurons and neural precursors in the tentacles, in scattered ectodermal cells along the body column and in case of CBPβ, also in the mouth and upper pharynx. Detailed histological analyses suggest that these two genes are often co-expressed and participate in the differentiation and maintenance of the apical nervous system (Sullivan et al., 2008).

3.- What role for the phylum-specific genes in neurogenesis and nematogenesis?

Two types of molecules are considered as putative phylum-specific actors, first the bioactive peptides, neuropeptides or epitheliopeptides that are often evolutionarily-conserved but most probably play phylum-specific roles in differentiation and developmental processes, and second the phylum-specific genes. Recently a peptide-gated Na-channel was found expressed at the root of Hydra tentacles, suggesting that fast transmission through neuropeptides already existed in ancient nervous systems (Golubovic et al., 2007). The
FIGURE 7: Regulatory genes involved in neurogenesis in bilaterians likely support neurogenesis and nematogenesis in Hydra polyps. A) MARKERS of NEUROGENESIS: Cnot) Neurons (nv) located at the root of tentacles in the adult polyp (left) and in the spots where tentacle rudiments (t.r.) emerge during budding (middle) and head regeneration (right, here at 52 hpa), express the ANTP-class homeogene cnot. Prdl-a) Sensory neurons and their progenitors located at the apical pole (top view) express the paired-like homeogenes prdl-a (white arrows). Right panel: macerated head tissues stained with Hoechst (blue), anti-prdla (red) and anti-α-tubulin (green). Gsx/cnox-2) Progenitors and apical neurons (nv, white arrow) located at the base of the head region express the ParaHox homeogene Gsx/cnox-2. During head regeneration, cnox-2 is up-regulated in proliferating precursors and neurons that differentiate in the regenerating tip, shown here at 32 hpa (arrow). Right panel: Apical neurons co-expressing cnox-2 transcripts (green) and β-tubulin (red). Msx) Neurons of the body column express the ANTP-class homeobox msx gene. Msx+ neurons are denser in the budding zone and restricted to the ectodermal layer (black arrow). HyCOUP-TF, prdl-b) A subset of sensory neurons in the body column express Hy-COUP-TF and the paired-like homeogene prdl-b (not shown). B) MARKERS of NEMATOGENESIS: Gsx/cnox-2, hyCOUP-TF and prdl-b genes are expressed in synchronously dividing nematoblasts (nb, thin black arrows) along the body column. Both hyCOUP-TF and prdl-b genes are repressed in the adult apical and basal regions (brackets) but also in the presumptive head region during budding (arrowheads) and head-regeneration (large arrow). These genes are expressed at distinct stages along the nematocyte pathway, with Gsx/cnox2 transcripts detected in precursors and hyCOUP-TF expressed in nematoblast clusters that start differentiating. Scale bars: 100 µm and 10 µm. For references see in the text. C) Silencing of Gsx/cnox-2 through RNAi leads to alterations of neurogenesis after repeated exposures to dsRNAs: In intact Hydra the apical nerve net is no longer visible (upper panels); after amputation (lower panels) the de-novo neurogenesis normally observed in head-regenerating tips (left, here at 40 hpa) is drastically reduced (outline, right). mo: mouth opening. Scale bars: 50 µm. D) Putative epistatic relationships in Hydra nematogenesis deduced from studies performed by (Lindgens et al., 2004; Miljkovic-Licina et al., 2007). Gsx/cnox-2 regulates directly or indirectly HyZIC expression in proliferating nematoblasts: note the complete disappearance of HyZIC transcripts (red) in cnox-2(RNAi) silenced cells (green). Scale bar: 10 µm.
neurogenic function of peptides is well known in cnidarians. Neuropeptides can modulate muscle activity (Grimmelikhuijzen et al., 1996) as hym-176 that triggers contraction of the ectodermal myoepithelial cells in Hydra (Yum et al., 1998). In fact the (eyeless) Hydractinia planulae exhibit dramatically increased phototaxis when exposed to RFamide indicating that RFamides, expressed by the neurosecretory cells in all cnidian species, whatever the stage of the life cycle, can modulate the non-neural behavior (Plickert and Schneider, 2004). The authors propose that the RFamide cells act as interneurons between photosensing cells and myoepithelial cells.

Beside physiological activity, neuropeptides can trigger or enhance neuronal differentiation as hym-355 (Takahashi et al., 2000), or head activator that also affects cell proliferation and head patterning processes (Schaller et al., 1989; Hobmayer et al., 1997). Similarly the neuropeptides LW-amides play a pivotal role in developmental processes as larval metamorphosis in Hydractinia (Plickert et al., 2003). Interestingly neuronal differentiation is also under the control of epithelial cells as among peptides that were identified in the systematic Hydra peptide project (Fujisawa, 2008), the epitheliopeptides belonging to the LPW family can inhibit nerve cell differentiation (Koizumi, 2002). In bilaterians, peptides are best known as neurotransmitters or hormonal regulators involved in physiological processes (Boonen et al., 2009); hence homologous functions in cell differentiation or developmental processes remain to be deciphered. As nematogenesis is a cnidian-specific process, genes that trigger nematocyte differentiation are frequently phylum-specific. A microarray analysis identified 51 genes as nematocyte-specific, most of them encoding putative secreted proteins expressed at distinct stages of the pathway (Hwang et al., 2007). Out of these 82% do not have bilaterian orthologs implying that beside conserved regulatory genes, nematocyte differentiation makes use of a large proportion of genes, which were not retained in bilaterians and whose origin could not be traced back so far.

A tentative integrative view of the early evolution of neurogenesis

Common molecular tools for the specification of the cnidian and bilaterian nervous systems

Several criteria support a common origin for neurogenesis in cnidarians, cnenophores and bilaterians: first the presence in non-bilaterian phyla of gene families orthologous to those that encode transcription factors with neurogenic functions shared by protostomes and deuterostomes, second their consistent cellular expression patterns in the cnidian nervous system or during its differentiation, third the loss and gain of function assays that affect the maintenance or the differentiation of the cnidian nervous system (even though only few gene families were tested so far) and fourth the heterologous assays that proved that the cnidian genes can affect neurogenesis when expressed in bilaterian developmental contexts. The functional dissection of the genetic cascades regulating the differentiation of the nervous system in cnidarians and cnenophores was only recently launched but the expression analyses currently available indicate that the ANTP-class (not, max, Gax), PRD-class (Pax, paired-like, Rx, repo, gsc, six), bHLH-class (Achaete scute, atonal), HMG-group (Sox2, SoxB2, SoxB, Sox2), winged-helix group (FoxB), MADS-box class (Mef2), zinc fingers (zic), nuclear receptors (COUP-TF, RXR), Runx/CBPβ and bZIP (CREB) transcription factors likely regulate neuronal differentiation since early eumetazoan evolution (for references see above), similarly to the bHLH family members in myogenesis (Muller et al., 2003; Seipel et al., 2004c). The reiterated and independent recruitment of orthologous genes and signaling pathways to perform similar functions in various phyla cannot be excluded and is actually discussed concerning eye evolution (Kozmik et al., 2008; Suga et al., 2008), but does not represent a parsimonious scenario.

What roles for the “neurogenic” genes in Porifera, a non-neuronal phylum?

The evolutionarily-conserved regulatory genes expressed in nematocytes and neuronal cells were in most cases identified in poriferas: the genes for the ANTP, Pax, POU, LIM-HD, Sox, nuclear receptor, Fox (forkhead), T-box, Mef2, Ets and bHLH transcription factors emerged and in many instances already diversified prior to the Porifera/eumetazoan split (Larroux et al., 2006; Jager et al., 2006; Richards et al., 2008). Similarly the main signaling pathways Wnt, TGF-β, RTK, Notch, Hedgehog, and Jak-Stat as well as the adhesion molecules are present in sponges (Nichols et al., 2006). Moreover the modulated expression of the bHLH (Richards et al., 2008) and NK-type (Gazave et al., 2008) genes during embryogenesis suggest a possible role in cell differentiation and region specification. This surprising finding according to which the origin of the neuronal genetic circuitry predated the occurrence of nerve cell differentiation, is intriguing. In fact the choanocytes were proposed to exert some sensory function, and as such might represent a proto-neuronal cell type (Gazave et al., 2008). Therefore, these gene families, which likely constitute the hallmark of metazoans, might already be committed to a “proto-neuronal” function. Alternatively, they might specify cell fate independently of their ability to differentiate mechanoreceptor and/or nerve cells. Two types of arguments can be proposed to explain the absence of neurogenic “success” for the poriferan “neurogenic” genes: firstly the absence of some essential neurogenic genes explaining that the genetic circuitry cannot be mounted properly; among those missing genes the ParaHox/Hox-like genes were never identified in Porifera and we saw that Gsx/cnnox-2 plays an essential role in the regulation of neuronal precursors in Hydra as well as in bilaterians, secondly the absence of some target structures, i.e. myofibers, might make the organization of a rudimentary genetic circuitry useless. However one cannot rule out the possibility that these genes represent a genuine neurogenic program originally acquired by the metazoan ancestor and secondarily lost in poriferans.

The early diversification of the regulatory gene families in eumetazoans and the emergence of neurogenesis in the Cnidaria-Bilateria ancestor

Interestingly most of these putative neurogenic gene families that encode transcription factors underwent an early wave of amplification in the last common Cnidaria Bilateria ancestor (Figure 6). This event likely preceded the emergence of the nervous system as evidenced by the few representatives present in sponges versus the large number of cnidian-bilaterian orthologous families belonging to the Homeobox, bHLH, bZIP, Wnt classes. Moreover the comparative analysis of the transcription
factor classes between one poriferan and two cnidarian genomes confirmed that several classes that exhibit an evolutionarily-conserved role in neurogenesis in bilaterians have emerged after the divergence of Porifera. This is the case of the Hox/ParaHox, Otx-like, Atonal/Twist gene families that are obvious candidates for having conducted the emergence of the nervous system. As the bilaterian orthologs also regulate neurogenic functions, we speculate that the combination of transcription factors that drove neurogenesis in the Cnidaria-Bilateria ancestor was iteratively used along evolution. In a limited number of cases, these “eumetazoan” families underwent a secondary wave of duplications after the divergence of cnidarians, in bilaterians, suggesting that the duplication of genes previously recruited for neurogenesis, led to the complexification of the neuronal structures thanks to paralogous genes.

Symbiosis might have contributed to the emergence of sophisticated sensing tools

Our current knowledge about the molecular tools supporting the various cellular differentiation pathways in distinct animal phyla tremendously increased over the past ten years with genomic sequencing, extended phylogenetic analyses of gene classes, cellular expression and functional analyses. This vast bag of informations allowed us to see emerging principles for understanding cell type specification. Detlev Arendt recently proposed three common principles that applied all along animal evolution to homologous cell types and sister cells (Arendt, 2008), 1) the multifunctionality of ancestral cells as the myoepithelial cnidian cells that carry out epithelial functions but also muscular contraction and electric conduction (Mackie and Passano, 1968), 2) the progressive segregation of the ancestral functions in more specialized cell types that abandon some of the ancestral functions by silencing the corresponding genetic functions, to carry out a more limited number of functions, 3) the divergence of some functions thanks to the duplication of the molecular tools such as the amplification of gene families within a given class.

The current set of data concerning neurogenesis in non-bilaterian species certainly obey these principles of linear diversification of cell types but the analysis of the origin and early evolution of neurogenesis also requires to take into account decisive processes that might constitute a fourth principle: the incorporation of foreign genetic material, either through horizontal gene transfer or through symbiosis. Few cases are currently documented but these certainly deserve attention. The analysis of the respective behaviors of the different cellular contingents in Hydra actually led to the proposal that the venom capsule (named nematocyst or cnidocyst) in nematocytes would result from a symbiogenetic process (Shostak, 1993). More recently Denker et al. showed that the horizontal transfer of a bacterial gene encoding a subunit of bacterial poly-γ-glutamate (PGA) synthase in the genome of the cnidian ancestor might have been decisive for the specification of the nematocyte weapon, the cnidocyst (Denker et al., 2008a). In fact only the receptor part of this highly sophisticated cell, the cnidocil, might be retained along evolution, sharing typical features with other mechano-sensory cells (Holstein and Hausmann, 1988), while structures homologous to the nematocyst capsule were not identified in other animal phyla so far, indicating that phylum-specific innovations might also stand alone, even when they contributed to the sustained evolutionary success of the phylum where they arose.

The second case where the importation of foreign material might have been decisive, concerns the specification of the eyes that combine photoreceptor and pigment cells since their origin (Gehring, 2004). As photoreception is widely distributed among living organisms, including in bacteria, Walter Gehring proposed that photoreception in the cnidian ancestor might result from a series of symbiotic transfers, a cyanobacteria into a red algae, a red algae into a dinoflagellate, the transformation of the chloroplast into an eye inside the dinoflagellate and finally the transfer of the dinoflagellate into a cnidian. A long way to go before seeing, but that certainly highlights the potency of such mechanisms to bring novelties in organisms that were less constrained than most bilaterian species.

What was the proto-neuronal cell from which nerve cells evolved?

If we assume that sharing molecular signatures signify a common history, then the nerve and muscle cells should be considered as sister cells in cnidarians. In fact members of the Six, Pax, bHLH, Sox, Pou gene classes are involved in both neurogenesis and myogenesis in bilaterians. In the Podocoryne jellyfish where both differentiation pathways were monitored during medusa budding and induced transdifferentiation, the analysis of the Six, C/EBP, MaftL, Atonal like 1, Achaete-scute 2 genes (Table 1) as well as the observation of the transient expression of neuronal markers during myogenesis (Seipel et al., 2004b; Seipel et al., 2004c; Stierwald et al., 2004) suggested that muscle cells and nerve cells derive from common myoepithelial cells. These molecular data actually fit with the three steps model proposed by George Mackie for the origin of neuromuscular transmission (Mackie, 1970) whereby muscle cells and nerve cells would have diverged from myoepithelial cells (see Figure 5a in Arendt, 2008). The scenario is as follows: Starting from a primordial myoepithelium capable of "neurid" conduction, the protomyocytes progressively detached from the basal side of the myoepithelium to sink into the interior; at the second step protoneurons evolve from the myoepithelium to connect the myocytes to the outside forming a group of electrically interconnected cells; at the third step, neurosensory cells and neurons evolved from the protoneurons, developing long processes that connected them to each other and to the myocytes by chemical polarized junctions. This scenario that is largely driven by the electrophysiological properties of the different cell types, is coherent and attractive. However it predicts that the origin of the neurosensory cells followed the emergence of protoneurons. In fact sensory-like cells appear to have predated by far the origin of the nervous system, already present in early metazoans as poriferae where they seem to use a proto-neural program, the Notch/Delta and bHLH pathway to differentiate (Richards et al., 2008). These results suggest that the program leading to the emergence of neurogenesis was multilayered, a pre-program being already available in the different cell types of the last common metazoan ancestor, therefore the differentiation of neurons could have arisen from myoepithelia as well as from sensory cells.
CONCLUSIONS & PERSPECTIVES

Most if not all the pieces of the puzzle that regulate the nervous system in bilaterians are present in cnidarians, but given the almost complete absence of functional analysis, the question of how these different pieces interact in cnidarians remains completely open. There are nevertheless clear differences between neurogenesis in cnidarians and bilaterians as for instance the origin of the neuronal precursors during early development: in cnidarians those were identified in the endoderm, from where they migrate towards the ectodermal layer, whereas in bilaterians the neural precursors are derived from the neural plate. Therefore, the origin of the neuronal precursors in cnidarians is an open question and needs to be further investigated.

Also some genes that were expected to be universal regulators of neurogenesis appear missing; the best example is engrailed that is apparently not expressed in cnidian genomes. Also some key genes for brain patterning in protostomes and deuterostomes appear to be submitted to looser constraints in cnidarians, as Otx that might support cell migration in developmental processes in hydrozoans, but be required for the formation of the nerve ring in Nematostella. Also some genes provide inconsistent expression patterns between different cnidian species, making difficult to identify the common themes among these developmental variations. Sampling more taxa in Cnidaria and Ctenophora will help unravel the core genetic mechanisms that allowed the emergence of neurogenesis and neuromuscular transmission.

The identification of epistatic interactions between cnidian regulatory genes is just embryonic. However, at least two candidate gene regulatory networks might be shared by cnidarians and bilaterians: one involved in neurogenesis along the axis, including the three ANTP-class homeobox genes and NKX2, possibly regulated by the BMP pathway and the second one, involved in eye specification with the Pax, Six, Dachsund and eye genes. Future approaches that combine high throughput genomic analyses and functional analyses in homologous and heterologous contexts should characterize the regulatory elements that drive neuronal cell differentiation in both cnidarians and bilaterians, establish a genetic hierarchy and subsequently deduce the level of conservation of the neurogenic circuitry across evolution. Ultimately such core robust genetic circuit could be implemented in non-neuronal species to induce neurogenesis and possibly modify the behavior of target cells as for instance ciliated cells (Jekely et al., 2008). Parallel efforts could induce muscles, photoreceptors and one can dream of sponges walking towards the light! Still these spectacular experiments would definitively prove that a given gene or pathway is indeed efficient but would leave unsolved the question of their ancestral recruitment in natural evolution.

There is a growing consensus about the absence of homology between the oral-aboral cnidian axis and the bilaterian axes (see above) and we believe that the emergence of novel cell types likely preceded the shared organization of a body axis between cnidarians and bilaterians. However the question of the mechanisms driving the emergence of novel cell types was much less investigated than those driving axis patterning, certainly for technical reasons. Therefore there is clearly much to be learnt about the molecular mechanisms that drove and maintained cellular novelties. Those were complexified between cnidarians and bilaterians, but the core processes were already at work in cnidarians. Also if the urbilaterian axis was not established in cnidarians yet, the organization of the oral pole might share some common rules with the anterior patterning in bilaterians and it would be of utmost interest to trace the phylogenetic relationships between the processes that allow the development of annular nerve rings in cnidarians and the central nervous system in bilaterians. Finally given the variety of the developmental contexts that can be tested beside sexual development in cnidarians, these approaches will certainly also highlight the intricate relationships between neurogenesis and patterning processes.

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