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# Neuronal Evolution : Analysis of Regulatory Genes in a First-Evolved Nervous System, the *Hydra* Nervous System

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## Abstract

Cnidarians represent the first animal phylum with an organized nervous system and a complex active behavior. The hydra nervous system is formed of sensory-motoneurons, ganglia neurons and mechanoreceptor cells named nematocytes, which all differentiate from a common stem cell. The neurons are organized as a nerve net and a subset of neurons participate in a more complex structure, the nerve ring that was identified in most cnidarian species at the base of the tentacles. In order to better understand the genetic control of this neuronal network, we analysed the expression of evolutionarily-conserved regulatory genes in the hydra nervous system. The PRD-CLASS homeogene, *prdl-b*, and the nuclear orphan receptor, *hyCOUP-TF*, are expressed at strong levels in proliferating nematoblasts, a lineage where they were found repressed during patterning and morphogenesis, and at low levels in distinct subsets of neurons. Interestingly, PRD-CLASS homeobox and COUP-TF genes are also expressed during neurogenesis in bilaterians, suggesting that mechanoreceptor and neuronal cells derive from a common ancestral cell. Moreover, the PRD-CLASS homeobox gene *prdl-a*, the ANTP-CLASS homeobox gene *msh*, and the thrombospondin-related gene *TSPI*, which are expressed in distinct subset of neurons in the adult polyp, are also expressed during early budding and/or head regeneration. These data strengthen the fact that two distinct regulations, one for neurogenesis and another for patterning, already apply to these regulatory genes, a feature also identified in bilaterian related-genes.

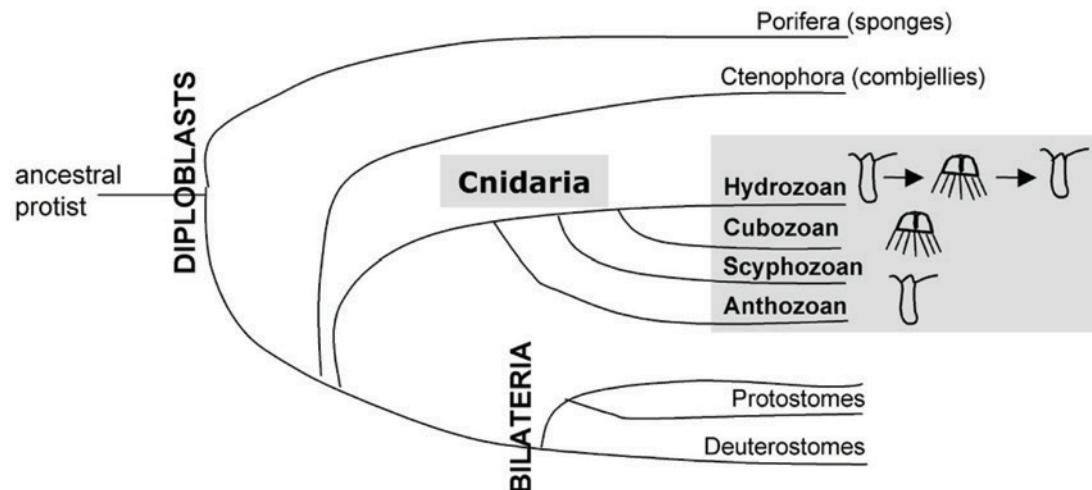
**Key words:** mechanoreceptor cells, origin of neuronal cells, neurogenesis and patterning, budding and regeneration

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## 1 Introduction

How did neuronal networks evolve from multicellular epithelial animals? How, in these simple organisms, did the emergence of neurogenesis affect patterning, i.e. developmental regulation of a specific shape? How asexual development, e.g. budding and regeneration, can specifically reshape an animal whatever its age? These are central questions to understand the establishment of the modules that underly the developmental processes of most bilaterian animal species. The Cnidaria phylum is supposed to have diverged about 700 Million years (My) ago, preceding the cambrian explosion, a period when most ancestors to bilaterian species arose Raff (1996), Nielsen (1997), Ayala and Rzhetsky (1998). Most cnidarians are marine animals that

display a radial symmetry, either as a polyp or as medusa Bouillon (1994). They are made up of two cell layers, ectoderm and endoderm, separated by an extracellular matrix named mesoglea. Cnidarian species distribute in four distinct groups, anthozoans (corals), scyphozoans and cubozoans (jellyfish), and hydrozoans, which alternate the polyp and the medusa shape in their life cycle (**Fig. 1**). Thanks to their nervous system, cnidarians are the simplest organisms in which movements are governed by a neuromuscular system Westfall (1996), specially their active feeding behavior, which rely on coordinated movements of their tentacles. In contrast, poriferans (sponges), which diverged earlier during evolution, feed by passive filtration. Although poriferans are capable of chemical



**Fig 1 :** Scheme depicting the position of cnidarians in the metazoan tree. Among cnidarians, anthozoans are exclusively polyps, cubozoans and scyphozoans are most often jellyfish, while hydrozoans usually alternate between the polyp and the jellyfish stages during their life cycle. Hydra is a freshwater hydrozoan, which displays the polyp stage exclusively. Among diploblasts, the respective positions of cnidarians and ctenophores is not well assigned.

conduction, they do not display any cell types exhibiting similarity with neurons Lawn et al. (1981), Leys et al. (1999). Therefore, cnidarians provide the most appropriate model systems to trace back the first-evolved nervous systems Anderson and Spencer (1989). In hydra, neurons represent about 3% of the total cell number, these nerve cells being either sensory or ganglia cells David (1973). Cell bodies of most sensory neurons are located within the ectodermal layer, while their processes reach the surface. These neurons are named sensory but, in jellyfish, they were shown to function as sensory-motoneurons, as they can establish bidirectional synapses with their target cells, namely myoepithelial cells, nematocytes Anderson (1985). In sea anemones, sensory neurons are associated with smooth muscle fibers, also suggesting that they behave as sensory and motoneurons Grimmelikhuijzen et al. (1989). In hydra, ganglia neurons are the most common type of nerve cells and spread among the mesoglea. Interestingly, neurons are organized as a nerve net, which can be visualised thanks to neuron-specific immunostaining: this nerve net is not uniform and distinct subsets of neurons with specific spatial distribution could be identified Grimmelikhuijzen et al. (1989), Koizumi et al. (1990). For example, the RFamide-expressing neurons are clearly more abundant in the hydra head and foot regions, while almost absent in the central part of the body column Grimmelikhuijzen and Westfall (1995). In addition to the nerve net, a dense anatomical structure, named the nerve ring was identified at the base of tentacles of some hydra species Koizumi et al. (1992). Similar ring structures were also identified in jellyfish : inner

and outer nerve rings at the bell margin of hydrozoan medusae Grimmelikhuijzen et al. (1989). Nerve rings might correspond to a simple form of cephalization involved in the coordination of behaviors Passano and McCullough (1965).

Beside neurons, another highly differentiated cell type plays a key role in capture of preys and defense in cnidarians. These cells, named nematocytes or cnidocytes (stinging cells), are abundant, representing 35% of all cell types David (1973) and actually gave their name to the Cnidaria phylum. Nematocytes are mechanoreceptor cells that contact preys through their cnidocil and discharge in few milliseconds the toxic content of a highly specialised thick-wall capsule named the cnidocyst Tardent (1995), Engel et al. (2002). The nematocyst's discharge works as a poison syringe, releasing large droplets of venom into the prey through an everting tubule. This venom will immobilize the prey, that hence will stimulate the feeding response, i.e. tentacle bending and mouth opening, by releasing the peptide glutathione, which acts as a feeding activator 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, the nematocytes are likely not only mechanoreceptor cells but also effector cells : it was demonstrated that nematocyst discharge could occur in the absence of nerve cells Aerne et al. (1991), proving thus that nematocytes can behave as autonomous mechanoreceptor-effector units.

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 Thurm (2001), Westfall et al. (2002). At the neurophysiological level, electrophysiological experiments performed on cnidarians neurons have demonstrated that « many of the basic synaptic mechanisms and properties that we associate with more “advanced” nervous systems, can be demonstrated in the Cnidaria » Spencer (1989). Recently, calcium and potassium channels were characterised in jellyfish with functional features similar to those measured in vertebrate counterparts Jeziorski et al. (1999), Grigoriev et al. (1999), while GABA receptors were pharmacologically identified Pierobon et al. (2001). Neurotransmitters like glycine Pierobon et al. (2001), nitric oxide Colasanti et al. (1997), endocannabinoid De Petrocellis et al. (1999) and glutamate Bellis et al. (1991) are likely playing a physiological role in the feeding response. However, despite morphological effects of dopamine synthesis inhibitors or dopamine antagonists in hydra Ostroumova and Markova (2002), the presence and function of bioamines as well as acetyl-choline remains to be proved. In fact, cnidarian nervous systems are strongly peptidergic Grimmlikhuijzen and Westfall (1995), which is likely a major difference with bilaterian nervous systems. All together, these structural, biochemical, electrophysiological and molecular data support the view according to which this “simple” cnidarian nervous system exhibits a rather sophisticated organization, with strikingly evolutionarily-conserved properties.

At the cellular level, hydra cells differentiate from three distinct stem cells; the ectodermal epithelial cells, the endodermal epithelial cells and the interstitial cells. Interactions between the epithelial and the interstitial cell lineages are highly regulatory, as for example the control of nerve cell differentiation by epitheliopeptides Fujisawa (2003). Neurons and nematocytes derive from a common stem cell in hydra, named the interstitial stem cell David (1973), Bode (1996). This stem cell also provides two other cell differentiation products, the gland cells and the gametes, when the animal follow the sexual cycle. These four differentiation pathways are clearly distinct, namely the nematocytes will undergo 4 to 5 synchronous syncytial cell cycle divisions, forming nests of nematoblasts into the ectoderm of the body column. Once they stop proliferating, the nematoblasts will differentiate their nematocyst

vacuole, which can be of four distinct types, and finally migrate to their definitive location, i.e. the tentacles for most of them Campbell (1988). This migration of nematocytes rely on contact guidance from surrounding tentacles Campbell and Marcum (1980). In the tentacles, several nematocytes will be embedded within large epithelial cells, named battery cells, and establish synapses with sensory neurons. In contrast, the nerve cell pathway is far more simple : cells committed to this pathway get stacked into G2 until a signal will let them divide and terminally differentiate Schaller et al. (1989). In this paper, we investigated the expression of a set of genes that encode highly evolutionarily-conserved regulatory proteins, either transcription factors or signalling molecule, both at the cellular level and during two morphogenetic processes, budding and regeneration.

## 2 Materials and Methods

### 2.1 *Hydra culture and regeneration experiments :*

*Hydra vulgaris* (Hv, Irvine strain) was cultured in hydra medium (HM: 1 mM NaCl, 1 mM CaCl<sub>2</sub>, 0.1 mM KCl, 0.1 mM MgSO<sub>4</sub>, 1 mM Tris pH 7.6) and fed 5 days a week with hatched *Artemia nauplii*. After a two days starvation period, animals were bisected at mid-gastric position and left for regeneration at 19°C.

### 2.2 *In situ hybridization and imaging*

For each gene, 15 animals were analysed for each condition (budless, regenerating time points, budding stages) and experiments were repeated several times. Riboprobes were labelled with either digoxigenin (DIG) or fluorescein following the supplier recommendations (Roche) and *in situ* hybridizations were performed on whole mount animals, following the procedure described in Grens et al. (1995). Double *in situ* were performed according to Mitgutsch et al. (1999). During the NBT/BCIP and/or Fast Red (Sigma) staining, appearance of the gene expression patterns was followed on Stemi SV11 stereomicroscope (Zeiss). The staining was then stopped by post-fixation in 4% paraformaldehyde and the animals were pictured with a Coolpix 995 camera (Nikon). Subsequently, selected animals were mounted with glycerol and examined on an Axioplan 2 microscope equipped with a Plan-Apochromat 40x objective (Zeiss). For cell-type determination, animals were washed after post-fixation in PBS, stepwise transferred from 10% sucrose/PBS to 50% sucrose/PBS and embedded in OCT

compound (Sakura) overnight at 4°C. Cryosections (10 µm) were then performed on Cryocut 1800 (Reichert Jung) and mounted on slides in mowiol Longin et al. 1993). Images were captured through an Axiocam camera with the Openlab System Software (Improvision). Sorting out of the images was processed with the FotoStation Pro4.5 software (FotoWare) and final artwork was done with the Photoshop software (Adobe).

### 3 Results

#### 3.1 *prdl-a*, *msh*, and *TSP1* are regulatory genes expressed during neurogenesis and patterning in hydra

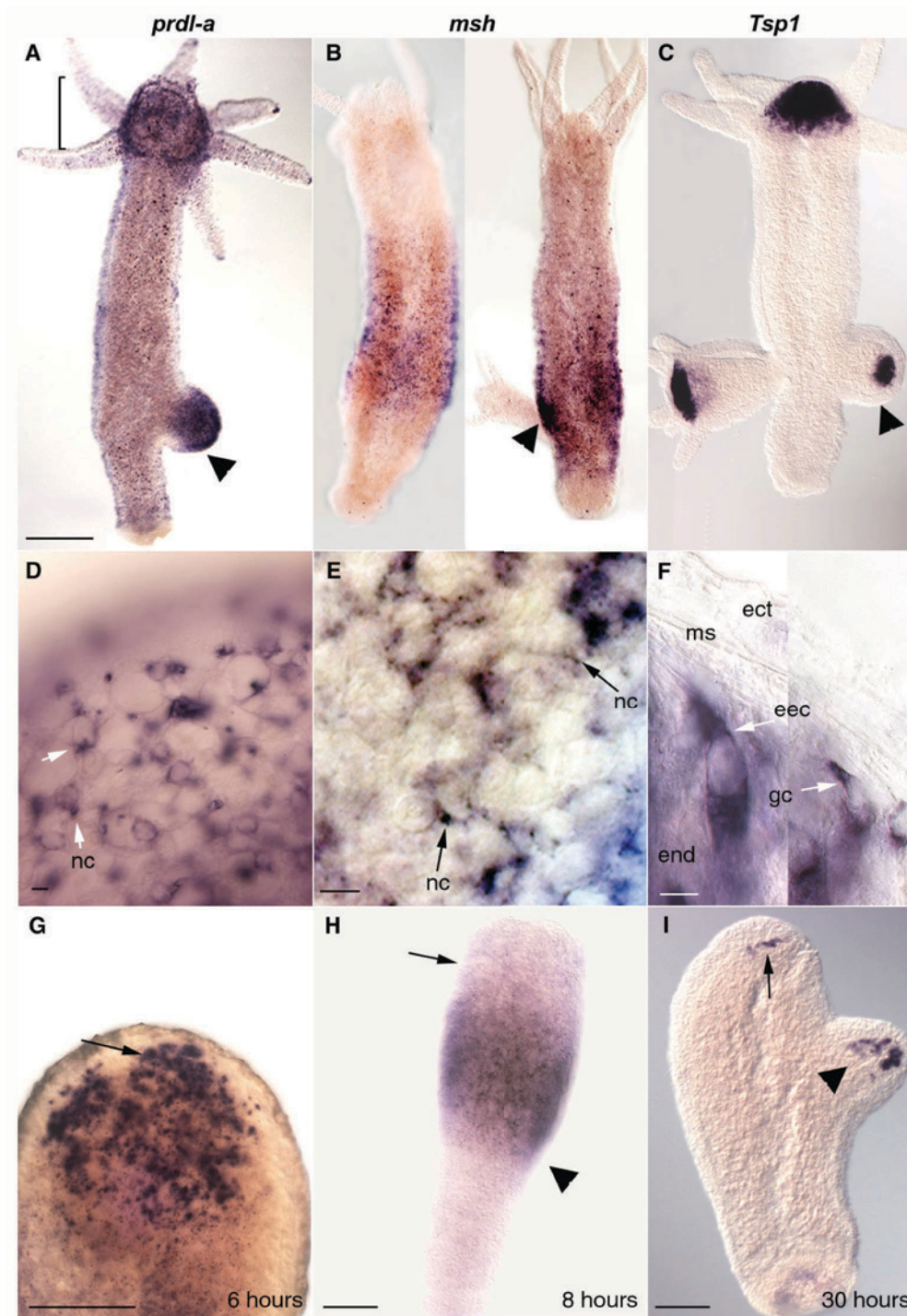
In previous work, we showed that the PRD-CLASS homeogene *prdl-a*, related to the paired-like *aristaleless* family Galliot et al. (1999), is expressed in precursors to nerve cells and neurons of the apical region Gauchat et al. (1998). Further examination of the *prdl-a* expression pattern showed that this gene is indeed predominantly expressed in neurons located in the ectodermal layer of the apical region but also in the body column, in scattered cells that show interstitial and nerve cell morphology (**Fig. 2A, 2D** and **Fig. 3G**). In the head region, the *prdl-a* expressing cells display a typical nerve net shape (**Fig. 2D**). In contrast, the ANTP-CLASS homeogene, *msh* Schummer et al. (1992), Gauchat et al. (2000) exhibits an expression pattern restricted to the ectodermal layer of the central part of the body column (**Fig. 2B**). A closer examination showed that *msh*-expressing cells were nerve cells (**Fig. 2E**) with a higher density in the bud spot, the circular area in the parental body column from which the future bud will grow (**Fig. 2B, 2H**, arrowheads).

Recently, we characterised a gene encoding thrombospondin-type 1 repeats Adams and Tucker (2000) and thereafter named *TSP1* Miljkovic-Licina et al. (in preparation). *TSP1* is strongly expressed in the endodermal layer of the most apical region of the hydra head, which surrounds the mouth opening, named the hypostome (**Fig. 2C**). Interestingly, we could detect *TSP1* expression in two distinct cell lineages: in endodermal epithelial cells and in ganglia nerve cells (**Fig. 2F**). When we analysed the expression of these genes during patterning and morphogenetic processes, i.e. budding and regeneration, we confirmed that *prdl-a* is transiently expressed in the endodermal layer of

the head regenerating stump Gauchat et al. (1998), here shown 6 hours after mid-gastric bisection (**Fig. 2G**). These endodermal cells that transiently express *prdl-a* during early regeneration are large, roundish, do not display processes and consequently do not belong to the nerve cell lineage but probably to the epithelial cell lineage (**Fig. 2G**, and not shown). This first endodermal wave of expression will vanish during the next 12 hours and be replaced by expression in the subsequent ectodermal cell layer of the regenerating stump during the second day Gauchat et al. (1998), Galliot and Schmid (2002). Similarly, during the budding process, *prdl-a* is expressed as soon as stage 2 Otto and Campbell (1977) in the endodermal layer Gauchat et al. (1998) and at subsequent stages in the ectoderm (**Fig. 2A**, arrowhead). The fact that in two distinct contexts (adult stage versus head regeneration) the same gene is expressed in two distinct cell layers, and two distinct cell lineages, proves that its expression is submitted to two distinct temporo-spatial regulations.

In contrast, *msh*-expressing cells, which show a high density in the bud spot (**Fig. 2B, 2H**, arrowheads), at the initiation stage of budding, seemingly belong to the very same ectodermal neuronal lineage. In addition, *msh* is clearly repressed during head and foot regeneration processes (**Fig. 2H**, arrow), proving that initiation of budding and early steps of regeneration do not rely on identical genetic cascades Miljkovic-Licina et al. (in preparation). Finally *TSP1* also shows a temporo-spatially regulated expression during budding and head regeneration: *TSP1* expression started to be detected in few endodermal cells of the head regenerating tip, between 24 and 30 hours after mid-gastric amputation (**Fig. 2I**, arrow). This timing in the expression during head regeneration is very similar to that observed for the ANTP-class homeogene *cnox-2* Gauchat et al. (2000), hence *TSP1* and *cnox-2* can be considered as head-specific « early-late » regeneration genes. During budding, *TSP1* transcripts were detected from stage 3 Otto and Campbell (1977) and onwards, in the most distal endodermal cells of the bud (**Fig. 2I**, arrowhead and not shown). In both contexts, either head regeneration or budding, *TSP1* expression was detected about 16 to 20 hours before tentacle rudiments became visible. This concomitant timing in gene expression suggests that at that early/late stage, the same genetic cascades are used for head formation during budding and regeneration.





**Fig 2 :** *Prdl-a*, *msh* and *Tsp1* transcripts are detected in the hydra nervous cell lineage. A) Transcripts of the PRD-class homeobox gene, *prdl-a* are mostly abundant in ectodermal cells of the hypostome (most apical area surrounding the mouth opening indicated with a bracket) and the presumptive head region of the growing bud (arrowhead, here stage 5 according to Otto and Campbell (1977)). B) The ANTP-class homeobox gene, *msh*, is expressed in the ectodermal layer of the central region of the body column. In the budding spot (arrowhead), a higher density of *msh*-expressing cells was noted. C) The thrombospondin-like gene, *Tsp1* is strongly expressed in endodermal cells of the adult hypostome and the presumptive head during budding (arrowhead, bud stage 5). D) Magnified view of the nerve net formed by *prdl-a* expressing cells in the hypostome. Cell bodies of *prdl-a* expressing neurons are indicated with white arrows. E) Magnified view of *msh*-expressing neurons. White arrows as in D. F) The *Tsp1* gene is expressed in ganglia cells of the head region (left panel) and endodermal epithelial cells (right panel). G) Transient *prdl-a* expression in endodermal cells of the head-regenerating stump 6 hours after bisection (arrow). H) During head regeneration, *msh* is repressed in the regenerating stump (arrow) while expression persists in the budding zone and the bud spot (arrowhead). I) *Tsp1* expressing cells can be detected in the endodermal layer of both the regenerating stump 30 hours after bisection (arrow) and in the growing bud (arrowhead). ect: ectodermal layer; eec: endodermal epithelial cell; end: endodermal layer; gc: ganglia cells; ms: mesoglea; nc: nerve cells. Bars correspond to 1 mm (A, B, C), 10  $\mu$ m (D, E, F), 300  $\mu$ m (G, H, I).

### 3.2 *prdl-b* and *hyCOUP-TH* are regulatory genes expressed in both nematocyte and nerve cell lineages in hydra

Previous work had shown that at least three regulatory genes that are involved in neurogenesis in bilaterians are expressed in proliferating nematoblasts in hydra. The first one, *CnASH*, belongs to the basic helix-loop-helix transcription factor family and is related to the *Achaete* and *Scute* genes that showed proneural activity in *Drosophila* Grens et al. (1995). The second is the MADS-box transcription factor, *SRF* (Serum Response Factor) that is expressed in interstitial cells as well as dividing nematoblasts Hoffmann and Kroiher (2001). The third one is a paired-like homeogene related to the *aristaleless* family, encoding a homeodomain highly similar to that of *prdl-a* and therefore named *prdl-b* Gauchat et al. (1998), Galliot et al. (1999).

The strong expression of *prdl-b* in nests of synchronously dividing nematoblasts along the body column (**Fig. 3A**, arrows), was confirmed on paraffin sections and cell-specific Northern analysis Gauchat et al. (1998). Moreover *prdl-b* expression was no longer detected in regions where nematocytes start to differentiate and migrate, i.e. the upper body column (**Fig. 3A**, bracket), the head and foot regions. During budding, *prdl-b* expression cannot be detected in the bud spot area (**Fig. 3A**, arrowhead) and in the presumptive head region of the growing bud (**Fig. 3D**, arrowhead). During regeneration, *prdl-b* expressing cells were absent in the head- and foot-regenerating stumps (**Fig. 3B, 3C**, arrows).

In order to refine the *prdl-b* expression pattern at the cellular level, we performed a double labelling with a DIG-labelled *prdl-a* probe and a fluorescein-labelled *prdl-b* probe (**Fig. 3E-H**). Fluorescent detection of riboprobes is more sensitive than brightfield detection after colorimetric staining (Stern, 1998). Using that former type of detection, we could detect beside strong, large spots corresponding to nests of nematoblasts (**Fig. 3E**, white arrowhead), numerous single *prdl-b* expressing cells (**Fig. 3E**, white arrows). Closer examination on magnified views proved that a large number of these single cells display a typical nerve cell morphology (**Fig. 3F**, arrows) and that a subset of these neurons co-expressed *prdl-a* and *prdl-b* (**Fig. 3G, 3H**, black arrows).

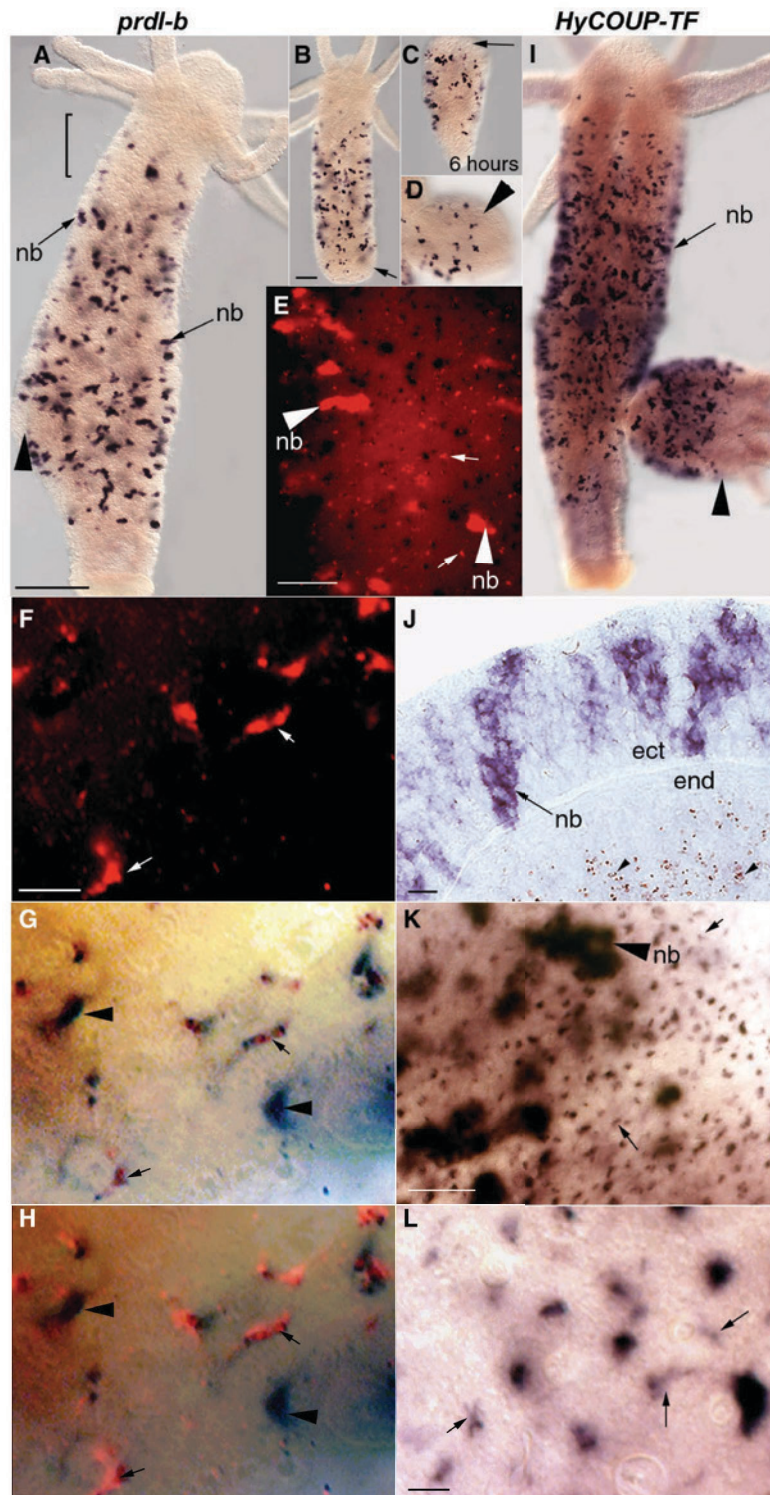
The orphan COUP-TF nuclear receptors form a highly evolutionarily-conserved family with COUP-TF homologues identified in a wide range

of metazoans from cnidarians to protostomes and deuterostomes Escriva et al. (1997), Langlois et al. (2000), Grasso et al. (2001), Devine et al. (2002), Gauchat et al. (submitted). These transcription factors show an extensive degree of homology in their DNA-binding as well as ligand-binding domains. Interestingly, COUP-TF genes are implicated both in neurogenesis and/or CNS patterning during the embryonic life and in neurophysiology of the adult nervous system Pereira et al. (2000), Cooney et al. (2001). We analysed the expression and the developmental regulation of the hydra COUP-TF homologue, named *hyCOUP-TF* and found that it was strongly expressed in the nematocyte lineage (**Fig. 3I,J**), displaying a pattern very similar to that of *prdl-b*. Cryosections proved that these nests of cells that were located within the ectoderm corresponded to synchronously dividing nematoblasts (**Fig. 3J**). Likewise *prdl-b*, we found *hyCOUP-TF* expression repressed in the apical and basal extremities of the adult polyp, in the bud spot where budding is initiated and in regenerating stumps Gauchat et al. (submitted). However, when animals were overstained, we detected in addition to the nests of *hyCOUP-TF* expressing nematoblasts numerous single *hyCOUP-TF* expressing cells located in the endodermal layer (**Fig. 3K, 3L**). A subset of these single *hyCOUP-TF* expressing endodermal cells could be identified as nerve cells (**Fig. 3L**, arrows), proving that as in the case of *prdl-b*, *hyCOUP-TF* is co-expressed in both nematocyte and the neuronal cell lineages.

## 4 Discussion

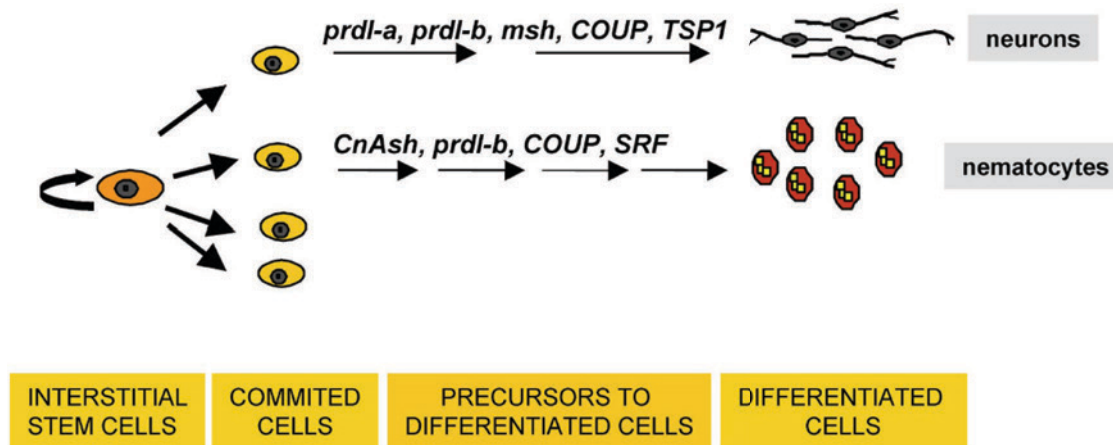
### 4.1 Co-expression of identical regulatory genes in the neuronal and nematocyte pathways suggests a common ancestral origin

Distinct neuropeptide gene families were characterized in hydra Yum et al. (1998), Takahashi et al. (2000), Hansen et al. (2000), Hansen et al. (2002) and their respective expressions were noted in specific subsets of neurons, where, in some cases, coexpression could be observed Hansen et al. (2000), Hansen et al. (2002). In the nematocyte pathway, the regulatory genes *cnASH* Grens et al. (1995), *prdl-b* Gauchat et al. (1998), *hyCOUP-TF* Gauchat et al. (submitted) as well as genes coding for structural proteins like spinalin Koch et al. (1998) or NOWA Engel et al. (2002) displayed very similar expression patterns, with expression recorded in synchronously-dividing nematoblasts but absent in



**Fig 3 :** The *prdl-b* and *hyCOUP-TF* genes are expressed in both the nematocyte and the neuronal lineages. A-D) The paired-like homeobox gene *prdl-b* is strongly expressed in nests of synchronously-dividing nematoblasts along the body column (arrows) but repressed in the budding area at stage 2 (A, arrowhead) and in the presumptive head region of the growing bud (D, arrowhead). During regeneration (B,C), *prdl-b* transcripts cannot be detected in the foot- (B, arrow) and head- (C, arrow) regenerating stumps. E-G) Double-labelling of the *prdl-b* (red) and *prdl-a* (dark purple) expressing cells. Beside large nests of nematoblasts (nb), *prdl-b* expression was observed (arrows) in small single cells (E, arrows), corresponding to a subset of neurons (F, white arrows). Some neurons co-expressed *prdl-b* and *prdl-a* (indicated by arrows in F-H) while in addition *prdl-a* was detected in interstitial cells (arrowheads). F : fluorescent detection ; G : DIC view; H: merged view. I,J) The orphan nuclear receptor *hyCOUP-TF* is expressed in nests of synchronously-dividing nematoblasts (arrows). J) transversal section at the level of the body column. Arrowheads indicate digestive vacuoles. K,L) Magnified area showing two distinct *hyCOUP-TF* expressing cell populations : the nests of nematoblasts (nb, arrowheads) and small single cells (arrows). L) Among the small single cell population, neurons can be identified (arrows). Bars correspond to 1 mm (A, I), 300  $\mu$ m (B-D), 120  $\mu$ m (E), 80  $\mu$ m (K), 20  $\mu$ m (F-H, J, L).





**Fig 4 :** Scheme depicting the neuron and nematocyte lineages in hydra after Bode (1996). The regulatory genes expressed in each lineage have been indicated : *prdl-a* in interstitial cells and apical neurons (Gauchat et al., 1998); *msh* in neurons of the budding zone (this work); *TSP1* in apical ganglia cells and endodermal epithelial cells (this work); *prdl-b* in nematoblasts and neurons of the body column Gauchat et al. (1998), this work; *hyCOUP-TF* in nematoblasts and neurons Gauchat et al. (submitted); *cnASH* in nematoblasts Grens et al. (1995); *SRF* in interstitial cells and nematoblasts Hoffmann and Kroihner (2001). Genes coding for neuropeptides or structural proteins were omitted.

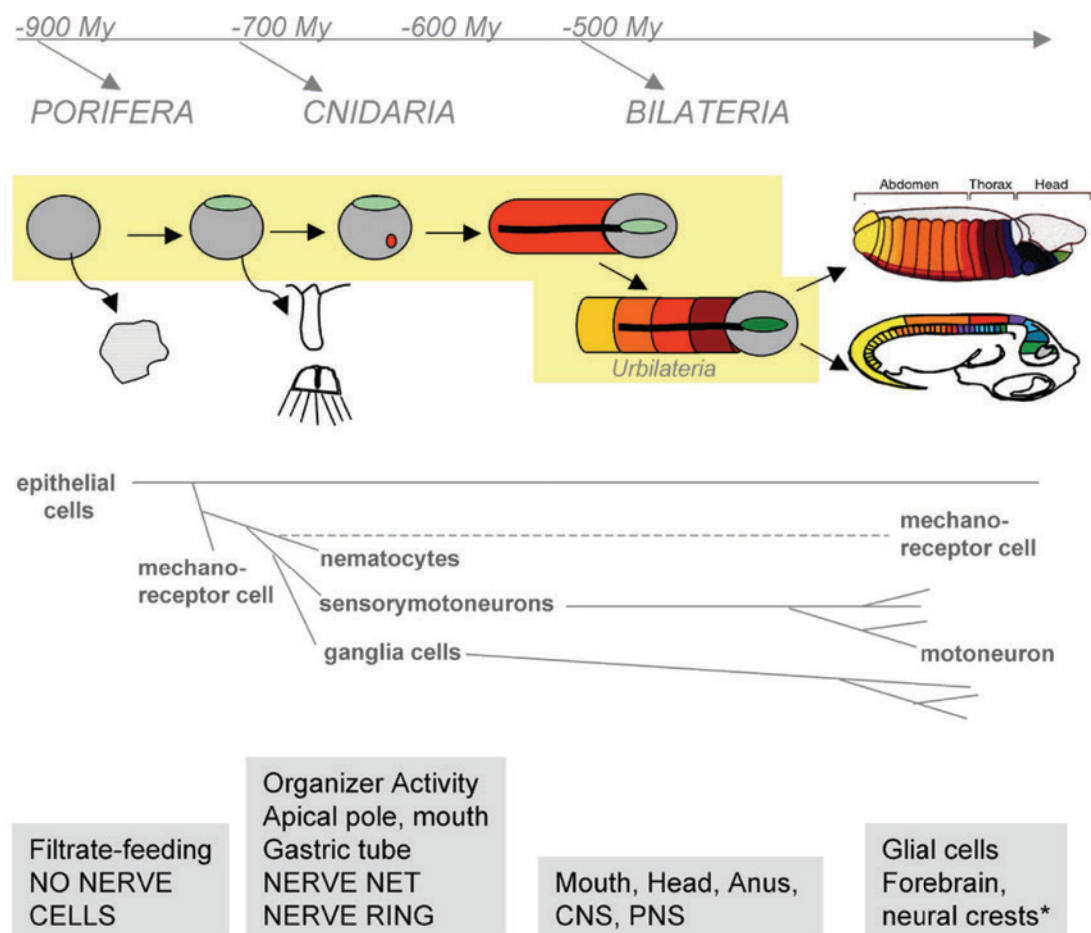
differentiating or migrating nematocytes. Recently, it was clearly demonstrated that the NOWA protein started to be produced in differentiating nematocytes before being incorporated in the outer wall of mature nematocysts, suggesting a strictly spatially-controlled switch from transcriptional to translational control in this lineage Engel et al. (2002). However, co-expression of the same gene in the neuron and nematocyte lineages was never reported so far. *Prdl-b* and *hyCOUP-TF* provide two distinct examples of genes coding for transcription factors that display such co-expression. Given that neurons and nematocytes derive from a common stem cell in hydra, and that the *paired-like* and *COUP-TF* gene families are both involved in neurogenesis in many distinct phyla, one might postulate that these cell types evolved from a common ancestral nerve cell, whose differentiation was under the control of these genes (Figs. 4 and 5).

#### 4.2 Do mechanoreceptor cells represent ancestral nerve cells?

Numerous evolutionary steps were required before reaching the complex organisation displayed by the central nervous systems of bilaterians and origin of nerve cells remains enigmatic. According to the distribution of the different channels that were recorded in eukaryotic cells, mechanosensitive channels were likely among the first ones to appear Kung (1989). The cnidocil apparatus of nematocytes, which react to mechanical as well as chemical stimulus, display structural and functional similarities with

mechanoreceptor cells from insects and mechanoreceptor hair cells of the vertebrate's inner ear Holstein and Hausmann (1988), strongly suggesting that the nematocytes represent a progenitor of higher metazoan mechanoreceptor cells. In this work, we show that the nematocyte and the neuronal cell pathway share regulatory genes that exhibit a high level of conservation along evolution. We propose a scenario in which differentiation of mechanoreceptor cells would have preceded that of neuronal cell types in evolution. Nerve cells are supposed to have arisen in multicellular organisms from epithelial cells, who became able to transmit external information (pressure, light, sounds, chemicals) through chemical and electric signals to their neighboring cells Mackie, (1970), Anderson (1989).

Among cells that fulfill this definition, nematocytes are peculiar as they behave as receptor-effector cells: they can sense chemical and mechanical stimulus, transduce these signals, and react to them through nematocyst discharge in a completely autonomous fashion. This fast and autonomous response could be hallmarks of very primitive nerve cells, nematocytes being a derived cnidarian by-product of these ancestral "neuro-epithelial" cells. At subsequent evolutionary steps, the same stem cell could have differentiated neuronal cells with prolonged processes that started to establish connection (synapses) with their surrounding cells, myoepithelial cells for example, and involve them in the response to the stimulus. At a latter stage, neuronal cells would progressively connect to each other and start organizing in a network, to form as observed in



**Fig 5 :** Scheme depicting a possible view of the origins of neurogenesis, head patterning and axes development from Porifera to Bilateria. Evolutionary time with approximative dates (not on scale) is indicated on the top. Putative ancestors from which modern animals derive are in yellow boxes. In sponges, passive filtration of nutrients occurs in the absence of nerve cells and cell body is not polarised. Appearance of mechanoreceptors and neurons along evolution represent a unique and single event that took place before divergence of Cnidaria but after that of Porifera. In cnidarians, active feeding requires a mouth (single opening) surrounded by clustered nerve cells, and tentacles harboring mature mechanoreceptor cells. This apical pole might correspond to a primitive head (green area). Animal species representing intermediate steps between these two stages are not known. According to the radial head model Shankland and Seaver (2000) some trunk precursors escaped from the radial symmetry (red spot) and developed ancestral trunk and abdomen structures (red tube), including a second opening (anus) at the extremity of the digestive tract (thick black line). At the subsequent stage, the Urbilaterian ancestor De Robertis and Sasai (1996) shares with most bilaterian animals a set of regulatory genes that provide during development positional information along the antero-posterior (e.g. Hox genes) and dorso-ventral axes. For clarity, only two modern embryos were depicted as deriving from the urbilaterian ancestor: the fly embryo on the top and a vertebrate embryo on the bottom. CNS : central nervous system, PNS : peripheral nervous system. \*Neural crests are vertebrate-specific.

cnidarians, a dominating structure localized in the head region at the base of the tentacles, named the nerve ring. This latter structure would allow a coordinated behavior. As a consequence, the nematocyte lineage can be regarded as a sophisticated derived lineage, representative of the ancestral sensory cells that appeared before the Cnidaria diverged.

#### 4.3 Did neurogenesis and patterning activity evolved simultaneously?

Multicellular organisms displaying mechanoreceptor and/or neuronal cell differentiation but no polarity along their body are not known; moreover the reverse situation is also true, animal species

displaying a pole with a specific organized activity but no neurons are not known. One exception might be the carnivorous sponges (Cladorhizidae) that differentiate at one extremity filament epithelial cells with hook-shaped spicules used to catch their prey and digest them in the absence of any aperture Vacelet and Boury-Esnault (1995). However, it is not proved that this localised differentiation event results from organizer activity, i.e. from "a piece of tissue able to create an organisation field" Spemann and Mangold (2001). Therefore, in the absence of evolutionary intermediates, one might consider that neurogenesis and patterning activity co-evolved since they appeared and were selected in the same

evolutionary period of time, before Cnidaria divergence.

Genetic cascades involved in developmental processes are highly conserved among bilaterians Gilbert (2000), but also from cnidarians to bilaterians Galliot (2000), Spring et al. (2002), Holstein et al. (2003). Among genes that show head-specific regulation, *prdl-a* displays a very unique dual regulation: On one hand in the hydra adult polyp, *prdl-a* is strictly expressed in neurons located in the ectodermal layer, mainly in the apical region, on the other hand during early hours of head regeneration, *prdl-a* is transiently expressed in a distinct cell lineage, likely epithelial endodermal cells of the stump, at a time and at a place where raising organizer activity was mapped by transplantation experiments MacWilliams (1983).

Consequently, we identified *prdl-a* as an « early » regeneration gene Gauchat et al. (1998), Galliot and Schmid (2002). Interestingly, in mouse embryos, several regulatory genes including paired-like homeogenes like *Hesx1*, *Otx*, *Gsc* exhibit a similar dual regulations with a first wave in the anterior visceral endoderm and then in the sus-jacent ectoderm which will differentiate the forebrain. Moreover, embryological and genetic manipulations have proved that these genes participate in head organizer activity Thomas and Beddington (1996), Stern (2001). These results suggested firstly that inductive interactions between two cell layers might be an ancestral process leading to organizer activity in developing animals, secondly that genes involved in neurogenesis and organizer activity in first-evolved animals kept tightly-linked regulation from cnidarians to bilaterians Galliot and Miller (2000). The establishment of an active feeding behavior at one pole of the animal would thus represent an ancestral stage to the further development of a more complex head. The fact that regulatory genes expressed in both nematocyte and neuronal lineages are not involved in patterning or morphogenetic processes in hydra, supports the view according to which differentiation of ancestral neuro-epithelial cells preceded the establishment of organizer and patterning activity in animals.

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