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NEUROGENESIS IN CEPHALOPODS: "ECO-EVO-DEVO" APPROACH IN THE CUTTLEFISH *SEPIA OFFICINALIS* (MOLLUSCA-CEPHALOPODA)

Sandra Navet[1], Sébastien Baratte[2, 3], Yann Bassaglia[2, 4], Aude Andouche[2], Auxane Buresi[2], and Laure Bonnaud[1, 2]

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ABSTRACT

Cephalopods are new evolutionary and ecological models. By their phylogenetic position (Lophotrochozoa, Mollusca), they provide a missing master piece in the whole puzzle of neurodevelopment studies. Their derived and specific nervous system but also their convergence with vertebrates offer abundant materials to question the evolution and development of the nervous system of Metazoa (evo-devo studies). In addition, their various adaptions to different modes of life open new fields of investigation of developmental plasticity according to ecological context (eco-evo-devo approach). In this paper, we review the recent works on cephalopod nervous developmental investigations. We show how cephalopods, and especially *Sepia officinalis*, an animal of economical interest, can be used as suitable models to extend our knowledge on cephalopod ecology and on nervous system evolution among molluscs.

I. INTRODUCTION

Evolution of the nervous system is one of the key features of functional adaption of metazoans to their environment: peripheral and central nervous systems associated to sensorial structures constitute the network of perception and integration of internal and environmental factors. These complex interacttions and relationships between the nervous elements result from developmental processes and have been selected during evolution as they confer an adaptive advantage. In the general concern of Evo-Devo investigations, the ecological dimension of the development is essential in the light of new knowledge on genome plasticity [54]. Actually, since development is also influenced by non-genetic parameters, as environmental variations or epigenetic processes, the functional and adaptive context of organisms to their environment has to be integrated into a new field to study evolution of metazoans, called Eco-Evo-Devo.

Nervous system (NS) organisation of numerous metazoans is well known but efforts on development are restricted to chordates and ecdysozoans: the mouse, *Drosophila*, *Caenorhabditis* being the most extensively explored evo-devo models [2]. To elaborate hypotheses on evolution of the structures and functions, additional models belonging to Lophotrochozoa are essential as they display a diversity of anatomical structures and physiological characteristics. Among them, in molluscs, cephalopods beyond their economic interest, constitute new biological models in an evolutionary and comparative perspective. First of all, few lophotrochozoans possess the brain and the camerular eyes as anatomical convergent structures with vertebrates and their highly developed nervous system is used as physiological comparative model for vertebrates (Fig. 1A). Second, cephalopods exhibit specific derived characters (synapomorphies) among molluscs that are worth being better explored: a very muscular mantle, arms and funnel derived from the foot, and cerebralisation of the central nervous system (Fig. 1A) [8]. Their development is also particular: unlike other lophotrochozoans, they are all present with a discoidal clivage and their development is direct. They do not show a veliger larva and no metamorphosis apparently occurs. The embryo develops inside a protective egg surrounded by black envelopes (Fig. 1B, C). Third, beside these unique structures, the cephalopod taxa show a wide range of nervous system variations linked to their different modes of life (pelagic, necto-benthic and/or benthic).

Paper submitted 08/23/11; revised 08/15/13; accepted 08/20/13. Author for correspondence: Laure Bonnaud (e-mail: bonnaud@mnhn.fr).
[1] *Université Paris Diderot-Sorbonne Paris Cité, Paris, France.*
[2] *Muséum National d'Histoire Naturelle, UMR BOREA MNHN CNRS 7208 IRD 207 UPMC, Paris, France.*
[3] *Université Paris Sorbonne-Sorbonne Universités, Paris, France.*
[4] *Université Paris Est Créteil-Université Paris Est, Créteil, France.*

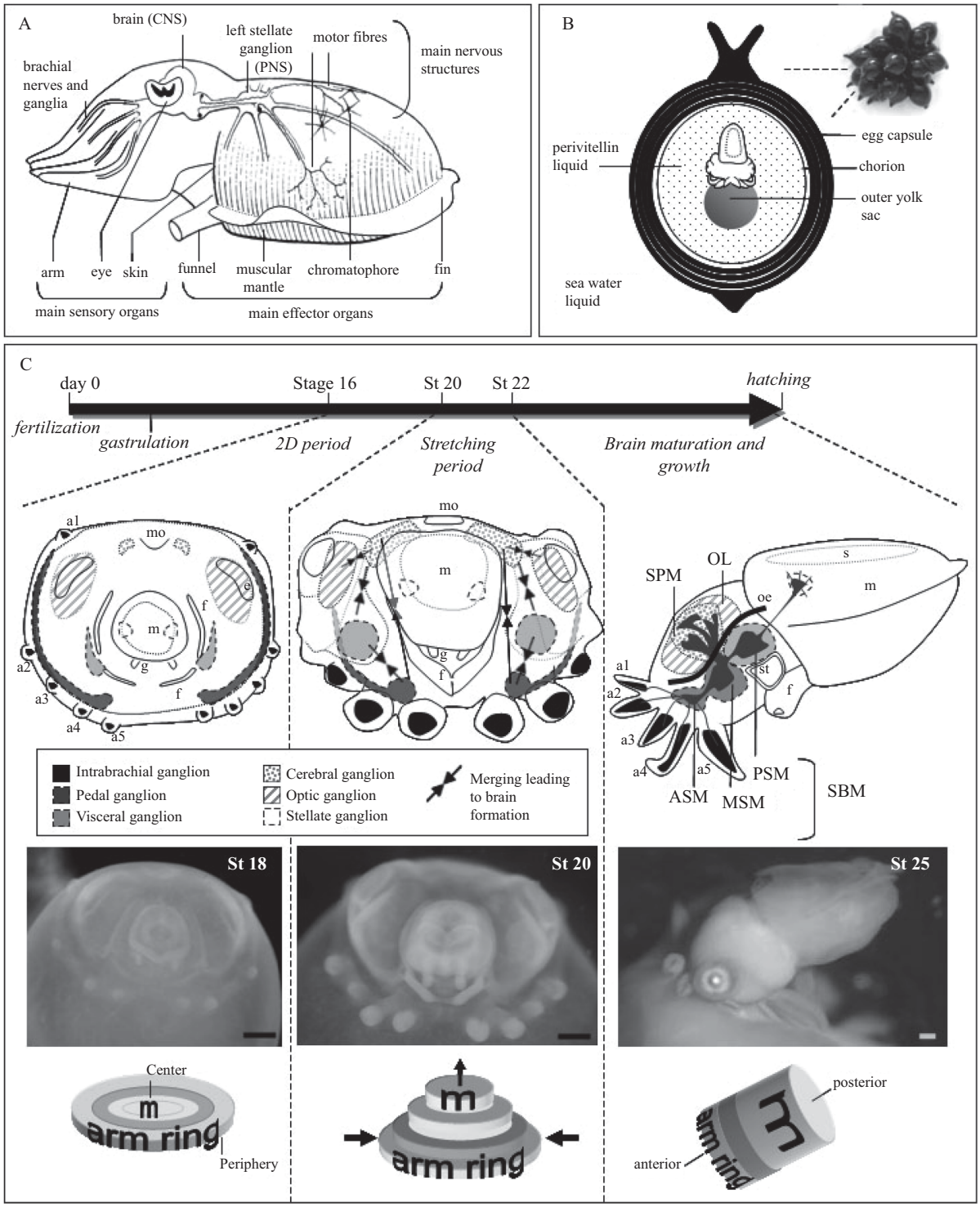

Fig. 1. Main features of *Sepia officinalis* anatomy and development. A- Diagram of a *Sepia officinalis* adult illustrating the main structures and organs involved in the interaction of the organism with its environment. B- Egg elements surrounding the *Sepia* embryo during its direct development. C- Main steps of *Sepia officinalis* development. Top: sketches of *Sepia* embryos during the three main periods of organogenesis with an emphasis on the development of the nervous structures (ganglia and brain lobes). Center: Diagrams of *Sepia* embryos at stages 18, 20 and 25 (stages from Lemaire, [47]). Bottom: illustration of the "Chinese lantern"-like development of *Sepia officinalis*, allowing the transition from a disk-shaped embryo to an adult-shaped embryo. Peripheral structures of the disk-shaped embryo, like arm buds, become anterior organs (from an ecological point of view) and central structures, like the mantle, become posterior organs. a1, a2, a3, a4, a5: arms 1, 2, 3, 4 and 5; ASM: anterior subesophageal mass; CNS: central nervous system; e: eye; f: funnel; g: gill; m: mantle; mo: mouth; MSM: middle subesophageal mass; oe: oesophagus; OL: optic lobe; PNS: peripheral nervous system; PSM: posterior subesophageal mass; s: shell; SBM: subesophageal mass; SPM: supraesophageal mass; st: statocyst. Scale bar: 200 µm.

In this paper, we 1) describe the cephalopod nervous system and link it in an ecological and evolutionary context, 2) explain why *Sepia officinalis* is a suitable model for the NS development exploration (evo-devo), 3) summarize the molecular data available on the NS development and show the specificity of this control by a comparative approach, 4) state the development of the embryo *Sepia officinalis* in regard to its environment (eco-evo-devo).

II. THE CEPHALOPOD NERVOUS SYSTEM AS A CENTRAL CHARACTER TO STUDY THE EVOLUTION OF BILATERALIA

1. Structure and Evolution of the Nervous System

The NS of cephalopod is composed by 1) a peripheral nervous system (PNS) with stellate ganglia, nervous cords of the arms/tentacles and several sparse ganglia and 2) a central nervous system (CNS) with a brain enclosed in a cartilaginous capsule and optic lobes (Fig. 1A). PNS constitutes 2/3 of the nervous cells of the total nervous system. The stellate ganglia, specific of cephalopods, are crucial for the neuromuscular laterality: they are the relay for the giant fibers innervating locomotory muscles and the chromatophores nerves pass through them (Fig. 1A) [95].

The anatomical structures of adult CNS have been described in numerous cephalopods (*Octopus vulgaris* [86]; *Loligo vulgaris* [52, 96-99], *Sepia officinalis* [12], *Idiosepius paradoxus* [81]). The brain comprises the supra- and suboesophageal masses (SPM and SBM, Fig. 1C right) disposed between the eyes around the oesophagus. They have clear internal and external features allowing the division into 25 major lobes, for some of which further subdivisions have been recognized, making nearly 40 lobes altogether [13, 78]. Different functions can now be attributed to each lobe largely as a result of experiments carried out on *Octopus*, *Sepia* and *Loligo* [98].

Cephalopod nervous system has been extensively studied as a comparative model to vertebrates [37, 52, 86, 94, 96, 97]. It represents an important material for neurocytology, electrophysiology and biophysics, the most known being the giant axons of the squid [33, 38, 74]. Learning and memory capabilities are well developed in cephalopods and many works have explored their performance and vertebrate-like behaviour [34, 37, 76, 99, 100]. With regard to anatomical structure comparisons, the convergence status of centralized nervous system between vertebrate and cephalopod is obvious [75]. This convergence allows access to differences or similarities which appeared during evolution in the development of structures with functional equivalence (i.e. analogy).

But how such a "supra-organized" nervous system appeared in cephalopods? The molluscan nervous system shows an extreme diversity and the evolutionary relationships concerning its orientation and polarization are not yet clarified. The cephalopod nervous system is certainly the most sophisticated within Mollusca [14]. Nevertheless, it still shows a

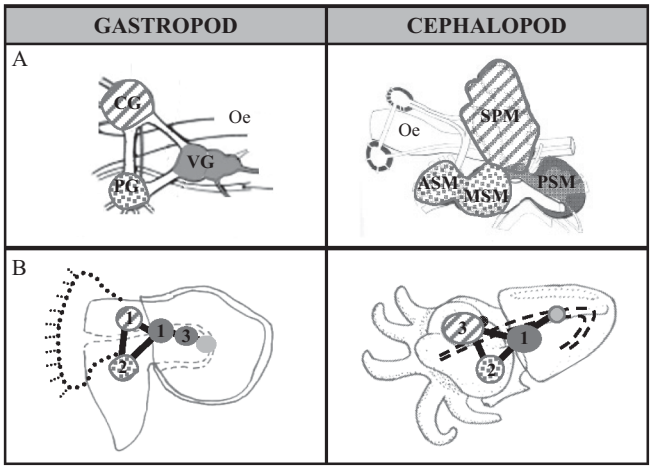

Fig. 2. Comparison of the relative position of the ganglia brain between gastropod and cephalopod brain. A: brain in adult B: ganglia location in gastropod pretorsionnal veliger (left- from [36]) and in *S.officinalis* stage 24 embryo (right- [3, 15]) Numbers indicate the sequence of maturation for each ganglion. ASM: anterior subesophageal mass; CG: cerebral ganglion; MSM: middle subesophageal mass; oe: oesophagus; OL: optic lobe; PG: pedal ganglion. PSM: posterior subesophageal mass; SBM: subesophageal mass; SPM: supraesophageal mass; VG: visceral ganglion.

molluscan basic design (bauplan), as in gastropods (Fig. 2A) with a set of paired ganglia: the cerebral, pedal and visceral-pleural ganglia. The left and right of each pair are linked by a commissure whereas connectives run in anterior/posterior direction between the ganglia. In cephalopods, this basic design has been modified in various ways. The ganglia are grouped and the CNS is cerebralized and arranged around the oesophagus in two main masses (Fig. 1C, Fig. 2A). Their brain is far larger than that of other molluscs approaching that of vertebrates-fish [63].

2. Nervous System Organisation and Mode of Life

The structure of the brain and the proportion of the lobes differ between cephalopod taxa [48]. The greatest centralization (cerebralization) among cephalopods is found in octopods [60]. Some conclusions can be drawn in adult about the significance of the patterns of connectivity between organization of the brain and habitat [19, 85, 98]. The relative size of the brain lobes change after hatching with morphological developments, those in some lobes being quite marked [31, 87]. Several of the changes have been correlated with changes in behaviour, habitat and habits in both *S. officinalis* and *Octopus vulgaris* [59]. In *Sepia* juveniles, memorization abilities increase during post-hatching development [26, 27, 51]. The mode of life differences between hatchling/adult individuals was accompanied in evolution by deep modifications. The maturation of the nervous system pre- and post-hatching can be interpreted as an adaptive response of the juvenile to environment and concerns both the CNS and the neuromuscular complex involved in the locomotion and patterning functions. For instance, the camouflage, one of the

systems to escape predators, results from complex interactions of elements located in the skin. Chromatophores constitute the main component for the establishment of the patterns. They are neuromuscular organs and are under the direct control of the brain; their control is clearly bilateral (review in [53]). The lobes identified as controlling the chromatophores (chromatophore lobes, anterior and posterior) are located in the sub-oesophageal mass. The differences in colored pattern behaviour are associated with different habits [34]. Benthic species, *Octopus* and *Sepia* show a highly complex chromatophore network by comparison with pelagic species (*Loligo*). Interestingly, the setting up and organisation of anterior chromatophore lobe are more complex in *Sepia* and *Octopus* than in *Loligo* [48].

Differences observed in the organisation of the PNS and neuromuscular complex are linked to the mode of life and the performance in fast-jet propulsion, the second system to escape predators. The giant cells and fibers constitute a relay between brain and muscles and insure a simultaneous bilateral transmission inducing the muscular mantle contraction; the first order cells and fibers are located in the brain and connected to the second ones that reach the stellate ganglia and connect the third order cells. The fibers are partially or totally fused (giant axon of the squid) leading to a more or less efficient propulsion. Accordingly, this system has been described essentially in pelagic (*Loligo*) or necto-benthic species (*Sepia*) [93, 98].

III. DEVELOPMENT AND EVOLUTION OF *SEPIA OFFICINALIS*

The development of several cephalopods has been described precisely for the first time by Naef in early 20th century [55]. It was only recently that the development of cephalopods has been considered in an evolutionary perspective [7-10]. Among them, *S. officinalis*, the European cuttlefish, has been considered as a putative model in the evo-devo field [3-5, 29, 35, 46, 56, 57, 89] because of 1) its necto-benthic mode of life in both juveniles and adults; 2) the extensive knowledge on its nervous system and behaviour; 3) the knowledge of its embryogenesis and the ease to observe the development *in-ovo*; 4) the facility to collect eggs and to keep individuals in laboratory-controlled conditions.

1. Embryogenesis in *Sepia officinalis*

In the evo-devo field, the developmental processes are informative on the evolution and adaptive changes. In molluscs, the veliger larva, a synapomorphy, is free and spends time until metamorphosis in direct contact with the environment (Fig. 2B). The metamorphosis modifies considerably the orientation of the organism : in gastropods, a close group of cephalopods, torsion and spiralisation lead to deep modifications of the internal organization. Among specificities, cephalopods show a direct development, the embryo being protected by envelopes. It is supposed that the development of cephalopods is a consequence of the loss of the veliger stage.

Sepia officinalis has a necto-benthic mode of life both near the bottom and in midwater. Females generally mate and spawn in the intertidal zone [11, 73]. Eggs are attached in batches on hard substrata. Embryos are protected by an egg capsule composed of black envelopes (Fig. 1B). The development is 2 months to 3 months long depending on the temperature of the waters. Eggs are telolecithal and present a meroblastic discoidal cleavage unlike other molluscs in which an holoblastic spiral segmentation occurs. The organogenesis started at a stage defined as the 14^{th} (based on the Lemaire system, [47]) leading to a flat embryo (Fig. 1C, left) above the yolk. Progressively, the embryo takes volume and straightens (Fig. 1C, middle), until hatching at stage 30 (Fig. 1C, right). The newly hatched are identical to an adult and adopt the adult necto-benthic mode of life.

2. Neural Network Setting Up in *Sepia officinalis*

Development of nervous system in cephalopods has been studied first in *Loligo vulgaris* [50] and *Octopus vulgaris* [49] based on histological works. Shigeno *et al.* [78, 80] described the brain development of two other coleoids (*Todarodes pacificus* and *Sepioteuthis lessoniana*) and Yamamoto *et al*. [90] established the first atlas of neural structures development in *Idiosepius paradoxus*. These works suggested a global similarity in the development of the brains whereas the timing of the lobe formation and rearrangement is conditioned by the mode of life at hatchling as already mentioned above.

At the beginning of organogenesis (disk-shaped embryo: stages 15 to 20), presumptive areas of the cerebroid ganglia emerge on both sides of the future mouth and then develop toward the eyes. The visceral ganglia start developing as two little territories on both sides of the mantle and the pedal ganglia emerge between arms 4 and 5 (Fig. 1C, left). All these ganglia begin condensing and merging to each other from stage 23 to form the brain in the embryo's head (Fig. 1C, middle). The brain is finally arranged around the oesophagus (Fig. 1C, right, Fig. 2). As for the peripheral nervous system, both stellate ganglia begin to develop on the left and right sides of the mantle at the edges of the presumptive shell sac [5] and intrabrachial ganglia take place all along the arms' crown and develop into the arms (Fig. 1C).

All these studies are based on histological analysis and the comparison of nervous territories between gastropods and cephalopods is not facilitated by the fundamental differences in development (Fig. 2B). How did the nervous system take place during evolution from a simple ventral nervous system with sparse ganglia is an unresolved question that can be approached by the study of developmental genes. Molecular data are necessary 1) to elaborate the phylogenetic hypotheses on the evolution of the NS within the mollusc lineage 2) to determine the origin of the specificities of the cephalopods nervous system, 3) to bring response elements to understand why cephalopods exhibit so many nervous convergences with vertebrates, especially concerning cerebralisation.

IV. MOLECULAR CONTROL OF THE NERVOUS SYSTEM DEVELOPMENT

The development of the nervous system is a very complex process, starting from the induction of the neural stem cells and lasting until the differentiation of neuronal progenitor cells into functional neurons correctly connected. In cephalopods, additional questions are particularly crucial: how do ganglia develop, how do they migrate and merge as lobes and how do brain lobes mature. Several aspects must be considered in the study of the molecular control of the development. 1) The characterization of homologous genes and comparison of their structure are informative about the molecular evolution and functional constraints; 2) the determination of their role in the setting up of homologous or convergent structures brings data on the processes of conservation, diversification and/or recruitment of genes 3) the identification of the gene networks allows comparison of molecular pathways and variations in the regulation processes, potentially responsible for nervous phenotypes diversity.

1. Molecular Data Availability

Molecular data available for evo-devo studies were until recently concentrated on vertebrates and ecdysozoans groups. Around 60% of animal species concerned by at least one achieved/on-going/funded genome sequencing projects belongs to Deuterostomia (5% of the known Metazoa. Regarding molluscs (~10% of Metazoa), only 12 species are studied, and among them, 6 belonging to the gastropod genus *Lottia*. This lack of genomic data does not facilitate the identification of homologous genes without close reference and carry out broad comparative genomics approaches. Moreover there is a clear need to bring new models to the scientific community to fully appreciate the diversity and the evolution of the mechanisms underlying biodiversity.

Nevertheless, since several years, the sequencing of ESTs libraries from species of Lophotrochozoan is increasing, even if in molluscs they are often established from a specific organ or cell type. In cephalopods, ESTs libraries have been elaborated from *Octopus* eye [62], *Euprymna scolopes* light organ [20], *Loligo bleekeri* eye [92], and *Nautilus pompilius* eye [92]. The only ESTs library from organogenesis stages of a cephalopod embryo has been established for *Sepia officinalis* [6]. Thanks to these data, homologous genes, known in other metazoans to play a role in the nervous system development, have been tested in cephalopods. The necessity of cephalopod genome sequencing has been recently underlined [1].

2. Molecular Control of Neurogenesis in Cephalopod

The first molecular work was dedicated to the determination of the expression pattern of *Hox* gene family during the development of *Euprymna scolopes* [17, 46]. Hox genes generally show a collinear pattern of expression, anterior-class genes being expressed in more rostral domains than posterior-class ones. In *E. scolopes* however, there are modifications in this colinearity, linked to the development of morphological innovations and the modification of the body plan orientation (Fig. 1C, bottom). Some Hox genes are expressed in nervous ganglia showing their role in the establishment of the central nervous system [39]: *Lab* and *Hox3* in palliovisceral ganglia, *Antp*, *Lox4* and *Post2* in pedal ganglia [46].

Transcription factors and morphogens have also been explored during the development in several cephalopod species : *Euprymna scolopes* [29, 35], *Sepia officinalis* [4, 56], *Nautilus* [79], and *Loligo opalescens* [82]. We summarize hereafter some of our results obtained in *Sepia officinalis* embryos illustrating the similarities and differences in the molecular control of neurogenesis with other species.

1) Engrailed

Engrailed transcription factor is a key gene in the establishment of segment polarity in almost all metazoans [30], in neurogenesis [66] and in appendage development [72]. Highly conserved in protostomes and deuterostomes, extensive comparisons among taxa suggest that neurogenesis is likely the ancestral function of Engrailed and that subsequent recruitments have increased *engrailed* contributions [32, 66]. In molluscs, however, there is no strong data for the involvement of *engrailed* in neural development. By immunostaining, no Engrailed protein has been evidenced in the developing nervous system of the three cephalopod species: *Sepia officinalis* (Fig. 3A, [4]), *Nautilus pompilius* and *Idiosepius paradoxus* [79].

2) Pax6

Pax6 is a member of the paired-box family of transcription factors; it belongs to the Pax gene family [61]. In vertebrates and *Drosophila*, *Pax6* is expressed in the developing central nervous system and several optic structures [71, 84]. *Pax6* has also been characterized in annelids and molluscs including cephalopods (*Loligo opalescens* [82], *Euprymna scolopes* [35], and *Sepia officinalis* [56]). In this group, *Pax6* is expressed in the eye, in the gills in sensorial structures such as the suckers of the arms but also in the cerebroid ganglia of the future CNS. Unlike vertebrates, *Pax6* is probably involved in the setting up of the whole brain and its expression is not restricted to a dorsal area (Fig. 3B). No expression is observed in peripheral ganglia.

3) Shh

Shh belongs to the *hedgehog* family and is a diffusible morphogen [42]. In vertebrates and *Drosophila*, *Shh* is expressed in several regions of the brain [45, 64]. The data in the gastropod *Patella vulgata* show that *shh* is expressed in the ventral cord of the trochophore larvae and in sensorial cells [58]. On the contrary, in *S. officinalis*, expression pattern is very restricted and no staining is observed within the ganglia areas. *Shh* is expressed in tissues surrounding the whole optic area and around the arm buds (Fig. 3C, [56]). In vertebrates, *shh* has been shown to indirectly inhibit *Pax6*. This seems to be the case also in *Sepia officinalis* as these gene expressions

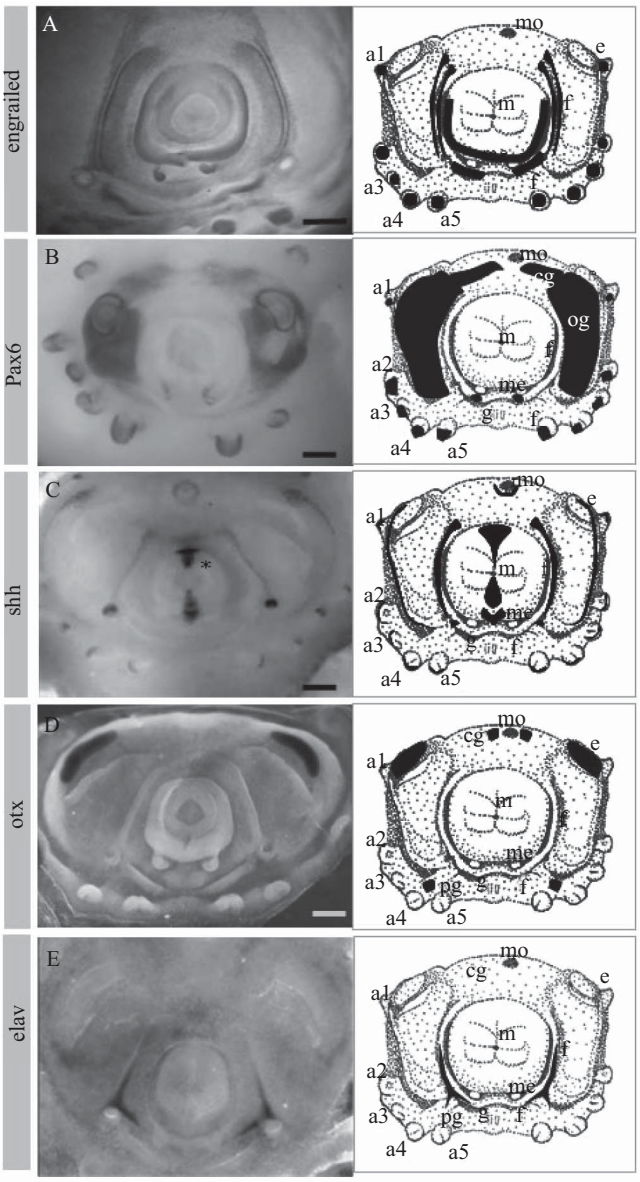

Fig. 3. Expression and locations of some neurogenetic factors during *Sepia officinalis* embryogenesis (pictures on the left, drawings of stage 20 on the right). A- *Engrailed* immunostaining at stage 17 (from [4]). At this stage, *Engrailed* is mainly located in the arm buds (a1 to a5), in the funnel primordia (f) and at the mantle edge (me), which suggests a limited role of *Engrailed* in *Sepia* neurogenesis; B- *Pax6 in situ* hybridization at stage 20 (from [56]). *Pax6* is expressed in the arm buds, the optic area and cerebral area, which suggests a role in the development of the brachial, optic (og) and cerebral (cg) ganglia; C- *Shh in situ* hybridization at stage 20 (from [56]). *Shh* is expressed in thin cell bands along the optic area, along the funnel area and in the arm buds. A median line of *shh* expressing cells (asterisk) suggests a role of *shh* in midline establishment in *Sepia*. D- *Otx in situ* hybridization at stage 17 (from [15]), showing expression in the eyes, in cerebral ganglia and pedal ganglia. E- *Elav in situ* hybridization at stage 17 (from [16]), showing an early expression in palliovisceral ganglia, a1, a2, a3, a4, a5: arms 1, 2, 3, 4 and 5, cg: cerebral ganglion, e: eye, f: funnel, g: gill, m: mantle, me: mantle edge, mo: mouth, og: optic ganglion, pg: pedal ganglion, pv: palliovisceral ganglion.

do not overlap during *Sepia officinalis* development.

4) Otx

Otx/orthodenticle like proteins are transcription factors known to have a very well conserved role in the development of photoreceptive organs and in the differentiation of anterior neural structures [39]. In *Sepia officinalis* embryos, *otx* expression is consistent with these functions, as staining can be found in the eyes from their early formation to hatching and in nervous ganglia destinated to become anterior parts of the adult central nervous system (cerebral, optic and pedal ganglia, Fig. 3D, [15]). Despite the fact that the central nervous system in *Sepia officinalis* arises from the aggregation of sparse ganglia and not from a unique central nervous tissue (as in vertebrates or insects), *otx* seems to have conserved its role for the determination of the anterior CNS.

5) Elav

Elav/hu family members are among the earliest markers for neural cells as they just exit the cell cycle and start to differentiate into neurons. This function has been evidenced in the main metazoan groups [18, 25, 43, 65, 91]. In *S. officinalis*, two *elav/hu* homologs have been characterized, *Sof-elav1* being the most neural-specific paralog. *Sof-elav1* expression is not similar and not coordinated in all the prospective ganglia ([16]. In particular, both palliovisceral ganglia show an early and massive *Sof-elav1* expression (Fig. 3E) whereas cerebral ganglia are the latest ones that express Sof-elav1 and exhibit differentiating neurons [16], This result contrasts with what is found in other gastropods where cerebral and pedal ganglia are the first ones that differentiate neurons, just before the visceral ones (Fig. 2). Such an evolution of the differentiation timing may help to explain the exceptional development of the supra-oesophageal mass (issued from the cerebral ganglia) within the cephalopod brains.

All the above mentioned results illustrate that the appearance of morphological innovations and/or derived characters, is inseparable from potential modifications of the genes involved either by a modification of their role or by their expression pattern. As a consequence, the gene relationships during the organogenesis are different from other metazoans showing the diversity of the regulation pathway and the plasticity of these developmental genes.

V. PHYSIOLOGICAL REGULATION AND MOLECULAR CONTROL DURING EMBRYOGENESIS

1. How to Perceive Environmental Variations?

Because of the importance of environmental signals for biological cycles, the molecular regulation in response to variations during development could confer adaptive advantage. The *Sepia* embryo is protected by envelopes and is bathed in a “buffered” environment (Fig. 1B). Nervous and muscular structures, allowing perception, analysis and reac-

tion to environment, are set up during development and permit an immediate functionality for benthic life at hatching.

By using antibody against TH, a key enzyme in the synthesis of dopamine, it has been shown that dopaminergic sensory neurons differentiate very early in the development and even before any ganglionic migration and brain formation (at around stage 20, [5]). These neurons exhibits dendrites and are probably already functional. An early differentiation of dopaminergic sensory neurons is frequent in veliger larvae and it has been suggested in some molluscs (gastropods and bivalves), that they are involved in the induction of metamorphosis [22, 23, 68, 69, 70], owing to their potential capacity to detect inductive chemical cues in the environment. Despite a direct development and protection by envelopes, the data on *Sepia* embryo strongly suggest the occurrence of an early embryonic sensory nervous system, likely related to the perception of external cues. Interestingly, cerebral ganglia that contribute to the main parts of the brain learning and sensory centres, show a late extensive *Sof-elav1* expression [16]. These delayed expressions in ganglia suggest that most ganglionic cells postpone differentiation after hatching, in relation to the environmental stimuli. In other molluscs, where larval stage is free, the larval nervous system predates the development of the definitive adult nervous system, and cerebral ganglia are among the first to mature (Fig. 2B). Some "non-cephalopod" molluscs (*Helisoma trivolvis*, *Lymnaea stagnalis*) undergo their whole larval stage and metamorphosis inside an egg capsule and have been recently shown to respond to conspecific chemical stimuli by retarding their development [83].

2. Physiological Regulation Processes in a Variable Environment

Given that *Sepia officinalis* lay eggs near the coast, embryos are often exposed at low tide: the early sensory system of *Sepia officinalis* embryos may intervene to regulate development in response to dehydration, oxygenation, or osmoregulation stresses. The protective envelopes limit the damage due to desiccation and can limit osmotic problems due to variation in salinity. As the embryo grows, the eggshell becomes thinner and permeable to seawater, which allows the supply of the various ions and respiratory gas required [9, 88]. The influence of salinity stress on embryonic development and hatching success in cephalopods has been evidenced by numerous authors [21, 24, 28, 67, 77]. A recent study on two species of cephalopods (*Loligo vulgaris* and *Sepia officinalis*) has shown a role of gills and Na^+/K^+-ATPase in the embryo before hatching, showing the importance of osmoregulation process during the development. Differences have nevertheless been evidenced between the two species studied probably in relation to their lifestyle [40]. The molecular mechanisms that are developed by the embryo to control the variations during the development remain to be determined in a comparative and evolutionary perspective.

In addition to the variations of salinity, modifications in temperature during the tide could affect the development. It is known that abnormal development occurs at high temperature [77] and this factor must be taken into account, as well as the ocean acidification, in the general context of global change. In a very nice work, Hu *et al*. [41] have explored the molecular control and the physiological regulation of the *Sepia* embryo after a long-term exposure to moderate environmental hypercapnia. They evidenced in late stage embryos a down-regulation of ion-regulatory and metabolic genes showing the sensitivity of the embryo to elevated seawater pCO_2. This finding has important consequences on the ability of the cuttlefish to adapt to these changes and may affect durably the biological cycle of this species.

VI. CONCLUSION

The *Sepia officinalis* species is a mollusc model allowing both developmental and physiological inferences in an evolutionary (and comparative) perspective and in the general context of the adaptation in aquatic environments. Recent global changes affect marine environment, such as global warming, increase of seawater pCO_2, but also seawater pollution by heavy metal or pharmacological compounds. As environmental cues are essential to the setting up of the correct regulation of the neuro-endocrine factors during development, it can be predicted that global changes will measurably affect (or are already affecting) embryogenesis and also reproduction (especially gonadal maturation). Because of their complex brain and of the convergences they shared with vertebrates, cephalopods stand therefore as essential and suitable models to investigate especially in a ecological perspective.

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