1   **A developmental perspective on the evolution of the nervous system**

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Abstract

The evolution of nervous systems in animals has always fascinated biologists, and thus multiple evolutionary scenarios have been proposed to explain the appearance of neurons and complex neuronal centers. However, the absence of a robust phylogenetic framework for animal interrelationships, the lack of a mechanistic understanding of development, and a recapitulative view of animal ontogeny have traditionally limited these scenarios. Only recently, the integration of advanced molecular and morphological studies in a broad range of animals has allowed tracing the evolution of developmental and neuronal characters on a better-resolved animal phylogeny. This has falsified most traditional scenarios for nervous system evolution, paving the way for the emergence of new testable hypotheses. Here we summarize recent progress in studies of nervous system development in major animal lineages and formulate some of the arising questions. In particular, we focus on how lineage analyses of nervous system development and a comparative study of the expression of neural-related genes has influenced our understanding of the evolution of an elaborated central nervous system in Bilateria. We argue that a phylogeny-guided study of neural development combining thorough descriptive and functional analyses is key to establish more robust scenarios for the origin and evolution of animal nervous systems.

Introduction

The nervous system is the set of cells, tissues and organs involved in perceiving and elaborating adequate responses to internal and external stimuli in animals (Schmidt-Rhaesa, 2007). As such, it is responsible for most animal behaviors, from simple directed muscular movements to abstract reasoning. Therefore, understanding how this organ system evolved and acquired the degree of sophistication displayed in many extant animals has been one of the most alluring questions in Biology. Despite the many advances in neurobiology, physiology, developmental and cell biology on this matter, unraveling the evolutionary history of the nervous system is still contentious (Arendt and Nübler-Jung, 1994, 1999; Arendt et al., 2016; Hejnol and Lowe, 2015; Holland et al., 2013; Holland, 2003; Lowe et al., 2006; Lowe et al., 2003; Northcutt, 2012; Pani et al., 2012; Tosches and Arendt, 2013). This is partially because animal phylogeny, and in particular the deepest nodes of the animal tree of life are debated (Dunn et al., 2008; Hejnol et al., 2009; Moroz et al., 2014; Ryan et al., 2013; Simion et al., 2017; Whelan et al., 2015) thus muddling our capacity to discern the primary origins of the nervous system (Jekely et al., 2015; Liebeskind et al., 2016; Moroz et al., 2014; Ryan, 2014). At the same time, most studies in nervous system development have focused on a handful of animal lineages, which has biased and limited our interpretation of how the nervous system diversified (Hejnol and Lowe, 2015). Herein, we review how the current status in animal phylogeny and the recent investigation of previously neglected animal taxa impact our understanding of the intricate story of the nervous system. We use these insights to evaluate very early ideas about the evolution of complex animal nervous systems and provide an updated developmental perspective. We focus this manuscript on the evolution of the central nervous system (CNS), which we define as the accumulation of neurons and axons usually positioned deep in the body and more or

60 less dissociated from more diffuse nerves and neurons that remain in connection with

61 the body wall (i.e. the peripheral nervous system or PNS) (Bullock and Horridge, 1965;

62 Schmidt-Rhaesa, 2007). Typically, the CNS consists of an anterior condensation, or

63 brain, and one or more longitudinal nerve cords. We conclude this review by putting

64 forward future lines of research that might contribute to elucidate disputed aspects of

65 neural evolution.

66

67 ***Traditional scenarios for bilaterian nervous system evolution***

68   Markedly narrative, traditional evolutionary scenarios aimed at delivering

69 plausible explanations for the origin and diversification of animal morphology (e.g. the

70 diversity of neural arrangements), while at the same time were used to define

71 phylogenetic relationships among animal lineages. This inevitably led to circular

72 reasoning (e.g. an animal lineage was placed in the phylogeny based on their

73 morphology, and at the same time their given phylogenetic position was used to justify

74 their morphology and evolution of a specific character), and endless debates between

75 the advocates (see i.e. Dougherty, 1963). In most of the cases, these scenarios focused

76 on defining linear transformations of adult body plans, with developmental biology only

77 playing a minor role. When embryos were considered, the argumentation was strongly

78 influenced by Haeckel's 'biogenetic law' (Haeckel, 1866), namely that ontogeny (i.e.

79 development) is a fast recapitulation of phylogeny. Embryos were not yet seen adaptive

80 and evolvable, but as mirrors of ancient adult forms that existed once in the past, which

81 were figuratively named after their corresponding developmental stage (e.g. 'Blastaea'

82 and 'Gastraea'). As we illustrate below with Reisinger's 'orthogon'-scenario (Reisinger,

83 1925), Sedgewick's and Balfour's 'oral nerve ring' scenario (Balfour, 1883; Sedgwick,

84 1884) and the 'nemertean' and 'annelid' scenarios by Hubrecht (Hubrecht, 1883, 1887)

85   and Dohrn (Dohrn, 1875) respectively, traditional scenarios proposed alternative

86   evolutionary transformations to explain the emergence of condensed neural cords from

87   a diffuse ectodermal nerve net composed out of basiepidermal interconnected neurons

88   (Hejnol and Rentzsch, 2015).

89

90   *The 'orthogon' scenario*

91   An orthogonal nervous system is defined by multiple pairs of longitudinal cords

92   distributed along the dorsoventral axis of the animal and connected with transverse

93   commissures (Richter et al., 2010). Such nervous system architecture is mainly present

94   in Platyhelminthes, but also in some representatives of other animal lineages (e.g.

95   annelids). The putative position of Platyhelminthes as one of the earliest branches of

96   Bilateria led Reisinger to propose that the condensation of a nerve-net like nervous

97   system, as found in Cnidaria, into an orthogonal CNS, as found in Platyhelminthes,

98   could explain the subsequent evolution of the different neuronatomies of bilaterian

99   animals (Fig. 1A) (Hanström, 1928; Reisinger, 1925; Reisinger, 1971; Steinböck,

100   1966). In this way, the loss of the dorsal longitudinal cords of the ancestral orthogon

101   would have originated the ventral cords found in many protostomian lineages, while the

102   loss of the ventral cords of the orthogon would explain the dorsal location of the nerve

103   cord in chordates (Fig. 1A). However, current molecular phylogenies do not place

104   Platyhelminthes as the sister group of Bilateria (Dunn et al., 2008; Kocot et al., 2016;

105   Laumer et al., 2015; Struck et al., 2014), but well-nested within Spiralia (see "The

106   nervous system in the animal phylogeny" section below; Fig. 2), a major bilaterian

107   clade that comprises animal groups with diverse neuroanatomies. Apart from

108   Platyhelminthes, only a few other lineages, such as some annelids and mollusks

109   (solenogastres, polyplacophorans and monoplacophorans) (Bullock and Horridge, 1965;

5

110   Schmidt-Rhaesa, 2007; Schmidt-Rhaesa et al., 2016), exhibit a more or less evident

111   orthogonal-like CNS (Fig. 2). It is thus unlikely that the platyhelminth orthogonal CNS

112   arrangement represents the ancestral neuroanatomy of Spiralia, because if it does, it was

113   then lost or heavily modified in most other spiralian taxa. Moreover, clear orthogonal

114   CNS are absent or at least unclear in other bilaterian taxa (Bullock and Horridge, 1965;

115   Schmidt-Rhaesa, 2007; Schmidt-Rhaesa et al., 2016), and the ancestral condition for

116   Xenacoelomorpha, the sister taxa to all remaining bilaterians (see "The nervous system

117   in the animal phylogeny" section below) is most likely a diffuse nerve net without any

118   neural condensations (Hejnol and Pang, 2016). Therefore, the presence of an orthogonal

119   arrangement of the neural condensations in the last common bilaterian ancestor is not

120   supported.

121

122   *The 'oral nerve ring' scenario*

123   Based on their observations of onychophoran development, Balfour (1883) and

124   Sedgwick (1884) proposed the idea that the oral nerve ring of anthozoan cnidarians (e.g.

125   sea anemones) directly corresponds to the longitudinal nerve cords of bilaterian animals

126   (Fig. 1B). In onychophorans (i.e. velvet worms), the ventral longitudinal cords develop

127   from the lateral ectoderm of the transient embryonic opening called mouth-anus furrow

128   (Sedgwick, 1884-1885), which the authors assumed to be the blastopore and equivalent

129   to the anthozoan mouth, which is also surrounded by a nerve ring (Schmidt-Rhaesa et

130   al., 2016). By stretching the anthozoan mouth along the cnidarian directive axis, the oral

131   nerve ring would turn into the ventrally located, paired longitudinal cords of many

132   protostomian (Fig. 1B), process recapitulated during onychophoran development

133   (Balfour, 1883; Sedgwick, 1884). This scenario was also extended to explain the

134   evolution of other bilaterian organs systems, such as coeloms, an alimentary canal, and

135   axial relationships (see as an example the 'Enterocoely' scenario and the amphistomy

136   concept; (Arendt et al., 2016; Remane, 1952)). However, recent investigations of

137   onychophoran development show that the opening that Balfour and Sedgwick

138   interpreted as a blastopore is instead an onychophoran-specific transient embryonic

139   structure, likely related to the high yolk content of these embryos (Janssen and Budd,

140   2017; Janssen et al., 2015). Furthermore, neural architectures that are different from a

141   ventral centralized nerve cord (e.g. dorsal cords) cannot be explained when the neural

142   tissue arises only from the blastoporal rim (Fig. 1B) and recent comparative

143   developmental studies demonstrate that the blastoporal behavior does not recapitulate

144   ancestral evolutionary events, but are the result of the specific molecular and cell fate

145   patterning of each embryo (Martín-Durán et al., 2016). From a phylogenetic

146   perspective, a diffuse nerve net is the most probable neuroanatomical character in the

147   last common cnidarian-bilaterian ancestor (Hejnol and Rentzsch, 2015; Schmidt-

148   Rhaesa, 2007), thus rendering the oral condensation of anthozoan cnidarians as a neural

149   specialization unrelated to the longitudinal nerve cords of bilaterians.

150

151   *The 'nemertean' and 'annelid' scenarios*

152   The 'nemertean' scenario (Hubrecht, 1883, 1887; Jensen, 1963) proposes

153   nemerteans as the starting point for the evolution of dorsal and ventral nerve cords in

154   other bilaterian lineages. Because nemerteans have lateral nerve cords (Fig. 2),

155   Hubrecht speculated that their movement to the dorsal side could lead to the dorsal

156   nerve cord of chordates, and the opposite movement would have originated the ventrally

157   centralized longitudinal cords of Protostomia (Fig. 1C). Similarly, Anton Dohrn (Dohrn,

158   1875) proposed a polychaete annelid as the closest relative of the chordates, explaining

159   the evolution of the dorsal nerve cord of chordates by an inversion of the dorsoventral

7

160   axis of the ancestral adult polychaete (Fig. 1D). However, no extant animal directly

161   resembles an ancient form, meaning that the last common ancestor of a clade does not

162   need to be similar to the species at the tips. Today, nemerteans and annelids are well

163   nested within Trochozoa, together with mollusks, brachiopods and phoronids, among

164   other lineages (Kocot et al., 2016; Laumer et al., 2015; Struck et al., 2014). The

165   diversity of neural architectures found in Trochozoa, and Spiralia generally (Fig. 2),

166   suggests that a relatively simple nervous system with ventral paired nerve cords, as

167   observed in meiofaunal taxa like gnatiferans, gastrotrichs and flatworms, is probably

168   ancestral for this group (Hejnol and Lowe, 2015; Struck et al., 2014), thus making very

169   unlikely the evolutionary scenarios proposed by Hubrecht and Dohrn, which implied

170   more elaborated CNS as ancestral conditions.

171

172   ***The nervous system in the animal phylogeny***

173   As described above, most scenarios for the evolution of the nervous system were

174   strongly influenced by pre-cladistic considerations of animal interrelationships.

175   However, our view of the animal tree of life has changed profoundly in the last two

176   decades, since the advent of molecular phylogenies (Dunn et al., 2014). Bilaterian

177   lineages –those with anteroposterior, dorsoventral, and left-right axes– have been

178   rearranged into three major monophyletic groups, Deuterostomia, Ecdysozoa, and

179   Spiralia (Fig. 2), with Deuterostomia (e.g. vertebrates) being the sister taxa to

180   Ecdysozoa (e.g. arthropods) and Spiralia (e.g. annelids) (Aguinaldo et al., 1997; Laumer

181   et al., 2015). Although the internal phylogeny of these three major clades still shows

182   some uncertainties (Dunn et al., 2014; Giribet, 2016), there is robust evidence placing

183   Xenacoelomorpha (e.g. acoel worms), which was previously placed within

184   Platyhelminthes, as the sister group to all remaining bilaterians (Cannon et al., 2016;

185 Ruiz-Trillo et al., 1999). The former Coelenterata (Cnidaria+Ctenophora) has little

186 support (Ryan et al., 2013; Simion et al., 2017; Whelan et al., 2015), and Cnidaria now

187 stands alone as the sister group to Bilateria. The position of Ctenophora is still

188 controversial (Shen et al., 2017; Simion et al., 2017), but most recent analyses place this

189 group as the sister lineage to all remaining animals.

190

191   The new animal phylogeny generates uncertainty around central questions on

192 nervous system evolution that were previously thought settled (Hejnol and Lowe, 2015;

193 Liebeskind et al., 2016; Ryan, 2014). Ctenophores have neurons and a diffuse nerve net,

194 which are absent in sponges and placozoans (Bullock and Horridge, 1965) (Fig. 2).

195 Therefore, the animal nervous system either has a single origin and got independently

196 lost in Porifera and Placozoa or evolved twice in Ctenophora and Cnidaria+Bilateria

197 (Jekely et al., 2015; Liebeskind et al., 2016; Moroz et al., 2014; Ryan, 2014; Ryan et al.,

198 2013; Simion et al., 2017) (Fig. 3). Unfortunately, developmental studies on ctenophore

199 neurogenesis that could shed light on this debate are very limited (Martindale and

200 Henry, 1999; Norekian and Moroz, 2016). Gene expression studies in the ctenophore

201 *Mnemiopsis leidyi* could so far not provide evidence for the homology of the

202 developmental pathways involved in neurogenesis in ctenophores (Pang and

203 Martindale, 2008; Schnitzler et al., 2014; Simmons et al., 2012). However, the absence

204 of core bilaterian neurogenic regulators in ctenophore gene repertoires (Moroz et al.,

205 2014; Ryan et al., 2013) and the failed attempt to identify neural cell types by using

206 typical pan-neural orthologs (Sebé-Pedrós et al., 2018) suggest that if homologous, the

207 gene networks controlling the specification and development of neurons might be

208 significantly different between these animals and cnidarians and bilaterians. Similarly,

209 the internal rearrangement of Bilateria has moved taxa that were once key to explain

210   nervous system diversification (e.g. platyhelminths, annelids, onychophorans,

211   nemerteans; see above) to internal nodes within the Ecdysozoa and Spiralia (Dunn et al.,

212   2014) (Fig. 2). This, together with the vast diversity of CNS anatomies within Bilateria

213   (Schmidt-Rhaesa et al., 2016), has blurred the identification of the ancestral bilaterian

214   neuroanatomy (Fig. 2), and in particular of the sequence of events that led to the

215   emergence of the vertebrate CNS, with an anterior brain and a medially condensed

216   dorsal nerve cord.

217

218   As some traditional ideas have proven wrong, the generally robust new

219   phylogenetic framework has set the foundations for a reinterpretation of animal

220   evolution (Dunn et al., 2014; Giribet, 2016). For instance, the placement of

221   xenacoelomorphs, which ancestrally solely possessed a diffuse basiepidermal nerve net

222   (Achatz et al., 2013; Hejnol and Pang, 2016; Raikova et al., 2016), as the intermediate

223   taxon between cnidarians and the remaining bilaterians decouples the evolution of a

224   CNS from the emergence of Bilateria and suggests that neural condensations have

225   evolved repeatedly within bilaterian animals.

226

227   ***The early development of the CNS in Bilateria***

228   With most traditional views on nervous system evolution falsified, a thorough

229   comparative investigation of neurogenesis under an unbiased phylogenetic framework

230   emerges as the alternative to reconstruct ancestral character states in the evolution of the

231   nervous system. Are there comparable, and perhaps homologous processes in bilaterian

232   neurogenesis that can illuminate the origin of a CNS? In most animal embryos, the

233   primary committed cells that will hierarchically give rise to all differentiated cell types

234   of an organism are spatially organized in relation to the animal-vegetal axis of the

235   oocyte, which is defined by the site of extrusion of the polar bodies (i.e. animal pole)

236   (Goldstein and Freeman, 1997; Martindale and Hejnol, 2009; von Baer, 1834). The

237   earliest events of cell fate specification occur during cleavage, so that precursor cells

238   immigrate and acquire their final embryonic destinations during gastrulation. Therefore,

239   it is possible to identify the prospective embryonic areas, or even cells, that will

240   eventually contribute to the formation of the nervous system at these very early

241   embryonic stages. This can be inferred from the expression of upstream neurogenic

242   genes (see below), but ideally cell fates should be demonstrated by actual cell tracking

243   techniques (Amat and Keller, 2013; Hejnol and Schnabel, 2006; Sulston et al., 1983).

244   Although limited to a handful of animal lineages, fate mapping gives a cellular

245   ontogenetic context to molecular data, thus improving evolutionary comparisons, and

246   can offer a general framework to understand common principles in nervous system

247   development in animals.

248

249   In ctenophores, neuronal and ectodermal cell fates have a common

250   developmental origin (Martindale and Henry, 1999), yet the specification and

251   development of neurons appear to be a rather late process in development (Moroz et al.,

252   2014; Norekian and Moroz, 2016). Differently from ctenophores, the cnidarian nerve

253   net develops from both the ectoderm and the endoderm in a process that starts early in

254   development, before the onset of gastrulation (Nakanishi et al., 2012; Richards and

255   Rentzsch, 2014) (Fig. 4). Intracellular injections of fluorescent dyes in early

256   blastomeres of the acoel *Neochildia fusca* demonstrate that the diffuse basiepidermal

257   nerve net shares a common developmental origin only with the ectoderm (Henry et al.,

258   2000) (Fig. 4). The sensory statocyst, however, appears to derive from, or at least

259   require the presence of, the vegetal macromeres (endomesoderm) for its differentiation

260 (Boyer, 1971). Indeed, the nervous system has a predominant ectodermal origin in all

261 Bilateria.

262

263   In Spiralia, cell lineage studies have greatly concentrated in those animal groups

264 that share the stereotypical quartet-spiral cleavage program (Hejnol, 2010; Henry and

265 Martindale, 1999). This is a broadly conserved early embryonic program, probably

266 ancestral to the whole Spiralia (Hejnol, 2010; Henry, 2014), where embryos get divided

267 in four quadrants, named A to D, each roughly forming the left, anteroventral, right, and

268 posterodorsal region of the animal respectively. The cell division from the 4- to the 8-

269 cell stage is asymmetric, with the four blastomeres at the vegetal pole being larger (i.e.

270 macromeres) than the cells at the animal top (i.e. micromeres). Before gastrulation,

271 macromeres bud off four tiers of micromeres, which are named 1 to 4 (to represent the

272 tier) and a to d (to represent the quadrant they come from) in the classic nomenclature

273 used to describe spiralian cell lineages (Conklin, 1897). Generally, in groups such as

274 polyclad flatworms, mollusks, annelids and nemerteans, the brain and anterior sensory

275 organs (e.g. the larval apical tuft and photoreceptors) originate from the first tier, or

276 quartet, of animal micromeres, which also form the head ectoderm (Henry and

277 Martindale, 1999) (Fig. 4). Other components of the nervous system, such as the nerve

278 cords, originate from derivatives of the second and third quartet micromeres (Henry and

279 Martindale, 1999). For instance, derivatives of the first quartet micromers ($1a^1$-$1d^1$)

280 form the brain in the annelid *Capitella teleta*, while the blastomere 2d gives rise to

281 dorsal parts of the brain and the ventral nerve cord, and the 3a cell forms isolated

282 neurons (Meyer et al., 2010; Meyer and Seaver, 2010). However, the first quartet

283 micromers also contribute to anterior regions of the ventral nerve cord in the annelid *P.*

284 *dumerilii* (Ackermann et al., 2005), and in the mollusk gastropod *Crepidula fornicata*

285 the visceral nerve cords arise from the 2b blastomeres (Hejnol et al., 2007; Lyons et al.,

286 2015). In the nemertean *Cerebratulus lacteus*, the larval nervous system originates from

287 1c, 1d, 2a, 2c, 2d, 3c and 3d (Henry and Martindale, 1998), and in the cyphonautes larva

288 of the bryozoan *Membranipora membranacea*, which has modified the spiral cleavage,

289 the apical organ forms from the 1a–1d blastomeres (Vellutini et al., 2017). Therefore,

290 there is a significant degree of interspecies variation underlying general spiralian

291 developmental plans. This, together with the lack of detailed cell lineage investigations

292 in gnathiferans (rotifers, gnathostomulids, and micrognathozoans) and gastrotrichs

293 makes difficult to confidently infer the exact cellular mode of nervous system

294 development for Spiralia.

295

296   The Ecdysozoa comprises three large monophyletic groups, namely

297 Scalidophora, Nematoida, and Panarthropoda (Dunn et al., 2014). There is virtually no

298 cellular data on the embryonic development of the nervous system in Scalidophora (i.e.

299 priapulid worms, kynorynchs and loriciferans), where only the expression of the

300 nervous system marker *otx* in the priapulid *Priapulus caudatus* indirectly suggests that

301 the circumoral brain originates from ectodermal cells at the introvert-trunk boundary

302 (Martin-Duran et al., 2012). This evident lack of knowledge stands in stark contrast

303 with the high-resolution cell lineage of the nervous system in Nematoda, in particular in

304 the developmental research system *Caenorhabditis elegans*. In this nematode, the

305 nervous system is mostly formed from the ectodermal founder cell AB, with some

306 contribution of the C blastomere, and even the mesodermal precursor MS (Sulston et

307 al., 1983) (Fig. 4). During gastrulation, AB precursors spread from anterior to posterior,

308 and trunk ventral neuroblasts finish the closure of the blastopore as they get internalized

309 by dorsally expanding epithelial cells (Sulston et al., 1983). Unfortunately, less is

310 known for most other nematode lineages, as well as for Nematomorpha, the sister group

311 of nematodes. Within Panarthropoda, cell lineage data in the tardigrade *Thulinia*

312 *stephaniae* demonstrates that the brain and the ventral nerve ganglia develop from

313 separate neural precursors that delaminate from the ectoderm (Hejnol and Schnabel,

314 2005). Direct cell tracking is missing in onychophorans, but morphological and gene

315 expression data indicate that delamination of neural progenitors from the neuroectoderm

316 forms the nervous system (Mayer and Whitington, 2009). A similar, more organized

317 and invariant process is seen in crustaceans and insects (Fig. 4), where isolated

318 neuroblasts that either delaminate (insects) or remain epithelial (crustaceans) divide

319 asymmetrically to form the neurons (Dohle et al., 2004; Whitington, 1996). In addition,

320 some parts of the nervous system (optic lobes, stomatogastric nervous system,

321 neuroendocrine system) involve the ingression of larger groups of neuroectodermal

322 precursors, a process that seems to be more prevalent in arthropod groups like

323 chelicerates and myriapods (Stollewerk and Chipman, 2006). As in Spiralia, the lack of

324 detailed studies in scalidophorans and nematodes with less derived nervous system

325 development hampers inferring the ancestral mode of nervous system development in

326 Ecdysozoa, and thus in Protostomia as a whole.

327

328   In Deuterostomia, direct tracing of the fate of individual blastomeres into

329 neurons has not been performed in hemichordates. However, the basiepidermal nerve

330 net probably derives from the animal mesomeres and macromeres in *Saccoglossus*

331 *kowalevskii*, which also form the ectoderm of the embryo (Colwin and Colwin, 1951)

332 (Fig. 4). In line with their diffuse basiepidermal nerve net, early neurogenic markers

333 like *soxB* and *elav* are ubiquitously expressed in the ectoderm of the gastrula of *S.*

334 *kowalevskii* (Cunningham and Casey, 2014; Lowe et al., 2003). A similar fate map is

335   observed in sea urchins (Cameron et al., 1987) (Fig. 4), but the canonical Wnt pathway

336   and the Nodal and BMP2/4 pathway restricts neurogenesis to the anterior and the ciliary

337   band neuroectoderm (Angerer et al., 2011). However, sea urchin larvae also form a

338   subset of their neurons from pharyngeal endodermal derivatives (Wei et al., 2011).

339   Chordates, on the other hand, exhibit a highly centralized nervous system, which

340   develops from the dorsal neural plate, of ectodermal origin (Lumsden and Krumlauf,

341   1996) (Fig. 4). In cephalochordates and vertebrates there is no invariant blastomere

342   lineage for the neural plate, but a defined set of blastomeres generates the neural plate in

343   ascidians, in accordance with their invariant cell lineage (Nicol and Meinertzhagen,

344   1988; Nishida, 1987). Therefore, the situation observed in hemichordates is reminiscent

345   of that of cnidarians and xenacoelomorphs, and might thus represent the ancestral

346   condition for Deuterostomia (Holland, 2003; Lowe et al., 2015), albeit this

347   interpretation is still debated (Arendt et al., 2016; Holland et al., 2013).

348

349   This brief outline of early bilaterian neurogenesis already highlights how diverse

350   the formation of the nervous system is in animal embryos. The uncertainties about the

351   homology between the ctenophore nervous system and the cnidarian/bilaterian neural

352   tissues (Fig. 3) limit drawing far-reaching conclusions regarding the ancestral metazoan

353   mode of neural development. However, the comparison between Cnidaria and Bilateria

354   strongly suggests that the neurogenic potential became restricted to the ectoderm at the

355   onset of Bilateria, and thus the rare cases of mixed germ-layer origins, like in

356   nematodes (Sulston et al., 1983) and echinoderms (Wei et al., 2011), are probably

357   secondary deployments of neurogenic programs in cellular lineages that give rise to

358   mostly mesodermal (as in nematodes) and endodermal (as in echinoderms) derivatives.

359   How the development of the nervous system became restricted to the ectoderm in

360   Bilateria is still unclear (Martindale and Hejnol, 2009), and will require of detailed

361   mechanistic investigations in key lineages of the animal phylogeny, such as cnidarians,

362   xenacoelomorphs, hemichordates, scalidophorans and gnatiferans. In this regard,

363   neurogenesis is largely unknown in many bilaterian taxa, which as we have already

364   pointed out hampers the reconstruction of homologous developmental processes for the

365   major bilaterian clades. Despite this paucity of knowledge, our current understanding of

366   cnidarian and bilaterian neurogenesis has allowed refuting most traditional scenarios for

367   CNS evolution (see above) and has set the grounds for a more accurate interpretation of

368   an increasing amount of molecular data in a growing number of bilaterian taxa.

369

370   ***The molecular patterning of the bilaterian nervous system***

371   The variation in the embryonic fate maps and cellular aspects of nervous system

372   development summarized above ultimately relies on modifications of the underlying

373   neurogenic processes. In this respect, the comparison of expression patterns of

374   orthologous genes involved in the specification and regionalization of bilaterian neural

375   tissues has transformed the study of CNS development and evolution (Arendt et al.,

376   2016; Hejnol and Lowe, 2015; Holland, 2015; Holland et al., 2013; Puelles and Ferran,

377   2012; Strausfeld and Hirth, 2013; Tosches and Arendt, 2013). Although in many cases

378   there is a lack of functional investigations of the genes used for comparisons, gene

379   expression data has been widely used to propose evolutionary scenarios and working

380   hypotheses (Arendt et al., 2016; Hejnol and Lowe, 2015; Holland et al., 2013; Lowe et

381   al., 2006; Lowe et al., 2003). For instance, the similar molecular profiles of the

382   arthropod mushroom bodies with anterior neural condensations in vertebrates, the

383   annelid *Platynereis dumerilii*, and other invertebrate taxa has grounded the hypothesis

384   that complex brain centers were already present in the last common bilaterian ancestor

385   (Tomer et al., 2010; Wolff and Strausfeld, 2015). As sequencing techniques and

386   molecular methods improved, this comparative molecular approach incorporated a

387   growing number of bilaterian lineages to CNS evolutionary discussions, thus

388   encompassing a significant proportion of the diversity of neural anatomies and

389   developmental modes found in Bilateria. As a result, many of the evolutionary

390   interpretations originally based on data from a few bilaterian lineages have been either

391   solidified or questioned (Hejnol and Lowe, 2015). In the following two sections, we

392   review how increased taxon sampling has affected the use of anteroposterior and

393   dorsoventral neuronal patterning to explain the evolution of the brain and nerve cords

394   respectively.

395

396   **1) Anteroposterior patterning**

397   As many other morphological features, the nervous system of most bilaterian

398   animals is regionalized along the anteroposterior axis (Bullock and Horridge, 1965).

399   This regionalization occurs at both the morphological and the functional level and gets

400   reflected on how upstream neural regulatory genes and downstream terminal

401   differentiation genes are deployed along the anteroposterior axis of the neural tissue. A

402   large number of transcription factors (e.g. *six3/6*, *foxQ2*, *otx*, *otp*, *fezf*, *pax6*, *pax2/5/8*,

403   *en*, *irx* genes, *Hox* genes) and signaling pathways (e.g. the Wnt/bcatening pathway,

404   Hedgehog pathway, FGF pathway, EGFR pathway) are differentially expressed along

405   the anteroposterior axis of the developing nervous system (Arendt et al., 2008; Holland

406   et al., 2013; Vermeren and Keynes, 2001). Importantly, the expression of these genes

407   often relates to anteroposterior neuroanatomical subdivisions, and as such, gene

408   expression data has been widely used to homologize nervous system compartments

409   across bilaterian lineages (Albuixech-Crespo et al., 2017; Arendt et al., 2008; Arendt

410 and Nübler-Jung, 1999; Arendt et al., 2016; Hirth et al., 2003; Hunnekuhl and Akam,

411 2014; Lowe et al., 2003; Marlow et al., 2014; Pani et al., 2012; Range, 2014; Sinigaglia

412 et al., 2013; Steinmetz et al., 2011; Tessmar-Raible et al., 2007; Tomer et al., 2010;

413 Tosches and Arendt, 2013).

414

415   One of the most relevant, and still debated examples of this comparative

416 approach affects the anteroposterior patterning of the vertebrate CNS and the evolution

417 of a complex brain. Early in vertebrate embryogenesis, the vertebrate neural plate

418 becomes molecularly patterned into the basic units that will form the CNS, namely the

419 forebrain, midbrain, hindbrain, and spinal cord (Puelles, 2013; Vermeren and Keynes,

420 2001). As development proceeds, secondary organizers such as zona limitans

421 intrathalamica (ZLI) and the isthmic organizer (IsO) subdivide and specialized these

422 primary neural regions. These areas, subregions, and organizers exhibit a combination

423 of developmental genes robustly conserved among vertebrate lineages (Fig. 5),

424 supporting the homology of the vertebrate CNS neuroanatomy (Puelles and Ferran,

425 2012). Consequently, the analysis of these molecular signatures in non-vertebrate

426 chordates (i.e. tunicates and amphioxus) and hemichordates, the closest chordate

427 outgroup, have been used to trace the evolutionary origins of the vertebrate CNS

428 (Albuixech-Crespo et al., 2017; Pani et al., 2012). The analysis of an extensive gene

429 dataset in the 7-somite mid neurula embryo of the European species *Branchiostoma*

430 *lanceolatum* has shown that the amphioxus neural plate is already anteroposteriorly

431 patterned, and that these primary areas have a direct topological correspondence with

432 vertebrate CNS compartments (Albuixech-Crespo et al., 2017). However, amphioxus

433 lacks the secondary organizers ZLI and IsO (Shimeld and Holland, 2005), whose

434 emergence in the stem lineage of vertebrates could explain how a primary amphioxus-

435   like neural plate pattern evolved into the more elaborated vertebrate CNS. Strikingly,

436   expression and functional data of ZLI and IsO markers in the hemichordate

437   *Saccoglossus kowalevskii*, together with the conservation of genomic *Cis*-regulatory

438   regions between hemichordates and vertebrates, has given compelling evidence that

439   these signaling centers predated chordates and the evolution of their complex brain

440   (Pani et al., 2012; Yao et al., 2016). In *S. kowalevskii*, however, the ZLI and IsO are

441   involved in the anteroposterior ectodermal pattern and the subdivision of the primary

442   body axis in proboscis, collar, and trunk (Pani et al., 2012). These findings thus propose

443   that the vertebrate CNS is a much recent innovation, evolved from the cooption of

444   preexisting ectodermal patterning gene regulatory modules that apparently got lost in

445   pre-vertebrate chordate lineages (Lowe et al., 2015; Pani et al., 2012) (Fig. 5).

446

447   Long-distance evolutionary comparisons of gene expression data have also been

448   used to homologize animal brains generally (Arendt et al., 2008; Arendt et al., 2016;

449   Hirth et al., 2003; Holland, 2015; Holland et al., 2013; Strausfeld and Hirth, 2013;

450   Tosches and Arendt, 2013). Indeed, the anteroposterior neural domains of transcription

451   factors such as *otx*, *pax2/5/8*, and *Hox* genes have been used to homologize the tripartite

452   organization of the arthropod brain (protocerebrum, deuterocerebrum and

453   tritocerebrum) with the forebrain, midbrain, and hindbrain of vertebrates (Hirth et al.,

454   2003). Similarly, comparable antibody immunoreactivity and gene expression data

455   suggested the shared ancestry of complex sensory-associated brain centers, as those

456   observed in arthropods, vertebrates, annelids, platyhelminths, and nemerteans (Tomer et

457   al., 2010; Wolff and Strausfeld, 2015). Generally, these hypotheses suffer from low, and

458   sometimes biased, taxonomic sampling, and thus it still remains to be seen how the

459   inclusion of bilaterian lineages that lack elaborated brain centers affects them. For

460   instance, immunoreactivity against pCaMKII, a protein enriched in arthropod

461   mushroom bodies, is broadly detected in the larval nervous system of priapulid worms,

462   whose nervous system comprises a simple circumoral nerve ring and a single caudal

463   bipolar neuron as ventral nerve cord (Martin-Duran et al., 2016). A similar case is seen

464   in the free-swimming larvae of the brachiopods *Terebratalia transversa* and

465   *Novocrania anomala* (Fig. 5), which have only poorly developed anterior

466   condensations, associated to an apical tuft in *T. transversa* (Santagata, 2011). In these

467   larvae, genes associated to brain signaling centers like the ZLI and IsO and neural

468   regional patterning like Hox genes are also expressed staggered along the

469   anteroposterior axis (Martín-Durán et al., 2016; Santagata et al., 2012; Schiemann et al.,

470   2017; Vellutini and Hejnol, 2016), but demarcating ectodermal domains rather than

471   different areas of the CNS. Furthermore, the expression of anteroposterior patterning

472   genes such as *six3/6*, *otx*, *foxQ2*, and *gsc* in bilaterian and cnidarian larvae suggest that

473   brain-related genes are also generally involved in (neuro)ectodermal patterning (Hejnol

474   and Martindale, 2008; Hiebert and Maslakova, 2015; Marlow et al., 2014; Martín-Durán

475   et al., 2015; Nederbragt et al., 2002; Sinigaglia et al., 2013; Steinmetz et al., 2011;

476   Wollesen et al., 2015). Therefore, scenarios that favor a complex brain as an ancestral

477   feature of Bilateria (Arendt et al., 2008; Arendt et al., 2016; Hirth et al., 2003; Holland,

478   2015; Holland et al., 2013; Strausfeld and Hirth, 2013; Tosches and Arendt, 2013), and

479   thus extensive simplification in most bilaterian lineages, are confronted with data

480   arguing for a convergent, stepwise evolution of bilaterian complex CNS architectures

481   (Pani et al., 2012).

482

483   **2) Dorsoventral patterning**

484   Bilaterian lineages such as arthropods and chordates share in addition the

485   presence of a medially unpaired condensed nerve cord (Bullock and Horridge, 1965;

486   Schmidt-Rhaesa, 2007) (Fig. 2). Whereas the nerve cord is located ventrally in

487   arthropods, it is dorsally positioned in chordates, which led the French naturalist

488   Geoffroy Saint-Hilaire to propose the homology between the arthropod ventral side and

489   the chordate dorsal side already on 1822 (Geoffroy Saint-Hilaire, 1822). Almost two

490   centuries later, the finding that similar genes and signaling pathways (e.g. BMP

491   pathway) were expressed in the embryonic territories giving rise to the nerve cords in

492   the fruitfly *D. melanogaster* and vertebrates revived those classic ideas, and in

493   particular those involving a dorsoventral inversion of the CNS in the stem lineage of

494   Chordata (Arendt and Nübler-Jung, 1994; De Robertis and Sasai, 1996). In this context,

495   orthologous members of the *NK2.1*, *NK2.2*, *NK6*, *pax6*, *pax3/7*, and *msx* gene families

496   are deployed in a similar fashion along the dorsoventral axis of the nerve cord in

497   insects, vertebrates and the polychaete annelid *P. dumerilii* (Denes et al., 2007) (Fig. 6).

498   In insects and vertebrates, the expression of these genes is required for the proper

499   patterning of the nerve cord (Cornell and Ohlen, 2000), and their expression is

500   associated with the location of distinct neuronal populations in these three bilaterian

501   lineages (Arendt et al., 2008). Although the upstream regulators and downstream

502   effectors of dorsoventral patterning genes differ between *Drosophila* and vertebrates

503   (Cornell and Ohlen, 2000; Winterbottom et al., 2010), the striking similarities observed

504   in the dorsoventral patterning of the nerve cord between insects, vertebrates, and the

505   annelid *P. dumerilii* solidified the idea that a CNS with a medially condensed ventral

506   nerve cord is ancestral for Bilateria (Arendt et al., 2008; Arendt et al., 2016; Denes et

507   al., 2007; Holland et al., 2013; Tosches and Arendt, 2013).

508

509   Investigations in hemichordates and nematodes have however challenged this

510   scenario (Kaul-Strehlow et al., 2017; Lowe et al., 2006; Lowe et al., 2003; Okkema et

511   al., 1997) (Fig. 6). The neural anatomy of these two lineages differs considerably from

512   that of vertebrates, insects, and annelids. Hemichordates have a diffuse nerve net

513   throughout the body, with one dorsal and one ventral nerve cord running along their

514   trunks (Bullock and Horridge, 1965). Nematodes, on the other hand, have a main

515   ventral and dorsal nerve cord, and additional pairs of lateral neurite bundles (Bullock

516   and Horridge, 1965). In the hemichordates *S. kowalevskii* and *Balanoglossus*

517   *misakiensis*, the genes *NK2.1*, *NK2.2*, *pax6* and *msx* do not exhibit a staggered

518   dorsoventral arrangement, but their expression is either confined to endoderm (e.g.

519   *NK2.2*) or to particular ectodermal areas along the anteroposterior axis (e.g. *NK2.1*,

520   *pax6*, and *msx*) (Kaul-Strehlow et al., 2017; Lowe et al., 2006; Lowe et al., 2003).

521   Similarly, only *pax6* and *msx* are expressed in connection to the nervous system in the

522   nematode *C. elegans*, (Chisholm and Horvitz, 1995; Du and Chalfie, 2001). Therefore,

523   it is unclear whether the different expression of dorsoventral patterning genes in

524   hemichordates and nematodes are derived situations, perhaps related to their different

525   neuroanatomies and life styles (Arendt, 2018; Denes et al., 2007), or suggest that the

526   similarities between lineages with a single medial nerve cord evolved convergently

527   (Lowe et al., 2006; Martin-Duran et al., 2018).

528

529   A recent study on the expression of dorsoventral nerve cord patterning genes in

530   xenacoelomorph worms and representatives of four major spiralian lineages has shed

531   new light into this debate (Martin-Duran et al., 2018). Character state reconstructions

532   suggest that a diffuse nerve net and one pair of ventral nerve chords are the most likely

533   ancestral neuroanatomies of Xenacoelomorpha and Spiralia respectively (Hejnol and

534 Lowe, 2015; Hejnol and Pang, 2016). However, trunk neuroarchitecture vary widely

535 within these two bilaterian lineages, with acoelomorph species showing independently

536 evolved neural condensations and spiralian lineages like annelids exhibiting medially

537 condensed unpaired nerve cords (Bullock and Horridge, 1965; Hejnol and Lowe, 2015;

538 Schmidt-Rhaesa, 2007) (Fig. 2). In line with this morphological diversity, the

539 expression of dorsoventral nerve cord patterning genes varies significantly among

540 xenacoelomorpha and spiralian lineages (Buresi et al., 2016; Forsthoefel et al., 2012;

541 Franke et al., 2015; Janssen, 2017; Mannini et al., 2008; Martin-Duran et al., 2018;

542 Martín-Durán et al., 2016; Vellutini et al., 2017), even between closely related species

543 like the annelids *Owenia fusiformis* and *Platynereis dumerilii* that share the presence of

544 a medially condensed unpaired nerve cord (Denes et al., 2007; Martin-Duran et al.,

545 2018) (Fig. 6). Remarkably, a similar case is found in Chordata, where non-vertebrate

546 chordates, such as amphioxus and tunicates, differ from vertebrates in the arrangement

547 of dorsoventral nerve cord patterning genes, yet all of them share the presence of a

548 dorsal neural tube (Holland et al., 1998; Ristoratore et al., 1999; Stolfi and Levine,

549 2011) (Fig. 6). It appears thus clear that dorsoventral nerve cord patterning and trunk

550 neuroanatomy has evolved independently in several animal lineages, and that the

551 diversity of expression arrangements of dorsoventral patterning genes is more the norm

552 than the exception in Bilateria, supporting the evolutionary scenario that poses the

553 similarities in dorsoventral patterning between vertebrates, *Drosophila* and some

554 annelids as a case of convergence (Lowe et al., 2006; Martin-Duran et al., 2018).

555 However, a more thorough investigation of the relationship between the dorsoventral

556 patterning genes and nerve cord architecture is needed, in particular regarding the actual

557 function of these transcription factors in the development of the nerve cords in most of

558 the bilaterian lineages studied to date.

559

Conclusions

561   The advances in reconstructing animal relationships combined with a

562   mechanistic understanding of nervous system development in a handful of animal taxa

563   has proven essential to falsify traditional scenarios for nervous system evolution. This

564   progress has also demonstrated that the evolutionary history of the nervous system is

565   more tortuous than previously thought, with the probable convergent evolution of

566   neurons, condensed brain centers, and longitudinal nerve cords. However, freeing our

567   interpretation of nervous system evolution from preconceived recapitulative scenarios

568   has also allowed to state clear questions and research lines that will eventually help to

569   clarify evolutionary events at specific nodes of the animal phylogeny. How similar are

570   the neurons of ctenophores to those of cnidarians and bilaterians? How did the

571   neurogenic potential become mostly restricted to the ectoderm in bilaterians? Is this

572   event related to the cooption of ancestral ectodermal patterning mechanisms to the

573   development of centralized neural structures? How do conserved molecular patternings

574   generate divergent neural architectures? Are there similar developmental principles

575   between the multiple cases of nervous system condensation that can inform us of

576   common evolutionary trajectories in the diversification of animal morphology? These

577   and many other questions will ultimately require embracing animal diversity and strive

578   for acquiring a functional understanding of the developmental implications of the

579   observed molecular neural patternings. On the long run, they will pay us off with a more

580   insightful view on one of the greatest questions in science.

581

587

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Figures

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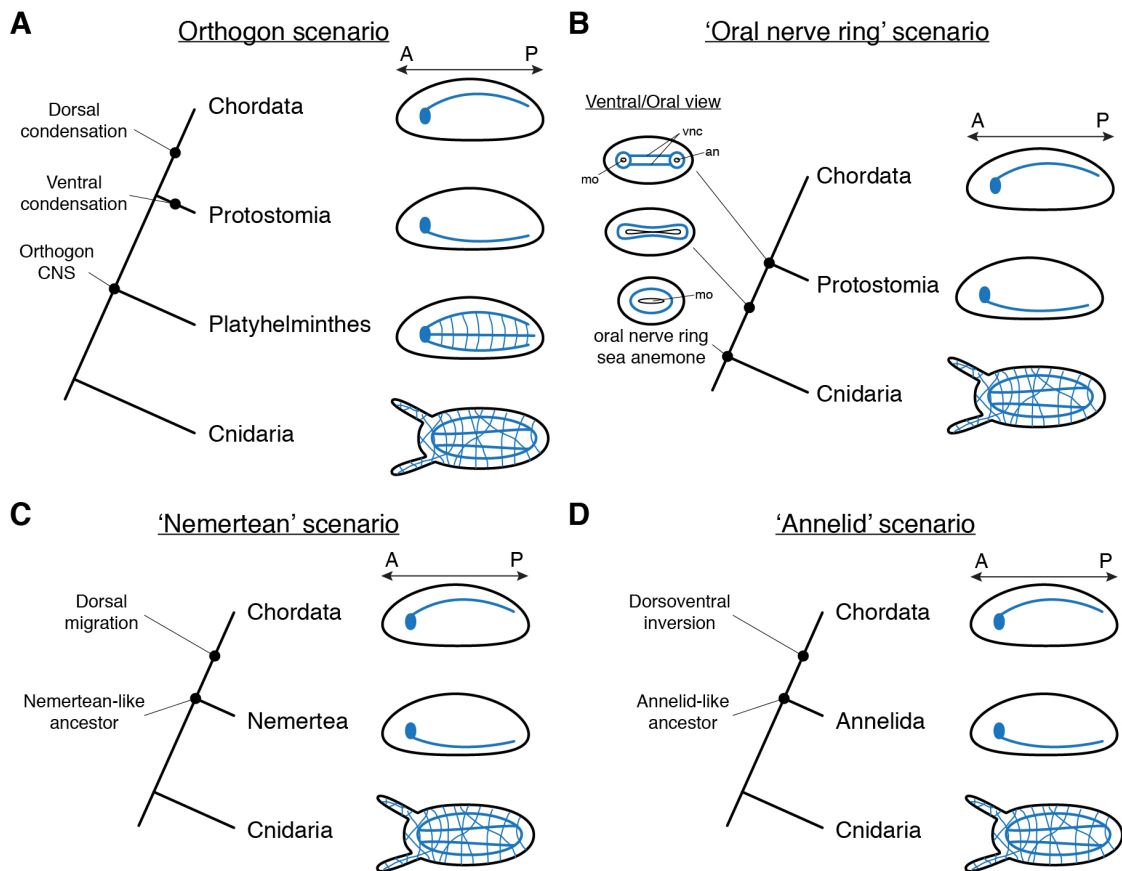
Figure 1. Traditional scenarios for the evolution of a centralized nervous system.
(**A**) In the orthogon scenario, the ancestral bilaterian was a flatworm-like animal with an
orthogonal arrangement of the nervous system. Dorsal and ventral nerve cords evolved
by the subsequent loss of ventral and dorsal nerves of the orthogon, respectively. (**B**)
The 'oral nerve ring' scenario explains the evolution of ventral paired nerve cords by
the elongation and fusion in the middle of the oral nerve ring condensation of extant sea
anemones. (**C**) In the nemertean scenario, the evolution of the chordate CNS occurs
from a nemertean-like ancestor, by movement to the dorsal side of the ventrolateral
nerve cords. (**D**) In the annelid scenario, the chordate CNS evolves from an annelid-like
ancestor that flips over its dorsoventral axis. Drawings are not to scale, and the CNS is
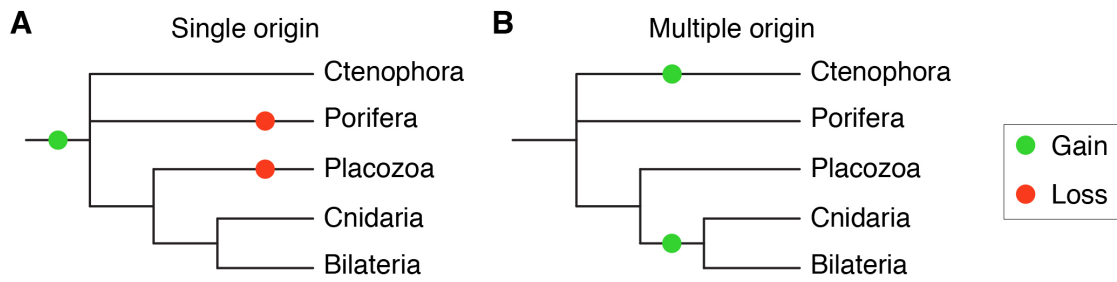in blue. See main text for references. An, anus; mo, mouth; vnc, ventral nerve cords.

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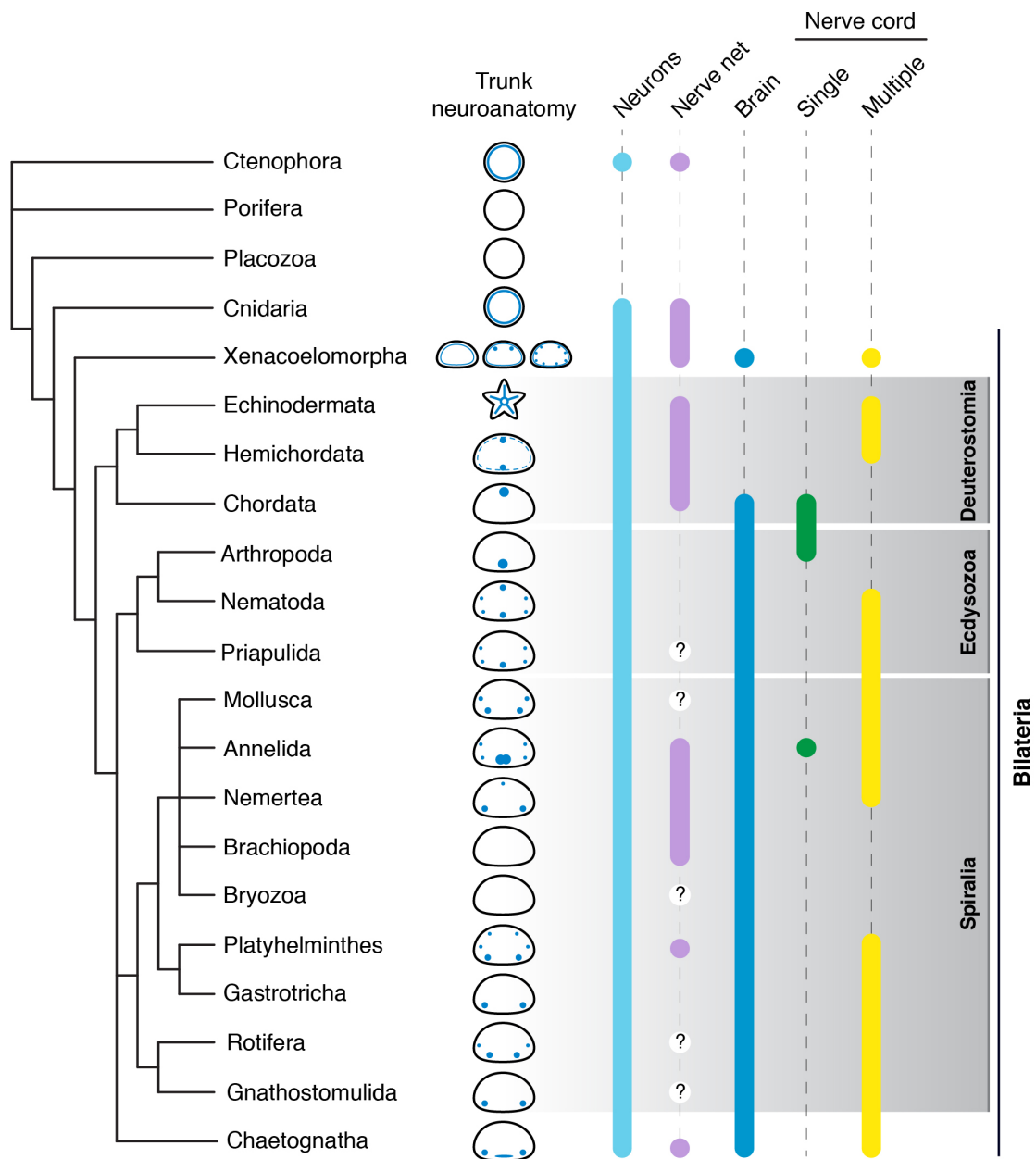
Figure 2. The diversity of neural anatomies in Metazoa. Distribution of neuroanatomical characters in representative metazoan lineages under the current phylogenetic relationships supported by molecular data (Dunn et al., 2014). Neuroanatomy is based on (Schmidt-Rhaesa, 2007).

42

**Figure 3. The evolution of the nervous system**. Possible evolutionary scenarios for the evolution of neurons and a nervous system in Metazoa given the current position of Ctenophora. (**A**) If ctenophore, cnidarian and bilaterian nervous systems are homologous, neurons got independently lost in sponges and placozoans. (**B**) Alternatively, the nervous systems of ctenophores and cnidarians+bilaterians evolved convergently.

Figure 4. The diversity of neuroectodermal fate maps in Bilateria. Schematic fate maps of representatives of the major bilaterian lineages and cnidarians, with special emphasis on the origin of the nervous system and endoderm. In the cnidarian *N. vectensis*, the neurons that form the diffuse nerve net (red lines) develop from both the ectoderm and endoderm (purple). In Bilateria, the vast majority of neurons develop

1002　　separate from the endoderm. The mode of specification and spatial position of neuronal

1003　　progenitors is variable in Bilateria, even among lineages that share a common

1004　　developmental program, such as spiral cleaving embryos (Trochozoa). Drawings are not

1005　　to scale. Ma, macromeres; me, mesomeres.

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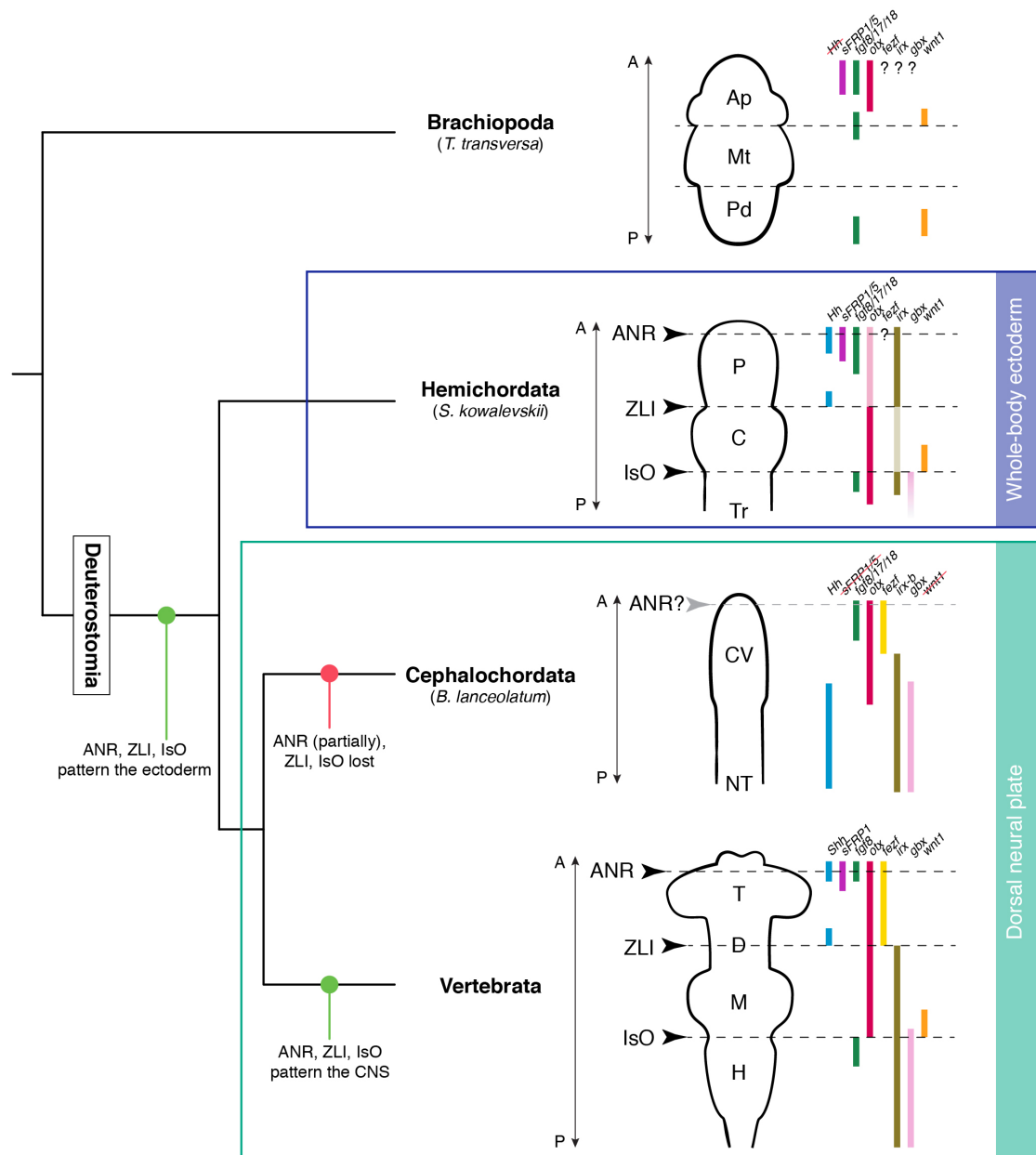
Figure 5. The evolution of anteroposterior neuronal patterning in Deuterostomia.

Schematic representation of the anteroposterior expression domains of genes involved in patterning the vertebrate CNS and defining the major brain signaling centers in brachiopods (outgroup), hemichordates, cephalochordates, and vertebrates. The similarities in expression patterns and functional interrelationships of these genes between hemichordates and vertebrates suggest that the anterior neural ridge (ANR), the zona limitans intrathalamica (ZLI) and the isthmic organizer (IsO) are conserved deuterostomian signaling centers involved in general ectodermal patterning. These

1016 organizers were partially lost in cephalochordates and urochordates, and coopoted into

1017 neuroectodermal/brain patterning in vertebrates. Drawings are not to scale. Question

1018 marks indicate unknown expression and red crossed text indicates that gene expression

1019 is not related to the ectoderm and/or the nervous system. See main text for references.

1020 Ap, apical lobe; C, collar; CV, cerebral vesicle; D, diencephalon; H, hindbrain; M,

1021 midbrain; Mt, mantle lobe; NT, neural tube; P, proboscis; Pd, pedicle lobe; T,

1022 telencephalon; Tr, trunk.

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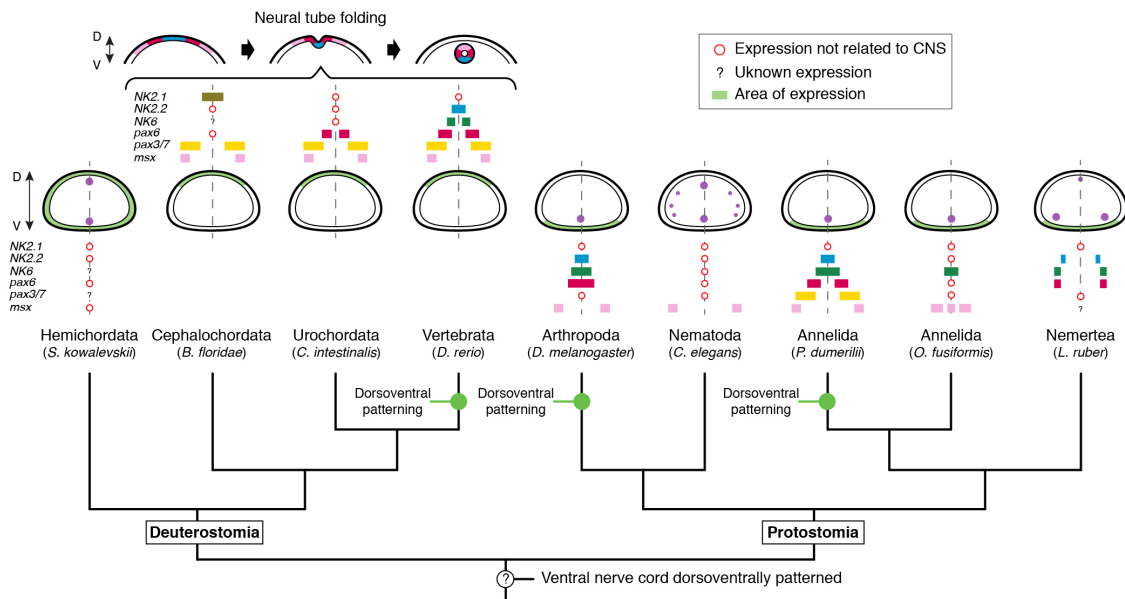
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Figure 6. The evolution of dorsoventral nerve cord patterning in Bilateria. The

genes *NK2.1*, *NK2.2*, *NK6*, *pax6*, *pax3/7*, and *msx* exhibit a similar combinatorial

expression along the dorsoventral axis of vertebrates, arthropods, and the annelid *P.*

dumerilii. This combinatorial expression is associated with the molecular patterning of

the medially condensed nerve cord of these three bilaterian lineages and has been

argued to support the presence of a single ventral nerve cord in the last common

ancestor of Protostomia and Deuterostomia. However, a similar dorsoventral expression

of these genes is absent in hemichordates, nematodes, and many spiralian taxa, such as

the nemertean *L. ruber* and the annelid *O. fusiformis*, which similar to *P. dumerilii*, also

shows a medially condensed unpaired nerve cord. Similarly, cephalochordates and

urochordates, which do have a single dorsal nerve cord, do not exhibit the dorsoventral

nerve cord patterning of vertebrates. These data favors more parsimonious scenarios

that propose that the similarities in molecular patterning between vertebrates,

Drosophila and the annelid *P. dumerilii* evolved by convergence.

1039