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HYPOTHESIS

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Origin of the chordate central nervous system – and the origin of chordates

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Abstract Contrary to traditional views, molecular evidence indicates that the protostomian ventral nerve cord plus apical brain is homologous with the vertebrates' dorsal spinal cord plus brain. The origin of the protostomian central nervous system from a larval apical organ plus longitudinal areas along the fused blastopore lips has been documented in many species. The origin of the chordate central nervous system is more enigmatic. About a century ago, Garstang proposed that the ciliary band of a dipleurula-type larva resembling an echinoderm larva should have moved dorsally and fused to form the neural tube of the ancestral chordate. This idea is in contrast to a number of morphological observations, and it is here proposed that the neural tube evolved through lateral fusion of a ventral, postoral loop of the ciliary band in a dipleurula larva; the stomodaeum should move from the ventral side via the anterior end to the dorsal side, which faces the substratum in cephalochordates and vertebrates. This is in accordance with the embryological observations and with the molecular data on the dorsoventral orientation. The molecular observations further indicate that the anterior part of the insect brain is homologous with the anterior parts of the vertebrate brain. This leads to the hypothesis that the two organs evolved from the same area in the latest common bilaterian ancestor, just anterior to the blastopore, with the protostome brain developing from the anterior rim of the blastopore (i.e. in front of the protostome mouth) and the chordate brain from an area in front of the blastopore, but behind the mouth (i.e. behind the deuterostome mouth).

Key words Chordate · Embryology · Nervous system · Phylogeny

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Introduction

It has generally been assumed that the dorsal sides of all bilateral animals are directly comparable, i.e. homologous, and that the dorsal chordate central nervous system (CNS) is therefore not homologous with the apical-ventral protostome CNS. However, new observations on a number of orthologous dorsoventral patterning genes indicate that the dorsal nervous system of vertebrates is indeed comparable to the ventral nerve cord of arthropods (Nübler-Jung and Arendt 1994; Arendt and Nübler-Jung 1994).

The idea of comparing arthropod and vertebrate nervous systems goes back to the beginning of the 19th century when Geoffroy-Saint-Hilaire (1822) compared the dorsal nervous system of a vertebrate and the ventral nerve cord of a crayfish, but he also compared the vertebral column with the cuticle of insects, and in the pre-evolutionary period such similarities were not seen as results of common descent. Nevertheless, the idea has been taken up from time to time, but has not met much acceptance (review in Nübler-Jung and Arendt 1994). More recently, observations on the morphology and embryology of pterobranchs, enteropneusts and chordates led Malakhov (1977) to the conclusion that the chordates are "upside-down enteropneusts", but his paper has apparently been completely overlooked by western biologists. Nübler-Jung and Arendt (1994, 1996) and Arendt and Nübler-Jung (1994, 1997) concluded that a combination of morphological, embryological and molecular data from annelids, arthropods and chordates indicate that chordates are inverted protostomes. Finally, Nübler-Jung and Arendt (1996) reached the conclusion that the inversion could have happened between enteropneusts and chordates. Bergström (1997) also found support for the last point of view. This interpretation of the chordates results in some nomenclatural problems with the dorsoventral orientation of the chordates, and in the following I will use the terms chordate-dorsal (c-dorsal) for the neural side and chordate-ventral (c-ventral) for the abneural side of the chordates to avoid confusion.

The “inversed” orientation of the chordates is in disagreement with the traditional interpretation of a number of morphological observations. The purpose of this paper is to propose a scenario for early chordate evolution which accommodates the morphological and molecular evidence, to highlight possible discrepancies between morphological and molecular results, and to point to investigations which may throw new light on the remaining questions.

Morphological evidence

The ontogeny of many protostomes, such as annelids and molluscs, indicates that their brain develops from the larval apical organ or from adjacent lateral cell groups (Lacalli 1984; Cragg 1996). In groups such as arthropods and nematodes, which lack the ciliated apical organ, the apical component is difficult to identify, and it is obvious that perioral and postoral parts of the CNS have moved forward and constitute the major part of the brain (Snodgrass 1938). The paired or fused ventral nerve cords, which are connected with the brain through circumoesophageal connectives, develop ontogenetically from a pair of midventral, longitudinal ectodermal zones. The ontogeny of some annelids, onychophorans and nematodes demonstrates that the blastopore becomes divided to form mouth and anus by fusion of the lateral blastopore lips and that the differentiating ventral nerve cords are located along the fused lips (Nielsen 1995).

The ontogeny of non-chordate deuterostomes, such as phoronids, echinoderms and enteropneusts, unequivocally shows that the larval apical organ degenerates at metamorphosis and that new, less well-defined central nervous centres take over (Nielsen 1995). The embryology of echinoderms (Hörstadius 1973) and enteropneusts (van der Horst 1927–39) shows that the blastopore directly becomes the anus and a new mouth breaks through from the bottom of the archenteron.

In the chordates, gastrulation is highly oblique, with extensive infolding of presumptive endodermal and mesodermal cells at the dorsal blastopore lip, along the c-dorsal side of the embryo (Keller 1975). There is no lateral blastopore closure as proposed by Arendt and Nübler-Jung (1997); the slit-like closure is the neurulation. Bergström (1997) compared the chordate neurulation resulting in the formation of the neuropore with the lateral blastopore closure of the protostomes resulting in the formation of the adult mouth; however, the careful cell-lineage studies of both ascidians and anurans (Nishida 1987; Eagleson and Harris 1989; Drysdale and Elinson 1991) show that the mouth develops from an invagination at the frontal side of the neural fold and thus anterior to the neuropore. The opening of the ciliated duct of the urochordate neural gland (sometimes called hypophysis) is formed as a secondary opening between the pharynx and the neural tube, and therefore is not homologous with the neuropore (Kowalevsky 1871; Ruppert 1990). The vertebrate adenohypophysis (Rathke’s pouch) appears to be homologous with Hatschek’s pit-and-groove in amphioxus; this structure is derived from the left anterior coelom and not associated with the neuropore (Ruppert 1997b).

The planktotrophic larvae of echinoderms and enteropneusts have ciliary bands of almost identical shape and structure, with separate cilia on monociliary cells, and function as “upstream-collecting” bands in filter-feeding (Strathmann and Bonar 1976; Nielsen 1987). Ultrastructural studies of starfish larvae have demonstrated a characteristic organization, including neurociliary innervation, of the ciliary band (Lacalli et al. 1990; Lacalli and West 1993; Lacalli 1996c), and some of these cells and their arrangement resemble cells in the neural tube of young amphioxus larvae (Bone 1959). This lead Lacalli and West (1993; see also Lacalli 1996a,b) to the conclusion that the neural tube could have evolved through fusion of the aboral sides of a larval ciliary band as that of the dipleurula larva [the presumed larval type of the common deuterostome ancestor (Nielsen 1995); this is in agreement with Garstang’s theory described below].

The origin of the chordate central nervous system and its possible morphological homology with that of the protostomes have not been discussed in the light of the new interpretation.

Garstang’s dipleurula theory

The origin of the chordate central nervous system has been a matter for discussion for more than a century (see e.g. Gee 1996). The most accepted hypothesis is that of Garstang (1894, 1928), which derives the chordate CNS from the ciliary band of a dipleurula (auricularia) larva, where the band moves dorsally, finally fusing middorsally and enclosing the entire aboral surface of the larva including the anterior apical pole with the apical organ as well as the posterior blastopore. This hypothesis is supported by the well-known formation of the neurenteric canal through fusion of the posterior part of the neural folds around the blastopore and by detailed ultrastructural similarities between the ciliary bands of larval echinoderms and enteropneusts and the neural tube of amphioxus (see above). Garstang’s interpretation places the chordate CNS on the dorsal side of the larva, but this is not in accord with the molecular data and new interpretation outlined above. Nielsen (1995) pointed out that some observations on chordate ontogeny contradict Garstang’s idea:

1. Cell lineage studies have shown that the apical pole of the eggs of urochordates (Nishida 1987), amphioxus (Tung et al. 1962) and vertebrates (Vogt 1929) does not become incorporated into the neural tube but moves to the c-ventral side of the embryo behind the mouth (Nielsen 1995: Fig. 51.1).
2. Garstang’s theory implies that the relative position of apical pole and mouth is inversed between enteropneusts and chordates.

3. The gill slits of enteropneusts develop behind the oral area of the tornaria, whereas according to Garstang's theory they should develop in the oral area of the ancestral dipleurula larva; this seems to imply either that the gill slits are not homologous in the two groups or that the gill slits have moved through the ciliary band in chordates; both possibilities appear improbable.

Both Lacalli (1996a) and Nielsen (1997) have attempted to reconstruct the ancestral deuterostome as an "upside-down protostome", but it now seems clear that the inversion has taken place in the ancestral chordate instead.

A new hypothesis for the origin of the chordate central nervous system

The enteropneusts are usually regarded as the sister group of the chordates based on morphological data (e.g. Maisey 1986; Schram 1991, 1997; Nielsen 1995; Nielsen et al. 1996), and some molecular results support this interpretation (Holland et al. 1991; Garey et al. 1996) although other authors disagree, without pointing unequivocally to one alternative phylogeny (Wada and Satoh 1994; Halanych 1996; Castresana et al. 1998). However, both Winnepenninckx and Backeljau (1996) and Naylor and Brown (1998) have demonstrated that refined methods of analysing base sequences give phylogenetic trees which can be quite different from those produced by ordinary analyses, and which in some cases are more in accordance with traditional, morphology based results.

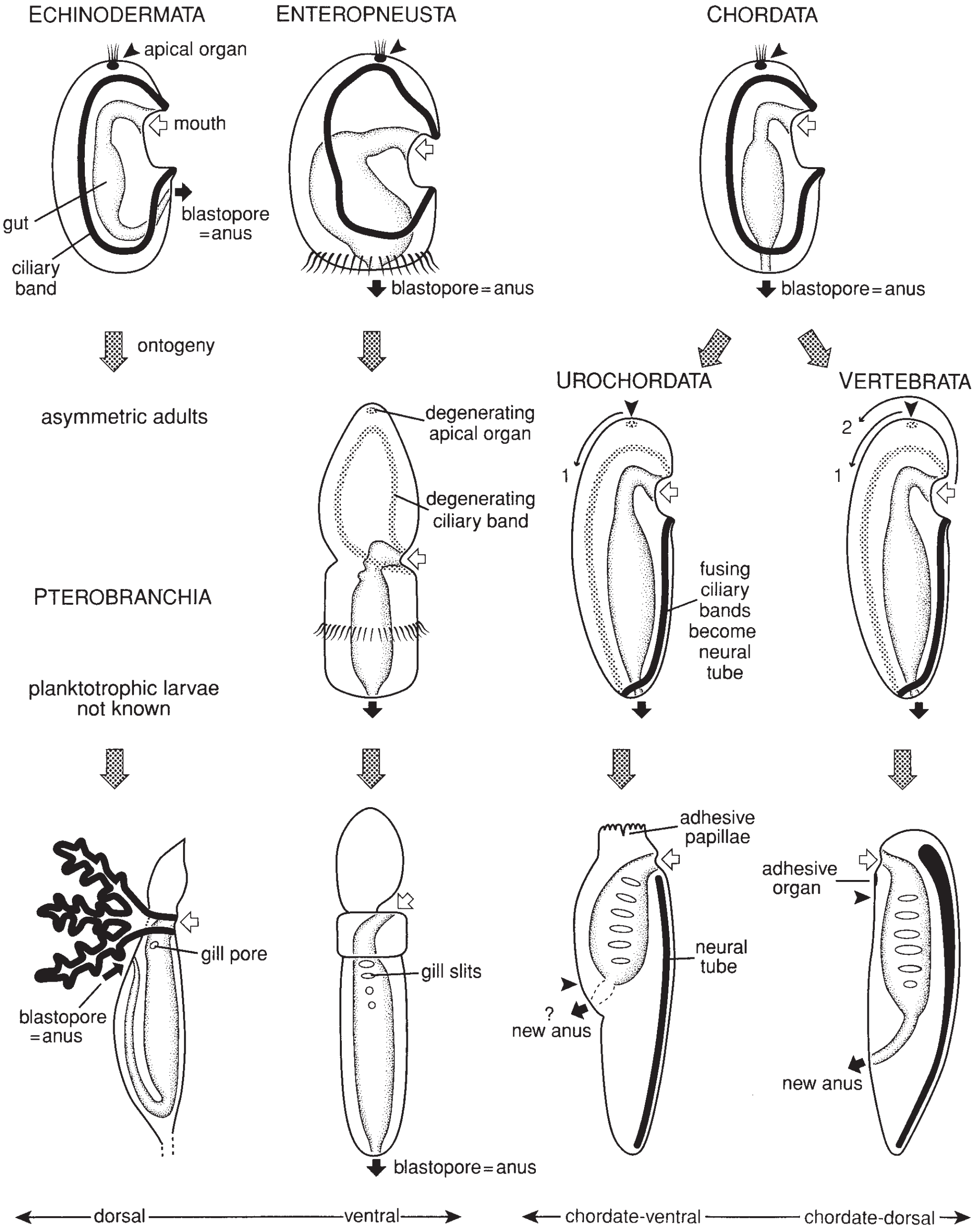
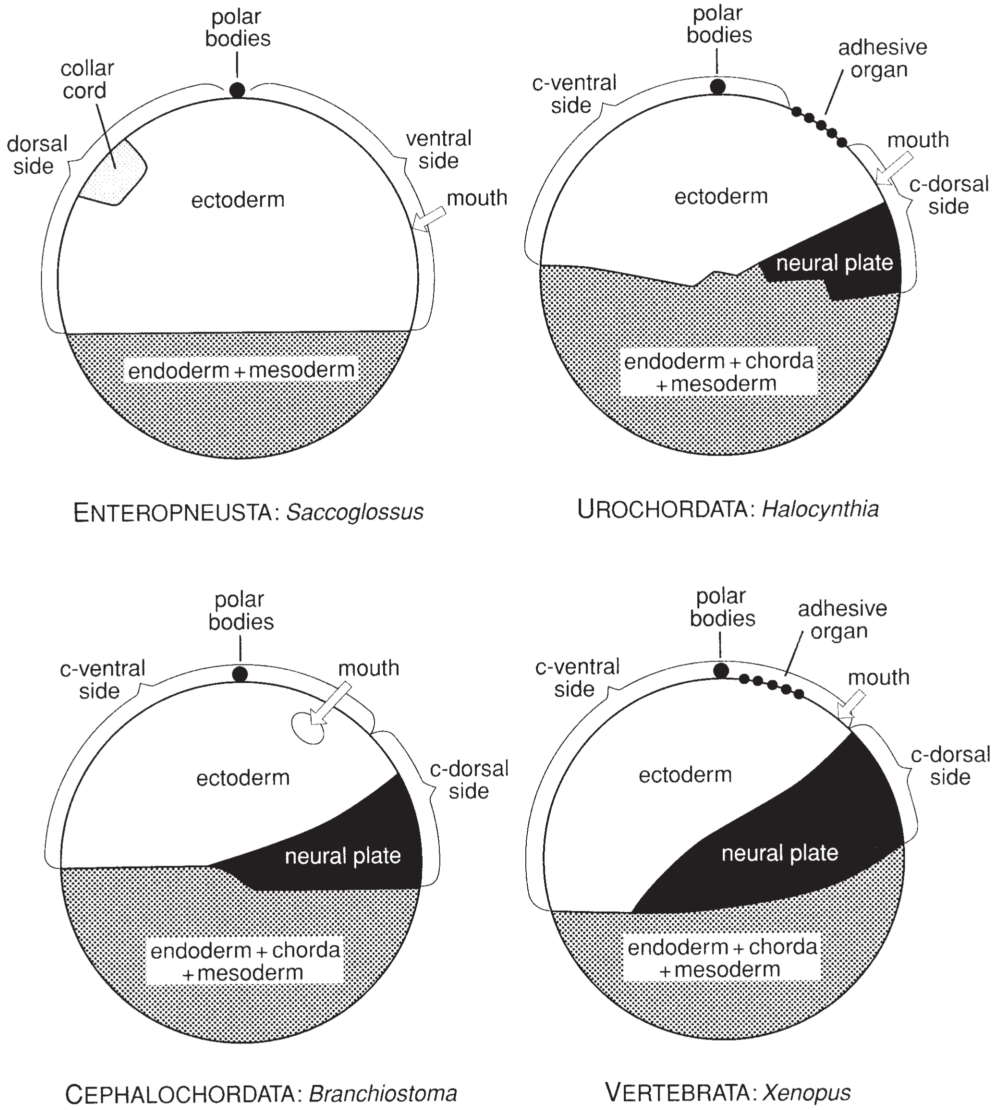


Fig. 1 Schematic representations of larvae and adults of deuterostome phyla. The drawings of the chordates express the hypothesis proposed in this paper. The urochordates are represented by a tadpole larva. *Arrows 1 and 2* denote movements of the apical pole and the mouth, respectively. The apical pole is indicated by *arrowheads*, the mouth by *white arrows*, and the anus by *black arrows*. The intraepithelial nervous system of the adult enteropneust (see Fig. 5) has been omitted. The position of the new anus in the ancestral urochordate larva is uncertain, because it is unknown when the atrium evolved



ENTEROPNEUSTA: *Saccoglossus*

UROCHORDATA: *Halocynthia*

CEPHALOCHORDATA: *Branchiostoma*

VERTEBRATA: *Xenopus*

Fig. 2 Fate maps of enteropneusts and chordates. The dorsoventral orientation of the chordate eggs are modified from Nielsen (1995) and Keller (1975) (*Xenopus*) in accordance with the hypothesis proposed in this paper

Both enteropneusts and the closely related echinoderms have dipleurula larvae and it therefore appears probable that the ancestral chordate had a similar larval stage (Nielsen 1995). If the chordate CNS should develop from the ventral side of a dipleurula-like larva it would have to be along the mid-ventral line between mouth and blastopore. The observations of Lacalli and West (1993) and Lacalli (1996b; see above) indicate that the chordate CNS has evolved from fused ciliary bands, and I propose that it evolved through fusion of the longitudinal parts of the postoral neotroch loop, posteriorly extending just behind the blastopore, while the remaining part of the neotroch degenerated (Fig. 1). This would result in a neural tube with an anterior neuropore behind the mouth and a posterior neurenteric canal.

The proposed formation of the CNS in the hypothetical chordate ancestor is in accordance with parts of Garstang's hypothesis and with Lacalli's observations in that the CNS and the neurenteric canal are formed by the fusion of two aboral sides of the ciliary band of a dipleurula larva, but it differs in that only the postoral loop of the band is involved and that the neural tube accordingly is formed on the ventral (= c-dorsal) side of the larva. It implies that the apical pole of the embryo remains outside the infolding of the nervous system and that the relative positions of chordate brain, mouth and apical pole remain unchanged during ontogeny. Also, the gill slits are not required to cross the larval ciliary band during evolution. It is further implied that the dorsal tongue bars of enteropneusts and the c-dorsal tongue bars of the chordates are not homologous, and several differences in their structures have been pointed out previously that support this theory (Ruppert 1997a). Pterobranchs and enteropneusts have an anterior extension of the gut called the stomochord which is often interpreted as a homologue of the notochord although this is questioned by other authors (see discussion in Ruppert 1997a). The illustration in Nübler-Jung and Arendt (1996) indicates the presence in the enteropneust of a pygochord, a ventral longitudinal extension of the intestine (van der Horst 1929–36); it is found only in the Ptychoderidae and its function is unknown, but Willey (1899) proposed that it could be a notochord homologue. These homologies are indeed quite uncertain, and it is interesting that both a dorsal and a ventral structure in enteropneusts have been proposed as homologues of the notochord.

The development, including cell lineage, of enteropneusts, ascidians, amphioxus and vertebrates is well known through a number of studies, and comparisons between the hypothesis proposed above (Fig. 1) and the actual observations of ontogeny give the following results (Figs. 2, 3).

Enteropneust development (Colwin and Colwin 1951) shows an uncomplicated process without any movements of apical pole or mouth, and this is also the case in the other non-chordate deuterostomes which have been studied (Nielsen 1995). This type of development is therefore assumed to be the ancestral developmental type within the deuterostomes.

Ascidian embryology (Julin 1904; Nishida 1987) shows that the apical pole moves to a c-ventral position at the base of the tail of the tadpole larva. *The mouth develops on the c-dorsal side just in front of the neuropore*, i.e. just in front of the neural plate, in complete agreement with the hypothesis. An attachment organ in the shape of three small papillae develops anteriorly between the apical pole and the mouth. The orientation of the adult ascidian with the incurrent opening (mouth) facing away from the substratum is obtained through a subsequent rotation of the gut during larval metamorphosis (Seeliger 1893–1907).

In amphioxus (Tung et al. 1962), the apical pole also moves to the c-ventral side, but the mouth develops from the left side which makes it difficult to draw any firm conclusions.

Vertebrate development shows considerable variation, but amphibian embryology appears to be the least modified type. Comparative studies of annelid and echinoderm embryos have shown that the relative positions of prospective areas of the eggs/blastulae are constant over a very large variation of developmental types (Anderson 1973; Wray and Raff 1990) so it appears reasonable to use amphibians with total cleavage as representative of all vertebrates. In *Bombinator* (Vogt 1929) and *Xenopus* (Keller 1975; Drysdale and Elinson 1991), the mouth forms from an area just in front of the anterior part of the neural fold, and both the apical pole and mouth area move to the c-ventral side, with the apical pole becoming situated behind the mouth. As in the ascidian tadpole, an adhesive organ develops between mouth and apical pole, but it is not known whether this is of phylogenetic significance. Some earlier theories (Garstang 1928) have included a sessile stage in the evolution of the chordates, so this should be investigated further.

It appears that the ontogeny of enteropneusts, urochordates, amphioxus and vertebrates (Fig. 3) are in accordance with the hypothesis theory proposed here, i.e. that the chordate CNS evolved from the postoral loop of the ciliary band in a dipleurula larva.

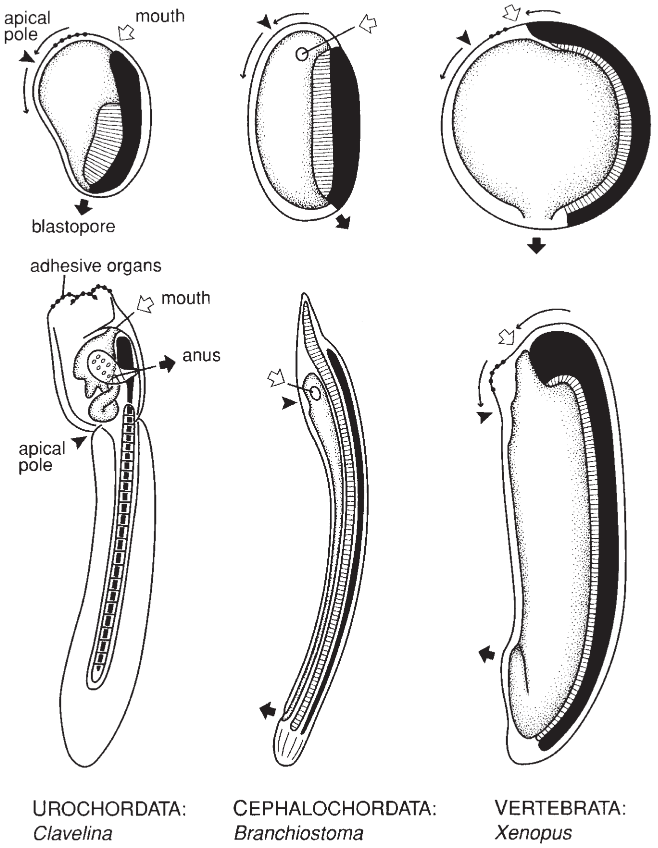


Fig. 3 Late embryos and larvae of chordates with indications of observed movements of the apical pole (the place where the two first cleavage furrows intersect at the position of the polar bodies) and mouth (or the area from which the mouth develops at a later stage) based on blastomere markings. The neural tube of the *Clavelina* larva is seen through the urochord because the tail is twisted 90° at the base. Modified from Nielsen (1995) and Hausen and Riebesell (1991; *Xenopus*)

It has been proposed that the protostomes have the longitudinal body axis at an angle to the primary, i.e. apical-blastoporal, axis (as indicated through the lateral blastopore closure and formation of the ventral nerve cord), whereas the deuterostomes have the longitudinal axis parallel to the primary axis (Nielsen 1995). This makes it difficult to relate the dorsoventral orientation of the two groups on morphological evidence. However, the hypothesis proposed above and the molecular results indicate that the ventral nerve cord of insects and the c-dorsal CNS of vertebrates develop from similar areas of the embryo, and this supports the traditional dorsoventral orientation of protostomes and non-chordate deuterostomes.

Discussion and perspectives

The new interpretation of the chordate CNS implies both similarities and differences between protostome and chordate nervous systems (Figs. 4, 5). According to the trochaea theory (Nielsen 1979, 1985) the ventral nerve cord(s) of the protostome ancestor was formed from the fused lateral blastopore lips which originally carried the

Fig. 4 Diagrams of nervous systems of hypothetical ancestral protostomes and chordates according to the hypotheses presented in this paper. The apical organ and the parts of the ciliary bands (and adjacent zones of cells) which should become transformed into the central nervous system are indicated in *black*, those that should disintegrate in *grey*

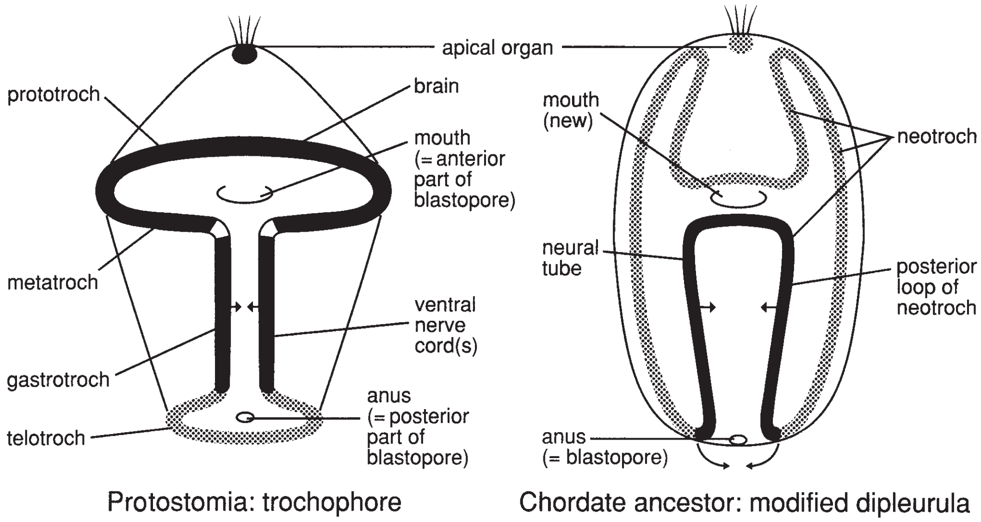
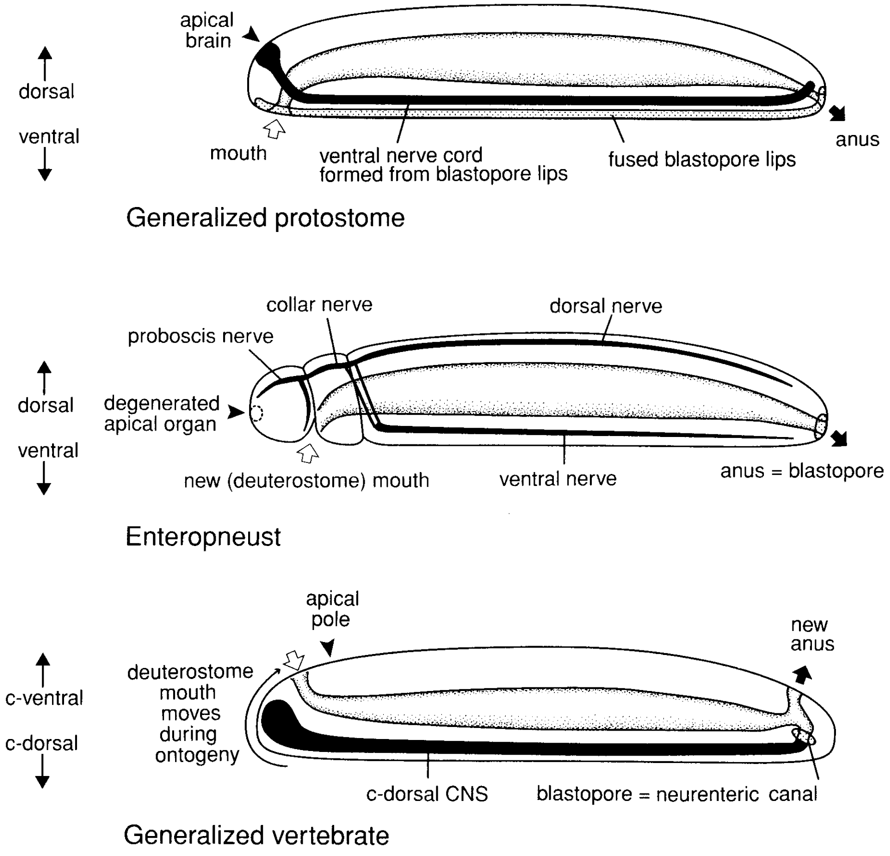


Fig. 5 Comparisons of blastopore fates, positions of apical organs/poles, gut openings and central nervous systems in a generalized protostome, enteropneust and vertebrate. The protostomes have a central nervous system (CNS) with an apical brain, circumoesophageal connectives and a paired or fused midventral nerve cord, often with a small loop around the anus. The enteropneusts have a nervous system without a well defined brain (Knight-Jones 1952; Cameron and Mackie 1996); the larval apical organ degenerates at metamorphosis. The vertebrates have a cordate-dorsal (c-dorsal) CNS which develops posterior to the area from which the mouth subsequently develops; the area of the future mouth initially lies just in front of the neural fold (on the c-dorsal side), but differential growth during ontogeny gradually displaces it to the c-ventral side



lateral parts of the archaeotroch (the ring of compound cilia around the blastopore in the trochaea larva of the ancestral protostome). It appears that the paired neural zones fuse in systematic groups lacking a gastrotroch (e.g. nematodes and large annelids), whereas the two cords remain separate in groups with a narrow gastrotroch (e.g. small polychaetes), and widely separated in the molluscs, which have the wide gastrotroch on the foot specialized for locomotion (Nielsen 1995). The protostome ventral nerve cord(s) thus originated from zones bearing ciliary bands, and this is also proposed for the chordates. However, there are several important differences between the protostome ventral nerve cord and the chordate neural tube. The protostome nerve cord evolved from cells associated with cells carrying downstream-collecting ciliary bands with compound cilia on multiciliate cells (Nielsen 1987); the fusion was along the ventral midline of the blastopore, i.e. over the oral field, and the anterior part of the brain developed from the apical organ or at least from the apical region (Lacalli 1984), i.e. from the aboral field. In contrast, the chordate neural tube evolved from cells bearing upstream-collecting ciliary bands with separate cilia on monociliate cells; the fusion was along the ventral (c-dorsal) midline between the blastopore and the new (deuterostome) mouth, and this implies that no part of the chordate brain evolved from the apical area.

Developing arthropod (insect) and vertebrate (mouse) brains express orthologous genes in similar spatial patterns (review in Arendt and Nübler-Jung 1996) which is a strong indication of homology of the brains of the two groups. According to the hypothesis proposed here, the chordate brain is exclusively postoral, with the mouth developing anterior to the neural fold. However, it should be emphasized that the deuterostome mouth appears to be a new opening and therefore not homologous with the protostome mouth, which is the anterior part of the blastopore. The apical pole of the chordate egg moves to the ventral side during ontogeny, indicating that the chordate brain has no apical component. The oesophagus penetrates the arthropod brain, and numerous embryological studies show that the protostome brain is preoral, and at least in some phyla, in part derived from the apical organ. At first sight, it seems difficult to regard the two structures as morphologically homologous (Bolker and Raff 1996), because of their different position relative to the mouth, but it is actually the mouths which are not homologous. The protostome brain could be characterized as pre-blastoporal (+ apical) and the chordate neural tube is situated between the anterior side of the blastopore and the apical organ, so the two structures may after all have comparable positions and be homologous. This would imply that the various patterning genes had already become operational in the latest common (bilateral) ancestor of protostomes and deuterostomes, probably correlated with the establishment of the new antero-posterior axis.

Nervous concentrations in the shape of tubular ectodermal infoldings like the chordate neural tube are known from other non-chordate deuterostomes, such as echinoderms (Ubisch 1913) and enteropneusts (Dawydoff 1948). However, the pentaradial symmetry of the echinoderm nervous system and its function without a centralized brain (Cobb 1988) makes comparisons with chordates futile. The enteropneust collar cord does not have any special concentration of nerve cells and does not function as a brain. There are intraepithelial median nerves along the dorsal side of the proboscis and through the mesosomal collar, paired lateral nerves from the posterior side of the collar to the ventral side and unpaired nerves along the dorsal and ventral metasomal midline (Knight-Jones 1952; see Fig. 5). A through-conduction pathway with giant fibres is present in proboscis, collar, lateral and ventral trunk nerves (Bullock 1945), and this has been compared to the cordate brain and spinal cord (Nübler-Jung and Arendt 1996), but its homology with the chordate CNS seems very dubious (Cameron and Mackie 1996). The possible homology of the midventral epithelial zone (between mouth and anus) of enteropneusts with the chordate CNS on one side and with the (pre-blastoporal) protostome brain on the other side should be investigated both with molecular and morphological methods. It will be difficult to obtain an experimental proof of the origin of the chordate CNS from the postoral loop of the neotroch in a dipleurula larva, because the living chordates do not have ciliated primary larvae. A search for endostyle-specific (Ogasawara and Satoh 1998) and gill-slit-specific genes (Tanaka et al. 1996) in enteropneusts could perhaps give additional information about homologies. Molecular studies of the role of the apical pole/organ in the development of the CNS in protostomes, enteropneusts and chordates could perhaps give important information about key differences in the evolution of the brain of major bilaterian lineages.

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References

Anderson DT (1973) Embryology and phylogeny in annelids and arthropods. Pergamon Press, Oxford

Arendt D, Nübler-Jung K (1994) Inversion of dorsoventral axis. Nature 371: 26

Arendt D, Nübler-Jung K (1996) Common ground plans in early brain development in mice and flies. BioEssays 18: 255–259

Arendt D, Nübler-Jung K (1997) Dorsal or ventral: similarities in fate maps and gastrulation patterns in annelids, arthropods and chordates. Mech Dev 61: 7–21

Bergström J (1997) Origin of high-rank groups of organisms. Paleontol Res 1: 1–14

Bolker JA, Raff RA (1996) Developmental genetics and traditional homology. BioEssays 18: 489–494

Bone Q (1959) The central nervous system in larval acraniates. Q J Microsc Sci 100: 509–527

Bullock TH (1946) The anatomical organization of the nervous system of enteropneusts. Q J Microsc Sci 86: 55–111

Cameron CB, Mackie GO (1996) Conduction pathways in the nervous system of *Saccoglossus* sp. (Enteropneusta). Can J Zool 74: 15–19

Castresana J, Feldmaier-Fuchs G, Pääbo S (1998) Codon reassignment and amino acid composition in hemichordate mitochondria. Proc Natl Acad Sci USA 95: 3703–3707

Cobb JLS (1988) A preliminary hypothesis to account for the neural basis of behaviour in echinoderms. In: Burke RD, Mladenov PV, Lambert P, Parsley RL (eds) Echinoderm biology. Balkema, Rotterdam, pp 565–573

Colwin AL, Colwin LH (1951) Relationships between the egg and larva of *Saccoglossus kowalevskii* (Enteropneusta): axes and planes; general prospective significance of the early blastomeres. J Exp Zool 117: 111–137

Cragg SM (1996) The phylogenetic significance of some anatomical features of bivalve veliger larvae. In: Taylor J (ed) Origin and evolutionary radiation of the Mollusca. Oxford University Press, Oxford, pp 371–380

Dawydoff C (1948) Classe des Entéropneustes. (Traité de Zoologie, vol 11) Masson, Paris, pp 369–453

Drysdale TA, Elinson RP (1991) Development of the *Xenopus laevis* hatching gland and its relationships to surface ectoderm patterning. Development 111: 469–478

Eagleson GW, Harris WA (1989) Mapping of the presumptive brain regions in the neural plate of *Xenopus laevis*. J Neurobiol 21: 427–440

Garey JR, Krotec M, Nelson D, Brooks J (1996) Molecular analysis support a tardigrade-arthropod association. Invert Biol 115: 79–88

Garstang W (1894) Preliminary note on a new theory of the phylogeny of the Chordata. Zool Anz 17: 122–125

Garstang W (1928) The morphology of the Tunicata, and its bearings on the phylogeny of the Chordata. Q J Microsc Sci 72: 51–187

Gee H (1996) Before the backbone. Chapman and Hall, London

Geoffroy-Saint-Hilaire E (1822) Considérations générales sur la vertèbre. Mém Mus Hist Nat 9: 89–119

Halanych K (1996) Convergence in the feeding apparatuses of lophophorates and pterobranch hemichordates revealed by 18 S rDNA: an interpretation. Biol Bull 190: 1–5

Hausen P, Riebesell M (1991) The early development of *Xenopus laevis*. Springer, Berlin Heidelberg New York

Holland PWH, Hacker AM, Williams NA (1991) A molecular analysis of the phylogenetic affinities of *Saccoglossus cambrensis* Brambell & Cole (Hemichordata). Philos Trans R Soc London Ser B 332: 185–189

Horst van der CJ (1927–39) Hemichordata. Bronn's Klassen und Ordnungen des Tierreichs, 4. Band, 4. Abt., 2. Buch, 2. Teil. Akademische Verlagsgesellschaft, Leipzig, pp 1–737

Hörstadius S (1973) Experimental embryology of Echinoderms. Oxford University Press, Oxford

Julin C (1904) Recherches sur la phylogenèse des Tuniciers. Z Wiss Zool 76: 544–611

Keller RE (1975) Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. Dev Biol 42: 222–241

Knight-Jones EW (1952) On the nervous system of *Saccoglossus cambrensis* (Enteropneusta). Philos Trans R Soc London Ser B 236: 315–354

Kowalevsky A (1871) Weitere Studien über die Entwicklung der einfachen Ascidien. Arch Mikrosk Anat Entwicklungsmech 7: 101–130

Lacalli TC (1984) Structure and organization of the nervous system in the trochophore larva of *Spirobranchus*. Philos Trans R Soc London Ser B 306: 79–135

Lacalli TC (1996a) Dorsoventral axis inversion: a phylogenetic perspective. BioEssays 18: 251–254

Lacalli TC (1996b) Landmarks and subdomains in the larval brain of *Branchiostoma*: vertebrate homologs and invertebrate antecedents. Israel J Zool 42: S131-S146

Lacalli TC (1996c) Mesodermal pattern and repeats in the starfish bipinnaria larva, and related patterns in other deuterostome larvae and chordates. Philos Trans R Soc London Ser B 351: 1737–1758

Lacalli TC, West JE (1993) A distinctive nerve cell type common to diverse deuterostome larvae: comparative data from echinoderms, hemichordates and amphioxus. Acta Zool (Stockholm) 74: 1–8

Lacalli TC, Gilmour THJ, West JE (1990) Ciliary band innervation in the bipinnaria larva of *Pisaster ochraceus*. Philos Trans R Soc London Ser B 330: 371–390

Maisey JG (1986) Heads and tails: a chordate phylogeny. Cladistics 2: 201–256

Malakhov VV (1977) The problem of the basic structural plan in various groups of Deuterostomia. Zh Obshch Biol 38: 485–499

Naylor GJP, Brown WM (1998) Amphioxus mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparisons of sequences. Syst Biol 47: 61–76

Nielsen C (1979) Larval ciliary bands and metazoan phylogeny. Fortschr Zool Syst Evolutionsforsch 1: 178–184

Nielsen C (1985) Animal phylogeny in the light of the trochaea theory. Biol J Linn Soc 25: 243–299

Nielsen C (1987) Structure and function of metazoan ciliary bands and their phylogenetic significance. Acta Zool (Stockholm) 68: 205–262

Nielsen C (1995) Animal evolution: Interrelationships of the living phyla. Oxford University Press, Oxford

Nielsen C (1997) The phylogenetic position of the Arthropoda. In: Fortey RA, Thomas RH (eds) Arthropod relationships. The Systematics Association, London, pp 11–22

Nielsen C, Scharff N, Eibye-Jacobsen D (1996) Cladistic analyses of the animal kingdom. Biol J Linn Soc 57: 385–419

Nishida H (1987) Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. Dev Biol 121: 526–541

Nübler-Jung K, Arendt D (1994) Is ventral in insects dorsal in vertebrates? Roux's Arch Dev Biol 203: 357–366

Nübler-Jung K, Arendt D (1996) Enteropneusts and chordate evolution. Curr Biol 6: 352–353

Ogasawara M, Satoh N (1998) Isolation and characterization of endostyle-specific genes in the ascidian *Ciona intestinalis*. Biol Bull 195: 60–69

Ruppert EE (1990) Structure, ultrastructure and function of the neural gland complex of *Ascidia interrupta* (Chordata, Ascidiacea): clarification of hypotheses regarding the evolution of the vertebrate pituitary. Acta Zool (Stockholm) 71: 135–149

Ruppert EE (1997a) Introduction: Microscopic anatomy of the notochord, heterochrony, and chordate evolution. In: Harrison FW (ed) Microscopic anatomy of invertebrates, vol 15. Wiley-Liss, New York, pp 1–13

Ruppert EE (1997b) Cephalochordata (Acrania). In: Harrison FW (ed) Microscopic anatomy of invertebrates, vol 15. Wiley-Liss, New York, pp 349–504

Schram FR (1991) Cladistic analysis of metazoan phyla and the placement of fossil problematica. In: Simonetta AM, Conway Morris S (eds) The early evolution of Metazoa and the significance of problematic taxa. Cambridge University Press, Cambridge, pp 35–46

Schram FR (1997) Of cavities – and kings. Contr Zool 67: 143–150

Seeliger O (1893–1907) Die Appendicularien und Ascidien. Bronn's Klassen und Ordnungen des Tierreichs, 3. Band (Suppl.), 1. Abt. Akademische Verlagsgesellschaft, Leipzig, pp 1–1280

Snodgrass RE (1938) Evolution of the Annelida, Onychophora, and Arthropoda. Smithson Misc Collect 97(6): 1–159

Strathmann R, Bonar D (1976) Ciliary feeding of tornaria larvae of *Ptychodera flava* (Hemichordata: Enteropneusta). Mar Biol (Berl) 34: 317–324

Tanaka KJ, Ogasawara M, Makabe KW, Satoh N (1996) Expression of pharyngeal gill-specific genes in the ascidian *Halocynthia roretzi*. Dev Genes Evol 206: 218–226

Tung TC, Wu SC, Tung YYF (1962) The presumptive areas of the egg of amphioxus. Sci Sin 11: 629–644

Ubisch LV (1913) Die Entwicklung von *Strongylocentrotus lividus (Echinus microtuberculatus, Arbacia pustulosa)*. Z Wiss Zool 106: 409–448

Vogt W (1929) Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II. Teil. Gastrulation und Mesodermbildung bei Urodelen und Anuren. Arch Entwicklungsmech Org 120: 384–706

Wada H, Satoh N (1994) Details of the evolutionary history from invertebrates to vertebrates, as deduced from sequences of 18 S rDNA. Proc Natl Acad Sci USA 91: 1801–1804

Willey A (1899) Remarks on some recent work on the Protochordata, with a condensed account of some fresh observations on the Enteropneusta. Q J Microsc Sci 42: 223–244

Winnepenninckx B, Backeljau T (1996) 18 S rRNA alignments derived from different secondary structure models can produce alternative phylogenies. J Zool Syst Evol Res 34: 135–143

Wray GA, Raff RA (1990) Novel origins of lineage founder cells in the direct-developing sea urchin *Heliocidaris erythrogramma*. Dev Biol 141: 41–54