ELSEVIER

Mechanisms of Development 61 (1997) 7–21

Review Article

Dorsal or ventral: similarities in fate maps and gastrulation patterns in annelids, arthropods and chordates

Detlev Arendt*, Katharina Nübler-Jung

Institut für Biologie I (Zoologie), Albertstrasse 21 A, 79104 Freiburg, Germany

Received 20 August 1996; revised version received 14 October 1996; accepted 14 October 1996

Abstract

The idea that chordates, during their evolution, have inverted their dorsoventral body axis has recently gained substantial support. It has been shown that various dorsoventral patterning genes that are evolutionarily conserved between insects and vertebrates are expressed dorsally in insects, and ventrally in vertebrates, or vice versa. The ventral body side of insects thus seems to correspond to the dorsal body side of vertebrates, and these are the nerve cord-bearing, *neural* body sides in both groups. In order to exclude that the inverted polarity of gene patterning activity is purely accidental, we compare here vertebrate and invertebrate blastula fate maps and their gastrulation patterns in the framework of early gene expression. From this comparison it appears that the neural body sides, 'ventral' in annelids or arthropods, and 'dorsal' in chordates, develop at similar positions with respect to the initial egg asymmetry. In addition, the formation of the neural body sides involves similar movements during gastrulation. We further suggest that the deuterostome gastrulation seen in today's chordates can be derived from a more ancestral gastrulation pattern seen in today's annelids and arthropods, and that the ventral midline cells of insects correspond to the dorsal midline cells of vertebrates.

Keywords: Fate maps; Gastrulation patterns; Neural body side; Dorsoventral axis inversion; Midline cells; Homology

1. Introduction

Orthologous genes with key functions in early development provide a new tool for interphyletic comparisons. On the basis of gene expression patterns we have recently proposed that the ventral side of insects corresponds to the dorsal side of vertebrates, and that chordates have inverted their dorsal-ventral (D-V) body axis during their evolution (Arendt and Nübler-Jung, 1994; Nübler-Jung and Arendt, 1994). A pair of orthologous genes, *decapentaplegic* in *Drosophila* and *BMP-4* in vertebrates, are expressed dorsally in insects and ventrally in vertebrates, where they exert, respectively, dorsalizing and ventralizing functions (reviewed in Hogan et al., 1994). Another pair of orthologous dorsal-ventral patterning genes, that act antagonistically to *dpp* and *BMP-4*, has now been discovered (*short gastrulation* in *Drosophila* and *chordin* in *Xenopus*), and further supports the idea of D-V axis inversion during chordate evolution (François and Bier, 1995; Holley et al., 1995; reviewed in Hogan, 1995; Jones and Smith, 1995). The conserved system of dorsoventral patterning already existed in ancestral bilaterians for which the name 'Urbilateria' has recently been proposed (De Robertis and Sasai, 1996). The idea that insects and vertebrates share rather similar body plans, and that the ventral side of insects corresponds to the dorsal side of vertebrates was first articulated in 1822 by Geoffroy St. Hilaire ('unité de plan'; reviewed in Nübler-Jung and Arendt, 1994). Some 50 years later, Anton Dohrn proposed that vertebrates evolved from an annelid-like ancestor by dorsal-ventral inversion, and that the annelid and vertebrate central nervous systems (CNS) be homologous structures ('annelid theory'; Dohrn, 1875; reviewed in Nübler-Jung and Arendt, 1994). These homologies could then extend from vertebrates to insects, as predicted by Geoffroy St. Hilaire. In keeping with this idea, the insect and vertebrate brain and nerve cord develop according to a common ground plan (Arendt and Nübler-Jung, 1996).

Comparable structures found in distinct animal groups are considered homologous if a common precursor structure existed in their most recent common stem species. Indicative of a common evolutionary origin, the concerned

* Corresponding author. Tel.: +49 761 2032595; fax: +49 761 2032596.

0925-4773/97/$17.00
PII S0925-4773(96)00620-X

structures often share specific qualities (Remane, 1952). In this sense, the specific expression and function of orthologous genes can indicate homology. Gene expression alone, however, is not sufficient to establish homology, because the patterning activity of ancestral genes might have been co-opted independently in separate lines of evolution (see e.g. Dickinson, 1995). A homologous relationship becomes more likely if the compared structures or embryonic anlagen also form at comparable sites within a comparable spatial reference system (Remane, 1952). Here, we utilize both molecular and embryological data to show that this is the case for the dorsal body region of chordates, as compared to the ventral body region of annelids and arthropods. We compare ancestral cleavage patterns in annelids, arthropods and chordates, and we show that in the blastula we find the same sequence of prospective epidermal, neural, mesodermal and endodermal blastomeres along the animal-vegetal (an-veg) axis of the egg. These conserved fate maps are established by similar molecular mechanisms in insects and in vertebrates. In spite of the conserved position between the mesoderm and the prospective epidermis, however, the prospective *neural* region of the blastula is then later termed 'ventral' in annelids, and 'dorsal' in vertebrates. These findings strongly support the concept of D-V inversion during early chordate evolution. During gastrulation the later *neural* body sides of annelids and vertebrates form in a similar manner. We therefore suggest that the deuterostome gastrulation mode seen in vertebrates evolved from an ancestral gastrulation pattern with mouth and anus arising from opposite ends of a longitudinal blastopore, as observed in extant polychaete annelids. Finally, we propose homology of the insect and vertebrate midline cells. These cells specifically mark the median line along which the lateral blastopore margins of a longitudinal blastopore had fused in their putative last common ancestor, and along which the nerve cord develops in both groups.

2. Comparison of blastula fate maps

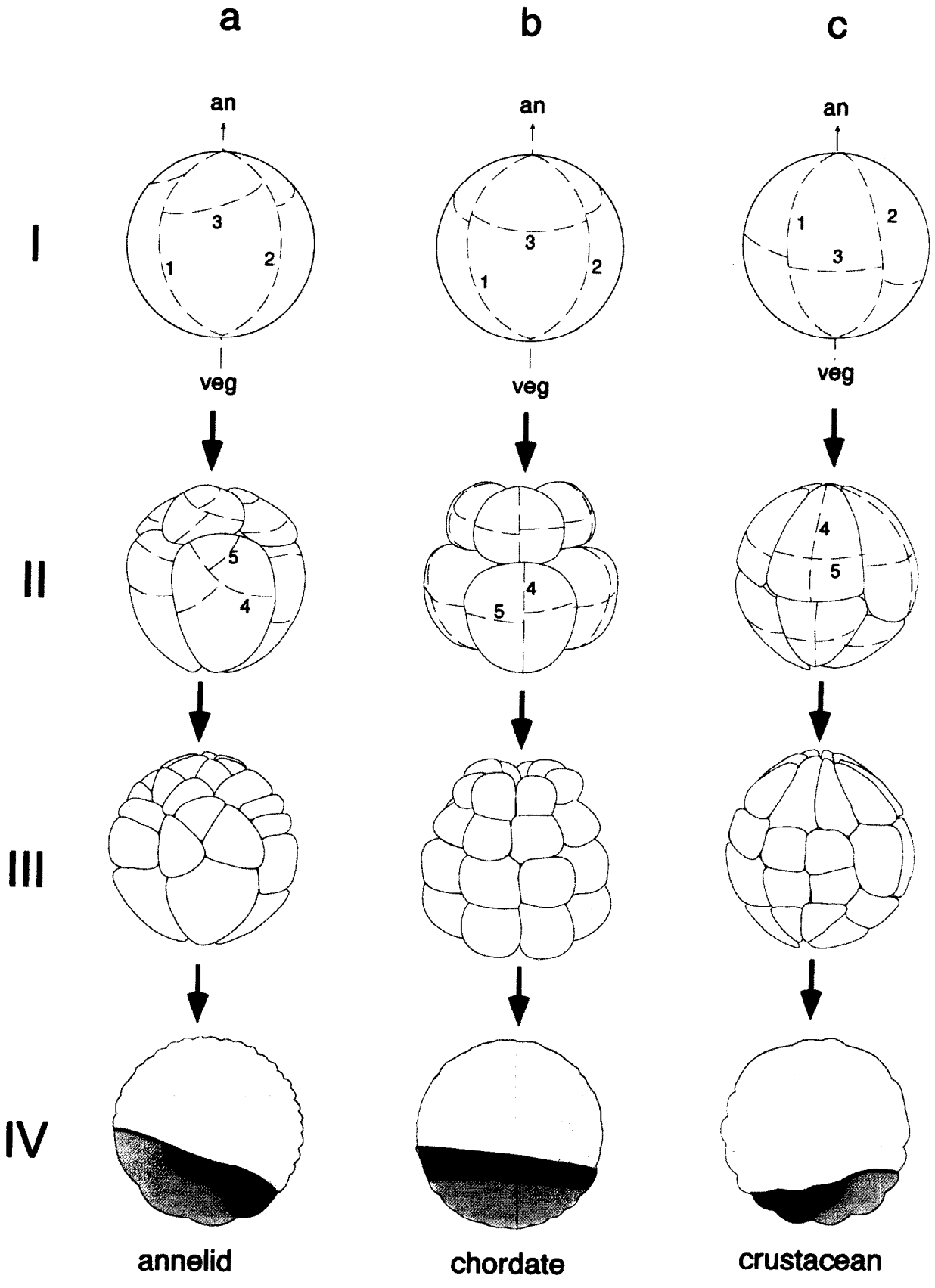
2.1. Cleavage generates a comparable sequence of epidermal, neural, mesodermal and endodermal blastomeres

The egg of most animals shows radial symmetry with a single an-veg axis. The animal pole of the egg is where polar bodies form during meiosis, and where the nucleus of the zygote often lies. The vegetal half of the egg is often richer in yolk. An-veg polarity is ancestral for the eggs of annelids, chordates and arthropods. It is found, for example, in polychaetes, in *Branchiostoma* and in crustaceans (Fig. 1). An-veg polarity also characterizes the egg of the more primitive apterygote insects, such as the *Collembola*. The egg of pterygote insects shows bilateral symmetry with two perpendicular axes, the later anterior-posterior (A-P) and dorsal-ventral (D-V) axes of the embryo. This latter situation is likely to be phylogenetically derived (Anderson, 1972).

During cleavage, an egg rapidly divides into numerous smaller cells (blastomeres) that eventually form a sphere known as blastula. In the majority of forms with aquatic larvae, the cleavage furrows extend through the entire egg (total or holoblastic cleavage). In contrast, terrestrial forms often produce enormous amounts of yolk as a built-in nutrient supply, and cleave their eggs only superficially (e.g. pterygote insects, reptiles and birds). Many crustaceans and the apterygote *Collembola* start with total cleavage to later continue with superficial cleavage (Anderson, 1973).

Cleavage patterns have often been used for phylogenetic considerations. For example, the total cleavage of crustaceans has been considered as derived from spiral cleavage (Anderson, 1973). Fig. 1 outlines resemblances between the overall pattern of spiral cleavage in a polychaete annelid and of the almost radial cleavage of a crustacean. We show that these resemblances can be extended to the cleavage of lower chordates (e.g. *Branchiostoma*). In all these groups, the first two cleavage planes pass through the animal and the vegetal egg poles (Fig. 1I). The third cleavage plane then lies approximately perpendicular to the first and the second, thus producing an animal and a vegetal quartet of blastomeres (Fig. 1II). From the third cleavage onwards, there is another common regularity in that each new cleavage plane forms perpendicular to the former (Fig. 1II,III). From approximately the sixth cleavage onwards, the orientation of cleavage planes becomes irregular. At the end of cleavage, the blastula fate maps show a similar sequence of prospective epidermal, neural, mesodermal and endodermal groups of blastomeres with respect to the an-veg axis of the egg (Fig. 1IV). Blastomeres around the animal pole will form the epidermal ectoderm (and anterior brain), those below the neuroectoderm, and the mesoderm. The most vegetal blastomeres form the endoderm. In the chordates, there is a ring of mesoderm (Fig. 1IVb). Anderson (1973) suggested that such a mesodermal ring be ancestral also for annelids

Fig. 1. Cleavage and blastula fate maps showing contributions of blastomeres to specific tissues. (a) *Podarke* (*Polychaeta, Annelida*); after Anderson (1973) and Dorresteijn et al. (1993). (b) *Branchiostoma* (*Acrania, Chordata*); after Conklin (1932); Tung et al. (1962). (c) *Polyphemus* (*Cladocera, Crustacea*); after Kühn (1913). (I) Dashed lines on the egg surface indicate where the first two meridional (1,2) and the third equatorial (3) cleavage planes will cut through the egg. (II) Eight-cell-stage. The fourth and fifth cleavage planes lie perpendicular to each other (4,5). (III) Thirty-two-cell-stage. (IV) Blastula. Bold line separates prospective external tissues (epidermal ectoderm, blue; neuroectoderm, yellow) from tissues that will be internalized during gastrulation (mesoderm, red; endoderm, green). Note that in polychaete annelids blastomeres at the animal pole will contribute to the cerebral ganglia. Prospective anterior tissues shown to the left. an, animal; veg, vegetal. For the orientation of the future dorsal-ventral axes see Fig. 2.


a
b
c
an
an
an
I
3
1
2
3
1
2
1
2
3
veg
veg
veg
II
5
4
4
5
4
5
III
IV
annelid
chordate
crustacean

and arthropods, because this would best explain that in polychaetes (Fig. 1IVa) and in crustaceans (Fig. 1IVc) the prospective mesoderm locates to opposite A-P positions with respect to the endoderm.

A similar sequence of tissue qualities forms along the D-V axis of the insect egg. This is most obvious in the pterygote isopterans (*Kalotermis*) that form a midventral stripe of endodermal cells, flanked by the mesoderm, neuroectoderm and epidermal ectoderm (Striebel, 1960; reviewed in Anderson, 1972). In the apterygote *Thysanura* and in most pterygote insects, there is a midventral area of mesodermal cells enclosed by the prospective ectoderm. Prospective endodermal cells, however, attain an internal position during blastoderm formation in apterygotes (Jura, 1972), and form from the extreme anterior and posterior of the egg in most pterygote insects (cf. Fig. 3a).

2.2. *Blastula fate maps predict an inverse assignment of 'dorsal' and 'ventral' in annelid and amphibian embryos*

The conserved distribution of epidermal ectoderm and neuroectoderm within the animal half of the embryo foreshadows the later D-V axis of the embryo, as shown for the blastula of a polychaete annelid (Fig. 2a) (Anderson, 1973) and of an amphibian (Fig. 2b) (Keller, 1975). In spite of their conserved distribution, however, prospective epidermal and neural regions of the annelid and amphibian blastulae show an inverse assignment of 'dorsal' and 'ventral' after gastrulation. For example, the region of the epidermal ectoderm is then termed 'dorsal' in annelids, and 'ventral' in chordates, and the reverse is true for the neuroectoderm. The assignment of 'dorsal' and 'ventral' in the ectoderm with respect to the an-veg axis thus seems to be inverted in amphibians as compared to annelids (Fig. 2). Since, by definition, 'ventral' is the side that is usually oriented towards the substrate, this inverse assignment can easily be accounted for, if chordates turned upside down during their evolution, so as to orient their former dorsal side towards the substrate (Arendt and Nübler-Jung, 1994). For simplicity, and in order to avoid confusion over annelid, arthropod and chordate former or present dorsal and ventral body sides, we now utilize the term 'neural' for their later nerve cord-bearing side. This term allows an unambiguous identification of corresponding body sides in these groups independent of their actual orientation with respect to the substrate. In both amphibians and annelids prospective 'neural' tissues (e.g. neuroectoderm) then locate to similar regions in the blastula.

2.3. *Conserved molecular mechanisms transform the initial egg asymmetry into a similar sequence of prospective tissues*

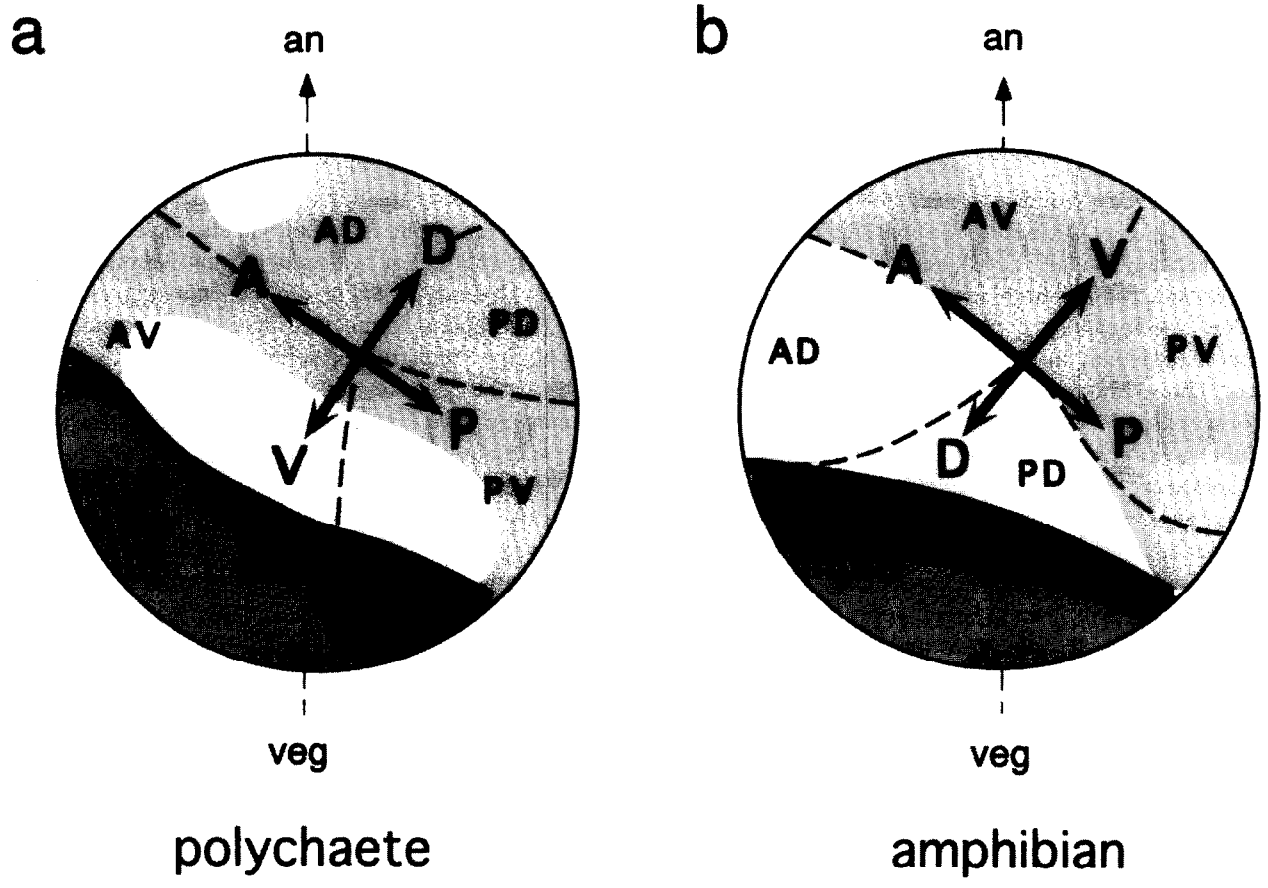
Fig. 3 illustrates that D-V patterning genes conserved between insects and vertebrates specifically lay down the characteristic sequence of prospective epidermal, neural and mesodermal blastomeres seen at blastula stages. Given that a similar sequence of prospective tissue qualities forms along the an-veg axis of the egg in annelids, arthropods and chordates (cf. Fig. 1), it seems likely that an-veg patterning is an ancestral function of these genes.

Within the *Drosophila* blastula ectoderm, the *decapentaplegic* (*dpp*) secreted protein promotes the formation of epidermal tissue but represses the formation of neuroectoderm (Fig. 3a) (Ferguson and Anderson, 1992a; Ferguson and Anderson, 1992b; Wharton et al., 1993). A similar situation is seen in *Xenopus*, where a *dpp* orthologue, *BMP-4*, is active in animal blastomeres, and also has anti-neurogenic activity (Fig. 3b) (Fainsod et al., 1994; Maéno et al., 1994; Suzuki et al., 1994; Xu et al., 1995; Hawley et al., 1995; Sasai et al., 1995; Suzuki et al., 1995; Wilson and Hemmati-Brivanlou, 1995; Jones et al., 1996). In both insects (Shimell et al., 1991; Childs and O'Connor, 1994) and vertebrates (Maéno et al., 1993; Fukagawa et al., 1994) the specific activation of the *dpp*-protein may similarly require the activity of a metalloprotease encoded by *tolloid*-orthologues. The activity of *dpp*-orthologues is antagonized by another conserved pair of secreted molecules, *short gastrulation* in *Drosophila* (François et al., 1994; Holley et al., 1995) and *chordin* in *Xenopus* (Sasai et al., 1994; Holley et al., 1995; Sasai et al.,

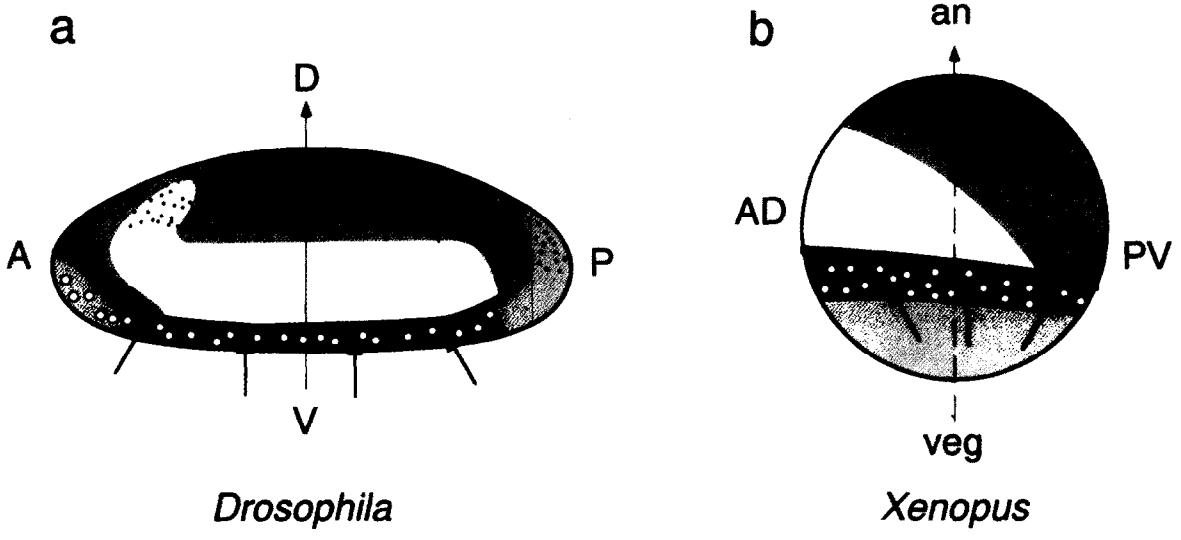
Fig. 2. Inverted assignment of 'dorsal' and 'ventral' in the ectoderm with respect to the an-veg axis in (a) *Podarke* (*Polychaeta, Annelida*; after Anderson, 1973) with respect to (b) *Xenopus* (*Amphibia, Vertebrata*; after Keller, 1975). Distribution of prospective anterior-dorsal (AD), anterior-ventral (AV), posterior-dorsal (PD) and posterior-ventral (PV) tissues after gastrulation (separated by broken lines) in blastula fate maps viewed from the left side. Red arrows indicate orientation of the future A-P and D-V body axes in the ectoderm. Bold lines separate prospective external tissues (epidermal ectoderm, blue; neuroectoderm, yellow) from prospective internal tissues (mesoderm, red; endoderm, green). an, animal; veg, vegetal. For the fate maps, compare Fig. 1.

Fig. 3. Regionally specific activity of *GATA*-orthologues (black dots), *decapentaplegic*-orthologues (stippled) and *snail*-orthologues (white dots) in *Drosophila* (a) and *Xenopus* (b) at the onset of gastrulation (viewed from the left side). Bold line separates prospective external tissues (epidermal ectoderm, blue; neuroectoderm, yellow) from tissues that will be internalized during gastrulation (mesoderm, red; endoderm, green). Small arrows stand for mesoderm-inducing signals. A, anterior; P, posterior; AD, anterior-dorsal; PV, posterior-ventral. Fate maps after (a) Hartenstein et al. (1985), Hartenstein (1993) and (b) Gilbert (1994). Note that, beside its mesodermal expression, *snail* is also expressed in a subset of prospective endoderm (anterior midgut anlage in *Drosophila*, suprablastoporal endoderm overlying the mesoderm in *Xenopus*; Ray et al., 1991; Mayor et al., 1993). Expression of *Drosophila decapentaplegic* disappears from the prospective forebrain and the posterior midgut anlage after gastrulation (Ray et al., 1991). For additional references see text.

2


a
an
AD
D
A
AV
PD
P
V
PV
veg
polychaete
b
an
AV
A
V
AD
PV
D
PD
P
veg
amphibian

3


a
D
A
P
V
Drosophila
b
an
AD
PV
veg
Xenopus

1995). Finally, genes likely to act downstream of *dpp* have also been conserved during evolution. For example, in both *Drosophila* and *Xenopus* (Kelley et al., 1994; Walmsley et al., 1994) a *GATA* orthologue is expressed within the *dpp* domain (Fig. 3). In *Drosophila*, *dpp* mutants fail to express *dGATAa/pannier* (Winick et al., 1993; Ramain et al., 1993); and in *Xenopus*, *BMP-4* may induce *GATA-2* (Walmsley et al., 1994). Interestingly, the *Drosophila dGATAa/pannier* protein also represses neurogenesis (Ramain et al., 1993), as do the *dpp*-orthologues in *Drosophila* and in vertebrates.

The specification of mesodermal cells also involves similar strategies. In the amphibian blastula, vegetal signals induce the equatorial ring of mesoderm (Fig. 3b) (Nieuwkoop, 1969), and in *Drosophila*, the specification of the mesoderm is induced by signals that had originally emanated from the ventral follicle cells during oogenesis (Fig. 3a) (reviewed in Chasan and Anderson, 1993). The position of signaling follicle cells along the later ventral side of the egg thereby corresponds to the position of the midventral stripe of endodermal cells in isopterans, and thus to the vegetal position of mesoderm-inducing endoderm in vertebrates. Given that endodermal cells localize to the vegetal pole in most bilaterians, it seems possible that the *Drosophila* mode of mesoderm induction has been evolutionarily derived from a vertebrate-like situation, where mesoderm-inducing signals emanate from the vegetal endoderm. This would explain why downstream effects of the signaling are rather similar in insects and in vertebrates. For example, expression of *snail* orthologues is induced in the future mesoderm, in *Drosophila* (Ip et al., 1992), and in *Xenopus* (Mayor et al., 1993). During gastrulation, *snail* expression occurs in involuting or invaginating cells in both insects (Leptin, 1991; Sommer and Tautz, 1994) and vertebrates (Nieto et al., 1992; Smith et al., 1992; Hammerschmidt and Nüsslein-Volhard, 1993; Mayor et al., 1993), suggesting that these genes have retained a conserved function in mesoderm formation (Fig. 3a,b).

In conclusion, similar molecular mechanisms help to establish a similar sequence of prospective mesoderm, neuroectoderm and epidermal ectoderm along the an-veg axis of the embryo. The anlagen of the neural body sides in vertebrates and invertebrates thus form from similar positions in the topological and molecular framework of the early embryo. We now show that they also undergo comparable movements during gastrulation.

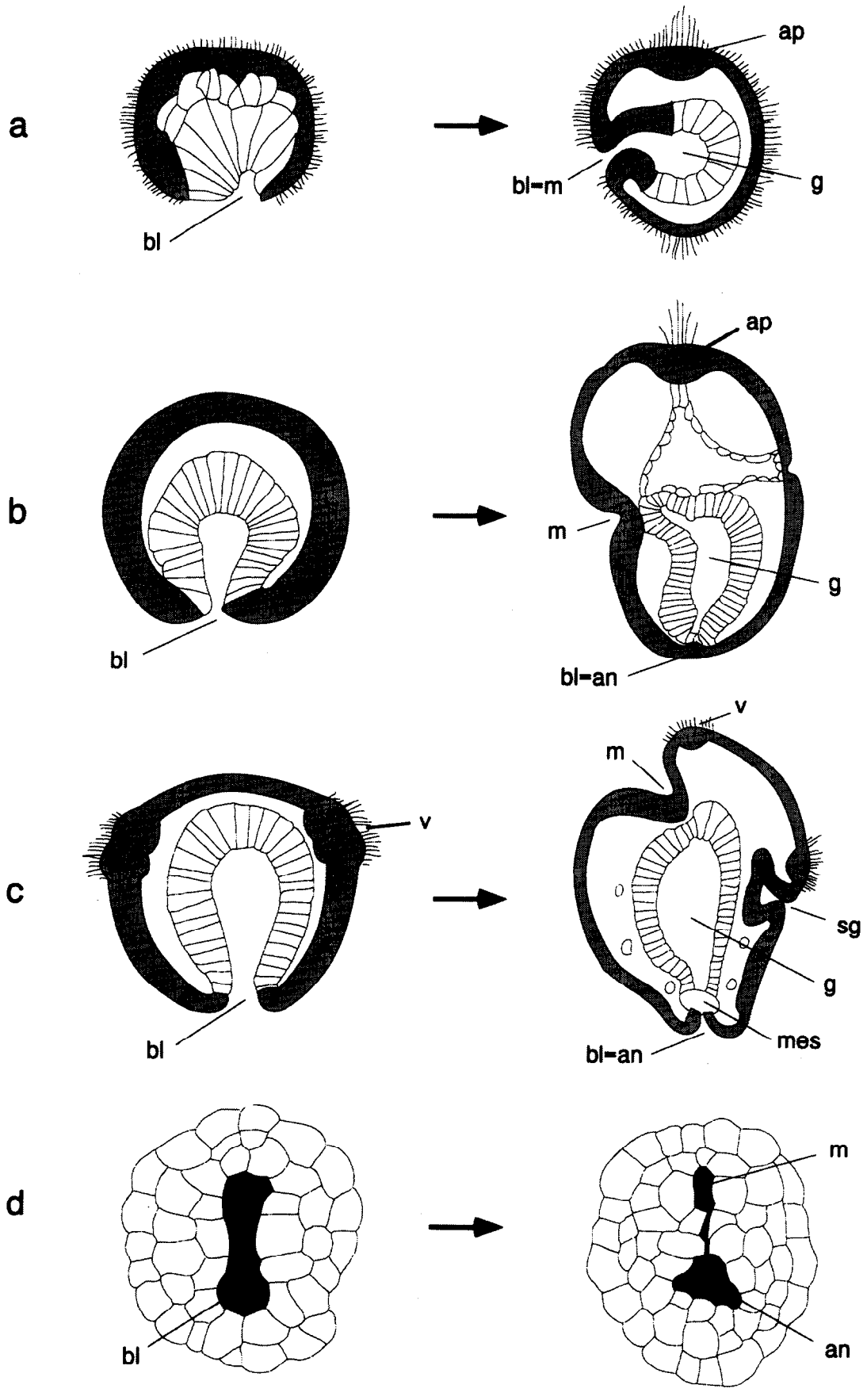
3. Comparison of gastrulation patterns

3.1. A slit-like blastopore foreshadows the longitudinal body axis

Gastrulation generally involves an internalization of vegetal blastomeres through the blastopore. Animals differ, however, in the fate of their blastopore after gastrulation. For example, in nemertines the blastopore forms the mouth while an anus breaks through secondarily (protostomy, 'mouth first'; Fig. 4a). In contrast, in enteropneusts the blastopore forms the anus while a mouth breaks through secondarily (deuterostomy, 'mouth second'; Fig. 4b). In the beginning of this century, these differences in ontogeny were considered so fundamental that they were given a phylogenetic significance. According to Grobben (1908), animals with bilateral symmetry (*Bilateria*) divide into two widely separated phylogenetic groups, the '*Protostomia*' and the '*Deuterostomia*' (Claus et al., 1932), and such views have prevailed into textbooks with slight modifications (see e.g. Hyman, 1940; Brusca and Brusca, 1990; Gruner, 1993). The chordates, hemichordates and echinoderms then belong to the '*Deuterostomia*', whereas annelids, arthropods, molluscs and platyhelminths belong to the '*Protostomia*' (although most of them do not show a true protostome pattern of development, see Fig. 4c,d) (Fioroni, 1980; see also Willmer, 1990; Nielsen, 1995).

As depicted in Fig. 4a,b, after gastrulation the former blastopore lies anteriorly in a prototypical protostome, and posteriorly in a deuterostome. This led Grobben to conclude that the longitudinal A-P body axes of the '*Protostomia*' and of the '*Deuterostomia*' represent convergent (non-homologous) features that evolved independently (Fig. 5a). In contrast to this view, however, the colinear expression of the phylogenetically conserved *Hox*-cluster genes in various bilaterians, strongly suggest that '*Protostomia*' and '*Deuterostomia*' have inherited their A-P body axes from their last common ancestor (Slack et al., 1993). This view is further supported because the anterior, respectively, posterior body regions of insects and vertebrates express orthologous patterning genes, like e.g. the *orthodenticle* and *empty spiracles* genes in the anterior (Dalton et al., 1989; Finkelstein et al., 1990; Boncinelli et al., 1993), and *caudal* genes in the posterior (Mlodzik et al., 1985; Frumkin et al., 1991; Joly et al., 1992; Meyer and Gruss, 1993). Given that the A-P body axis is a conserved feature of bilaterian animals, the evolution of

Fig. 4. Divergent fates of the blastopore in '*Protostomia*' and '*Deuterostomia*'. (a) Prototypical protostomy in *Procephalothrix*, *Nemertini* ('*Protostomia*'). The blastopore moves ventrally and forms the mouth. (b) Deuterostomy in *Balanoglossus*, *Enteropneusta* ('*Deuterostomia*'). The blastopore forms the posterior anus. The mouth forms secondarily by fusion of the gut (g) with the mouth (m). (c) Deuterostomy in *Viviparus*, *Gastropoda* ('*Protostomia*'). (a–c) Lateral view; the animal pole is up. (d) Amphistomy in *Polygordius*, *Polychaeta* ('*Protostomia*'); vegetal view. The lateral blastopore margins fuse along the future ventral midline. The blastopore gives rise to both mouth and anus. Shaded, ectoderm; an, anus; ao, apical organ; bl, blastopore; g, gut; m, mouth; sg, shell gland; v, velum. (a,c) after Fioroni (1980); (b) after Van der Horst (1939); (d) after Woltereck (1904).


a
bl
ap
bl=m
g
b
bl
ap
m
g
bl=an
c
v
bl
v
m
sg
g
mes
bl=an
d
bl
m
an

protostome and deuterostome organisms must have been possible without changing the A-P polarity of the embryo.

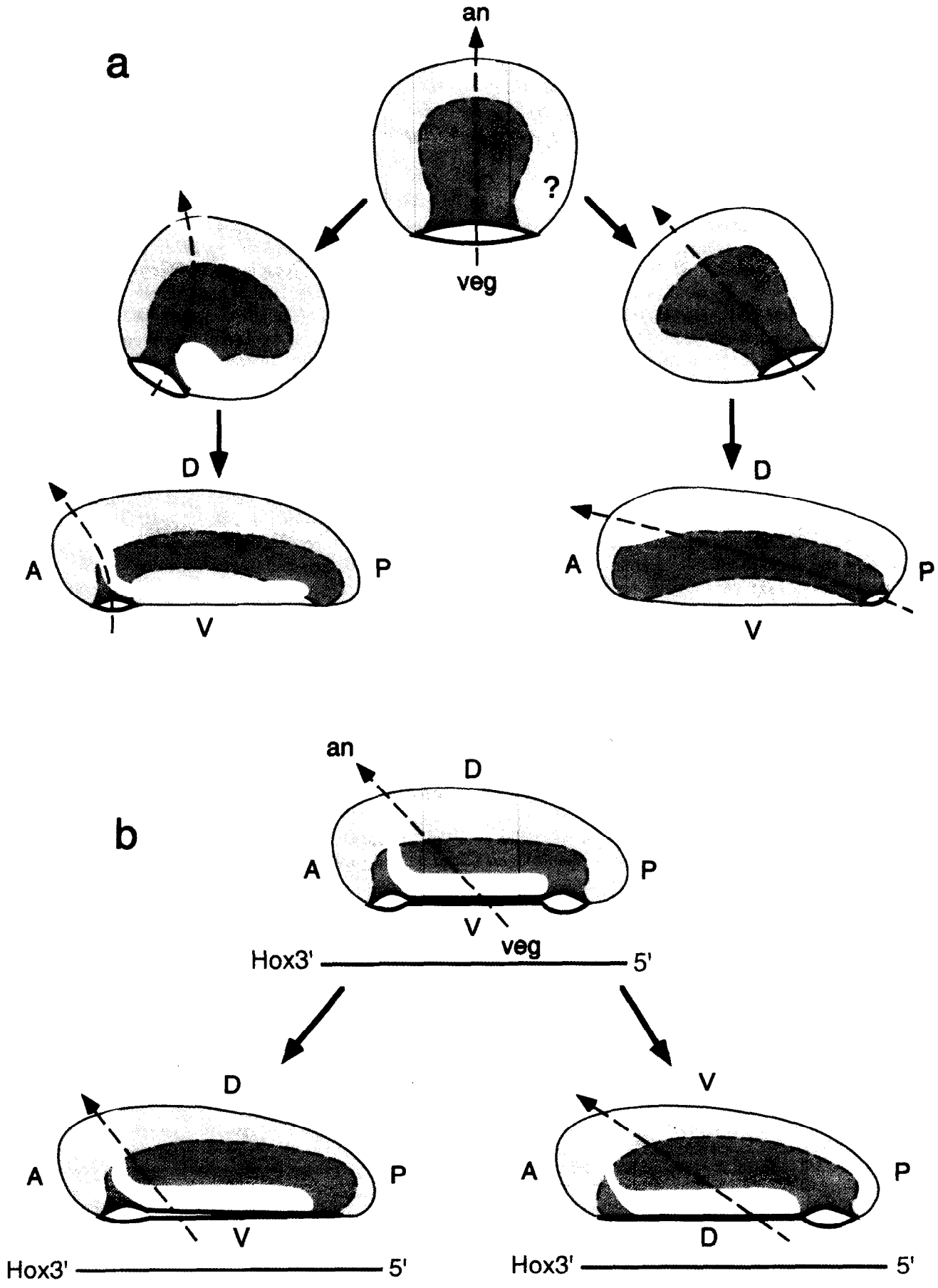
Fig. 5b shows a modified concept for the evolution of protostome and deuterostome animals, whereby the longitudinal body axis is being maintained. Here, the common ancestor of protostome and deuterostome animals already formed a digestive tube with a mouth and an anus. During its gastrulation, the blastopore lies neither anterior nor posterior, but spans the whole length of the body (see also Jägersten, 1955; Remane, 1967; Siewing, 1976; Fioroni, 1980; Gruner, 1993), as was first suggested by Sedgwick (1884). Today, such an elongated blastopore is observed e.g. in polychaete annelids (Fig. 4d) (see e.g. Anderson, 1973; Dorresteijn et al., 1993), where the lateral blastopore lips advance around the vegetal hemisphere to finally fuse along the midline of the *neural* body side, and thereby form the gut with its anterior and posterior opening. The longitudinal blastopore thus parallels the A-P orientation of the later gut, and therefore foreshadows the A-P body axis. The two openings anterior and posterior to the line of fusion form the later mouth and anus. We refer to this type of gastrulation pattern as amphistomy. Amphistomy is found in various polychaetes (e.g. *Polygordius*; Fig. 4d) and in onychophorans (Siewing, 1976; Fioroni, 1980). From an amphistome situation we can conceptually derive both protostomy and deuterostomy, while maintaining the longitidinal body axes, with the posterior part of the longitudinal blastopore slit closing in protostomes, and its anterior part closing in deuterostomes (Fig. 5b). This rather simple derivation of deuterostomy from an amphistome situation would also help to explain the unexpected deuterostomy seen in the mollusc *Viviparus* (Fig. 4c) and in the polychaete *Eunice*, that both belong to Grobben's taxon '*Protostomia*' (reviewed in Fioroni, 1980). A deuterostome gastrulation mode thus has evolved independently at least twice within the '*Protostomia*', as well as at least once at the base of the '*Deuterostomia*'. It appears that deuterostomy evolved from an amphistome pattern in each case.

3.2. Textbook drawings turn the vertebrate embryo onto its belly

Assuming that amphistomy is ancestral for deuterostome chordates, is there any reminiscence of this amphistome gastrulation left in today's chordates' deuterostome gastrulation? To obtain deuterostomy from an amphistome situation, the blastopore could close from anterior to posterior to form the anus at the embryo's posterior end (Fig. 5b). And indeed, during chordate gastrulation, the anterior/dorsal lip of the closing blastopore moves from anterior to posterior, while the posterior/ventral lip retains its posterior position. This is clearly seen in ascidians, where the anterior/dorsal blastopore lip grows to the posterior end of the embryo during gastrulation (Conklin, 1905). Note that in the lower chordates, as well as in fish and amphibians, the 'dorsal blastopore lip' can as well be called 'anterior blastopore lip', because the prospective anterior tissues and the prospective dorsal axial tissues locate to the same side of the animal hemisphere (Fig. 2b) (cf. Conklin, 1932). In a gastrulating amniote, it is Hensen's node (which corresponds to the anterior/dorsal blastopore lip: see e.g. Izpisúa-Belmonte et al., 1993) that moves from anterior to posterior along the later A-P body axis. A similar movement can also be observed in amphibians (Keller et al., 1992).

In usual textbook drawings of amphibian gastrulation, however, this posteriorly directed movement of the anterior/dorsal blastopore lip goes unnoticed because of the manner in which gastrulation of the embryo is depicted. The process of gastrulation is usually shown through a series of drawings which hold the anterior/dorsal blastopore lip at a constant position as a reference point. With this as a fixed point it is then necessary to turn other regions of the gastrulating embryo such that the an-veg axis, together with the future body axes, become rotated (Fig. 6a) (cf. Gilbert, 1994). Yet this representation holds still the region of the embryo undergoing much cell movement, while turning the regions that see the least amount of movements. In Fig. 6b, we instead depict the gastrulating amphibian by holding as a fixed point of reference those

Fig. 5. Two divergent concepts for the evolution of protostomy (to the left) and of deuterostomy (to the right) from a common ancestor (middle). All stages after gastrulation. Bold lines indicate blastopore margins. Blue, epidermal ectoderm; yellow, neuroectoderm; green, endoderm. A, anterior; P, posterior; D, dorsal; V, ventral. (a) '*Protostomia-Deuterostomia* concept': interpretative drawing after Grobben (1908) and Claus et al. (1932). The common ancestor of the '*Protostomia*' and of the '*Deuterostomia*' was viewed to resemble a double-layered, gastrula-like organism with a blastopore-like opening. (Left) In the '*Protostomia*', the blastopore shifts towards an anterior-ventral position and forms only the mouth. The primary an-veg axis is thus 'bent towards the (future) ventral side' (Grobben, 1908), so that the longitudinal A-P axis of the embryo forms approximately rectangular to the former an-veg axis. (Right) In the '*Deuterostomia*', the blastopore forms the anus. The A-P axis thus corresponds to the former an-veg axis. Dashed arrows represent the former an-veg axis of the egg. Note that when comparing the '*Protostomia*' and the '*Deuterostomia*' the A-P axes show different orientations with respect to the an-veg axes. Grobben leaves open the question whether the common ancestor had a localiized neuroectoderm. (b) Modified concept taking into account the conserved colinear expression of the *Hox*-genes in the '*Protostomia*' and in the '*Deuterostomia*' (indicated by Hox3'-5'). The putative common ancestor forms a longitudinal blastopore cleft leaving mouth and anus at opposite ends (amphistomy). (Left) In most protostome animals, the blastopore cleft is restricted towards the anterior and forms only the future mouth. (Right) In deuterostome animals, the blastopore cleft is restricted towards the posterior and forms only the future anus. D, V refer to the inverted chordate situation. Note that the A-P body axis of the putative common ancestor is maintained in both protostome and deuterostome animals.


a
an
?
veg
D
A
P
V
D
A
P
V
b
an
D
A
P
V
veg
Hox3'
5'
D
A
P
V
Hox3'
5'
V
A
P
D
Hox3'
5'

regions with the least amount of cell movements, such as, for example, the forebrain region close to the animal pole. The future body axes of the embryo are then held constant (Fig. 6b). This representation shows how the anterior/dorsal blastopore lip moves towards the posterior end of the embryo (Fig. 6b,c) (Keller et al., 1992, their Fig. 3). As a matter of course, depicting gastrulation in this way results in the embryo lying somewhat awkwardly, though significantly, on its back.

3.3. The annelid and chordate neural body side forms by epiboly, with lateral cells moving towards the neural midline

In the blastula of a polychaete annelid, the neuroectoderm of the later ventral nerve cord maps to the lateral blastopore margins (Figs. 2 and 7a). During gastrulation, these lateral blastopore margins move around the vegetal hemisphere, thereby enclosing endodermal and mesodermal cells to finally meet along the midline. The two halves of the neuroectoderm then fuse to form the *neural* body side (Fig. 7a). Such a movement of ectodermal cells in animal-vegetal direction is called epiboly (see e.g. Gilbert, 1994).

In the amphibian blastula the neuroectoderm maps to the anterior and lateral blastopore margins (Figs. 2b and 7b). During gastrulation, the anterior/dorsal blastopore lip moves around the vegetal hemisphere towards the posterior, while the posterior/ventral lip shows only scarce movements. The lateral blastopore lips, however, also move and close around the vegetal hemisphere, so that the blastopore opening is progressively constricted towards the posterior. The future neural plate thereby narrows in the lateral-medial direction, and elongates in the anterior-posterior direction (convergence and extension; see e.g. Keller et al., 1992). Epiboly during amphibian gastrulation thus involves both an anterior to posterior, as well as a lateral to medial movement of neuroectodermal tissues around the internalizing vegetal hemisphere (Fig. 7b).

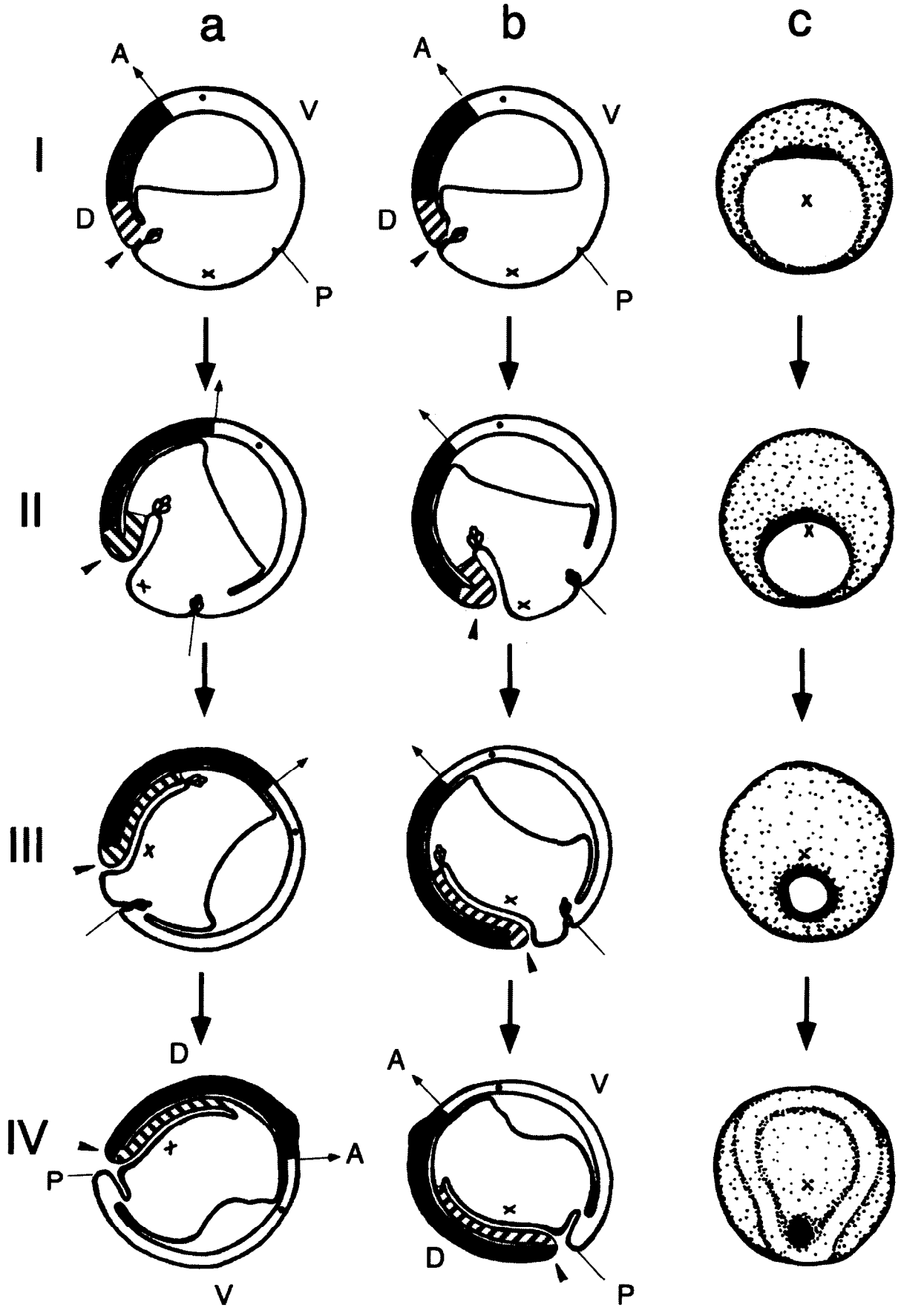
In gastrulating annelids and amphibians the *neural* body side thus forms in a similar way, that is by epiboly around the internalizing vegetal hemisphere. Lateral cells thereby move towards the prospective midline of the *neural* body side in both groups. Cellular mechanisms of epiboly also seem to be rather similar between annelids and vertebrates (Smith et al., 1996), in that in leeches and in polychaetes, as well as in fish the cytoskeleton of the vegetal cytoplasm is involved in generating forces that pull the blastopore margins towards the vegetal pole (Trinkaus, 1984; Strahle and Jesuthasan, 1993; Solnica-Krezel and Driever, 1994; Smith et al., 1996).

3.4. Homology of insect and vertebrate midline cells

In both insects and vertebrates a specific population of midline cells marks the median line of their *neural* body sides that corresponds to the line of fusion of the lateral blastopore lips in the amphistome situation (Fig. 7a). Naef (1927), after an analysis of cell movements during newt gastrulation, had already proposed that the midline of the amphibian neural plate corresponds to the line of fusion of the lateral blastopore margins in an amphistome ancestor. In keeping with this idea, molecular data now reveal that the insect and vertebrate midline cells have more in common than their comparable positions (Arendt and Nübler-Jung, 1996). For example, in both insects and vertebrates midline cells of the CNS share a common signaling molecule called *netrin*, that plays a key role in the guidance of commissural axons (Klämbt et al., 1991; Davies, 1994). An orthologous *netrin* gene is expressed in the ventral nerve cord in nematodes (Colomarino and Tessier-Lavigne, 1995). Another pair of orthologous genes expressed in insect and vertebrate midline cells are *short gastrulation* in *Drosophila* which is confined to the midline cells by germ band extension (François et al., 1994), and its *Xenopus* counterpart, *chordin*, which resolves to midline cells that form the prechordal plate and the notochord (Sasai et al., 1994; Holley et al., 1995; Sasai et al., 1995; Schmidt et al., 1995).

Moreover, insect and vertebrate midline cells are similar in that they have mesodermal as well as ectodermal properties. In *Drosophila*, the midline cells are said to derive from the mesoderm (Beer et al., 1987), yet develop neural properties (reviewed in Wright, 1995). In vertebrates, the population of midline cells may comprise the ectodermal floorplate as well as the mesodermal notochord, as deduced from the common expression of *fork head*-related and *hedgehog*-related genes in both structures (Echelard et al., 1993; Krauss et al., 1993; Roelink et al., 1994; Arendt and Nübler-Jung, 1996). This ambiguity could be reminiscent of an amphistome gastrulation pattern. In the amphistome situation, prospective midline cells lie along the

Fig. 6. Different manners of depicting amphibian gastrulation. (a) In usual textbook drawings the anterior blastopore margin (arrowhead) is held at a fixed position as a reference point, such that the later A-P body axis (arrow) together with the animal (dots) and vegetal (crosses) poles become rotated. (b) Alternatively, the A-P body axis, as well as the animal and vegetal pole regions are kept fixed, such that the embryo comes to lie on its back. (c) The vegetal view shows how the anterior blastopore margin moves from anterior to posterior around the (internalizing) vegetal hemisphere, leaving behind the neural plate. Movies nicely showing this movement are available on the Internet (http://www.library.wisc.edu/guides/biology/demo/frog2/gastxen/xlgast.mov). Black, prospective forebrain; shaded, neuroectoderm; striped, future notochord. A, anterior; P, posterior; D, dorsal; V, ventral. (a,b) modified after Keller et al. (1992); (c) after Balinsky (1981).


a
b
c
I
II
III
IV
A
V
D
P

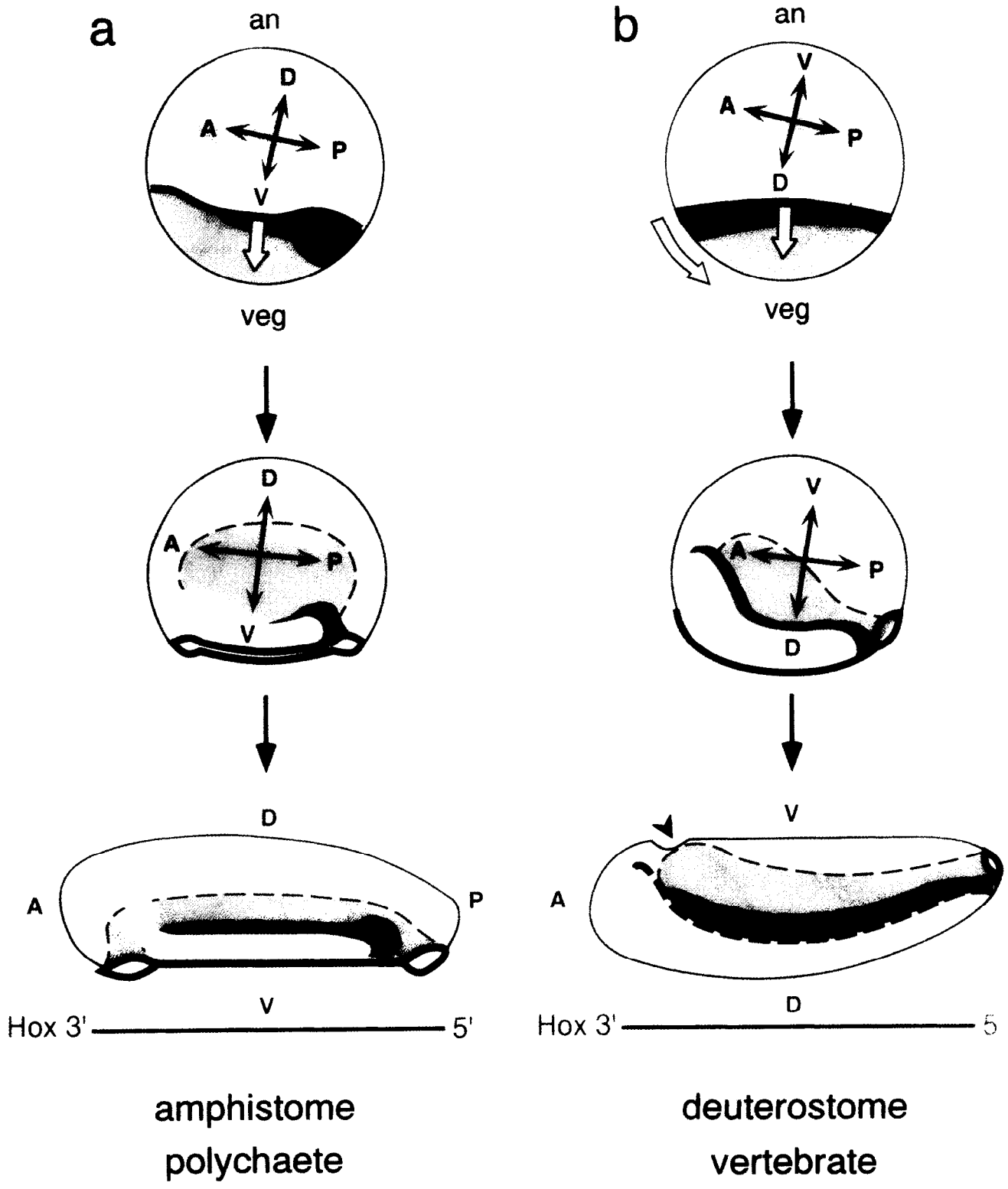


Fig. 7. Similar formation of *neural* body sides by epiboly around the former vegetal hemisphere (open arrows). Comparable developmental stages at late blastula (upper panels), shortly after gastrulation (middle) and at the phylotypic stage (lower panels). In the amphistome polychaete (a, *Polygordius*) the lateral blastopore margins (bold line) fuse along the later *neural* midline. In the deuterostome vertebrate (b, *Xenopus*) the bold line is the line along which the lateral blastopore margins probably fused in ancestral chordates to form the midline of the neural plate. During neurulation, the midline is internalized and forms the median floor plate of the neural tube (dashed bold line in lower panel). Note that the definite mouth forms on the later ventral side (arrowhead; see Nübler-Jung and Arendt, 1994). Blue, epidermal ectoderm; yellow, neuroectoderm; red, mesoderm; green, endoderm. Red arrows indicate orientation of the future A-P and D-V body axes in the ectoderm. *Hox* gene expression depicted by (Hox3'-5').

closing lateral blastopore margins, where they form an interface between the external ectoderm and the internalized mesoderm. The double affinity of insect and vertebrate midline cells to mesoderm and to ectoderm could reflect such an intermediate position, and lends additional support to the proposal that amphistomy is ancestral for the deuterostome chordates.

4. Conclusion

In annelids, arthropods and chordates cleavage generates a similar sequence of epidermal ectoderm, neuroectoderm, mesoderm and endoderm along the an-veg axis of the egg (Fig. 1). In spite of its conserved position between the mesoderm and the prospective epidermis, the prospective *neural* region of the blastula will later be called 'ventral' in annelids, and 'dorsal' in vertebrates (Fig. 2). We have proposed before that this inverse situation be the result of an inversion of the D-V axis during chordate evolution (Arendt and Nübler-Jung, 1994). To avoid misleading comparisons of body sides in annelids, insects and vertebrates, we use the term '*neural*' for their later nerve-cord bearing body side.

Based on a comparison of gastrulation patterns, we propose that the last common bilaterian ancestor of insects, annelids and vertebrates formed a longitudinal blastopore (Fig. 5b). During gastrulation the lateral blastopore margins approached each other to eventually fuse along the midline of the prospective *neural* body side, leaving open anteriorly the mouth and posteriorly the anus. This 'amphistome' gastrulation mode is still seen in extant polychaetes. Reminiscent of such an amphistome gastrulation mode may be that during chordate gastrulation lateral cells move towards the midline of the *neural* body side (Fig. 7). Given that amphistomy be ancestral for insects, annelids and vertebrates alike, these *neural* midline cells would then mark the ancestral line of fusion of the lateral blastopore lips.

Taken together, the comparison of fate maps and of gastrulation patterns reveals that developmental strategies such as the formation of the dorsal-ventral body axis from an initial an-veg asymmetry in the egg, and the various modes of gastrulation seen in annelid, arthropod and chordate embryos are surprisingly similar. Interphyletic comparisons may therefore allow us to eventually propound a 'model embryogenesis' valid for various bilaterians, and may in turn lead to a better understanding of more divergent modes of embryogenesis that evolution has derived from this general theme.

Acknowledgements

We thank H. Jäckle, J.P. Forjanic, K. Sander, U. Schmidt-Ott and an unknown reviewer for very helpful comments and suggestions during the elaboration of our ideas.

References

Anderson, D.T. (1972) The development of hemimetabolous insects. In Counce, S.J. and Waddington, C.H. (eds.), Developmental Systems: Insects, Vol. 1, Academic Press, London.

Anderson, D.T. (1973) Embryology and Phylogeny in Annelids and Arthropods, Pergamon Press, Oxford.

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

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

Balinsky, B.I. (1982) An Introductyion to Embryology, 5th. edn. Saunders, Philadelphia, PA.

Beer, J., Technau, G. and Campos-Ortega, J.A. (1987) Lineage analysis of transplanted individual cells in embryos of *Drosophila* melanogaster. IV. Commitment and proliferative capabilities of mesodermal cells. Roux's Arch. Dev. Biol. 196, 222–230.

Boncinelli, E., Gulisano, M. and Broccoli, V. (1993) *Emx* and *Otx* homeobox genes in the developing mouse brain. J. Neurobiol. 24, 1356–1366.

Brusca, R.C. and Brusca, G.J. (1990) Invertebrates, Sinauer, Sunderland, MA.

Chasan, R. and Anderson, K.V. (1993) Maternal control of dorsal-ventral polarity and pattern in the embryo. In Bate, M. and Martinez-Arias, A. (eds.), The Development of *Drosophila melanogaster*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 387–423.

Childs, S.R. and O'Connor, M.B. (1994) Two domains of the *tolloid* protein contribute to its unusual genetic interaction with *decapentaplegic*. Dev. Biol. 162, 209–220.

Claus, C., Grobben, K. and Kühn, A. (1932) Lehrbuch der Zoologie, 10th edn., Verlag Julius Springer, Berlin.

Colomarino, S.A. and Tessier-Lavigne, M.(1995) The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons. Cell 81, 621–629.

Conklin, E.G. (1905) Mosaic development in ascidian eggs. J. Exp. Zool. 2, 145–224.

Conklin, E.G. (1932) The embryology of amphioxus. J. Morphol. 54, 69–151.

Dalton, D., Chadwick, R. and McGinnis, W. (1989) Expression and embryonic function of *empty spiracles*: a *Drosophila* homeobox gene with two patterning functions on the anterior-posterior axis of the embryo. Genes Dev. 3, 1940–1956.

Davies, A.M. (1994) Chemoattractants for navigating axons. Curr. Biol. 4, 1142–1145.

De Robertis, E.M. and Sasai, Y. (1996) A common plan for dorsoventral patterning in Bilateria. Nature 380, 37–40.

Dickinson, W.J. (1995) Molecules and phylogeny: where's the homology? Trends Genet. 11, 119–121.

Dohrn, A. (1875) Der Ursprung der Wirbelthiere und das Princip des Functionswechsels, Verlag von Wilhelm Engelmann, Leipzig.

Dorresteijn, A.W.C., O'Grady, B., Fischer, A., Porchet-Henneré E. and Boilly-Marer, Y. (1993) Molecular specification of cell lines in the embryo of *Platynereis* (*Annelida*). Roux's Arch. Dev. Biol. 202, 260–269.

Echelard, Y., Epstein, D.J., St. Jaques, B., Shen, L., Mohler, J., McMahon, J.A. and McMahon, A.P. (1993) *Sonic hedgehog*, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75, 1417–1430.

Fainsod, A., Steinbeisser, H. and De Robertis, E.M. (1994) On the function of *BMP-4* in patterning the marginal zone of the *Xenopus* embryo. EMBO J. 13, 5015–5025.

Ferguson, E.L. and Anderson, K.V. (1992a) *decapentaplegic* acts as a morphogen to organize dorsal-ventral pattern in the *Drosophila* embryo. Cell 71, 451–461.

Ferguson, E.L. and Anderson, K.V. (1992b) Localized enhancement and repression of the activity of the TGF-β family member, *decapentaple-*

gic, is necessary for dorsal-ventral pattern formation in the *Drosophila* embryo. Development 114, 583–597.

Finkelstein, R., Smouse, D., Capaci, T.M., Spradling, A.C. and Perrimon, N. (1990) The *orthodenticle* gene encodes a novel homeodomain protein involved in the development of the *Drosophila* nervous system and ocellar visual structures. Genes Dev. 4, 1516–1527.

Fioroni, P. (1980) Zur Signifikanz des Blastoporus-Verhaltens in evolutiver Sicht. Rev. Suisse Zool. 87, 261–272.

François V., Solloway, M., O'Neill, J.W., Emery, J. and Bier, E. (1994) Dorsal-ventral patterning of the *Drosophila* embryo depends on a putative negative growth factor encoded by the *short gastrulation* gene. Genes Dev. 8, 2602–2616.

François V., Bier, E. (1995) *Xenopus chordin* and *Drosophila short gastrulation* genes encode homologous proteins functioning in dorsal-ventral axis formation. Cell 80, 19–20.

Frumkin, A., Rangini, Z., Ben-Yehuda, A., Gruenbaum, Y. and Fainsod, A. (1991) A chicken *caudal* homologue, *CHox-cad*, is expressed in the epiblast with posterior localization and in the early endodermal lineage. Development 112, 207–219.

Fukagawa, M., Suzuki, N., Hogan, B.L.M. and Jones, C.M. (1994) Embryonic expression of mouse Bone Morphogenetic Protein-1 (BMP-1), which is related to the *Drosophila* dorsoventral gene *tolloid* and encodes a putative Astacin metalloendopeptidase. Dev. Biol. 163, 175–183.

Gilbert, S.F. (1994) Developmental Biology, 4th edn., Sinauer, Sunderland, MA.

Grobben, K. (1908) Die systematische Einteilung des Tierreichs. Verh. Zool. Bot. Ges. Wien 58, 491–511.

Gruner, H.-E. (1993) Möglichkeiten der Grossgliederung des Tierreichs. In Kaestner, Lehrbuch der speziellen Zoologie. Bd. 1: Wirbellose Tiere. 1. Tl. Fischer Verlag, Jena.

Hammerschmidt, M. and Nüsslein-Volhard, C. (1993) The expression of a zebrafish gene homologous to *Drosophila snail* suggests a conserved function in invertebrate and vertebrate gastrulation. Development 119, 1107–1118.

Hartenstein, V. (1993) Atlas of *Drosophila* development. In: Bate, M. and Martinez-Arias, A. (eds.), The Development of *Drosophila melanogaster*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 387–423.

Hartenstein, V., Technau, G.M. and Campos-Ortega, J.A. (1985) Fate-mapping in wild-type *Drosophila melanogaster* III. A fate map of the blastoderm. Roux's Arch. Dev. Biol., 194, 213–216.

Hawley, S.H.B., Wünnenberg-Stapleton, K., Hashimoto, C., Laurent, M.N., Watabe, T., Blumberg, B.W. and Cho, K.W.Y. (1995) Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction. Genes Dev. 9, 2923–2935.

Hogan, B.L.M. (1995) Upside-down ideas vindicated. Nature 376, 210–211.

Hogan, B.L.M., Blessing, M., Winnier, G.E., Suzuki, N. and Jones, C.M. (1994) Growth factors and development: the role of TGF-β related polypeptide signalling molecules in embryogenesis. Development Suppl. 1994, 53–60.

Holley, S.A., Jackson, P.D., Sasai, Y., Lu, B., De Robertis, E.M., Hoffmann, F.M. and Ferguson, E.L. (1995) A conserved system for dorsal-ventral patterning in insects and vertebrates involving *sog* and *chordin*. Nature 376, 249–253.

Hyman, L.S. (1940) The Invertebrates, Vol. 1: Protozoa through Ctenophora, McGraw-Hill, New York.

Ip, Y.T., Park, R.E., Kosman, D., Yazdanbakhsh, K. and Levine, M. (1992) Dorsal-twist interactions establish *snail* expression in the presumptive mesoderm of the *Drosophila* embryo. Genes Dev. 6, 1518–1530.

Izpisúa-Belmonte, J.C., De Robertis, E.M., Storey, K.G. and Stern, C.D. (1993) The homeobox gene *goosecoid* and the origin of organizer cells in the early chick blastoderm. Cell 74, 645–659.

Jägersten, G. (1955) On the early phylogeny of the Metazoa. Zool. Bidr. Uppsala 30, 321–354.

Joly, J.-S., Maury, M., Joly, C., Duprey, P., Boulekbache, H. and Condamine, H. (1992) Expression of a zebrafish *caudal* homeobox gene correlates with the establishment of posterior cell lineages at gastrulation. Differentiation 50, 75–87.

Jones, C.M. and Smith, J.C. (1995) Revolving vertebrates. Curr. Biol. 5, 574–576.

Jones C.M., Dale, L., Hogan, B.L.M., Wright, C.V.E. and Smith, J. (1996) Bone morphogenetic protein-4 (BMP-4) acts during gastrula stages to cause ventralization of *Xenopus* embryos. Development 122, 1545–1554.

Jura, C. (1972) Development of apterygote insects. In Counce, S.J. and Waddington, C.H. (eds.) Developmental Systems: Insects. Vol. 1. Academic Press, London, pp. 49–94.

Keller, R.E. (1975) Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layer. Dev. Biol. 42, 222–241.

Keller, R., Shih, J. and Sater, A. (1992) The cellular basis of the convergence and extension of the *Xenopus* neural plate. Dev. Dyn., 193, 199–217.

Kelley, C., Yee, K., Harland, R. and Zon, L.I. (1994) Ventral expression of *GATA-1* and *GATA-2* in the *Xenopus* embryo defines induction of hematopoietic mesoderm. Dev. Biol. 165, 193–205.

Klämbt, C., Jacobs, R.J. and Goodman, C.S. (1991) The midline of the *Drosophila* CNS: model and genetic analysis of cell lineage, cell migration and development of commissural axon pathways. Cell 64, 801–815.

Krauss, S, Concordet, J.P. and Ingham, P.W (1993) A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. Cell 75, 1431–1444.

Kühn, A. (1913) Die Sonderung der Keimesbezirke in der Entwicklung der Sommereier von *Polyphemus pediculus* de Geer. Zool. Jahrb. Abt. Anat. Ontog. Tiere 35, 243–340.

Leptin, M. (1991) *twist* and *snail* as positive and negative regulators during *Drosophila* mesoderm development. Genes Dev. 5, 1568–1576.

Maéno, M., Xue, Y., Wood, T.I., Ong, R.C. and Kung, H.-F. (1993) Cloning and expression of cDNA encoding *Xenopus laevis* bone morphogenetic protein-1 during early embryonic development. Gene 134, 257–261.

Maéno, M., Ong, R.C., Suzuki, A., Ueno, N. and Kung, H.-F. (1994) A truncated bone morphogenetic protein 4 receptor alters the fate of ventral mesoderm to dorsal mesoderm: roles of animal pole tissue in the development of ventral mesoderm. Proc. Natl. Acad. Sci. USA 91, 10260–10264.

Mayor, R., Essex, L.J., Bennett, M.F. and Sargent, M.G. (1993) Distinct elements of the *xsna* promoter are required for mesodermal and ectodermal expression. Development 119, 661–671.

Meyer, B.I. and Gruss, P. (1993) Mouse *Cdx-1* expression during gastrulation. Development 117, 191–203.

Mlodzik, M., Fjose, A. and Gehring, W.J. (1985) Isolation of *caudal*, a *Drosophila* homeo box-containing gene with maternal expression, whose transcripts form a concentration gradient at the pre-blastoderm stage. EMBO J. 4, 2961–2969.

Naef, A. (1927) Notizen zur Morphologie und Stammesgeschichte der Wirbeltiere. 14. Über Blastoporusverschluss und Schwanzknospenanlage bei den Anamniern. Zool. Jahrb. Abt. Anat. Ontog. Tiere 49, 357–390.

Nielsen, C. (1995) Animal Evolution: Interrelationships of the Living Phyla. Oxford University Press, Oxford.

Nieto, M.A., Bennett, M.F., Sargent, M.G. and Wilkinson, D.G. (1992) Cloning and developmental expression of *Sna*, a murine homologue of the *Drosophila snail* gene. Development 116, 227–237.

Nieuwkoop, P.D. (1969) The formation of the mesoderm in urodelean amphibians. II. The origin of dorsoventral polarity of the mesoderm. Roux's Arch. Dev Biol. 162, 341–373.

Nübler-Jung, K. and Arendt, D. (1994) Is ventral in insects dorsal in

vertebrates? A history of embryological arguments favouring axis inversion in chordate ancestors. Roux's Arch. Dev. Biol. 203, 357–366.

Ramain, P., Heitzler, P., Haenlin, M. and Simpson, P. (1993) *pannier*, a negative regulator of *achaete* and *scute* in *Drosophila*, encodes a zinc finger protein with homology to the vertebrate transcription factor *GATA-1*. Development 119, 1277–1291.

Ray, R.P., Arora, K., Nüsslein-Volhard, C. and Gelbart, W.M. (1991) The control of cell fate along the dorsal-ventral axis of the *Drosophila* embryo. Development 113, 35–54.

Remane, A. (1952) Die Grundlagen des natürlich Systems. Geest und Portig, Leipzig.

Remane, A. (1967) Die Geschichte der Tiere. In: Heberer, G. (ed.), Die Evolution der Organismen I. Gustav Fischer Verlag, Stuttgart, pp. 589–677.

Roelink, H., Augsburger, A., Heemskerk, J., Korzh, V., Norlin, S., Ruiz i Altaba, A., Tanabe, Y., Placzek, M., Edlund, T., Jessell, T.M. and Dodd, J. (1994) Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of *hedgehog* expressed by the notochord. Cell 76, 761–775.

Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L.K. and De Robertis, E.M. (1994) *Xenopus chordin*: a novel dorsalizing factor activated by organizer-specific homeobox genes. Cell 79, 779–790.

Sasai, Y., Lu, B., Steinbeisser, H. and De Robertis, E.M. (1995) Regulation of neural induction by the *Chd* and *BMP-4* antagonistic patterning signals in *Xenopus*. Nature 376, 333–336.

Schmidt, J.E., Suzuki, A., Ueno, N. and Kimelman, D. (1995) Localized BMP-4 mediates dorsal/ventral patterning in the early *Xenopus* embryo. Dev. Biol. 169, 37–50.

Sedgwick, A. (1884) On the origin of metameric segmentation and some other morphological questions. Q. J. Microsc. Sci. 24, 43–82.

Shimell, M.J., Ferguson, E.L., Childs, S.R. and O'Connor, M.B. (1991) The *Drosophila* dorsal-ventral patterning gene *tolloid* is related to human Bone Morphogenetic protein 1. Cell 67, 469–481.

Siewing, R. (1976) Probleme und neuere Erkenntnisse in der Grosssystematik der Wirbellosen. Verh. Dtsch. Zool. Ges., 1976, 59–83.

Slack, J.M.W., Holland, P.W.H. and Graham, C.F. (1993) The zootype and the phylotypic stage. Nature 361, 490–492.

Smith, D.E., Franco Del Amo, F. and Gridley, T. (1992) Isolation of *Sna*, a mouse gene homologous to the *Drosophila* genes *snail* and *escargot*: its expression pattern suggests multiple roles during postimplantation development. Development 116, 1033–1039.

Smith, C.M., Lans, D. and Weisblat, D.A. (1996) Cellular mechanisms of epiboly in leech embryos. Development 122, 1885–1894.

Solnica-Krezel, L. and Driever, W. (1994) Microtubule arrays of the zebrafish yolk cell: organization and function during epiboly. Development 120, 2443–2455.

Sommer, R.J. and Tautz, D. (1994) Expression patterns of *twist* and *snail* in *Tribolium* (Coleoptera) suggest a homologous formation of mesoderm in long and short germ band insects. Dev. Genet. 15, 32–37.

Strahle, U, and Jesuthasan, S. (1993) Ultraviolet irradiation impairs epiboly in zebrafish embryos: evidence for a microtubule-dependent mechanism of epiboly. Development 119, 909–919.

Striebel, H. (1960) Zur Embryonalentwicklung der Termiten. Acta Trop. 17, 193–260.

Suzuki, A., Thies, R.S., Yamaji, N., Song, J.J., Wozney, J.M., Murakami, K. and Ueno, N. (1994) A truncated bone morphogenetic protein receptor affects dorsal-ventral patterning in the early *Xenopus* embryo. Proc. Natl. Acad. Sci. USA 91, 10255–10259.

Suzuki, A., Shioda, N. and Ueno, N. (1995) Bone morphogenetic protein acts as a ventral mesoderm modifier in early *Xenopus* embryos. Dev. Growth Differ. 37, 581–588.

Trinkaus, J.P. (1984) Mechanisms of *Fundulus* epiboly: a current view. Am. Zool. 24, 673–688.

Tung, T.C., Wu, S.C. and Tung, Y.Y.F. (1962) The presumptive areas of the egg of amphioxus. Sci. Sin. 11(5), 629–644.

Van der Horst, C.J. (1939) *Hemichordata*. In Dr. H.G. Bronns Klassen und Ordnungen des Tierreiches, Bd. 4. Akademische Verlagsgesellschaft, Leipzig, pp. 277–326.

Walmsley, M.E., Guille, M.J., Bertwistle, D., Smith, J.C., Pizzey, J.A. and Patient, R.K. (1994) Negative control of *Xenopus GATA-2* by activin and noggin with eventual expression in precursors of the ventral blood islands. Development 120, 2519–2529.

Wharton, K.A., Ray, R.P. and Gelbart, W.M. (1993) An activity gradient of *decapentaplegic* is necessary for the specification of dorsal pattern elements in the *Drosophila* embryo. Development 117, 807–822.

Willmer, C.H. (1990) Invertebrate Relationships, Cambridge University Press, Cambridge.

Wilson, P. and Hemmati-Brivanlou, A. (1995) Induction of epidermis and inhibition of neural fate by BMP-4. Nature 376, 331–333.

Winick, J., Abel., T., Leonard, M.W., Michelson, A.M., Chardon-Loriaux, I., Holmgren, R.A., Maniatis, T. and Engel, J.D. (1993) A *GATA* family transcription factor is expressed along the embryonic dorsoventral axis in *Drosophila melanogaster*. Development 119, 1055–1065.

Woltereck, R. (1904) Beiträge zur praktischen Analyse der *Polygordius*-Entwicklung nach dem 'Nordsee-' und dem 'Mittelmeer-' Typus. I. Der für beide Typen gleich verlaufende Entwicklungsabschnitt. Vom Ei bis zum jüngsten Trochophora-Stadium. Roux's Arch. Dev. Biol. 18, 377–403.

Wright, D. (1995) Neurons from the wrong side of the tracks? J. NIH Res. 7, 34–3.

Xu, R.-H., Kim, J., Taira, M., Zhan, S., Sredni, D. and Kung, H.-F. (1995) A dominant negative bone morphogenetic protein 4 receptor causes neuralization in *Xenopus* ectoderm. Biochem. Biophys. Res. Commun. 212, 212–219.