

The mouth, the anus, and the blastopore—open questions about questionable openings

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Gastrulation is one of the major events during the embryogenesis of an animal. In addition to the formation of the germ layers it is often the time when the future axial properties and digestive openings become apparent, and it is not surprising that this event plays an important role in hypotheses regarding metazoan evolution. A major difference between these theories concerns the structure of the alimentary canal and the relationship of its openings to the blastopore of the last common bilaterian ancestor. Here we review competing theories of bilaterian evolution and evaluate their plausibility in the light of recent insights into metazoan phylogeny and development.

4.1 Gastrulation as a process determining multiple body plan characteristics

The evolution of an internal germ layer enabled the compartmentalization of the body of multicellular animals (Metazoa) into a digestive cavity (endoderm) and an outer layer, the integument (ectoderm). The developmental process that separates the inner from the outer cell populations is called gastrulation. During gastrulation, cells or cell layers are internalized and later form the digestive epithelium and often also germ cells. Bilaterians have in addition a third germ layer, the mesoderm, which either separates from the endoderm or ingresses as independent precursors. Gastrulation not only generates distinct cell types, it also establishes the organismal axes from a pre-existing animal–vegetal embryonic axis. One can thus picture gastrulation as

a fundamental event in every organism as it determines the main body features during development. Consequently, gastrulation plays a major role in hypotheses regarding the transition from a radially symmetrical diploblastic animal to a bilaterally symmetrical triploblastic organism with mesoderm, the last common bilaterian ancestor.

4.2 The blastopore as the site of internalization

In most animals the site of internalization of gastrulating cells is limited to a specific area, the blastopore. The fate of this site is often not only the area of germ layer specification, it sometimes becomes the connection of the endodermal digestive cavity to the ectoderm and thus to the animal's environment. This connection is usually called either the 'mouth' if the organism has only a single opening to the gut or if it is the anterior opening of a through gut, or the 'anus' when it corresponds to the posterior opening and functions as a site of excretion. Grobden (1908) subdivided the bilaterian clade into the taxa Protostomia and Deuterostomia based on the fate of the blastopore becoming either the mouth or the anus. Grobden claimed that in most protostomes ('first mouth') the blastopore becomes the mouth, and the anus is formed secondarily at a different site, later during embryogenesis. In contrast to protostomes, in deuterostome embryos the site of gastrulation becomes the anus while the mouth is formed at a different site in the animal hemisphere of the embryo (Figure 4.1). The blastopore becoming the anus appears to be ancestral for the

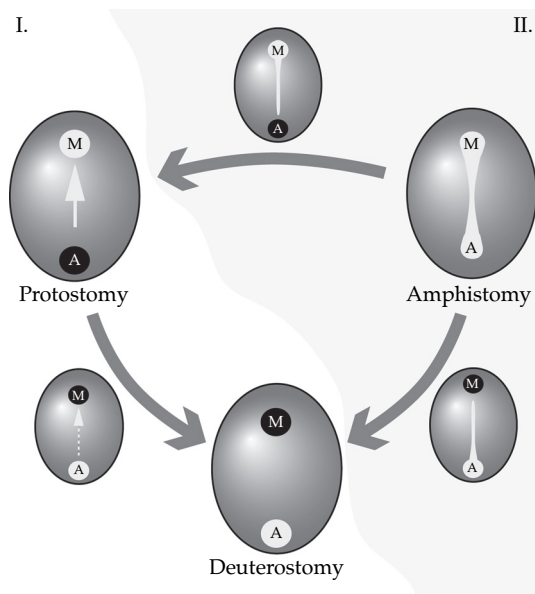


Figure 4.1 Protostomy, deuterostomy, and amphistomy and evolutionary scenarios about the direction of change. In protostomy, the blastopore (bright in all figures) is displaced from posterior to antero-ventral by morphogenetic movements (bright arrow), and gives rise to the mouth (M). The anus (A), if present, is formed at a different site at the posterior end of the embryo. In deuterostomy the blastopore stays at the posterior site of the embryo and either closes or gives rise to the anus. The mouth is formed at a different site from the former blastopore in the anterior of the embryo. In amphistomy, the blastopore elongates and closes laterally with both ends giving rise to the mouth and anus. One scenario (I) for the evolution of deuterostomy is using the protostomy as ancestral, proposing the abbreviation of the anterior movement of the blastopore (dotted bright arrow) by a molecular separation of the mouth determination from the blastopore (intermediate stage small). The other scenario (II) claims the amphistomy as being ancestral, from which deuterostomy and protostomy evolved by closure of the blastopore from the posterior to anterior direction (protostomy) or from the anterior to posterior direction (deuterostomy).

deuterostomes, but the situation in the protostomes is unclear due to its extreme variability in different forms (Table 4.1) and because the phylogenetic relationships of several key taxa are not known. In some cases a large maternal investment of yolk (e.g. hexapods, cephalopods, and onychophorans) influences the gastrulation process, similar to what is found in most amniote embryos.

However, even in embryos with small quantities of yolk, the fate of the blastopore is highly

variable (see Table 4.1) and the reconstruction of the ancestral type of gastrulation is further hindered by intrataxon variation within larger clades such as annelids, nematodes, arthropods, and nemerteans (Table 4.1). The variability of the relationship between the blastopore and its possible future fates—mouth or anus—has led Ulrich (1951) to dismiss the term Protostomia and rename it Gastroneuralia, based on the ventral localization of nerve cords in these taxa. However, as it is not clear if a ventrally centralized nervous system is part of the ground pattern of the Protostomia, it is premature to rename this clade.

4.3 The fate of the blastopore and its role in scenarios of bilaterian evolution

The significance of transitions from the cnidarians to the Bilateria, which possess an antero-posterior and a dorsoventral axis, might be one of the biggest controversies in zoology over the last centuries. In cnidarians and ctenophores, two groups that diverged prior to the origin of the Bilateria, the blastopore gives rise to a single opening (mouth–anus) of the gastric digestive cavity, while in many bilaterians two openings are present, both of which can be formed independently from the blastopore. The scenarios under debate for explaining this transition differ in various details, such as the presence of a coelom or a larval stage. However, the formation of a through gut and the fate of the blastopore to the openings are central to understanding bilaterian body plan evolution.

4.3.1 The gastraea theory and the ancestral lateral closure of a slit like blastopore

The gastraea theory (Haeckel, 1872, 1874) proposes a hypothetical metazoan ancestor similar to the gastrula stage of recent animals. A fundamental assumption in the future variations of Haeckel's theme of the gastraea, for example the bilaterogastraea theory (Jägersten, 1955) and the trochaea theory (Nielsen and Nørrevang, 1985), is the extension of the ventral blastopore along the antero-posterior body axis. In these theories, a lateral closure of this elongated blastopore, which stays open at its ends,

Table 4.1 Blastopore fates in bilaterian taxa

Taxon	Fate of blastopore	References
Gastrotricha	Protostomy (<i>Lepidodermella</i> , <i>Turbanella</i>)	<i>Lepidodermella</i> (Sacks, 1955), <i>Turbanella</i> (Teuchert, 1968)
Nematoda	Protostomy (<i>Tobrilus</i> , <i>Ascaris</i>), blastopore closure (<i>Tylenchida</i>)	<i>Tobrilus</i> (Schierenberg, 2005), <i>Ascaris</i> (Boveri, 1899), <i>Tylenchida</i> (Malakhov, 1994)
Nematomorpha	Deuterostomy (<i>Paragordius</i>)	<i>Paragordius</i> (Montgomery, 1904)
Mollusca	Protostomy (<i>Crepidula</i> , <i>Patella</i>), deuterostomy (<i>Viviparus</i>)	<i>Crepidula</i> (Conklin, 1897; Hejnol <i>et al.</i> , 2007), <i>Patella</i> (Dictus and Damen, 1997), <i>Viviparus</i> (Dautert, 1929)
Priapulida	Blastopore closure?	<i>Priapulid</i> (Wennberg <i>et al.</i> , 2008)
Kinorhyncha	?	
Loricifera	?	
Platyhelminthes	Protostomy (<i>Planocera</i> , <i>Hoploplana</i>)	<i>Planocera</i> (Surface, 1907), <i>Hoploplana</i> (Boyer <i>et al.</i> , 1998)
Gnathostomulida	?	
Rotifera	Protostomy (<i>Asplanchna</i> , <i>Calidina</i> , <i>Philodina</i>)	<i>Asplanchna</i> (Lechner, 1966), <i>Calidina</i> (Zelinka, 1891), <i>Philodina</i> (AH, unpublished)
Entoprocta	Protostomy (<i>Pedicellina</i>)	<i>Pedicellina</i> (Marcus, 1939)
Nemertea	Protostomy (<i>Procephalotrix</i>), deuterostomy (<i>Drepanophorus</i>)	<i>Procephalotrix</i> (Iwata, 1985), <i>Dephanophorus</i> (Friedrich, 1979)
Annelida	Protostomy (<i>Polygordius</i>), deuterostomy (<i>Eunice</i>), blastopore closure (<i>Capitella</i>)	<i>Polygordius</i> (Woltereck, 1904), <i>Eunice</i> (Åkesson, 1967), <i>Capitella</i> (Eisig, 1898)
Sipunculida	Protostomy (<i>Phascolosoma</i>)	<i>Phascolosoma</i> (Gerould, 1906)
Cycliophora	?	
Phoronida	Protostomy (<i>Phoronopsis</i>)	<i>Phoronopsis</i> (Rattenbury, 1954)
Brachiopoda	Protostomy (<i>Terebratulina</i>)	<i>Terebratulina</i> (Conklin, 1902)
Onychophora	Mouth and anus form at different site of blastopore (<i>Peripatopsis</i>)	<i>Peripatopsis</i> (Manton, 1949)
Tardigrada	Protostomy (<i>Thulinia</i>)	<i>Thulinia</i> (Hejnol and Schnabel, 2005)
Arthropoda	Protostomy (<i>Cyprideis</i>), deuterostomy (<i>Meganctiphanes</i>)	<i>Cyprideis</i> (Weygoldt, 1960), <i>Meganctiphanes</i> (Alwes and Scholtz, 2004)
Chaetognatha	Deuterostomy (after blastopore closure)	<i>Sagitta</i> (Hertwig, 1880)
Xenoturbella	?	
Hemichordata	Deuterostomy (<i>Balanoglossus</i>)	<i>Balanoglossus</i> (Heider, 1909)
Echinodermata	Deuterostomy (<i>Synapta</i>)	<i>Synapta</i> (Selenka, 1876)
Cephalochordata	Deuterostomy (<i>Branchiostoma</i>)	<i>Branchiostoma</i> (Cerfontaine, 1906)

? Indicates unknown.

gives rise to a mouth and anus in the bilaterally symmetrical ancestor with a through gut. This process has been termed ‘amphistomy’ by Arendt and Nübler-Jung (1997). The same concept was the foundation of Remane’s enterocoely theory (Remane, 1950), which begins with a cnidarian polyp transforming into an ‘ur-bilaterian’ by stretching along its directive axis. Remane’s theory assumes the simultaneous evolution of the mouth and anus, and predicts that the coeloms of this hypothetical ancestor are formed from the common gastric pouches of an anthozoan-like polyp (Remane, 1950). These

theories thus predict a rather morphologically complex bilaterian ancestor, which is consistent with what has also been proposed on the basis of similarities of the expression of some developmental genes (Carroll *et al.*, 2001; Arendt, 2004). The two common themes of these theories are: (1) the simultaneous evolution of the mouth and anus, and (2) that the blastopore gives rise to both openings in the common ancestor. Many authors suggest that a slit-like blastopore is ancestral for the Bilateria and argue that a variety of extant animals such as onychophorans, polychaetes, insects and some nematodes

appear to show this kind of gastrulation (Sedgwick, 1884; Arendt and Nübler-Jung, 1997; Nielsen, 2001; Malakhov, 2004). The most elaborate explanation of the evolution of gastrulation processes which supports the gastraea-based theories is delivered by Arendt and Nübler-Jung (1997). These authors explain the evolution of deuterostomy by a closure of the slit-like blastopore from anterior to posterior, leaving an opening, which becomes the anus (Figure 4.1). Accordingly, protostomy evolved by a closure of the blastopore from posterior to anterior leaving a mouth open (Figure 4.1). Indeed, some bilaterians show a ventrally elongated blastopore that follows this pattern (e.g. *Polygordius*).

4.3.2 The acoeloid-planuloid theory and the ancestry of protostomy

Competing with 'gastraea'-based theories is a different view that does not require the simultaneous evolution of mouth and anus to establish bilaterality. The starting points of these hypotheses were pioneered by von Graff (1891), who proposed a paedomorphic planula larva, similar to that of recent cnidarians, which adopted a benthic lifestyle and flattened along one body axis, giving rise to the bilateral symmetry of the Bilateria (Hyman, 1951; Salvini-Plawen, 1978). The authors suggest that the former posterior blastopore—which gives rise to the mouth—is shifted to the ventral body side, to facilitate uptake of food from the ventral surface. This condition is represented by extant acoel and nemertodermatid flatworms, which have only a ventral opening to their digestive cavity. A posterior position of the mouth is found in the nearly radially symmetrical acoel *Diopisthoporus*, which is thought to reflect the ancestral planula-like condition (Beklemishev, 1969; Reisinger, 1970) of some feeding anthozoan planula larvae (Widersten, 1973).

According to these acoeloid-planuloid theories, the last common bilaterian ancestor was a rather simple benthic, probably meiofaunal, worm lacking a through-gut, coeloms, and excretory organs (Hejnol and Martindale, 2008b).

How do proponents of the acoeloid-planuloid theory explain the variation of the gastrulation types in the Bilateria and how this is related to the

evolution of the anus? The most thorough thoughts about this problem are presented by Salvini-Plawen (1978, 1980). He points out that in many spiralian embryos the vegetal (=posterior) blastopore gets displaced into the antero-ventral direction (e.g. in molluscs, annelids, nemerteans, and polyclad flatworms), and thus recapitulates the evolutionary process (protostomy). The anus is thought to have evolved independently from the blastopore in multiple lineages, which is also reflected by the late developmental formation of the anus in many protostomes. The deuterostome condition is explained with an evolutionary 'abbreviation' of the anterior movement of the blastopore (Figure 4.1). The mouth, instead of moving anteriorly in the form of the blastopore, is immediately formed at its final location (Beklemishev, 1969; Salvini-Plawen, 1980) and the blastopore either closes completely, such as in chaetognaths and nemerteans, or stays open and forms the anus, as in nematomorphs, deuterostomes, and several crustaceans (Table 4.1).

4.4 Recent progress in molecular systematics and developmental biology and their impact on the problem

Both competing scenarios differ in their assumption about which type of gastrulation is ancestral for the Bilateria. In gastraea-based theories the lateral closure of the blastopore—or 'amphistomy'—with the simultaneous evolution of the orifices would deliver the state from which the diversity of gastrulation can be derived. In the acoeloid-planuloid theory, protostomy is ancestral, including an independent evolution of an anal opening. Since both hypotheses have their roots in a time when the metazoan phylogeny was speculative, a proper phylogenetic framework is required to determine the direction of evolutionary change.

4.5 A new animal phylogeny

Recent progress in molecular biology, computer technology and the development of new phylogenetic reconstruction algorithms have improved the ability to determine animal relationships with the use of molecular data (Philippe and Telford, 2006; Dunn

et al., 2008). In addition to the seminal publication of Aguinaldo *et al.*, (1997) which established the subdivision of the Bilateria into three large clades, Ecdysozoa, Lophotrochozoa, and Deuterostomia, further resolution of metazoan relationships has been accomplished by increased taxon sampling and the use of phylogenomic approaches (Dunn *et al.*, 2008). A major result pertinent to understanding the role of gastrulation in body plan evolution is the placement of the acoels as the sister group to the remaining Bilateria (Ruiz-Trillo *et al.*, 1999; Bagnuà and Riutort, 2004). Their position has been corroborated by multiple independent molecular approaches (see Telford *et al.*, 2003, for example). Our current understanding places the nemertodermatids as sister to Bilateria, and Acoela as sister to that group, thus breaking the monophyly of the Acoelomorpha (Jondelius *et al.*, 2002; Wallberg *et al.*, 2007). Having the acoel flatworms at the base of the Bilateria has important implications for our understanding of the evolution of organ systems (Hejnol and Martindale, 2008b). The similarity of the body plan of acoels and nemertodermatids, both possessing only one opening to their digestive system and an orthogonal nervous system and lacking a through gut and nephridia, clearly support a simple acoeloid bilaterian ancestor, which was previously proposed on the basis of morphological data (Hyman, 1951; Salvini-Plawen, 1978; Haszprunar, 1996a). These data do not support the gastraea theory of Haeckel or the transformation of a coelom bearing ur-bilaterian from a sessile cnidarian polyp (Remane, 1950).

4.6 'Amphistomy'—a common theme in bilaterian development?

While molecular phylogenetic results support the acoeloid-planuloid theory, the recently improved resolution of the metazoan relationships with the use of the phylogenomic approach has implications for accepting the 'amphistomy' hypothesis (Dunn *et al.*, 2008). If one assumes gastrulation with a slit-like blastopore is in the ground pattern of the protostomes and deuterostomes (together named Nephrozoa after Jondelius *et al.*, 2002), one would expect a broad distribution of a lateral closure of a slit-like blastopore in the Bilateria (Arendt and

Nübler-Jung, 1997). The cases for which amphistomy are most commonly cited are onychophorans, the polychaete *Polygordius*, and the nematode *Pontonema* (e.g. Arendt and Nübler-Jung, 1997; Nielsen, 2001). In drawings from the early research on the onychophoran *Peripatus capensis* (Figure 4.2a), it indeed seems that a large extended blastopore closes laterally and both ends stay open and give rise to the mouth and anus (Balfour, 1883; Sedgwick, 1885). In contrast, Kennel (1885) draws a different picture for *Peripatus edwardsii*, showing that the opening that gives rise to the mouth and anus is separate from the blastopore, which is positioned more posteriorly (Figure 4.2a). The most thorough analysis of onychophoran gastrulation (Manton, 1949) corroborates Kennel's findings for several onychophoran species and describes the immigration of the mesoderm and germ cells at the posterior blastopore which is never in contact with either mouth or anus. The syncytial development of the yolky onychophoran embryos seems to be a rather derived adaptation to their terrestrial lifestyle, as is the case in other terrestrial arthropods (e.g. hexapods and myriapods), and does not represent an example of an 'amphistomic' type of gastrulation. Another taxon often referred to as being 'amphistomic' is the polychaete annelids. The traditional example is the description of the development of *Polygordius* (Woltereck, 1904). A close examination of the original work shows that the extended blastopore first closes laterally, but instead of leaving both ends open, only the anterior edge gives rise to the mouth (Figure 4.2b). The anus is formed later in development one cell row posterior (Figure 4.2b) to the former 'seam' of the blastopore (Woltereck, 1904). Thus, the development of *Polygordius* follows a protostomic pattern rather than amphistomy. Even if a polychaete can be shown to possess a prototypical amphistomy form of gastrulation, it is difficult to be sure that it is a plesiomorphic character, since we observe a high variation in gastrulation patterns in polychaete annelids. Both protostomy (Mead, 1897) and deuterostomy (Åkesson, 1967), have been described in polychaetes as well as in other trochozoan taxa including nemertean (Friedrich, 1979; Iwata, 1985) and molluscs (Fioroni, 1979; see Table 4.1).

A similar variation of gastrulation is found in the third taxon for which amphistomy has been

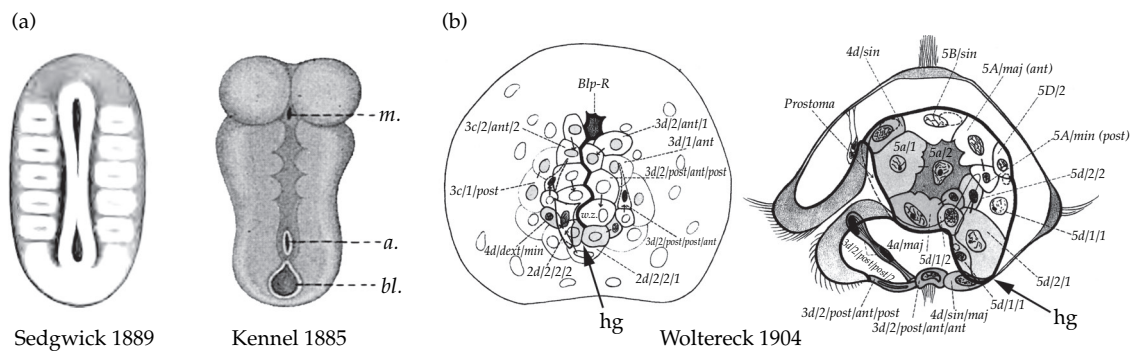


Figure 4.2 Original drawings of the development of the onychophoran *Peripatus* and the annelid *Polygordius*. (a) The drawing of Sedgwick (left) shows the supposed 'amphistomic' gastrulation in the onychophoran *Peripatus capensis*. On the right the drawing from the study of Kennel on *Peripatus edwardsii*, which shows the blastopore (bl.) separate from the secondary opening, which gives rise to the mouth (m.) and anus (a.). (b) Original drawings of the gastrulation of *Polygordius*, a supposed amphistomic polychaete. The drawing on the left shows the closure of the blastopore (dark black line in the original) leaving the mouth open (prostoma). The hindgut (hg) forms at a different site from the blastopore (arrow).

described, the Nematoda (Malakhov, 1994). Apart from a clear case of blastocoelic protostomy in *Tobrilus* (Schierenberg, 2005) the site of immigration of the two entoderm cells becomes the future mouth in most nematodes, which is separate from the later immigration site of the mesodermal precursors which are descendants from different lineages (Schierenberg, 2006).

The developmental stage for which amphistomic gastrulation has been described in *Pontonema* is much later than the immigration of the E precursors, which form the endoderm—a process which is usually referred to as gastrulation in nematodes (Schnabel *et al.*, 1997; Sulston *et al.*, 1983; Voronov and Panchin, 1998).

Again, even if the cursory study of *Pontonema* is correct, it is not clear what type of gastrulation the last common ancestor of nematodes had, because the sister group of nematodes, the Nematomorpha (Dunn *et al.*, 2008) gastrulate by deuterostomy (Montgomery, 1904; Inoue, 1958). Tardigrades, which form the sister group to the Arthropoda and Onychophora (Dunn *et al.*, 2008), gastrulate by immigration of mesodermal and endodermal precursor cells and show protostomy (Hejnol and Schnabel, 2005), with no evidence of enterocoely as has been assumed by early investigators (Marcus, 1929).

If amphistomy was an ancestral pattern, giving rise to both deuterostomy and protostomy, it must have been lost independently in nearly every larger protostome clade. Taken thus, the developmental diversity of bilaterians described today gives little support for either amphistomic gastrulation or Haeckel's gastraea. Both concepts seem to deliver a feasible evolutionary scenario for the human mind but are not represented in living organisms.

4.7 'Abbreviated' protostomy as a model for the variability of gastrulation?

Attempts to explain variations in bilaterian gastrulation patterns from the point of view of the acoeloid-planuloid theory are based on the movement of the vegetal blastopore in the antero-ventral direction, which then gives rise to the mouth in protostomic animals (Figure 4.1). In the case of deuterostomy, the mouth forms at a separate site and no such movement can be observed. Salvini-Plawen (1980) explains the multiple independent origins of deuterostomy in several animal lineages with an evolutionary 'abbreviation' of the antero-ventral movement by means of the mouth forming directly in the anterior part, the former animal hemisphere of the embryo. This includes a spatial

separation of the molecular mechanisms determining the mouth from the site of gastrulation. It is clear from the developmental studies on ctenophores and cnidarians that the mouth and the blastopore have a common origin (Goldstein and Freeman, 1997). In both animals the blastopore gives rise to the single opening of the digestive cavity. Thus, it is not surprising that many genes which have been assigned to gastrulation and foregut development, for example *brachyury*, *gooseoid*, and *forkhead*, are expressed at the cnidarian and ctenophore blastopore (Scholz and Technau, 2003; Martindale *et al.*, 2004; Matus *et al.*, 2006a; Yamada *et al.*, 2007). It is difficult, however, to dissect the role of these genes, since the blastopore has overlapping functions in mouth formation, axis determination, and germ layer specification.

It is important to point out that the site of gastrulation has changed along the animal–vegetal egg axis in the stem lineage of the Bilateria. While cnidarians and ctenophores gastrulate at the animal pole, bilaterians, including acoels, gastrulate at the vegetal pole. Since bilaterians form their mouths at the animal hemisphere, this indicates an ancient separation of the determining factors of mouth formation and site of germ layer specification. The molecular separation of signalling centres might explain the variation of the relationships between mouth and blastopore in the Bilateria by a facilitation of movements of signalling centres. The absence of molecular data from a broader range of taxa limits detailed conclusions at this time, but in animals in which the mouth is formed at a separate site from the blastopore (deuterostomy) (including asteroids, echinoids, hemichordates, and chaetognaths), the gene *brachyury* is expressed in both locations, indicating the spatial separation of a former common expression domain at the blastopore (Peterson *et al.*, 1999; Shoguchi *et al.*, 1999; Takada *et al.*, 2002). Furthermore, recent work indicates that the mouth in protostomes and deuterostomes—although formed by variable developmental processes—is homologous, based on the shared arrangement of the expression domains of *gooseoid* and *brachyury* (Arendt *et al.*, 2001). A specific mouth signalling system which would enable a detailed explanation of how the position of the mouth becomes specified in the Bilateria has not

yet been identified, but the dissociation of the blastopore from the position of the mouth already occurred in the bilaterian stem lineage.

The fate map of the acoel *Neochildia fusca* shows that the mouth is formed at a site different from the blastopore (Henry *et al.*, 2000) and the mouth of *Isodiametra pulchra* is formed long after blastopore closure (Ladurner and Rieger, 2000). Our own studies of *brachyury* and *gooseoid* expression in the acoel *Convolutriloba longifissura* supports the homology of the acoel mouth with the protostome and deuterostome mouth (Hejnol and Martindale, 2008a). The fate map of the acoel *Neochildia fusca* furthermore shows that the vegetal part of the embryo gets shifted in an antero-ventral direction by the increased proliferation of descendants of micromere 1a versus those of its ventral counterpart micromere 1b (Henry *et al.*, 2000). This mirrors the observations made by fate map studies in spiralian taxa, like polyclads (Boyer *et al.*, 1998), molluscs (Dictus and Damen, 1997; Hejnol *et al.*, 2007), annelids (Ackermann *et al.*, 2005), and nemertean (Maslakova *et al.*, 2004a) in which the dorsal side of the embryo proliferates more than the ventral regions (see also discussion in van den Biggelaar *et al.*, 2002). This shifting of the vegetal part of the embryo by proliferation of dorsal cells might be an ancestral bilaterian feature which was lost in the deuterostome lineage where the position of the mouth is specified independently from the blastopore. Unfortunately, the relationship of the blastopore to the primary egg axis has not been investigated in a large number of ecdysozoan taxa and appears to be highly variable and obscured by yolk content.

4.8 Has the anal orifice evolved several times convergently?

If the mouth is homologous in all animals and was the earliest opening to the digestive cavity, when did the anal opening evolve and do all anal openings share a common ancestry? The outgroups of the Nephrozoa—ctenophores, cnidarians, acoels, and nemertodermatids—as well as several other key bilaterian taxa lack an anal opening. All Platyhelminthes possess only a mouth and the presence of one or more dorsal anal pores in the

branched gut system of some polyclads is a derived character (Ehlers, 1985). Gnathostomulids and some rotifers (e.g. *Asplanchna*) lack an anus and *Xenoturbella*, which in the current view forms the sister taxon to the Ambulacraria or to all remaining Deuterostomia (Bourlat *et al.*, 2003, 2006; Perseke *et al.*, 2007; Dunn *et al.*, 2008), also possesses only one opening to the digestive cavity (Westblad, 1949).

This lack of an anus, however, can be interpreted as either loss from a stem species with a through gut (as seems to be the case for the brachiopods and many parasitic forms) or that the anus has evolved later and independently in several lineages. Although it is not parsimonious that the anus evolved multiple times, its functional advantage and the differences in development and morphology could be evidence for an independent evolutionary origin (Beklemishev, 1969; Schmidt-Rhaesa, 2007).

The anus is morphologically very diverse between the protostome taxa. For example, gastrotrichs do not possess an ectodermal hindgut like most bilaterians; instead the anus is formed by a direct and often temporary connection of the endoderm to the outside (Ruppert, 1991b). Such a temporary anus is also present in Micrognathozoa (Kristensen and Funch, 2000) and in the gnathostomulid *Haplognathia* (Knauss, 1979). Another variation of anal morphology found in many lineages is a combined opening of the anus with the gonopore, a so-called cloaca. Cloacas are present in the ecdysozoan nematodes, nematomorphs, tardigrades, and rotifers. Acoels and nemertodermatids lack an anus but have a male gonopore at the posterior end of the body formed by an involution of the ectoderm. *Brachyury* is expressed in the hatchling of the acoel *Convolutriloba longifissura* in the posterior ectoderm, which later gives rise to the adult male gonopore (Hejnol and Martindale, 2008a). This might indicate that the anus of some taxa, as in the Platyzoa and Ecdysozoa, might have been derived from a connection between the endoderm and the ectoderm of the gonoduct. Thus the last common nephrozoan ancestor might have had only an antero-ventral mouth and a posterior male gonopore similar to what is found in acoels and nemertodermatids (Hejnol and Martindale, 2008a). Of the larger animal clades, the Trochozoa show

the highest morphological similarity of their anal openings: most of them possess an ectodermal hindgut, which ends in an opening that is separate from the gonoducts. In most trochozoans the hindgut is formed after the mouth as a secondary ectodermal involution at a site separate from the blastopore. Despite this evidence for the homology of the trochozoan anus, it remains unclear if the anus is homologous in all bilaterians and thus part of the nephrozoan ground pattern. The independent evolution of the anus might have been a result of the extension of the body length in several lineages, since a blind gut is mostly present in smaller animals and parasitic forms. However, it is too early to draw a conclusion because important nodes in metazoan phylogeny are still not resolved. It will be important to see if the Platyzoa, a taxon comprising Rotifera, Gnathostomulida, Platyhelminthes, and Gastrotricha, turns out to be a true monophyletic group or a long-branch artefact (see Chapter 6).

4.9 Conclusion

We are far from understanding the evolution of gastrulation mechanisms in the different animal lineages. As in phylogenetic systematics where broader taxon sampling helps to reconstruct phylogeny, it is necessary to follow the same approach in comparative developmental biology to answer the questions raised from studies of a handful of arbitrarily selected animal species. The new molecular approaches to deliver the necessary phylogenetic framework, as well as detailed analyses of the development of more animal taxa using modern cell lineage studies and molecular developmental approaches, will deliver insights into the evolution of the important organ systems.

4.10 Acknowledgements

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