

# Origin and evolution of endoderm and mesoderm

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**ABSTRACT** Germ layers are defined as cell layers that arise during early animal development, mostly during gastrulation, and that give rise to all tissues and organs in adults. The evolutionary origin of the inner germ layers, endoderm and mesoderm, and their relationship have been a matter of debate for decades. In this review we summarize the major modes of endoderm and mesoderm formation found in Metazoa and possible evolutionary scenarios to reconstruct the ancestral state. In the second part, we address the question whether endoderm as well as mesoderm are homologous among Bilateria. In this regard, we propose that the comparative analysis of some crucial transcription factors involved in the early specification and differentiation of these germ layers might provide cues for the level of homology. We focus on four classes of genes: the Zn-finger gene *GATA 4-6*, the bHLH gene *twist*, the Krüppel-like Zn-finger gene *snail* and the T-box gene *brachyury*. The role of each of these genes in mesendoderm formation is summarized and we propose that the specific function of each of these genes in endoderm and mesoderm formation evolved from the regulation of basic cellular features, such as cell adhesion, cell motility, cytoskeleton and cell cycle.

**KEY WORDS:** *mesendoderm, gastrulation, metazoa, axis formation, Brachyury, twist, snail, gata*

## Introduction

More than 30 of the 35 described animal phyla develop from three germ layers: the outer ectoderm and the two inner layers, endoderm and mesoderm. Besides these triploblastic animals, few phyla exist, that consist of two germ layers, endoderm and ectoderm, only. These diploblastic organisms all arose very early during animal evolution. The evolutionary origin of endoderm and mesoderm is closely linked to the origin of metazoa. i.e. the transition from protists to metazoa, since multicellularity is accompanied with the division of labor of the cells: those that are specialized in digestion become located in the inner side of the organism and those that are specialized in protection, locomotion and sensing the environment remain on the outer side of the organism. Further, since the next major transition from diploblastic and radially symmetrical to the triploblastic bilaterally symmetrical animals also marks the origin of the second body axis, the emergence of the mesoderm is also linked to the evolution of axis formation in metazoa. The two inner layers, endoderm and mesoderm, arise during or tightly linked to gastrulation. Hence, in order to understand the evolution of endoderm and mesoderm, we have to understand gastrulation and its molecular regulation. In the first part of this review, we will therefore summarize different modes of gastrulation, that have been evolved in the different phyla. In the

second part we will examine the role of some crucial conserved genes involved in the formation of endoderm and mesoderm in Bilateria and discuss, whether the evolutionary origin can be traced back by analysing these genes in basal metazoa.

## What are germ layers: a definition and some cautionary notes

Germ layers are distinct cell layers that form very early during embryonic development and that give rise to all tissues of the adult. For instance ectoderm will give rise to the nervous system and the epidermis, endoderm to the midgut (and some inner organs in vertebrates) and mesoderm to blood, muscle tissue and in vertebrates to bones and some inner organs. However, we should strictly avoid any sort of teleological definition of the germ layers. For instance: since muscles are a derivative of the mesoderm, all cells that eventually give rise to muscles must be mesoderm. Although this might be true in this particular case, we will get into trouble with the neural crest for example (see below). Also, a few organs exist that are composed of cells that derive from two germ layers, e.g. the pancreas in vertebrates.

Besides this cautionary note, we should be aware that the terminology "endoderm, mesoderm, ectoderm" is not correct in the strict sense. The ending *-derm* is commonly used in connection

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with differentiated tissues, mostly of epithelial type. In contrast, the ending *-blast* indicates a proliferating, not differentiated tissue. Hence, it might appear more appropriate to speak of mesoblast, endoblast and ectoblast instead as a proliferating cell layer during embryogenesis of the animals with different prospective fates (Salvini-Plawen and Splechtna, 1979). The terminology is even more confusing as in Cnidaria, ectoderm and endoderm indeed define the epithelial layers of the adults, although at least in the polyp form the endodermal and ectodermal cells are constantly dividing and can adopt different fates. Even in higher animals ectoderm and endoderm derivatives are often also constantly proliferating, however, differentiation is usually restricted to a particular fate. Despite these terminological inconsistencies, we will, however, continue to use the words endoderm, mesoderm and ectoderm in this review to define the germ layers.

If we want to conclude on the evolutionary origin of the germ layers, we have to use a firm phylogeny as a base of interpretation. Therefore in this review, we will not discuss the interesting findings from some animal groups (e.g. Ctenophores), because their phylogenetic position is uncertain and the interpretation of the findings might be hampered. Also, any interpretation should consider the rather trivial fact, that we try to reconstruct the common ancestor, inferred from the situation in today's phyla, which are composed of ancestral and derived features. Hence, we have to think in trees, not in lineages.

## Gastrulation: the embryological stage of germ layer formation

Gastrulation means the formation of a gastric cavity, the archenteron, in which food can be digested later in the adults. In most animals, endoderm formation goes hand in hand with the formation of the third germ layer, the mesoderm. However, this is not a *conditio sine qua non* of gastrulation and indeed mesoderm formation can be temporally separated from the formation of a gastric cavity. Also, and importantly, the diploblastic Cnidaria gastrulate without ever forming a mesoderm. Today, the term gastrulation is often used as a synonym of endoderm and mesoderm formation. It is, however, important to keep the distinction in mind. In his famous Gastraea-theory, Haeckel (1874) postulated that a gastrula-like organism was at the origin of the metazoa. While he initially used the sponges as a model for this gastrula-like organism, he later changed his view and interpreted the Cnidaria *Hydra* as a today's representative of this level of organisation (see Salvini-Plawen and Splechtna, 1979 for discussion). Other authors modified Haeckel's Gastraea-theory and postulated that this Urmetazoa was not radially symmetrical but had an anterior-posterior and a dorso-ventral body axis (e.g. Bilaterogastraea-theory; Jägersten, 1955). During gastrulation cells have to enter the inner space of an early embryo and eventually form an endodermal epithelial layer. Animals of different phyla use a variety of different cellular mechanisms to fulfill this task and these modes of gastrulation seem to be completely unrelated at first glance.

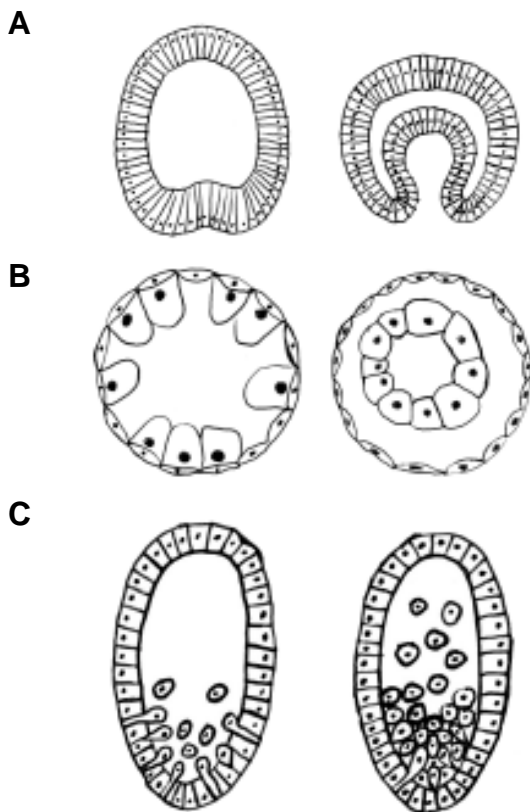
### Modes of gastrulation (endoderm formation)

**Invagination / Epiboly.** Although some authors differ between these two mechanisms, in both cases, one half of the epithelial blastula involutes usually from the vegetal pole, leading to two epithelial sheets (Fig. 1A). This mechanism was proposed by Haeckel (1874) and others as the ancestral mode of gastrulation. Invagination is found and well studied in sea urchins (Hardin, 1996; reviewed in Etensohn, 1999; Wessel and Wikramanayake, 1999). Epiboly is found in yolk-rich embryos and involves the migration and proliferation of the cells of the animal pole over the yolk-rich vegetal cells (reviewed in Arendt and Nübler-Jung, 1997). Prominent examples of epiboly are the amphibian *Xenopus* and the zebrafish.

**Delamination.** In this gastrulation mode, the inner layer is formed by cell division of the epithelial layer of the blastula in a radial plane (Fig. 1B). This mode was favored as the ancestral mode of gastrulation by Lankester (see discussion in Salvini-Plawen and Splechtna, 1979), who developed his planula theory from this idea. Delamination is, however, rather rare and found in a few cnidarians (e.g. *Geryonia*).

**Immigration.** Immigration of individual cells can occur from all sides of the blastula (multipolar immigration) or preferentially from one side of the early embryo (polar immigration) (Fig. 1C). In many cases, this mode initially does not lead to an epithelial organization of the endoderm, but can instead lead to a unorganized inner cell mass, the stereogastrula, which only later organizes into an endodermal epithelium. It is found for example in some Cnidaria (Hydrozoa) and birds.

It is an old question, which of these mechanisms of gastrulation represents the ancestral mode. Several authors argue, that invagi-



**Fig. 1. Major modes of endoderm formation during gastrulation. (A)** Invagination, **(B)** delamination and **(C)** polar immigration. Modified after Sewing (1969).

nation, which is integral part of Haeckel's gastraea theory was the original form of gastrulation, from which the others evolved (Wolpert, 1992). In line with these arguments, invagination is often found in Anthozoa, which are considered the basal group within the Cnidaria. Given that gastrulation in sponges is very different from all other metazoa (for review see Fell, 1997) one might argue, that the Anthozoa-like Cnidaria were the first animals in evolution that "invented" gastrulation. Interestingly, however, all of the above mentioned different mechanisms of gastrulation can be found in different Cnidaria (reviewed by Tardent, 1978). This suggests, that even if invagination was the ancestral mode of gastrulation, the selection pressure did not act on the mechanism itself, but on the result: the embryo has to make an endoderm, but it does not matter how (Wolpert, 1992).

As pointed out above, gastrulation (i.e. endoderm formation) is often, but not always linked to the formation of the mesoderm. Again, in the animal kingdom, we find a variety of cellular mechanisms in the formation of mesoderm. The two major mechanisms found in basal protostomes and deuterostomes are summarized below.

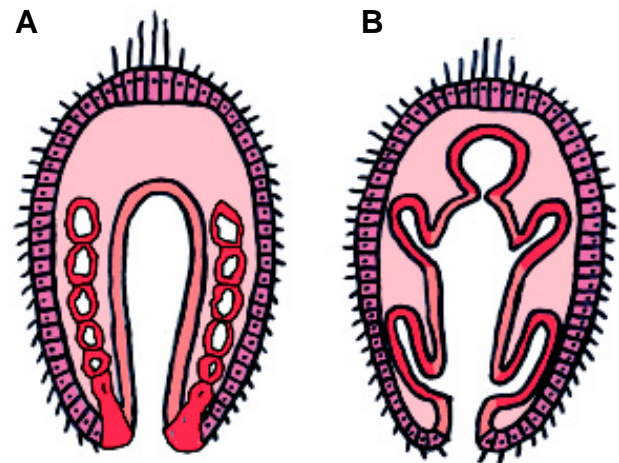
### Modes of mesoderm formation

**Schizocoely.** This form of mesoderm formation starts with an immigration of cells that form a mesenchymal tissue between ectoderm and endoderm, which then forms a cavity inside, the coelom, by introducing clefts between the cells, which eventually line up into an epithelial sheet (Fig. 2A). Schizocoely is found in several Spiralia, where most of the mesoderm develops from the descendants of the 4d cell. This cell enters the inner side of the embryo from the posterior margin of the blastopore and proliferates in two sacs on both sides of the developing gut.

**Enterocoely.** Epithelial mesoderm develops directly from endodermal pouches, which are budding at specific places of the archenteron, finally fuse and separate from the endoderm (Fig. 2B). This mode is found in lower deuterostomes, i.e. echinoderms and hemichordates, where these sac-like cavities lined by an epithelium form the different forms of coeloms: hydro-, axo-, and somatocoel in echinoderms or proto-, meso-, and metacoel in hemichordates. In Remane's Enterocoely theory, which has been further developed by Siewing, this mode of mesoderm formation has been proposed to be ancestral (Remane, 1963; Siewing, 1980).

### The blastopore: signalling center and specialized region for the formation of germ layers

The blastopore is a very special region of the early embryo. It is where the inner layers, endoderm and mesoderm involute and after gastrulation it marks the point, where ectoderm meets endoderm. In the polychaete *Platynereis dumerilii* and most other Spiralia, the blastopore forms a slit-like structure, where the anterior end develops into the stomodeum, the larval foregut and the posterior end develops into the proctodeum, the larval hindgut. The blastopore margin between the two opposing poles closes and defines the ventral midline. Thus, both mouth and anus derive in these organisms from the original blastopore. Since polychaetes are considered to represent a basal group within the protostomes



**Fig. 2. Major modes of mesoderm formation found in ciliary larvae of basal Protostomia and Deuterostomia.** (A) *Schizocoely*, as found in many Spiralia. Specific cells from the margin of the blastopore (4d) immigrate as single cells, proliferate and start making coelomic cavities in a segmental pattern by organizing into epithelia and forming clefts between the inner cells. (B) *Enterocoely*, as found in Hemichordates. Parts of the archenteron fold into epithelial pouches to form protocoel, mesocoel and metacoel. Colors indicate different germ layers: ectoderm (dark pink), mesoderm (red), endoderm (orange), ECM (rosé), archenteron and coelom (white). Modified after Salvini-Plawen and Splechtna (1979).

with a prototypical mode of development, many authors argue that this reflects the ancestral type of gastrulation for protostomes (reviewed in Nielsen, 2001; Arendt *et al.*, 2001). Interestingly, the comparison of molecular markers of the oral field and the blastopore in basal protostomes and basal deuterostomes suggests that the common ancestor, the Urbilateria, used the same set of genes to form mouth and anus from opposite sides of the blastopore (Shoguchi *et al.*, 1999; Arendt *et al.*, 2001; Lartillot *et al.*, 2002). Consequently, the major division between Deuterostomia and Protostomia is not marked by their different and independent way to form the mouth, as postulated by Grobben's concept (Grobben, 1908). Deuterostomy (i.e. the formation of the mouth by a secondary breakthrough of the archenteron) appears to be rather a derived mode of gastrulation, that can easily evolve from Protostomy. The morphogenetic movement of endoderm invagination occurs from the posterior pole solely, but the archenteron then fuses back to the original anterior end of the slit-like blastopore, which still expresses the conserved set of oral markers genes (Salvini-Plawen and Splechtna, 1979; Arendt *et al.*, 2001). Deuterostomy might therefore have evolved several times independently, and consistent with this view, it is found also among several Protostomes (e.g. some molluscs; Salvini-Plawen and Splechtna, 1979).

The blastopore of the Bilateria can be traced back to the Cnidaria, in particular those like the basal anthozoan *Nematostella vectensis*, which gastrulate by invagination and thus, have a defined blastopore (Hand and Uhlinger, 1992). The blastopore of the cnidarian embryo develops directly into the mouth of the polyp, the only opening of the cnidarian body (Scholz and Technau, 2003). In this blastopore / oral end of the Cnidaria, one finds expression of the same conserved set of transcription factor genes as in Bilateria (such as *Brachyury*, *gooseoid* and *forkhead*, see

below). This shows that the blastopore is specified by a conserved set of developmental regulator genes in all Eumetazoa (reviewed in Technau, 2001). Interestingly, most of these genes later in evolution are involved in the formation of the third germ layer, the mesoderm. In fact, cells, that contribute to the mesoderm, arise in many organisms from a specific subset of cells within the blastopore region. For instance, in *Spiralia*, the 4d cell, which gives rise to most of the mesoderm is located at the posterior margin of the blastopore and expresses at least transiently the blastopore marker genes. In vertebrates, the mesoderm arises from cells along the whole blastopore, the marginal zone, marked by the expression of the T-box gene *Brachyury* (Herrmann *et al.*, 1990).

The blastopore, however, not only marks the intersection between the two or three germ layers, it also acts as an organizer of the body axes. In *Hydra*, the hypostome, the homologous structure of the cnidarian blastopore, was shown very early to have organizer activity (Browne, 1909). Recent work has shown, that the hypostome expresses several of the genes, that are also expressed in the „Mangold-Spemann-Organizer“ of Amphibia, such as *Brachyury*, *Wnt* and *Chordin* (Technau and Bode, 1999; Hobmayer *et al.*, 2000; Hobmayer and Holstein, personal communication). The „Mangold-Spemann-Organizer“ in turn, which sets up the dorso-ventral axis in amphibians, is located at the dorsal blastopore lip, hence, part of the blastopore region.

### Patterning the germ layers: molecules that specify mesoderm and endoderm

Endoderm and mesoderm can separate in various ways from ectoderm. The variability of the cellular processes raises the question, whether these so differently produced germ layers are really of the same evolutionary origin. One way to approach this question is to study genes involved in endoderm and mesoderm formation in different systems. The underlying assumption is that there is a conserved set of genes, that triggers the different mechanisms, that finally lead to the same result. Of course, the role of these genes in these processes could also have evolved convergently. Yet, transcription factors are particularly useful tools to examine this question, since there is no *a priori* reason, why a particular transcription factor regulates the expression of genes involved in the specification and differentiation of germ layers and their morphogenetic behavior. In contrast to genes coding for structural proteins specific of a particular structure, there is no functional constraint on the role of a given transcription factor in the generation of a structure. Thus, if we find common expression patterns in a number of distantly related organisms we must conclude that the common ancestor of these organisms also used this gene in this context, i.e. the function of the gene is homologous.

Among the genes, that are commonly accepted as mesoderm markers and key players in mesoderm specification and patterning, are those coding for the transcription factors, *twist*, *snail*, *GATA* factors and *Brachyury*. *GATA* factors appear to play crucial roles in specifying mesendoderm in early development, later specifying endoderm, when mesoderm separates from endoderm. Especially *twist* and *snail* contribute to mesoderm formation in protostomes and deuterostomes. The role of *brachyury* in mesoderm formation is well established in vertebrates, and recent results also showed a contribution in mesoderm formation in protostomes. Many other genes are also important players in the formation of the inner germ

layers, but in the following section we will focus on these four genes and review their role during endoderm and mesoderm formation.

### GATA factors

*GATA*-factors are a family of zinc finger proteins with two highly conserved zinc fingers and adjacent basic domains. This DNA-binding domain binds to (A/T)*GATA*(A/G) sequences, which gave the name to these transcription factors (reviewed in Molkenin, 2000). So far, no *GATA* factors have been reported from non-bilaterians. While other transcription factors often form large families with many subfamilies, only 6 *GATA* factors have been isolated from vertebrates and only 3 were identified in *Drosophila*. The six vertebrate *GATA* factors are subdivided in two classes, *GATA* 1-3 and 4-6. Especially the *GATA* factors 4-6 play a central role in specifying the mesendoderm and in the differentiation of endodermal and mesodermal tissues in invertebrates and vertebrates (Shoichet *et al.*, 2000; reviewed in Patient and McGhee, 2002). In vertebrates they may act as master regulators of endoderm formation, under the control of Nodal signalling during early development (Fujikura *et al.*, 2002; Aoki *et al.*, 2002), while in later organogenesis they are regulated by BMP and FGF signalling (e.g. Rossi *et al.*, 2001). Thus, in vertebrates, *GATA* factors 4-6 are under control of signalling pathways that act in the early specification of endoderm and mesoderm. The best studied invertebrate model for the role of *GATA* factors is *C. elegans*, where different *GATA* factors act in several hierarchical cascades during the specification of the EMS cell, which gives rise to the endoderm and most of the mesoderm (Maduro *et al.*, 2001; reviewed in Maduro and Rothman, 2002). This common role of homologues of the *GATA* 4,5 and 6 factors in specification of the mesendoderm led Rodaway and Patient (2001) to propose that the mesendoderm is an ancient germ layer, that was specified in the Urbilateria by the same set of genes, with *GATA* 5 homologs high up in the hierarchy. This attractive hypothesis will certainly be tested by the analysis of more species, both among the Bilateria and also among the diploblastic non-bilaterians. The specificity of the different *GATA* factors most certainly is assured by the specific interaction with other transcription factors with overlapping but not identical expression domains (reviewed in Molkenin, 2000; Patient and McGhee, 2002). It will be most interesting to investigate to what extent this network of protein interactions has been conserved or modified throughout evolution.

### Twist

In *Drosophila* embryos the expression of the bHLH transcription factor *twist* is regulated by a nuclear gradient of the protein Dorsal (Thisse *et al.*, 1987; Ip *et al.*, 1992a). This leads to an expression of *twist* in the ventral blastoderm shortly before gastrulation. The *twist* expressing cells mark the ventral furrow, through which prospective mesodermal cells migrate into the embryo. *Twist* activates a number of transcription factors like *tinman* and *Mef2*, that are involved in patterning mesodermal tissue, especially the heart and muscles. *Drosophila twist* mutants fail to gastrulate indicating that it is not only necessary for mesoderm differentiation but also for the morphogenetic movement during gastrulation (Leptin and Grunewald, 1990; Leptin, 1991). Although *twist* acts in vertebrates after specification of the mesoderm (Wolf *et al.*, 1991; Chen and Behringer, 1995; Gitelman, 1997), it is crucially involved

in mesoderm differentiation. For example in chick embryos, this gene is expressed in the anterior head mesoderm and also in the somites, where it contributes to the patterning of mesodermal tissue like muscles (Tavares *et al.*, 2001). Interestingly, while high levels of Twist protein promote myogenesis in *Drosophila* (Baylies and Bate, 1996), myogenesis in vertebrates is repressed by Twist (Spicer *et al.*, 1996), indicating that Twist has opposite effects in vertebrates and insects. The results support the view that in vertebrates Twist prevents premature differentiation of myogenic cells. The different function of vertebrate and insect Twist is most likely due to the heterodimer formation with E proteins, thereby competing for the binding of E proteins to the myogenic bHLH protein MyoD. Interestingly, in *in vitro* or cell culture experiments *Drosophila* twist behaves like the vertebrate twist, indicating that it is the specific cellular context that is responsible for the different role *in vivo*. This shows that although a role of twist in regulating myogenesis has been conserved in Bilateria, the cellular context evolved and led to a change of the *in vivo* function of the protein. If we want to understand the evolution of twist gene function, we have to examine species that can give insights into the ancestral state. In this respect, it is interesting that the *twist* homolog from the mollusc *Patella vulgata*, a lophotrochozoan, is expressed in the ectomesoderm, but not in the endomesoderm, which derives from the 4d cell at the posterior pole of the embryo (Nederbragt *et al.*, 2002). Strikingly, in the diploblast *Podocoryne carnea*, a hydrozoan cnidarian, the *twist* homolog is expressed during the budding of the medusa in the entocodon, which will later differentiate into the striated and smooth muscles of the medusa (Spring *et al.*, 2000). This supports the view that *twist* genes were ancestrally involved in mesoderm differentiation in Bilateria, but not in early specification of the whole mesoderm. The further analysis of the protein interactions of twist in diploblasts and basal Bilateria is required to give insights into the evolution of this gene in the specification and differentiation of the mesoderm.

## Snail

Snail is a member of the Krüppel-like subfamily of zinc finger proteins, which usually acts as a transcriptional repressor (reviewed in Nieto, 2002). During *Drosophila* embryogenesis, expression of *snail* is regulated by nuclear Dorsal protein and by Twist. *snail* is expressed in the ventral blastoderm of *Drosophila* embryos before gastrulation in a somewhat broader domain than *twist* (Leptin, 1991). By repressing neuroectodermal genes, Snail favours the specification to mesoderm (Leptin, 1991). Snail plays a crucial role for the formation of the ventral furrow and the immigration of mesodermal cells into the embryo (Ip *et al.*, 1992b). While Snail also plays a crucial role in mesoderm formation in chordates (Thisse *et al.*, 1995; Langeland *et al.*, 1998; Sefton *et al.*, 1998; Fujiwara *et al.*, 1998), it is also expressed in the ectoderm, in particular during the formation of the neural crest (Nieto, 2002; del Barrio and Nieto, 2002; Knecht and Bronner-Fraser, 2002). Interestingly, in the mollusc *Patella vulgata*, two *snail* genes have been isolated that are both expressed in the ectoderm (Lespinet *et al.*, 2002). However, in the cnidarian *Podocoryne* the *snail* homolog is expressed during early embryonic development and later in the entocodon, together with *Brachyury* and *twist*, suggesting a role in myogenesis of the medusa (Spring *et al.*, 2002). The detailed functional as well as comparative analysis shows that Snail might

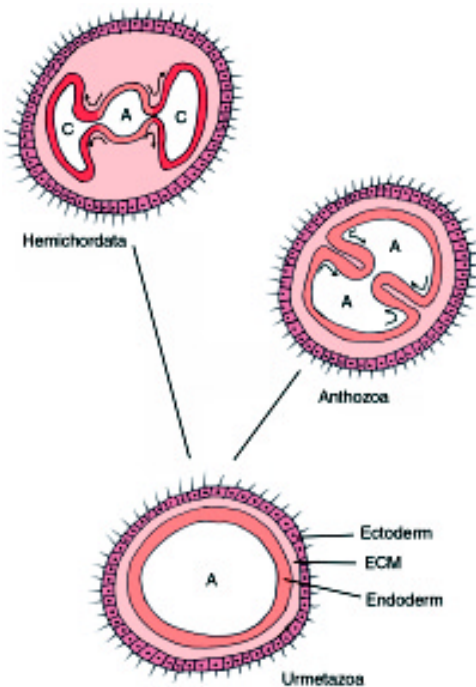
act as a master regulator of the epithelial mesenchymal transition (EMT) (Carver *et al.*, 2001; reviewed in Savagner, 2001). This involves the downregulation of specific Cadherins (Cano *et al.*, 2000) and activation of marker genes for migratory cells, such as RhoB and HNK-1 (del Barrio and Nieto, 2002). In general, *snail* is expressed in cells, that change their shape in such a way, that they contribute to the formation of a fold or a furrow in an epithelium or that detach from an epithelium to form mesenchymal tissue.

## Brachyury

More than 60 years after the *brachyury* mutant was described in mice (Dobrovskaja-Zavadskaja, 1927) the gene was identified by positional cloning (Herrmann *et al.*, 1990). Embryos with a homozygotic defect in this gene fail to elongate along the anterior-posterior axis and they do not develop mesodermal extraembryonic tissue, which leads to death *in utero*. In *Xenopus*, the T-box transcription factor *brachyury* is an early-immediate response gene of mesoderm inducers such as Activin or related TGF- $\beta$  molecules (Smith *et al.*, 1991) and its expression is maintained by FGF and Wnt3a signalling (Isaacs *et al.*, 1994; Schulte-Merker *et al.*, 1995; Liu *et al.*, 1999; Galceran *et al.*, 2001; Arnold *et al.*, 2000). Overexpression of *brachyury* in animal halves of *Xenopus* embryos leads to the dose-dependent differentiation of ventral to dorsal mesoderm (Smith *et al.*, 1991). On the other hand, *brachyury* plays a crucial role in the convergent extension movements during gastrulation that result in the elongation of the body axis. In vertebrates, Brachyury therefore seems to have a dual role in mesoderm specification and axis elongation (Beddington *et al.*, 1992). In basal deuterostomes and protostomes, *brachyury* is expressed around the blastopore (Shoguchi *et al.*, 1999; Arendt *et al.*, 2001; Gross and McClay, 2002). The opposing ends of the slit-like blastopore of the polychaete *Platynereis dumerilii*, that will develop into mouth and anus, show a stronger *brachyury* expression (Arendt *et al.*, 2001). In the deuterostome *Asterina*, a sea star, an additional *brachyury* expression domain is present, at the site, where the archenteron will break through to create the second body opening, the mouth (Shoguchi *et al.*, 1999; Croce *et al.*, 2000). Thus, the common ancestor of Protostomia and Deuterostomia, the Urbilateria, most likely also expressed *brachyury* around the blastopore and its derivatives, i.e. foregut and hindgut (Arendt *et al.*, 2001; for review see Technau, 2001). Strikingly, in embryos of the diploblastic sea anemone *Nematostella vectensis*, a basal Cnidaria, *brachyury* is also expressed around the blastopore and its derivatives, the endodermal mesenteries (Scholz and Technau, 2003). This suggests that the blastoporal expression of *brachyury* is ancestral for all Eumetazoa. Another T-box gene, expressed before gastrulation in the vegetal half of the *Xenopus* embryos, is *VegT*. Similar to *brachyury*, transcription of *VegT* is initiated by TGF- $\beta$  molecules. In vertebrates, VegT is one key molecule in first determining the mesoderm and later triggering the cellular processes, that lead to the separation of endoderm and mesoderm (Yasou and Lemaire, 1999; Clements and Woodland, 2003).

In *Xenopus*, two different behaviours have been described for mesodermal cells. Cells determined to become prechordal plate mesoderm migrate as single cells to their destinations and express the *brachyury* repressor *gooseoid* and not *brachyury*. Cells, that are designated to form posterior axial mesoderm express *brachyury*





**Fig. 3. Possible scenario of the evolutionary origin of mesoderm by enterocoely.** The common ancestor of Cnidaria and Deuterostomia is supposed to be diploblastic. In the diploblastic Anthozoa, mesenteries form by folding the endodermal epithelium towards the archenteron (A). The two layers remain separated by the mesogloea, a typical extracellular matrix (ECM). The strong longitudinal musculature of the anthozoan polyps differentiates in one side of the mesenteries, thereby breaking the radial symmetry. In hemichordates, folding of the endodermal epithelium occurs towards the outside, between endoderm and ectoderm to form the coelomic cavities (C). Hence, mesoderm formation by enterocoely is supposed to be a derivative of the epithelial folding during formation of endodermal mesenteries. The scenario predicts that similar sets of genes are involved in the morphogenesis of mesenteries in Anthozoa and mesodermal pouches in basal Deuterostomia.

and converge and extend to form the axial mesoderm. In fact, *brachyury* inhibits cell migration, and actively promotes convergent extension (Kwan and Kirschner, 2003). By comparison, *gooseoid*, which is expressed anteriorly of *brachyury* in the presumptive prechordal plate, promotes cell migration of single cells via interaction with fibronectin in the extracellular substratum. Thus, *brachyury* supports the formation of epithelial-like mesoderm (in the sense that cell-cell contacts are being kept), while *gooseoid* promotes mesenchymal mesoderm formation. This shows, that specific transcription factors act in mesoderm formation by regulating the cellular adhesive properties, and by controlling the cellular morphogenetic behavior during gastrulation. The dissection of morphogenetic properties and the differentiation potential of *brachyury* in the sea urchin *Lytechinus variegatus* suggests that involuting cells only transiently express *brachyury* while they move through the blastopore into the inside of the embryo. Blocking of Brachyury translation by a morpholino impairs gastrulation movements, but did not repress the expression of endoderm and mesoderm specific marker genes (Gross and McClay, 2002). This suggests that

one ancestral feature of *brachyury* is to regulate genes involved in morphogenetic movements during gastrulation, in particular of cells of the presumptive mesoderm. In vertebrates, Brachyury in addition regulates expression of target genes directly involved in mesoderm differentiation. In line with this, screens for downstream targets of Brachyury in *Xenopus*, the ascidian *Ciona* and sea urchins showed that in lower deuterostomes and urochordates Brachyury activates expression of genes regulating cytoskeleton, cell adhesion and cell cycle, while in *Xenopus*, a few members of signalling cascades such as *Wnt11* as well as other transcription factors (e.g. the homeobox genes *Bix 1-4*) have been identified (Tada *et al.*, 1998; Takahashi *et al.*, 1999; Di Gregorio and Levine, 1999; Tada and Smith, 2000; Rast *et al.*, 2002). It would be most interesting to see whether in basal metazoa the target genes of Brachyury are restricted to genes involved in morphogenesis or whether they also include genes involved in pattern formation, cell communication and signal transduction. So far, no direct target genes have been isolated from Cnidaria or other basal metazoa. However, the *Hydra Brachyury HyBra1* forms a synexpression group with *Wnt* and *Tcf*, suggesting that Brachyury might act in a feedback loop with the canonical Wnt pathway (Technau and Bode, 1999; Technau *et al.*, 2000).

In many Bilateria, endoderm and mesoderm involute together and have to be separated after gastrulation. Besides controlling mesodermal cell behaviours, *brachyury* also appears to be involved in the distinction between mesoderm and endoderm in the developing mesendoderm. In *Xenopus* animal cap assays brachyury homologs from a variety of bilaterians all induce mesoderm. However, Brachyury molecules from ascidians and *Drosophila*, in addition also induce endoderm. Interestingly, all bilaterian Brachyury proteins share a short conserved domain N-terminal of the T-box, except for ascidians and *Drosophila*, which lack this conserved motif. Domain swapping experiments showed that this conserved motif confers suppression of endoderm induction to the function of the protein (Marcellini *et al.*, 2003). It seems clear that ascidians and *Drosophila* both lost this motif independently. Interestingly, in both groups endoderm and mesoderm are formed at distinct places and do not derive from the same cell lineage. Hence, there was no need to separate endoderm from mesoderm anymore, which presumably led to a loss of the selective pressure on the maintenance of this motif. These findings provide circumstantial evidence for the existence of proteins that bind to the Brachyury protein and that might modulate the function of this transcription factor. Yet, no interaction partners of Brachyury have been reported to date. In summary, it will be most interesting to compare the genetic network of this gene in basal and higher metazoa. This will help us to understand how the function of the molecule evolved to the crucial role in mesoderm formation in vertebrates.

### The neural crest: ectodermal cells with mesodermal behaviours

The neural crest is generally regarded as an innovation of the vertebrates. More recently, cell population have been identified in the protochordate Amphioxus, that might be homologous to the neural crest in vertebrates (Holland and Holland, 2001; Yu *et al.*, 2002; Meulemans and Bronner-Fraser, 2002; Gostling and Shimeld, 2003). The neural crest is of ectodermal origin, located at the edges

of the neural plate. During neurulation, the neural tube forms in a process similar to endoderm invagination during gastrulation. The cells of the neural crest detach from the epithelium and start migrating as single cells in the mesenchyme of the mesoderm. Neural crest cells can adopt a large variety of different fates: they contribute to the formation of bones and cartilage, particular in the head, to melanocytes and they form the whole peripheral nervous system (Baker and Bronner-Fraser, 1997; reviewed in Knecht and Bonner Fraser, 2002). Thus, they give rise to tissues that are either considered of typical for mesoderm (e.g. bones, cartilage) or ectoderm (neurons). The transition from an epithelial to a mesenchymal state is reminiscent of that of migratory cells of the mesoderm in amphibians (e.g. the anterior mesoderm, which gives rise to the prechordal plate) and birds. In both processes – mesoderm formation and neurulation – the cells that go through EMT (epithelial mesenchymal transition) express *snail*. Thus it seems convincing, in the neural crest Snail regulates cell motility and cell adhesion, similar to its role in mesoderm formation.

## Conclusions

Although the advent of molecular developmental biology has shed some light in the molecular regulation of endoderm and mesoderm formation, its evolutionary origin remains an open question. Different modes of gastrulation can be found in different phyla or even within a single phylum, suggesting that there is no strong selective pressure on the cellular mechanism itself. However, comparative analyses suggest that invagination / epiboly might be ancestral. Similarly, it is not clear, whether mesenchymal or epithelial mode of mesoderm formation is ancestral. A mesenchymal mode of gastrulation is found in the polar immigration of endodermal cells in some Hydrozoa, the derived class of Cnidaria. Since the Anthozoans, the basal group among the diploblastic Cnidaria, form endodermal mesenteries by epithelial foldings, it is feasible, that this mode of evagination of an epithelium was also used in the Urbilateria in process of mesoderm formation (Fig. 3). Notably, muscle cells, the major derivative of the mesoderm, differentiate on one side of the mesenteries, which indicates an internal bilateral symmetry of these animals. However, this view requires further supporting data by the comparative analysis of the genes involved in gastrulation and of their molecular networks. We propose that genes that specify endoderm and mesoderm in Bilateria may have evolved their function from ancestral roles in regulating basic cellular features like cell motility, cell adhesion and proliferation. Further comparative and functional analysis of these genes in basal metazoans, in particular those, that lack mesoderm, will certainly provide clues for the evolution of endoderm and mesoderm.

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