Mesendoderm: An Ancient Germ Layer?

Minireview

Adam Rodaway* and Roger Patient†‡
*The Randall Centre
3rd Floor North
New Hunt's House
Guy's Campus
King's College London
London SE1 1UL
United Kingdom
†Institute of Genetics
University of Nottingham
Queen's Medical Centre
Nottingham NG7 2UH
United Kingdom

Formation of the three primary germ layers, ectoderm, mesoderm, and endoderm, is an early distinction between groups of cells in developing embryos. Our understanding of their generation in vertebrates has benefitted from the classical experiments of Nieuwkoop and his colleagues (referenced in Nieuwkoop, 1997), in which explants of tissue from the animal hemisphere of amphibian embryos (fated to form ectoderm) apposed to explants of vegetal tissue (fated to form endoderm) were induced to form mesoderm. These results have been widely interpreted as indicating that mesoderm forms at the interface between presumptive endoderm and presumptive ectoderm as a consequence of inductive signals from the former to the latter. However, recent data from nematodes and zebrafish suggest that endoderm and some portion of the mesoderm may derive from a bipotential layer of cells, called the mesendoderm. In addition, the genes involved in this process may be conserved.

In *C. elegans*, a nematode worm, the endoderm and much of the mesoderm derive from the mesendodermal EMS cell in the 4-cell embryo (Figure 1; referenced in Maduro et al., 2001). EMS is distinguished from its sister cell, P_2, by the absence of one maternal factor, PIE-1,

and the action of another, the transcription factor SKN-1. SKN-1 directly activates the expression of two nearly identical GATA factors, MED-1 and MED-2, which are essential for the specification of the EMS cell (Maduro et al., 2001). In addition, expressing either one of these GATA factors can induce mesendodermal differentiation in a non-EMS cell. EMS divides to give MS, a mesodermal precursor, and E, the sole progenitor of the endoderm. MS generates a variety of mesodermal cell types, including the posterior portion of the pharynx, an organ that has homologies to the heart, coelomocytes, which resemble macrophages, and a subset of body wall muscle. The two GATA factors therefore occupy a pivotal position as zygotic factors specifying a single precursor that gives rise to both mesoderm and endoderm.

In much closer relatives of the vertebrates, the sea urchins (echinodermata, a sister phylum to the chordates), the macromeres of the 16-cell embryo, and their daughters in the 32-cell embryo, are also mesendodermal, in that they give rise to both the endoderm and the majority of the mesoderm (referenced in Angerer and Angerer, 2000). What, then, is the evidence that vertebrates develop along similar lines?

Vertebrate Mesendoderm

Labeling of single cells at the mid-blastula stage in zebrafish has shown that endoderm and mesoderm, including blood, heart, and muscle, derive from cells in the 3–4 tiers nearest to the vegetal margin of the blastoderm (Warga and Nusslein-Volhard, 1999 and references therein). We will refer to these cells as mesendoderm (Figure 2A). The mesendodermal nature of these cells is also suggested, in the late blastula, by the coexpression of genes expressed by and active in the formation of mesoderm and endoderm: the transcription factors, *brachyury* (*bra*, also known as *no tail* (*ntl*) in the zebrafish) and *gata5* (*faust* in the zebrafish) (Rodaway et al., 1999). Perturbation of signaling at these early stages of development indicates common signal requirements for the expression of both *bra* and *gata5* in the mesendoderm (Rodaway et al., 1999). Yolk cell transplants induce expression
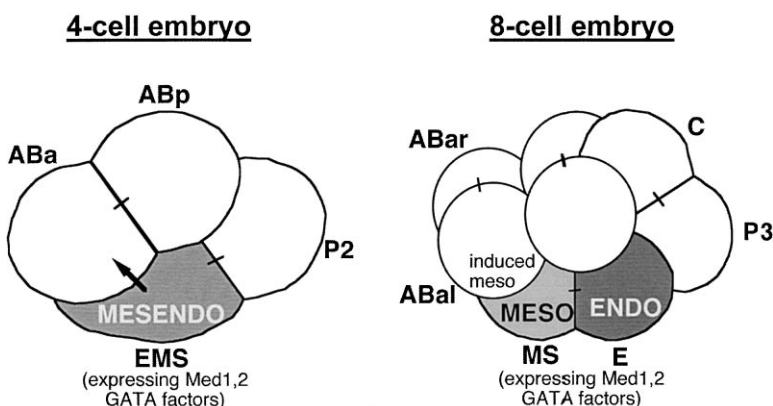

Figure 1. Formation of Mesoderm and Endoderm in *C. elegans*

In the 4-cell embryo, the mesendoderm consists of a single blastomere, EMS, which is the sister of P_2. The maternal transcription factor SKN-1 (in the absence of PIE-1) directly induces expression by EMS of two GATA factors, MED-1 and -2, which are necessary and sufficient to initiate the mesendodermal program. EMS divides to give an endodermal cell (E), and a predominantly mesodermal cell (MS), which both express MED-1 and -2. A Wnt signal from P_2 acting on EMS differentiates E from MS. The EMS cell (or possibly its daughters) is the source of a signal that induces mesoderm formation by ABal and ABar. Note that mesoderm (exclusively muscle) also arises from the C and P_3 cells.

‡E-mail: roger.patient@nottingham.ac.uk

A. Mid to Late Blastula
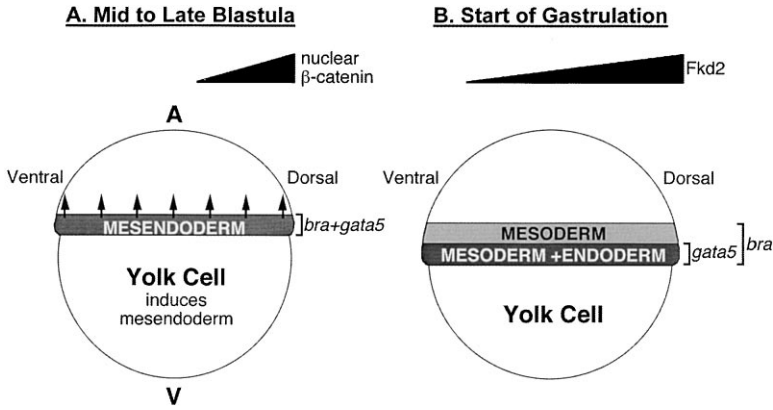
B. Start of Gastrulation

Figure 2. Formation of the Mesoderm and Endoderm in Zebrafish

(A) Mid to late blastula. The blastoderm is a cap of cells covering the animal portion of the extraembryonic yolk cell. A signal from the yolk cell induces mesendoderm, which coexpresses *bra* and *gata5*, in the 3–4 tiers of cells nearest to the vegetal margin of the blastoderm. A signal (arrows) passes from these cells to blastomeres more distant from the margin to induce further expression of *bra*, but not of *gata5*. β-catenin is concentrated in nuclei toward the dorsal side of the embryo. Animal (A) and vegetal (V) poles are indicated.

(B) Early gastrula. The mesendodermal cells in the margin become restricted to either mesoderm or endoderm. More endoderm forms dorsally, which is reflected in a gradient of expression of transcription factor *Fkd2* (expressed in gut precursors). However, endoderm forms around the entire margin (see text). The *bra* expressing mesoderm cells distal to the margin are fated to form predominantly somitic mesoderm.

of both genes in presumptive ectoderm, identifying the yolk cell as a likely source of inducing signals for both genes. Expression of dominant negative receptors shows that expression of both *gata5* and *bra* in the margin, where the blastoderm is closely apposed to the yolk cell, depends on TGF-β signaling, while a constitutively active activin receptor will induce the expression of both genes ectopically. Finally, mutant analysis indicates that likely candidates for the endogenous TGF-β signal include the nodal-related genes, *squint* and *cyclops*; however, there is at least one further yolk cell–derived signal able to induce mesoderm, because embryos missing both signals still form small amounts of mesoderm (Kimelman and Griffin, 2000).

In the frog, *Xenopus*, fate mapping also indicates that the cells of the marginal zone contain precursors for endoderm as well as mesoderm (Nieuwkoop, 1997 and references therein). The transcription factors *Mix.1* and *bra* are associated with endoderm and mesoderm development respectively, and are expressed in distinct groups of cells during gastrulation. However, prior to the onset of gastrulation these genes are expressed in the same cells (Lemaire et al., 1998). Furthermore, like *bra*, *Mix.1* is an immediate early target for TGF-β inductive signals. Thus, the marginal zone in *Xenopus* embryos contains precursors for both endoderm and mesoderm, and gene expression analysis indicates that they may derive, at least in part, from a bipotential mesendodermal population; however, a detailed understanding of the lineage relationships between mesoderm and endoderm in this region will require further fate mapping.

An Ancient Pathway

What is the evidence that GATA factors play the same role in zebrafish and *Xenopus* that they do in *C. elegans*? Zebrafish *gata5* is required for full endoderm and heart mesoderm formation (Reiter et al., 1999). Furthermore, its ectopic expression induces expression of both endoderm and heart mesoderm markers (Reiter et al., 1999, 2001). Lineage tracing shows that the induction is cell autonomous in both cases, so the induction of heart mesoderm does not appear to be a result of secondary induction by endoderm. In contrast, in *Xenopus*, before and during gastrulation, *GATA5* is not expressed in the early involuting cells of the mesendoderm (Weber et

al., 2000). Furthermore, ectopic expression of *GATA5* in presumptive ectoderm has only been shown thus far to induce endodermal marker expression. However, a closely related GATA factor, *GATA6*, is expressed in the involuting leading edge mesendoderm cells in *Xenopus* (referenced in Weber et al., 2000) and like *GATA5*, this gene is later expressed in heart and gut cells. In contrast to GATA5, GATA6 does not induce ectopic expression of endodermal genes (Weber et al., 2000). Whether it induces heart or blood mesoderm has not yet been tested. It therefore could be that, in *Xenopus*, two GATA factors are required to carry out the function of one in zebrafish.

Two GATA factors are involved in the specification of endodermal and mesodermal derivatives in the fruit fly *Drosophila*. Here the GATA transcription factor, *serpent*, is required for midgut (endoderm) as well as fat body and hemocyte (mesoderm) production (Rehorn et al., 1996), while another GATA factor, *pannier*, is required for heart formation (Gajewski et al., 1999). Since *Drosophila* is not thought to generate a mesendodermal precursor to endoderm and heart or blood mesoderm, it would appear that GATA function in the specification of these tissues is found even in the absence of mesendoderm formation. Furthermore, mutations in the *serpent* promoter that affect hemocyte production have no effect on endoderm formation, suggesting that the upstream regulation of GATA factor production differs between mesoderm and endoderm in this organism (Rehorn et al., 1996).

A Secondarily Induced Mesoderm?

In none of the animal models described does all the mesoderm derive from the mesendoderm. In *C. elegans* significant numbers of mesodermal cells, especially in body muscle, are derived from the three cells other than EMS in the 4-cell embryo (referenced in Maduro et al., 2001). Likewise in zebrafish, by the start of gastrulation, the mesendoderm continues to coexpress *bra* and *gata5*, but the adjacent 4 tiers of cells have begun to express *bra* and not *gata5* (Rodaway et al., 1999). These cells are fated to form predominantly somitic tissue, which also gives rise to body muscle (Warga and Nusslein-Volhard, 1999). Similarly, as *Xenopus* begins to gastrulate, *bra* is not expressed in the cells which will con-

tribute to the blood and prechordal plate, but is expressed a few cell diameters away from the blastopore lip, in a region fated to form somitic tissue and notochord (referenced in Kumano and Smith, 2000). Taken together, these observations suggest a distinction between two types of mesoderm early in gastrulation. One type, derived from the mesendoderm, is the source of blood- and heart-like precursors in nematodes and vertebrates alike. It also forms other mesoderm tissues, including some muscle. However, muscle also derives from the second type of mesoderm, which does not share a precursor with endoderm.

How is this non-mesendoderm-derived mesoderm induced? One view is that the distance from the vegetal/yolk-cell source of TGF-β signaling could effect differential responses, and several lines of evidence support dose dependence in the TGF-β induction of mesodermal types (Papin and Smith, 2000). Alternatively, the signal could be derived from the mesendodermal cells. Such a view would be consistent with data from the nematode for at least the AB cell, which depends on signaling from the EMS cell or its derivatives for mesoderm formation (referenced in Maduro et al., 2001; Figure 1). The secondary signal in vertebrates may involve nodal-related TGF-βs expressed in the mesendoderm (reviewed in Kimelman and Griffin, 2000). Alternatively it could be FGF, which can induce somitic muscle in presumptive ectoderm (Logan and Mohun, 1993). In support of this, blocking FGF signaling prevents the spread of *bra* expression beyond the tier of cells closest to the yolk cell in zebrafish (Rodaway et al., 1999). Furthermore, in *Xenopus*, blocking FGF signaling causes loss of somitic tissue in cells distal to the blastopore lip, and an expansion of blood—an observation which may also suggest that FGF is involved in restricting mesendoderm to the margin (Kumano and Smith, 2000).

How Is Mesendoderm Subdivided?

It seems that a population of cells (or a single cell in *C. elegans*) bipotential for mesoderm and endoderm formation may have arisen at an early stage in evolution, and that a conserved family of transcription factors, the GATA factors, is involved in its specification. Downstream targets of the GATA factors, and other gene products involved in the subsequent differentiation of these two germ layers, may also be conserved (Figure 3). Thus, in *C. elegans*, the homolog (*CEH-22*) of the vertebrate heart-associated transcription factor *Nkx2.5*, is required for formation of a heart-like structure, the pharynx (referenced in Maduro et al., 2001). Furthermore, *Nkx2.5* can substitute for the function of *CEH-22*, and both vertebrate and *C. elegans* genes are activated by GATA factors; this reinforces the idea that the similarities in these systems represent homologies. In both *C. elegans* and vertebrates, members of the HNF-3 and HNF-4 families of transcription factors are activated in the endoderm during its separation from the mesoderm, indicating further conservation of the genetic circuitry.

How is the mesendoderm apportioned between mesoderm and endoderm? In *C. elegans*, an inductive signal from P_2, which contacts EMS posteriorly, is responsible for polarizing EMS prior to its division, so that its posterior daughter adopts the E fate and its anterior daughter becomes MS. Genetic evidence indicates that Wnt signaling plays a central role in this induction of endodermal

A. Vertebrate (zebrafish)
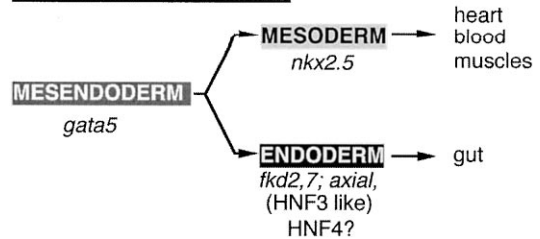
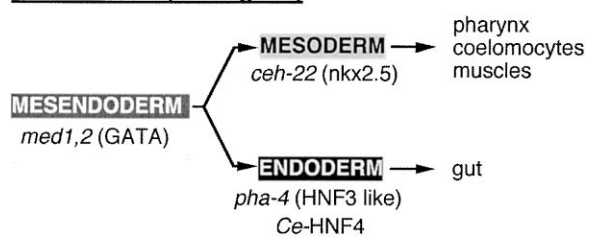## B. Nematode (C. elegans)

Figure 3. Conservation of Genes Involved in Mesendoderm Specification and Its Differentiation

Mesendodermal fate in both zebrafish and *C. elegans* is regulated by GATA factors. Separating the mesendoderm into mesoderm and endoderm involves downstream genes which are also conserved between the two species (HNF4 has not been reported yet in zebrafish, but it is implicated in endoderm development in other vertebrates. *pha-4*/HNF3 is also required for pharynx formation in *C. elegans*). The cell types produced by the mesoderm derived from the mesendoderm are also similar in vertebrates and nematodes.

fate (reviewed in Thorpe et al., 2000; Maduro et al., 2001). This signal acts, in concert with members of a diverged MAP kinase-like pathway, to inactivate the endoderm suppressive activity of POP-1, a LEF/TCF-related transcription factor. These findings raise the possibility that Wnt signaling may have a role in regulating endoderm formation in other taxa. In both ascidians (tunicates) and sea urchins, components of the Wnt signaling pathway act as a vegetalizing signal. In ascidians, β-catenin, whose translocation to the nucleus is a mediator of Wnt signaling, is able to convert presumptive notochord and epidermal cells into endoderm, while downregulation of nuclear β-catenin suppresses endoderm differentiation (Imai et al., 2000). In sea urchins the vegetal signaling mechanism is also triggered by nuclear localization of β-catenin in vegetal cells (reviewed in Angerer and Angerer, 2000). This is later reinforced by the zygotic expression of a Wnt gene, *SpWnt-8*. This signaling is required for the formation of the mesendoderm, and misexpression of *SpWnt-8* can convert ectoderm cells to endoderm. However, in contrast to *C. elegans*, the subdivision of the mesendoderm in sea urchins involves an inductive event that promotes mesoderm formation, involving *notch* signaling.

In vertebrates, with their complex and highly regulative development, the mechanism of separating mesendoderm into mesoderm and endoderm is less clear. In contrast to its vegetalizing role in invertebrate deuterostomes, it seems that the Wnt signaling pathway has been co-opted for use in dorso-ventral patterning. In the blastula, localization of β-catenin to nuclei on the prospective dorsal side of the embryo (Figure 2A) plays

a central role in formation of the dorsal axis. A possible role for the dorsalizing Wnt pathway in the segregation of the mesoderm and endoderm is suggested by zebrafish fate maps, which show more endoderm formed from the dorsal margin than the ventral. This distribution is reflected in expression of the HNF3-related protein, Fkd-2 (Warga and Nusslein-Volhard, 1999; Figure 2B). However, several genes involved in the formation of the endoderm are expressed symmetrically around the margin (Reiter et al., 2001), and some endoderm is formed from ventral regions. TGF-β/nodal signaling is also clearly involved in regulating endoderm formation (reviewed in Kimelman and Griffin, 2000), and in *Xenopus*, Wnt/β-catenin signaling can act in combination with TGF-β/nodal signaling to regulate genes expressed in dorso-anterior endoderm (Zorn et al., 1999). Thus, in vertebrates, the evidence for Wnt pathway signaling involvement is currently strongest for the specification of dorsally derived endoderm.

Conclusions and Prospects

The data reviewed here suggest that the mesendoderm may be an ancient germ layer specified by the conserved GATA family of transcription factors and with conserved downstream targets. They further suggest that this layer may contain only a subset of mesodermal precursors and that cells of the mesendoderm may induce adjacent cells to form the remaining mesodermal derivatives. The parallels drawn out here suggest that early development, at least in lower vertebrates, may have more in common with worms than might have been predicted. However, further fate mapping of single cells between blastula and gastrula stages will be needed to determine if there are truly bipotential mesendodermal cells in vertebrates. In addition, further signal perturbation experiments are necessary to explore the roles of Wnt signaling in vertebrate endoderm formation, of FGF in mesoderm formation, and the relationships between these pathways and the various TGF-β signals active in these processes.

Selected Reading

Angerer, L., and Angerer, R. (2000). Dev. Biol. *218*, 1–12.

Gajewski, K., Fossett, N., Molkentin, J.D., and Schulz, R.A. (1999). Development *126*, 5679–5688.

Imai, K., Takada, N., Satoh, N., and Satou, Y. (2000). Development *127*, 3009–3020.

Kimelman, D., and Griffin, K. (2000). Curr. Opin. Genet. Dev. *10*, 350–356.

Kumano, G., and Smith, W.C. (2000). Dev. Biol. *228*, 304–314.

Lemaire, P., Darras, S., Caillol, D., and Kodjabachian, L. (1998). Development *125*, 2371–2380.

Logan, M., and Mohun, T. (1993). Development *118*, 865–875.

Maduro, M.F., Meneghini, M.D., Bowerman, B., Broitman-Maduro, G., and Rothman, J.H. (2001). Mol. Cell *7*, 475–485.

Nieuwkoop, P.D. (1997). Cell. Mol. Life Sci. *53*, 305–318.

Papin, C., and Smith, J.C. (2000). Dev. Biol. *217*, 166–172.

Rehorn, K.-P., Thelen, H., Michelson, A.M., and Reuter, R. (1996). Development *122*, 4023–4031.

Reiter, J., Alexander, J., Rodaway, A., Yelon, D., Patient, R., Holder, N., and Stainier, D. (1999). Genes Dev. *13*, 2983–2995.

Reiter, J.F., Kikuchi, Y., and Stainier, D.Y.R. (2001). Development *128*, 125–135.

Rodaway, A.R.F., Takeda, H., Koshida, S., Price, B.M.J., Smith, J.C., Patient, R.K., and Holder, N. (1999). Development *126*, 3067–3078.

Thorpe, C.J., Schlesinger, A., and Bowerman, B. (2000). Trends Cell Biol. *10*, 10–17.

Warga, R.M., and Nusslein-Volhard, C. (1999). Development *126*, 827–838.

Weber, H., Symes, C., Walmsley, M.E., Rodaway, A.R.F., and Patient, R.K. (2000). Development *127*, 4345–4360.

Zorn, A.M., Butler, K., and Gurdon, J.B. (1999). Dev. Biol. *209*, 282–297.