

Shield formation at the onset of zebrafish gastrulation

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Summary

During vertebrate gastrulation, the three germ layers, ectoderm, mesoderm and endoderm are formed, and the resulting progenitor cells are brought into the positions from which they will later contribute more complex tissues and organs. A core element in this process is the internalization of mesodermal and endodermal progenitors at the onset of gastrulation. Although many of the molecules that induce mesendoderm have been identified, much less is known about the cellular mechanisms underlying mesendodermal cell internalization and germ layer formation.

Here we show that at the onset of zebrafish gastrulation, mesendodermal progenitors in dorsal/axial regions of the germ ring internalize by single cell delamination. Once internalized, mesendodermal progenitors upregulate E-Cadherin (Cadherin 1) expression, become increasingly motile and eventually migrate along the overlying epiblast (ectodermal) cell layer towards the animal pole of the

gastrula. When E-Cadherin function is compromised, mesendodermal progenitors still internalize, but, with gastrulation proceeding, fail to elongate and efficiently migrate along the epiblast, whereas epiblast cells themselves exhibit reduced radial cell intercalation movements. This indicates that cadherin-mediated cell-cell adhesion is needed within the forming shield for both epiblast cell intercalation, and mesendodermal progenitor cell elongation and migration during zebrafish gastrulation.

Our data provide insight into the cellular mechanisms underlying mesendodermal progenitor cell internalization and subsequent migration during zebrafish gastrulation, and the role of cadherin-mediated cell-cell adhesion in these processes.

Key words: E-Cadherin, Shield formation, Cell migration, Gastrulation, Zebrafish

Introduction

During vertebrate gastrulation, the three germ layers ectoderm, mesoderm and endoderm are formed from a common pool of blastodermal progenitor cells. This process involves the induction of different cell fates and the rearrangement of cells into distinct germ layers. Although the molecular nature of the signals determining cell fate has been extensively studied, the actual cellular rearrangements and molecules controlling these rearrangements have only begun to be analyzed.

Insight into the cellular mechanisms that regulate vertebrate germ layer formation at the onset of gastrulation comes primarily from studies in the amphibian *Xenopus* (for a review, see Winklbauer et al., 1996). Here, mesodermal and endodermal progenitor cells involute as a coherent sheet of cells at the blastopore lip. The involuting mesodermal and endodermal cell layers fold back onto the non-involuting sheet of ectodermal progenitors and move along this layer towards the animal pole of the gastrula. The layer of ectodermal progenitors is thought to facilitate and direct the migration of mesodermal progenitors by secreting both extracellular matrix proteins, such as Fibronectin, and guidance signals, such as Platelet-Derived Growth Factors (PDGFs) (Ataliotis et al., 1995; Davidson et al., 2002; Nagel et al., 2004).

Much less is known about the cellular rearrangements underlying the internalization and migration of mesendodermal

progenitor cells at the onset of zebrafish gastrulation (for a review, see Kane and Adams, 2002). In zebrafish, the first mesendodermal progenitors are induced at the margin of the blastoderm when the blastoderm starts to spread over the yolk cell (dome stage) (for reviews, see Kimelman and Schier, 2002; Warga and Stainier, 2002). When the blastoderm covers about half of the yolk cell (50% epiboly), the germ ring forms as a local thickening at the margin of the blastoderm. Germ ring formation is accompanied by convergence movements of blastodermal cells, leading to a compaction of cells at the dorsal side of the germ ring, where the embryonic organizer or ‘shield’ forms (Warga and Kane, 2003). This is also the time, when mesendodermal progenitors within the germ ring begin to internalize by moving first to the margin of the blastoderm, then downwards in direction of the yolk sac and eventually migrating back towards the animal pole of the gastrula (Warga and Kimmel, 1990).

How mesendodermal progenitor cells internalize in zebrafish is still not fully understood. Two possible modes of mesendodermal cell internalization have been proposed (Stern and Ingham, 1992; Trinkaus, 1996): (1) ‘involution’, describing the inward movement of mesendodermal progenitors cells as a cohesive sheet of cells; and (2) ‘ingression’, the internalization of single mesendodermal progenitors cells as they undergo an epithelial to mesenchymal

transition. Experiments in which single mesendodermal progenitors were transplanted into places of the gastrula where no other mesendodermal progenitors can be found have shown that these cells ingress in a cell-autonomous manner, suggesting that single-cell ingression is the primary mode for mesendodermal cell internalization (Carmany-Rampey and Schier, 2001; David and Rosa, 2001). Similarly, experiments demonstrating that mesendodermal progenitors in outer cell layers of the shield at the onset of gastrulation intermingle with ectodermal progenitors have been interpreted as evidence for mesendodermal progenitors undergoing single-cell ingression rather than involution (Shih and Fraser, 1995). By contrast, studies in which the movements of cells within the forming shield were analyzed have demonstrated that mesendodermal progenitor cells at the germ ring margin involute as a continuous stream of cells (D'Amico and Cooper, 1997; D'Amico and Cooper, 2001). It is likely that these contradictory views are due to the fact that the morphogenetic movements within the germ ring have not yet been looked at in sufficient resolution to be able to unambiguously distinguish between these two different modes of cell internalization.

The molecular pathways that control tissue morphogenesis at the onset of vertebrate gastrulation have only begun to be unraveled. The zebrafish Nodal-related genes *Squint* and *Cyclops* are required for germ layer and shield formation at the onset of gastrulation (Feldman et al., 2000; Feldman et al., 1998; Gritsman et al., 1999). Furthermore, inactivation of the Nodal antagonists *Lefty 1* and *Lefty 2* both during zebrafish and *Xenopus* gastrulation causes deregulation of Nodal signaling, expansion of mesendoderm and loss of ectoderm (Branford and Yost, 2002; Feldman et al., 2002). The expansion of mesoderm after *Lefty 1/Lefty 2* inactivation is accompanied by an extended period of mesendodermal cell internalization, and a failure of epiboly movements in zebrafish (Feldman et al., 2002) and exogastrulation in *Xenopus* (Branford and Yost, 2002). Similarly, activation of Nodal signaling in single cells transplanted into the blastoderm margin of maternal zygotic *one eyed pinhead* (*mz-oep*) mutants that are defective in receiving Nodal signals, or, alternatively, into the animal pole of wild-type embryos where endogenous Nodal signaling is low, causes these cells to autonomously express mesendodermal marker genes and to undergo cell internalization movements (Carmany-Rampey and Schier, 2001; David and Rosa, 2001). This indicates that Nodal signals are required and sufficient to induce both mesendodermal cell fate and the morphogenetic cell behaviors underlying mesendodermal cell internalization during vertebrate gastrulation.

The regulation of cell adhesion has also been shown to be required for germ layer formation at the onset of vertebrate gastrulation. Cadherins form a large group of cell-cell adhesion molecules regulating tissue morphogenesis in a variety of developmental processes (Tepass et al., 2000). During *Xenopus* and zebrafish gastrulation, the over or underexpression of *paraxial protocadherin* (*papc*) leads to defects in convergence and extension of the forming embryonic body axis (Hukriede et al., 2003; Kim et al., 1998; Yamamoto et al., 1998). Furthermore, when XB/U- or EP/C-Cadherin function is blocked at the onset of *Xenopus* gastrulation, both mesodermal progenitor cell involution and migration, as well as germ layer separation, are affected (Kuhl et al., 1996; Lee and Gumbiner,

1995; Wacker et al., 2000). Finally, inactivating E-Cadherin in *Xenopus* and zebrafish embryos causes variable defects before and during gastrulation, including impaired prechordal plate formation and migration in zebrafish and ectodermal lesions in *Xenopus* (Babb and Marrs, 2004; Levine et al., 1994). While these results provide clear macroscopic evidence for an involvement of Cadherins in tissue morphogenesis during vertebrate gastrulation, their function in vivo on a cellular level is much less understood.

In this study we have addressed two main questions. (1) What are the cellular mechanisms that underlie mesendodermal progenitor cell internalization and subsequent migration within dorsal/axial regions of the germ ring during early stages of zebrafish gastrulation? (2) How is Cadherin-mediated cell-cell adhesion involved in these processes? We provide evidence that axial mesendodermal progenitors delaminate as single cells and then migrate along the overlying epiblast (ectodermal) cell layer towards the animal pole of the gastrula. We further show that E-Cadherin is needed both in mesendodermal progenitors for elongation and efficient migration along the epiblast, and in epiblast cells for undergoing radial cell intercalation movements.

Materials and methods

Embryo maintenance

All embryos were obtained from zebrafish TL and AB wild-type lines. Fish maintenance and embryo collection was carried out as described (Mullins et al., 1994). Embryos were staged as previously described, grown at 31°C and manipulated in E3 zebrafish embryo media (Kimmel et al., 1995).

mRNA, morpholino and fluorescein-dextran injections

For mRNA synthesis the following constructs were used: pCS2-YFP, pCS2-GAP43-YFP and pCS2-Ndr2 (Rebagliati et al., 1998). mRNA was synthesized using the Ambion mMessage mMachine Kit. For mRNA overexpression, 120 pg of *YFP*, 40 pg of *GAP43-YFP* and 100 pg of *ndr2* mRNA was injected into one-cell-stage embryos. Time-lapse movies were recorded from embryos injected with either 120 pg of *YFP* and 40 pg of membrane bound- (*GAP43-YFP*) (see Movie 2 in supplementary material) or, alternatively, a combination of 40 pg of *GAP43-YFP* and 125 pl of 0.5% high molecular weight fluorescein-dextran (see Movies 1, 3 and 4 in supplementary material). For *e-cadherin morpholino* (MO) injections, 8 ng of a MO targeted against the 5' end of the *e-cadherin* cDNA (Babb and Marrs, 2004) was injected into one-cell-stage embryos. The specificity and efficiency of this MO has been tested previously (Babb and Marrs, 2004). We determined the efficiency of our own MO injections by measuring the total amount of E-Cadherin present in uninjected wild-type (control) and *e-cadherin* MO (8 ng)-injected embryos at shield and bud stage through western blotting (see Fig. S3 in supplementary material).

Embryo sectioning and immunostaining

Embryos at shield stage were fixed in 4% paraformaldehyde (PFA) at 4°C overnight, dehydrated and embedded in paraffin wax. For immunohistochemistry 10 µm sections were taken. Samples were hydrated and blocked with serum for 1 hour and incubated overnight in a humidity chamber with the following primary antibodies: E-Cadherin, 1:745; β-Catenin, 1:100, Sigma; pan-Cadherin, 1:100, Sigma. The primary antibodies were visualized by using a FITC-coupled secondary antibody (Molecular Probes, 1:2000).

Electron microscopy

Embryos were fixed in 4% paraformaldehyde, 2% glutaraldehyde in

0.1 M phosphate buffer overnight. They were postfixed in 1% osmium tetroxide (Science Services, Munich) for 1 hour, dehydrated through a graded series of ethanol and infiltrated in Embed-812 resin (Science Services, Munich) overnight. The samples were cured for 48 hours at 65°C. Ultrathin sections (70 nm thick) were cut on an Ultracut microtome (Leica Microsystems, Vienna). The samples were viewed in a Morgagni EM (FEI, Eindhoven).

In situ hybridisation

Whole-mount in situ hybridisation was performed as previously described (Barth and Wilson, 1995). For *gsc*, *ntl*, *fkf3* and *gata5* in situ hybridisation, antisense RNA probes were synthesized from partial sequences of the respective cDNA.

Embryo mounting, confocal imaging and image analysis

Embryos at shield stage were manually dechorionated and mounted in 0.8% low-melting point agarose in E3. For confocal time-lapse imaging, we used a Nikon TE 300 microscope and a BioRad Radiance 2000 Multiphoton Confocal Microscope with a 60× water immersion lens. For data acquisition, we used the LaserSharp 2000 program version 1.4 on a Windows NT-based PC. Images were taken by scanning a 207×207 μm area with 50 lines per second. The images were analyzed using Volocity (Improvision, UK) for cell movements and morphology, and ImageJ to measure the relative intensity of immunostaining in fixed tissue sections. The relative intensity of immunostaining for the antibodies used in this study (E-Cadherin, β-Catenin, pan-Cadherin) was determined by comparing the plot profiles of the plasma membrane staining between ectodermal and mesendodermal progenitors using ImageJ. For cell movement traces, we defined the cell nucleus as the center of the cell. We either determined the movement tracks of cells within the frame of our movies (blue tracks in Figs 2, 5), or, alternatively, within the germ ring margin itself (red tracks in Figs 2, 5). For the latter, we determined the position of the cell relative to the margins of the germ ring along the *x*-axis (animal-vegetal extent of the germ ring) and *y*-axis ('inner-outer' extent of the germ ring); for a schematic diagram of how the margins were defined see Fig. S1 in supplementary material. For analysis of significance between two mean values, Student's *t*-test was chosen, based on an unequal variance between the two mean values.

Cell culture

For mesendodermal and ectodermal progenitor cell cultures, cells from wild-type embryos injected with 100 pg of *cyclops* mRNA (mesendodermal) or *mz-oep* mutant embryos (ectodermal) were used. Otherwise, cultures were prepared as previously described (Montero et al., 2003). Images/time-lapse movies of the cultures were taken on a Zeiss Axioplan2 compound scope and processed using Volocity (Improvision, UK) imaging software.

Cell transplantations

Cells from donors fluorescently labeled with Rhodamine- or Fluorescein-dextran (Molecular Probes) and expressing membrane-bound (*GAP43*-) *YFP* mRNA were transplanted into unlabeled hosts, as described (Reim and Brand, 2002), and monitored using a dissecting scope (Nikon) or a Multiphoton Confocal Microscope (BioRad).

Results

Germ ring patterning and morphogenesis

To obtain insight in the cellular mechanisms underlying germ layer formation within dorsal/axial regions of the germ ring during zebrafish gastrulation, we first determined the organization and cellular rearrangements underlying mesendodermal (prechordal plate) cell internalization at early

stages of gastrulation. At the onset of gastrulation, the germ ring margin contains distinct populations of cells that express genes associated with ectodermal, mesodermal and endodermal cell identities (Gritsman et al., 2000). Irrespective of their actual fate, cells within the germ ring that have internalized are named 'hypoblast cells', whereas non-internalized cells are called 'epiblast cells'. In order to visualize the localization of the ectodermal, mesodermal and endodermal progenitor cells within the forming epiblast and hypoblast at the onset of gastrulation (60% epiboly), we determined the expression domains of marker genes delineating those different progenitor populations. Within dorsal/axial regions of the germ ring that give rise to the embryonic organizer or shield, hypoblast cells are positive for *gooseoid* (*gsc*), marking most anterior axial mesendodermal (prechordal plate) progenitors (Stachel et al., 1993), and for *gata5*, delineating endodermal precursors (Reiter et al., 1999) (Fig. 1A,B,E,F). By contrast, epiblast cells in marginal regions of the germ ring express posterior axial mesodermal marker genes such as *no-tail* (*ntl*) (Schulte-Merker et al., 1994), and further away from the margin neuroectodermal marker genes such as *forkhead3* (*fkf3*) (Odenthal and Nusslein-Volhard, 1998) (Fig. 1C,D,G,H). This suggests that within the shield at the onset of gastrulation, the hypoblast contains both anterior mesodermal and endodermal progenitors, while the epiblast is composed of neuroectodermal and, at its most marginal extent, posterior mesodermal progenitors.

To observe the dynamic rearrangement of these different populations of cells within dorsal/axial (shield) domains of the germ ring, we took high-resolution two-photon confocal time-lapse movies outlining the movements of single cells during early stages of gastrulation. For 'axial' domains of the germ ring, we recorded movies in the middle 2–4 cell diameters of the morphological recognizable shield. To visualize single cells within the germ ring margin, we labeled these cells with a mixture of fluorescein-dextran and membrane-bound GFP (*GAP43*-GFP). We distinguished between epiblast and hypoblast cells by their localization within the germ ring, their oppositely directed movements towards the vegetal and animal poles of the gastrula, respectively, and their different cellular organization, with epiblast cells being more tightly associated to each other and hypoblast cells exhibiting a more mesenchymal character. To follow the movements of single cells within the embryo, we assembled the movement tracks of these cells both relative to the position within the embryo (not taking into account changes in the shape and position of the germ ring itself), and relative to the outer surface of the germ ring (taking into account changes in the shape and position of the germ ring itself; for details see Materials and methods, and Fig. S1 in supplementary material). In the first case, the movement tracks provide insight into the overall movement of cells within the embryo, whereas in the second case, the movement of cells within their tissue is shown.

The germ ring starts to form when the blastoderm has spread about halfway over the yolk cell (50% epiboly), and becomes apparent by an accumulation of cells at the margin of the blastoderm all around the circumference of the blastula (Warga and Kimmel, 1990). This accumulation is triggered by cells close to the margin of the blastoderm slowing down their movement towards the vegetal pole of the blastula (epiboly movements) and, instead, moving as a continuous stream of

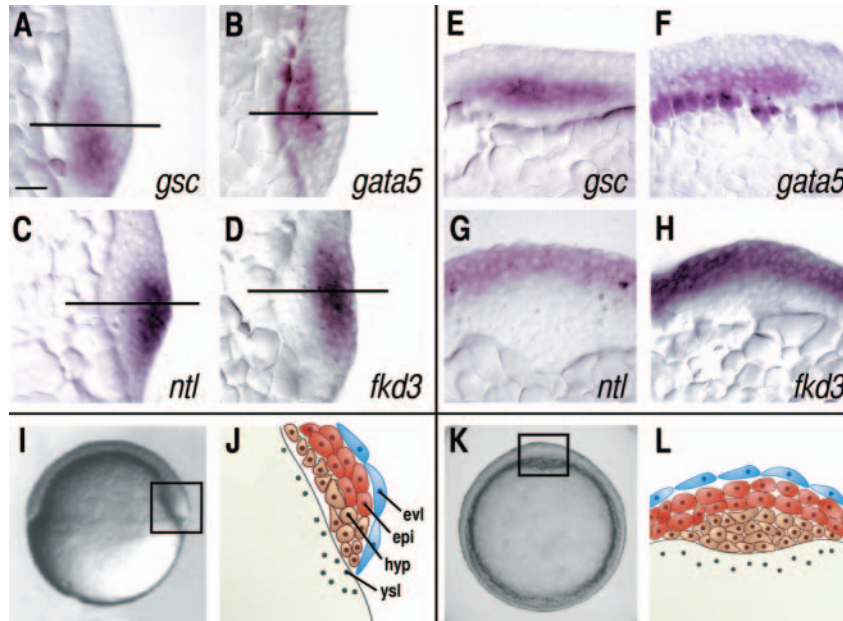


Fig. 1. Expression patterns of marker genes for (neuro)ectodermal, mesodermal and endodermal progenitor cells within the shield region at the onset of gastrulation (60% epiboly). Sagittal (A-D) and frontal (E-H) sections of the shield region demarcating the expression domains of *goosecoid* (*gsc*; A,E), *gata5* (B,F), *notail* (*ntl*; C,G) and *forkhead3* (*fkd3*; D,H). The horizontal lines in A-D indicate the level where the frontal sections in E-H were taken. (I-L) Orientation of the embryos (I,K) and schematic representations (J,L) of the shield region (boxed areas in I,K), representing sagittal (I,J) and frontal (K,L) views of the shield as shown in panels A-D and E-H, respectively. Please note that number of cell layers depicted in the schematic diagrams is lower than in the real embryo (for details about cell layer numbers, see Results). epi, epiblast; hyp, hypoblast; evl, enveloping cell layer; ysl, yolk syncytial layer. Scale bar in A: 100 μ m for A-H.

cells over the margin down towards the yolk cell, which causes the blastoderm to ‘fold-in’ at its margin; examples for the ‘folding-in’ of the blastoderm margin in dorsal/axial regions of the forming germ ring are shown in Fig. 2A-F (cells 1-3); for a complete picture of all cell tracks see Fig. S2A and Movie 1 in supplementary material).

As soon as a recognizable germ ring has formed, single mesendodermal (prechordal plate) progenitor cells within dorsal/axial domains of the germ ring segregate or ‘delaminate’ from their neighboring cells and become increasingly motile, eventually giving rise to a distinct hypoblast (mesendodermal) cell layer positioned between the yolk cell and overlying epiblast (ectodermal) cell layer (Movie 2 in supplementary material). Prechordal plate progenitor delamination is predominantly seen for cells at the tip of the germ ring directly adjacent to the yolk cell (as an example of a delaminating cell within the shield see cell 5 in Fig. 2G-I,K, and for a complete picture of all cell tracks see Fig. S2B in supplementary material). Notably, all of the prechordal plate progenitor cells – out of the 70 epiblast cells we analyzed in dorsal/axial of the germ ring – delaminated not more than 4–5 cell diameters away from the tip of the germ ring; this indicates that prechordal plate progenitor cell internalization is restricted to the marginal-most region of the germ ring. Moreover, prechordal plate progenitors positioned at the tip of the germ ring synchronously undergo delamination with direct neighbors showing similar cell behaviors (for examples of neighboring cells delaminating see the red tracks in Fig. S2B in supplementary material).

Once prechordal plate progenitors have delaminated, they move up towards the overlying epiblast (ectodermal germ layer), intercalate between each other at the forming interface between epiblast and hypoblast (mesendodermal germ layer), and eventually migrate along the epiblast in the direction of the animal pole of the gastrula (as an example for such a cell within the shield, see cell 6 in Fig. 2G-I,L; for a complete picture of all cell tracks, see Fig. S2B in supplementary material; and for a quantification of the rate of radial cell intercalations see Table

1). At the same time as the germ ring forms and prechordal plate progenitors begin to delaminate, ectodermal progenitors further away from the germ ring margin move from the deeper layers of the epiblast up towards the surface of the epiblast, ‘radially’ intercalate in between more superficially located cells, and move together with the germ ring margin towards the vegetal pole of the gastrula (as examples for such cells within the shield, see cells 1 and 4 in Fig. 2A-D,G-J; for a complete picture of all cell tracks, see Fig. S2A,B; and for a quantification of the rate of radial cell intercalations, see Table 1).

Germ layer adhesion and morphogenesis

Prechordal plate progenitors (hypoblast cells) in dorsal/axial regions of the germ ring migrate in close contact to the epiblast cell layer towards the animal pole. To do so, they might either directly contact epiblast cells or, alternatively, migrate on extracellular matrix deposited between the epiblast and hypoblast. To visualize structures on a subcellular level at the border region between epiblast and hypoblast in dorsal/axial domains of the germ ring, we took electron microscopy (EM) images of this region at the onset of gastrulation. We distinguished between presumptive epiblast and hypoblast cells by their localization within the germ ring and their different cellular organization; epiblast cells cluster more tightly together and form a clearly recognizable border to the underlying more loosely associated hypoblast cells (Fig. 3A). Analysis of these images shows that presumptive hypoblast and epiblast cells form multiple processes crossing the border between epiblast and hypoblast, thereby connecting cells from both layers directly (Fig. 3B-D). This indicates that prechordal plate progenitors (hypoblast) are in direct contact with the overlying ectodermal progenitors (epiblast) during their migration from the germ ring towards the animal pole. In addition, we frequently saw junctional complexes resembling tight junctions between cells both within the epiblast (Fig. 3E,F) and hypoblast (Fig. 3G,H). By contrast, we only found junctional complexes resembling adherens junctions between

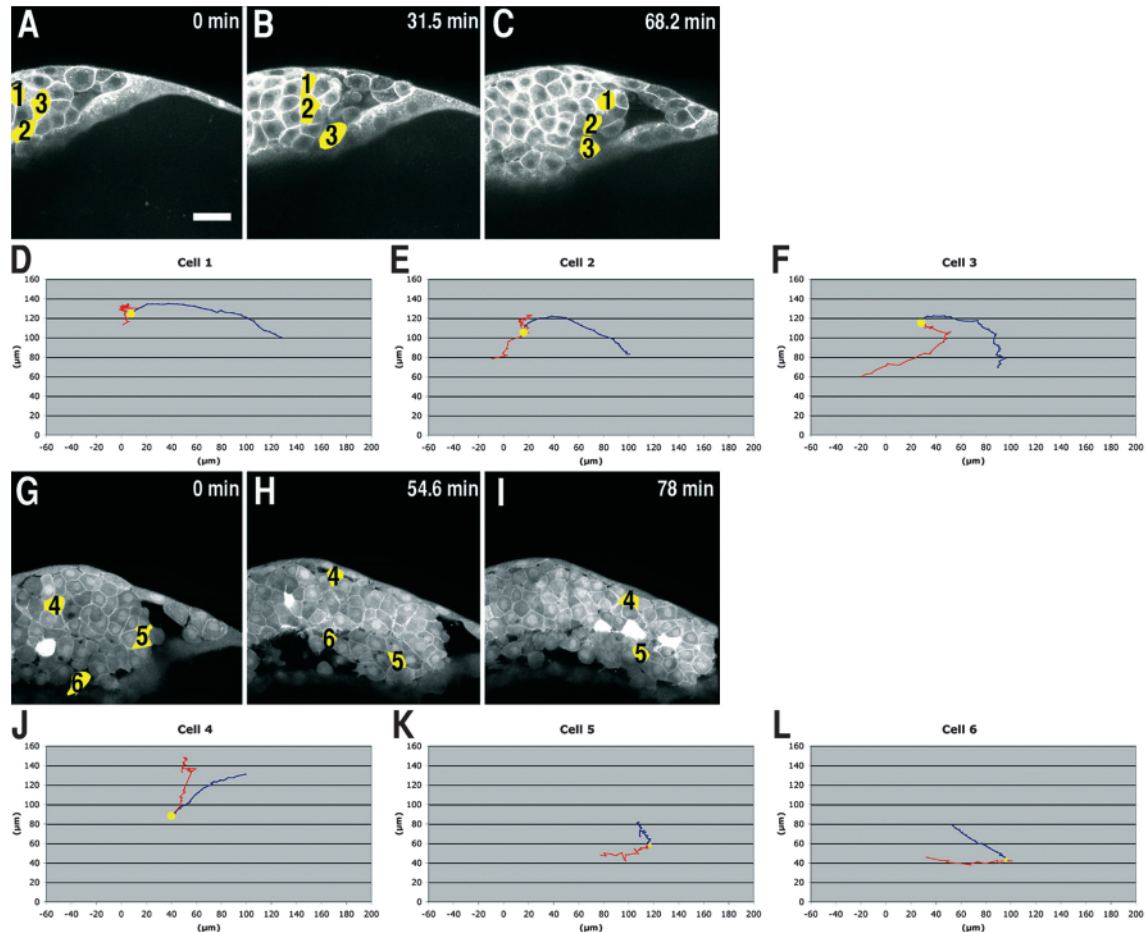


Fig. 2. Movement tracks of exemplary cells within the shield region during early stages of gastrulation. (A-C) Lateral (sagittal) views of the forming shield at 40% epiboly±0 minutes (A), +31.5 minutes (B) and +68.2 minutes (C). (D-F) Tracks of the three exemplary cells marked yellow in A-C. (G-I) Lateral (sagittal) views of the shield at 60% epiboly±0 minutes (G), +54.6 minutes (H) and +78 minutes (I). (J-L) Movement tracks of the three exemplary cells marked yellow in G-I. The cells were tracked in 2.6-minute time intervals for a total period of 68 (cells 1-3), 81 (cell 4), 66 (cell 5) and 60 (cell 6); note that this cell has already left the frame in I) minutes. For each exemplary cell, at least 10 other cells at similar positions within the germ ring from at least three independent movies were tracked. The blue tracks in D-F and J-L delineate the movement within the embryo, while the red tracks show the movement of the respective cell within the germ ring (for details see Materials and methods and Fig. S1 in supplementary material). The yellow dots (D-F, J-L) indicate the starting points of the cell tracks. Scale bar in A: 30 μm.

Table 1. Comparison of the movement speed, rate of radial cell intercalations and layering of germ layers in ectodermal and mesendodermal cells within the shield between wild-type and *e-cadherin* morphant embryos at early (6.0-7.5 hpf) and mid-gastrulation (7.5-8.5 hpf) stages

		Wild type	<i>e-cadherin</i> MO
Total speed*	Ectoderm	1.20±0.30 (6.0-8.5 hpf)	1.09±0.20§ (6.0-7.5 hpf) 0.90±0.20§ (7.5-8.5 hpf)
	Mesendoderm	1.50±0.30 (6.0-8.5 hpf)	1.48±0.30 (6.0-7.5 hpf) 1±0.2§ (7.5-8.5 hpf)
Rate of radial cell intercalations†	Ectoderm	1.90±0.39	1.48±0.57§
	Mesendoderm	2.40±0.60	1.51±0.63§
Layering of germ layers‡	Ectoderm	4-5 to 3-2	4-5 to 3
	Mesendoderm	5 to 2-1	5 to 3-4

*Movement speeds were calculated for the total movement of cells within the germ ring in μm/minute.

†Rate of radial cell intercalations was determined as the number of intercalation/cell/hour before a cell has reached the upper-most position within the epiblast (border epiblast/EVL) or hypoblast (border hypoblast/epiblast) measured from the onset of gastrulation (6 hpf) + 2.5 hours.

‡Layering of germ layers was determined as the number of cell layers within one germ layer at the onset of gastrulation (6 hpf) and at mid-gastrulation (8 hpf).

§Difference between wild-type and morphant cells is significant ($P<0.05$).

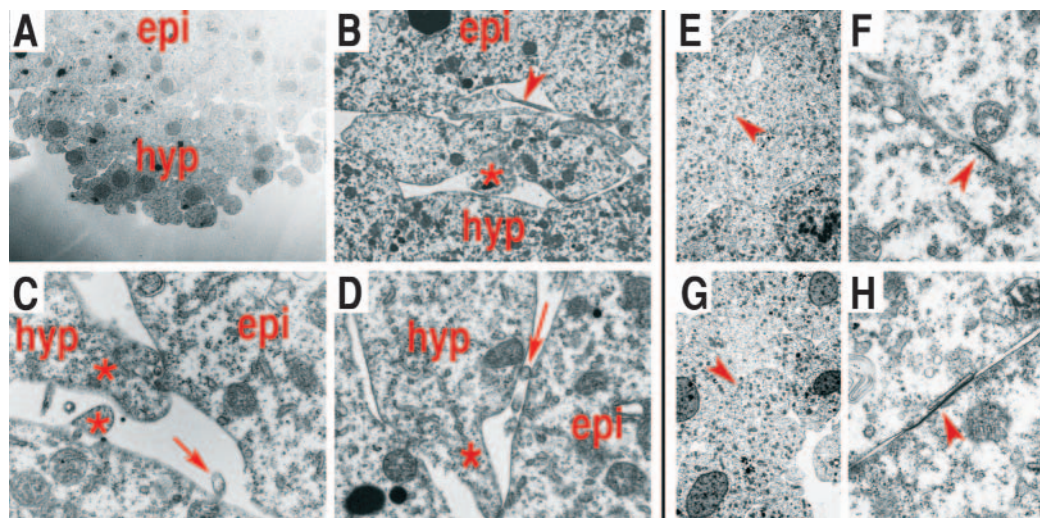
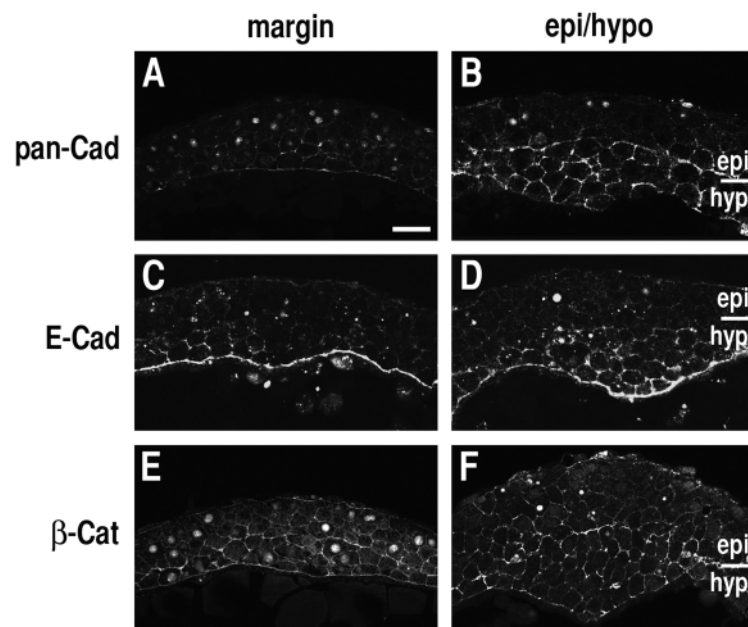


Fig. 3. Electron microscopy (EM) images of the border region between epiblast (ectoderm) and hypoblast (mesendoderm) at the onset of gastrulation (65% epiboly). (A) Frontal section of the shield region showing the apposition of epiblast and hypoblast tissues. (B) Multiple cellular processes crossing the border between epiblast and hypoblast. (C,D) Hypoblast cell process directly contacting an adjacent epiblast cell. (E-H) Structures resembling tight junctions between two epiblast (E,F) and hypoblast (G,H) cells at low (E,G) and high magnifications (F,H). Asterisks in B-D indicate cellular processes resembling pseudopods, arrowheads indicate structures resembling lamellipods and arrows indicate processes looking similar to filopods. Arrowheads in E,G point at the places within the epiblast and hypoblast, respectively, where the tight junctions shown in F,H were found. epi, epiblast; hyp, hypoblast.

cells of the enveloping cell layer, and not between epiblast or hypoblast cells (data not shown).

Cadherins regulate cell-cell contact and adhesion of various cell types during development (for a review, see Tepass et al., 2000). To test whether Cadherins might also be involved in controlling cell-cell adhesion at early stages of zebrafish gastrulation, we determined the distribution of Cadherins and Cadherin-associated molecules, such as β -Catenin, within the forming shield at the onset of gastrulation. Using antibodies recognizing multiple classical Cadherins (pan-Cadherin antibody) (Geiger et al., 1990), zebrafish E-Cadherin (Babb and Marrs, 2004) and β -Catenin, we observed Cadherin and β -

Catenin localization at the plasma membrane of both presumptive ectodermal (epiblast) and internalized prechordal plate (hypoblast) progenitors (Fig. 4; we distinguished between these two cell types on the basis of their different localization and distinct morphological features, see also previous paragraphs). Interestingly, while the expression of Cadherins and β -Catenin appear to be similar between epiblast and hypoblast close to the germ ring margin (Fig. 4A,C,E), only two to three cell diameters away from the margin, we could detect a two-times higher localization of Cadherins and β -Catenin within presumptive hypoblast cells when compared with adjacent epiblast cells (Fig. 4B,D,F). In addition, we also detected strong E-Cadherin staining at the border between hypoblast and the yolk cell (Fig. 4C,D).



E-Cadherin function within the germ ring

To determine whether E-Cadherin expression within the forming germ ring is needed for germ layer formation at the onset of gastrulation, we 'knocked down' E-Cadherin expression by injecting *morpholino* antisense oligonucleotides directed against E-Cadherin (*e-cadherin* MO). Recent studies have shown that 'knocking down' E-Cadherin using the same *e-cadherin* MO leads to variable morphogenetic defects before and during gastrulation, including reduced epiboly and convergent extension movements, but, at the same time,

Fig. 4. Localization of pan-Cadherin, E-Cadherin and β -Catenin in epiblast (ectoderm) and hypoblast (mesendoderm) cells within the shield at the onset of gastrulation (65% epiboly). Frontal sections of the shield region at the margin (A,C,E) and four to six cell diameters away from the margin (B,D,F) stained with an anti-pan-Cadherin (A,B), anti-E-Cadherin (C,D) and anti- β -Catenin (E,F) antibody. epi, epiblast; hyp, hypoblast. Scale bar in A: 40 μ m.

did not cause major defects in pattern formation during gastrulation (Babb and Marrs, 2004). To be able to study the morphogenetic function of *e-cadherin* during gastrulation, we used a concentration of the *e-cadherin* MO (8 ng/embryo) that did not cause any morphologically recognizable defects before the onset of gastrulation, or led to any obvious changes in patterning of the gastrula along its main axis of polarity (data not shown). To further control for the specificity of the morphant phenotypes observed, we compared *e-cadherin* morphant embryos to *weg* mutants that have recently been reported to represent loss-of function mutants of the zebrafish *cadherin 1* (*e-cadherin*) gene (Kane and Warga, 2004). All the main phenotypical features in respect to cell migration and morphology were found in both *e-cadherin* morphant embryos (see below) and *weg* mutants (data not shown), indicating that the morphant phenotype analyzed is specific to the (zygotic) function of E-Cadherin during gastrulation.

In order to visualize the function of E-Cadherin in controlling cellular movements at the onset of gastrulation, we

took high-resolution two-photon confocal time-lapse movies of the germ ring margin at the region of the shield in *e-cadherin* MO-injected embryos during early stages of gastrulation [6–8.5 hours post fertilization (hpf)]. The analysis of these movies showed that blastodermal cells further away from the blastoderm margin of *e-cadherin* MO-injected embryos exhibit reduced movements towards the surface of the blastoderm (reduced radial intercalation) and, together with the germ ring, in direction of the vegetal pole of the gastrula (reduced epiboly), when compared with uninjected wild-type cells during all stages analyzed (Table 1; see cells 1, 2 and 4 in Fig. 5A–E,G–J; see also Movies 3 and 4 in supplementary material; for a complete picture of all cell tracks, see Fig. S2C,D). By contrast, mesendodermal progenitors from *e-cadherin* MO-injected embryos appear to normally delaminate and move away from the germ ring margin but, at the same time, fail to efficiently move up towards the overlying epiblast and to intercalate between each other at the forming interface of epiblast and hypoblast (Table 1; as an example, see cell 3 in

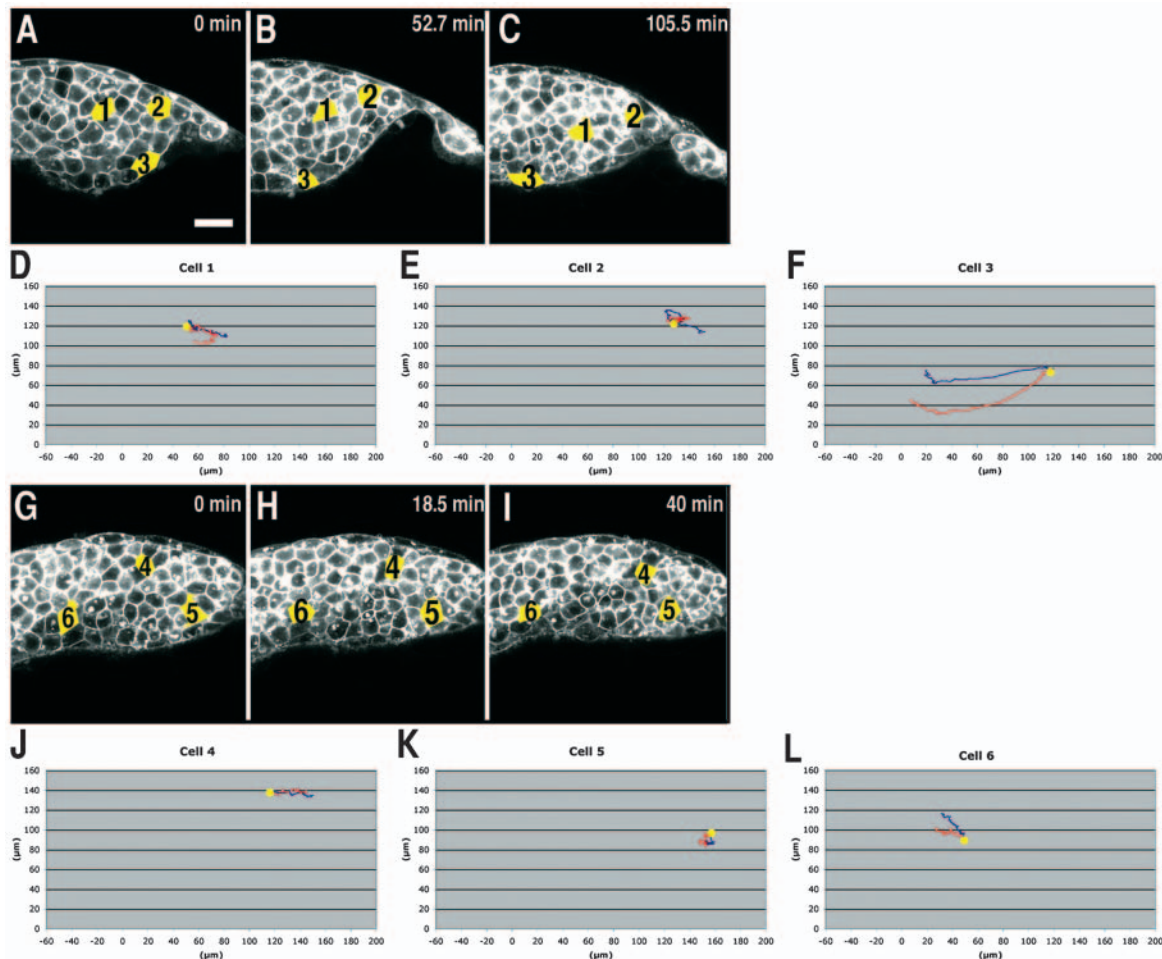


Fig. 5. Movement tracks of exemplary cells within the shield region of *e-cadherin* morphant embryos during early stages of gastrulation. (A–C) Lateral (sagittal) views of the shield at 6 hpf±0 minutes (A), +52.7 minutes (B) and +105.5 minutes (C). (D–F) Tracks of the three exemplary cells marked yellow in A–C. (G–I) Lateral (sagittal) views of the shield at 7.5 hpf±0 minutes (G), +18.5 minutes (H) and +40 minutes (I). (J–L) Tracks of the three exemplary cells marked yellow in G–I. The cells were tracked in 2.6-minute time intervals for a total period of 105 (cells 1–3) and 63 minutes (cells 4–6). For each exemplary cell, at least 10 other cells at similar positions within the germ ring from at least three independent movies were tracked. The blue tracks in D–F and J–L delineate the movement within the embryo, while the red tracks show the movement of the respective cell within the germ ring (for details see Materials and methods and Fig. S1 in supplementary material). The yellow dots in D–F,J–L indicate the starting points of the cell tracks. Scale bar in A: 30 μm.

Fig. 5A-C,F; see also Movie 3 and see Fig. S2C). In addition, when gastrulation has proceeded and a border has formed between the epiblast and hypoblast (7.5 hpf), mesendodermal cell migration along this border is reduced when compared with uninjected wild-type cells (Table 1; as examples see cells 5 and 6 in Fig. 5G-I,K,L; see also Movie 4 and Fig. S2D in supplementary material). This indicates that E-Cadherin function is required for radial intercalations of ectodermal and radial intercalation/anterior migration of mesendodermal progenitors within the shield during gastrulation.

To investigate whether these cell movement defects in *e-cadherin* morphant embryos are due to defective cell-cell adhesion in ectodermal and mesendodermal cells, we established primary cultures of ectodermal and mesendodermal progenitor cells and monitored the cell aggregation behavior throughout the first few hours in culture. Ectodermal cells were taken from maternal-zygotic *one-eyed-pinhead* mutant embryos (*mz-oep*), which lack most of their mesendodermal progenitors (Gritsman et al., 1999), whereas mesendodermal cells were taken from wild-type embryos overexpressing the mesendoderm-inducing Nodal signal Cyclops (Cyc) (Montero et al., 2003; Rebagliati et al., 1998). We confirmed the difference in cell fate between these two populations of cells by detecting strong expression of the anterior axial mesendodermal (prechordal plate) marker gene *gsc* in *cyc*-

injected mesendodermal, but not *mz-oep* ectodermal, cells (data not shown). In both ectodermal and mesendodermal cell cultures, cells rapidly assemble into distinct clusters of cells consisting of 20-100 cells (Fig. 6A,C,G,I). When E-Cadherin is inactivated in ectodermal or mesendodermal cells, they still bind to each other but are unable to cluster as efficiently as their wild-type counterparts (Fig. 6B,D,H,J). This difference in the ability to cluster between wild-type and *e-cadherin* morphant cells becomes particularly obvious when wild-type and morphant cells are co-cultured and wild-type cells form tight clusters that are surrounded by loosely associated cells from *e-cadherin* MO injected embryos (Fig. 6E,F,K,L and Movies 5, 6). Taken together, these data indicate that E-Cadherin is required for cell-cell adhesion in gastrulating ectodermal and mesendodermal cells.

E-Cadherin might not only be required for cell-cell adhesion within the germ layers but might also be needed for cell-cell adhesion and/or repulsion between the germ layers. In order to test whether E-Cadherin controls germ layer adhesion and/or repulsion, we prepared cultures containing both ectodermal and mesendodermal progenitor cells. In co-cultures of wild-type ectodermal and mesendodermal cells, these two cell-types sort into separate tightly clustered groups of cells, indicating that ectodermal and mesendodermal progenitors possess differential adhesive properties (Fig. 6M,N). When ectodermal

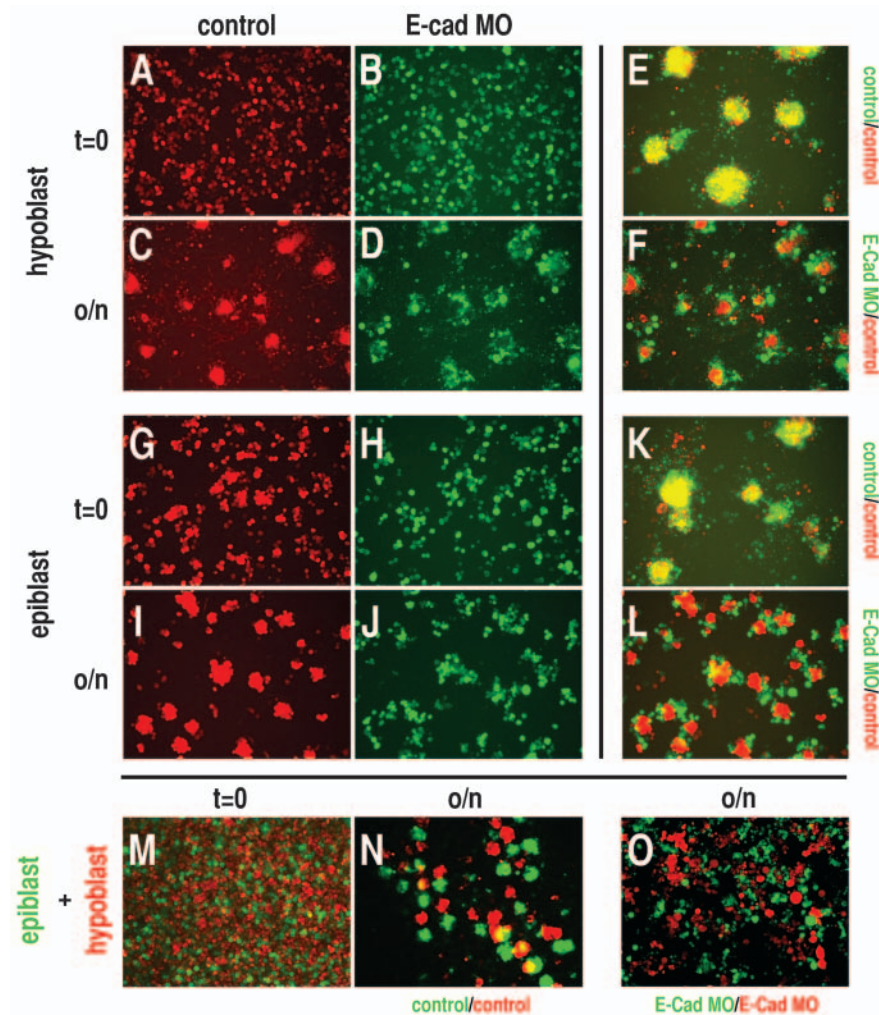


Fig. 6. Clustering behavior of cultured mesendodermal (hypoblast) and ectodermal (epiblast) progenitor cells from wild-type/*cyc*-overexpressing (hypoblast) and *mz-oep* (epiblast) embryos in the absence or presence of E-Cadherin. (A-D) Hypoblast cells from wild-type/*cyc*-overexpressing (A,C; red) and *e-cadherin* morphant/*cyc*-overexpressing (B,D; green) embryos, after plating out (A,B; t=0) and after 16 hours in culture (C,D; o/n). (E) Wild-type/*cyc*-overexpressing hypoblast cells labeled in red or green ('control/control') after 16 hours in culture (o/n). (F) Wild-type/*cyc*-overexpressing hypoblast cells (labeled in red) mixed with *e-cadherin* morphant hypoblast cells (labeled in green) after 16 hours in culture (o/n). (G-L) Epiblast cells from *mz-oep* (G,I; red) and *e-cadherin* morphant *mz-oep* (H,J; green) embryos, after plating out (G,H; t=0) and after 16 hours in culture (I,J; o/n). (K) *mz-oep* epiblast cells labeled in red or green ('control/control') after 16 hours in culture (o/n). (L) *mz-oep* epiblast cells (labeled in red) mixed with *e-cadherin* morphant *mz-oep* epiblast cells (labeled in green) after 16 hours in culture (o/n). (M-O) Wild-type/*cyc*-overexpressing hypoblast cells labeled in red mixed with *mz-oep* epiblast cells labeled in green, after plating out (M; t=0) and after 16 hours in culture (N,O; o/n); at 16 hours, mixed cultures are shown for control epiblast and hypoblast cells (N), and for epiblast and hypoblast cells injected with *e-cadherin* MO (O). Green, fluorescein; red, rhodamine.

and mesendodermal cells from *e-cadherin* morphant embryos are co-cultured, both ectodermal and mesendodermal cells show reduced clustering with themselves (see also above), but still retain the ability to sort into separate groups of cells (Fig. 6O). This suggests that although E-Cadherin is required for cell-cell adhesion within the germ layers, it is not an essential component determining the differential adhesiveness between ectodermal and mesendodermal cells.

To determine whether the defect in cell-cell adhesion of ectodermal and mesendodermal cells in the absence of E-Cadherin leads to alterations of their cellular morphology that might correspond to their cell movement phenotypes, we recorded and analyzed the shape of anterior mesendodermal (prechordal plate) and overlying ectodermal (epiblast) cells from wild-type and *e-cadherin* MO-injected embryos at early stages of gastrulation. At these early stages, the identity of prechordal plate progenitors appears to be unchanged in *e-cadherin* morphant embryos (Babb and Marrs, 2004). Although the overall appearance of epiblast cells looks indistinguishable between wild-type and *e-cadherin* MO-injected embryos (for details on cellular processes, see below), cells at the leading edge of the prechordal plate, close to the border between epiblast and hypoblast, are less elongated in *e-cadherin* MO-injected embryos than in wild-type siblings (circularity of wild-type cells was 0.69 ± 0.17 compared with 0.82 ± 0.12 in morphant cells; $P < 0.05$; Fig. 7A,B). These results suggest that E-Cadherin is needed for the elongation of cells at the leading edge of the prechordal plate close to the border between epiblast and hypoblast, a morphological feature previously shown to correlate with the migratory activity of prechordal plate cells at the onset of zebrafish gastrulation (Montero et al., 2003; Ulrich et al., 2003).

E-Cadherin might function in a cell-autonomous way within prechordal plate progenitors for cell elongation and migration, but might also be needed in a cell-non-autonomous way within surrounding cells of the prechordal plate and/or overlying epiblast to enable prechordal plate progenitors to elongate and migrate. In order to distinguish between these two possibilities, we transplanted single/small groups of wild-type prechordal plate progenitors (1–20 cells) into the forming prechordal plate of *e-cadherin* morphant embryos at shield stage and vice versa. While wild-type prechordal plate progenitors within the prechordal plate of *e-cadherin* morphant embryos in 11 out of 13 analyzed cases were indistinguishable from morphant cells (Fig. 7E,F), *e-cadherin* morphant cells within the prechordal plate of wild-type embryos in four out of 10 analyzed cases were less elongated than wild-type cells in similar positions (the remaining six cases showed similar but clearly milder phenotypes than the four severe cases; Fig. 7C,D). We also transplanted a mix of differentially labeled wild-type and *e-cadherin* morphant cells into the prechordal plate of wild-type embryos and monitored their position within the prechordal plate at the end of gastrulation. In 12 out of 32 analyzed embryos, the wild-type cells were positioned anterior of the morphant cells, whereas in the remaining 20 cases, wild-type and morphant cells either ended up in similar positions (18) or morphant cells were located anterior of wild-type cells (2; Fig. 7G,H). This suggests that E-Cadherin is both cell-non-autonomously, as well as cell-autonomously, required for prechordal plate cell elongation, process formation and migration.

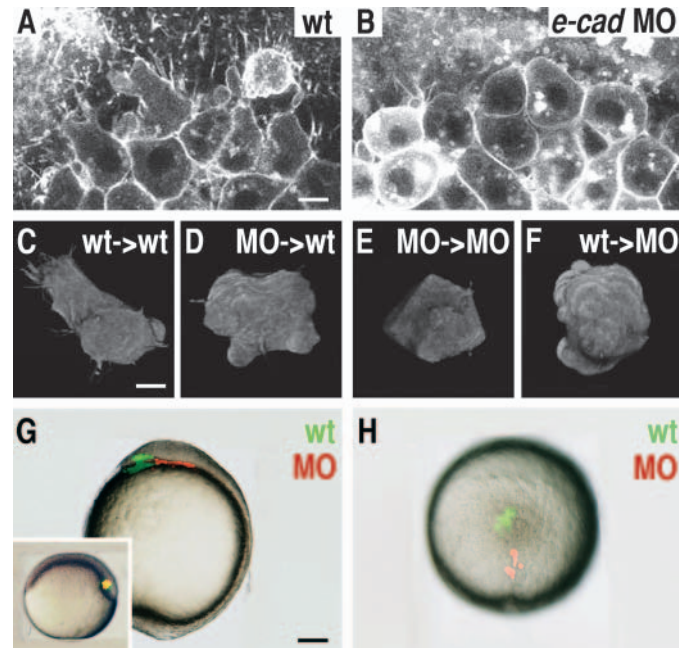


Fig. 7. Cellular morphology and position of anterior mesendodermal (prechordal plate) cells in wild-type, *e-cadherin* morphant and mosaic embryos at early and late stages of gastrulation. (A,B) Face-on view at the anterior edge of the prechordal plate of wild-type (A) and *e-cadherin* morphant (B) embryos at 6.5 hpf. (C,D) Three-dimensional reconstruction of a transplanted wild-type (C) and *e-cadherin* morphant (D) cell within the anterior prechordal plate of a wild-type embryo at 7 hpf. (E,F) Three-dimensional reconstruction of a transplanted *e-cadherin* morphant (E) and wild-type (F) cell within the prechordal plate of an *e-cadherin* morphant embryo at 7 hpf. (G,H) Position of transplanted wild-type (green) and *e-cadherin* morphant (red) cells within the prechordal plate of a wild-type embryo at 10 hpf; lateral view with anterior to the left (G), and animal view with anterior to the top (H). The inset in G shows the position of the transplanted cells (yellow) within the shield directly after the transplantation, with dorsal to the right. For all transplantation experiments (C–H), cells were transplanted at 6 hpf and the distribution (mixing) of the different cell types was monitored straight after the transplantation. MO, *e-cadherin* morphant cells; wt, wild-type cells. Scale bars: in A, 20 µm; in C, 10 µm; in G, 200 µm.

The reduced cell elongation and migration of prechordal plate cells at the border between epiblast and hypoblast in *e-cadherin* MO-injected embryos might be a consequence of more general defects in the formation of the interface between these tissues. We, therefore, analyzed specifically the border region between the epiblast and hypoblast within the shield at the onset of gastrulation (65% epiboly) in wild-type and *e-cadherin* morphant embryos. In *e-cadherin* MO-injected embryos, the border between epiblast and hypoblast within the shield is, although present and recognizable, morphologically less distinct when compared with uninjected control embryos (compare Movie 2 with Movies 3 and 4) suggesting that E-Cadherin controls the apposition of these layers. This assumption is further supported by the observation that the dense layer of cellular processes found in between the epiblast and hypoblast layers in wild-type embryos (see also Fig. 3) is

strongly reduced in *e-cadherin* MO-injected embryos, as detected both in confocal sections of this region (Fig. 8A,B) and in electron microscopy images of cells at the border between these tissues (Fig. 8C,D). By contrast, although the border epiblast and hypoblast was often indistinct in *e-cadherin* morphant embryos, we did not observe cells crossing this border, neither from the epiblast nor from the hypoblast (data not shown), nor is the differential sorting behavior of ectodermal and mesendodermal cells in culture affected when E-Cadherin function is diminished (Fig. 6M-O). This indicates that E-Cadherin is required for cell elongation and process formation at the border between epiblast and hypoblast, but is not needed to restrict the movement of cells between the forming germ layers.

Discussion

In this study, we show that mesendodermal progenitors within the forming shield internalize by single-cell delamination at the onset of zebrafish gastrulation. Once internalized, mesendodermal progenitors upregulate E-Cadherin (Cadherin 1) expression and migrate along the overlying layer of epiblast (ectodermal) cells, away from the germ ring towards the animal pole of the gastrula. In embryos where E-Cadherin function is compromised, mesendodermal progenitors fail to elongate, intercalate and, with gastrulation proceeding, also to efficiently migrate along the overlying epiblast, while epiblast cells themselves exhibit reduced radial cell intercalation movements.

Cellular mechanisms underlying germ ring formation, mesendodermal cell internalization and migration

Zebrafish gastrulation starts when the blastoderm covers about half of the yolk cell (50% epiboly) and the germ ring emerges as a local thickening at the margin of the blastoderm all around the circumference of the blastula (Warga and Kimmel, 1990). We show that the germ ring in zebrafish forms by cells at the blastoderm margin transiently slowing down their movement

towards the vegetal pole of the blastula (epiboly movements) and moving over the margin down towards the yolk cell where they accumulate. This movement resembles the 'folding-in' of a sheet of cells at its margin and shares some similarities with the involution movements of the mesodermal and endodermal germ layers at the onset of *Xenopus* gastrulation (for a review, see Winklbauer et al., 1996). However, in contrast to *Xenopus* involution, germ ring formation in zebrafish occurs before the first mesendodermal progenitors delaminate and appears to be at least partially independent of mesendodermal cell fate inducing signals such as Nodals (J.-A.M. and C.-P.H., unpublished). This suggests that the formation of the germ ring and the germ layers in zebrafish are separable events that involve different sets of cell fate inducing and morphogenetic signals.

Although the molecular pathways that trigger germ ring formation at the onset of zebrafish gastrulation have yet to be elucidated, much more is known about the signals responsible for mesendodermal cell fate induction and progenitor cell internalization. The Nodal-related genes *cyclops* and *squint* are required and sufficient to induce mesendodermal cell fate (Feldman et al., 2000; Feldman et al., 1998; Gritsman et al., 1999), and to trigger the morphogenetic processes leading to mesendodermal progenitor cell internalization (Branford and Yost, 2002; Feldman et al., 2002). Furthermore, the observation that Nodal signaling can induce cell internalization in a cell-autonomous way, has been interpreted as if single-cell ingress would be the predominant way by which zebrafish mesendodermal progenitors internalize (Carmany-Rampey and Schier, 2001; David and Rosa, 2001). In this study, we found that single mesendodermal (prechordal plate) progenitors delaminate at the margin of the germ ring close to the yolk cell, supporting the notion that mesendodermal progenitors ingress as single cells. Importantly, we did not observe ingress of cells further away from the germ ring margin (greater than four cell diameters) suggesting that ingress is restricted to a marginal region within the germ ring.

We also see – predominantly in paraxial regions of the germ ring – a continuous flow of cells originating within the

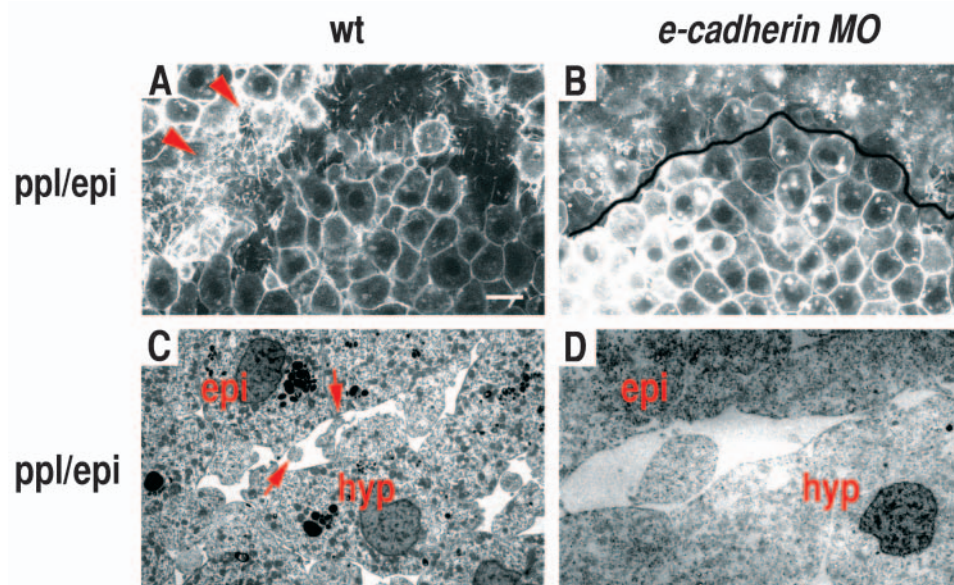


Fig. 8. Cellular organization of the border region between prechordal plate and overlying epiblast in wild-type and *e-cadherin* morphant embryos at the onset of gastrulation. (A,B) Border region between prechordal plate and overlying epiblast cells in wild-type (A) and *e-cadherin* morphant (B) embryos at 6.5 hpf. (C,D) Electron microscopy images (frontal views) of the border region between anterior prechordal plate cells and overlying epiblast cells in wild-type (C) and *e-cadherin* morphant (D) embryos at 6.5 hpf. Arrowheads in A and arrows in C indicate cellular processes at the interface between prechordal plate and epiblast. ppl/epi, border region between prechordal plate and epiblast. Scale bar in A: 30 μ m.

blastoderm further away from the germ ring margin, and moving over the margin towards the place where mesendodermal progenitors delaminate (J.-A.M. and C.-P.H., unpublished). This might create the impression that delaminating mesendodermal progenitors are part of a continuous 'involuting' sheet of cells, although cells within the germ ring are clearly not organized into sheets, neither in the epiblast nor in the hypoblast, and certainly not between epiblast and hypoblast. This indicates that single-cell ingression or 'delamination' at the margin of the germ ring, but not involution of a continuous sheet of cells, is the primary way by which mesendodermal progenitors internalize during zebrafish gastrulation.

Once internalized, mesendodermal (prechordal plate) progenitors move towards the interface between epiblast and hypoblast, and migrate along this interface in the direction of the animal pole. The epiblast, therefore, forms the substrate on which internalized prechordal plate progenitors migrate. As cells within the epiblast undergo radial cell intercalations that move these cells towards the vegetal pole of the gastrula, the prechordal plate progenitors migrate on a layer of cells that moves in the opposite direction to their own movement. In order to generate some net movement towards the animal pole, prechordal plate progenitors have therefore not only to reach the animal pole, but also to counteract the movement of the epiblast cell layer in the opposite direction.

The role of E-Cadherin-mediated cell adhesion for the formation of the germ layers

E-Cadherin has previously been shown to control tissue integrity and morphogenesis during vertebrate gastrulation (Babb and Marrs, 2004; Levine et al., 1994). In *Xenopus*, blocking E-Cadherin function using an anti-E-Cadherin antibody leads to defects in the integrity of the ectoderm during epiboly (Levine et al., 1994). Furthermore, 'knocking down' E-Cadherin translation in zebrafish results in variable defects before and during gastrulation, including delayed epiboly, reduced convergent extension of the body axis and impaired advancement of the prechordal plate towards the animal pole of the gastrula (Babb and Marrs, 2004). The results of our study provide insight into the cellular mechanisms by which E-Cadherin controls these different processes. We show that the epiboly defect in *e-cadherin* morphant embryos is most likely due to reduced radial intercalation movements of ectodermal progenitors in these embryos. Similarly, we present evidence that reduced cell elongation, process formation and anterior migration of prechordal plate progenitors is the cause for the posterior displacement of the prechordal plate previously observed in these embryos (Babb and Marrs, 2004). As E-Cadherin function has not yet been directly associated with any of these morphogenetic processes, either during zebrafish or *Xenopus* gastrulation, this is the first demonstration of such a function of E-Cadherin at early stages of gastrulation.

Internalizing prechordal plate progenitors in zebrafish have recently been suggested to undergo an epithelial to mesenchymal transformation (EMT), which is triggered by the nuclear localization of Snail, a transcriptional repressor of *e-cadherin* expression (Yamashita et al., 2004). We were therefore surprised to see that prechordal plate progenitors in our study upregulated rather than downregulated E-Cadherin expression. The most likely explanations for this discrepancy

are that either the downregulation of E-Cadherin is not a suitable readout for EMT in zebrafish, or, alternatively, that mesendodermal cells are actually not undergoing EMT. Supporting the latter notion that mesendodermal progenitors are not undergoing a 'classical' form of EMT are our own observations that neither epiblast cells nor hypoblast cells exhibit clear epithelial features such as an apical-basolateral polarity. Instead, both cell types move within their tissues, show dynamic changes in their cellular morphology and form multiple cellular extensions (Ulrich et al., 2003). The only obvious difference between epiblast and hypoblast cells is that hypoblast cells, in particular in paraxial regions of the germ ring, are more loosely associated and show a more prominent elongation in the direction of their individual cell migration. It therefore is quite likely that internalizing mesendodermal progenitor cells, rather than undergoing EMT, primarily change their general state of adhesiveness (e.g. through the upregulation of E-Cadherin expression), which allows them then to delaminate from the epiblast and take on a more mesenchymal appearance.

The differential expression of E-Cadherin between epiblast (ectodermal) and hypoblast (mesendodermal) progenitor cells might not only determine the different states in cellular motility between these two cell types, but might also allow mesendodermal progenitor cells to delaminate and remain separated from the pool of ectodermal progenitors. Evidence for a role of Cadherins in germ layer separation comes primarily from studies in *Xenopus* demonstrating that XB/U- and EP/C-Cadherin activity is required for the separation of the ectodermal and mesodermal germ layers at the onset of gastrulation (Wacker et al., 2000). By contrast, we did not observe any such defects in zebrafish embryos lacking E-Cadherin activity. The most likely explanation for this is that the function of E-Cadherin in germ layer separation is redundant to the function of other yet unidentified Cadherins expressed in the same territory. Future studies will have to address which other Cadherins are expressed within the zebrafish germ ring and how they functionally interact in regulating germ layer formation and separation at the onset of gastrulation.

Conclusions

With this study we provide the first detailed analysis of the cellular rearrangements underlying germ ring formation and mesendodermal progenitor cell internalization at the onset of zebrafish gastrulation. From this analysis we conclude: (1) that mesendodermal progenitor cells segregate or 'delaminate' as single cells within dorsal/axial regions of the germ ring margin; (2) that delaminated mesendodermal progenitors migrate along the overlying layer of non-internalizing ectodermal progenitors away from the germ ring towards the animal pole of the gastrula; and (3) that Cadherin-mediated cell-cell adhesion controls cell movements within the germ layers, and mesendodermal progenitor cell elongation and process formation at the interface between the germ layers. These observations provide a solid starting point from where to further analyze the cellular and molecular mechanisms underlying germ layer formation during zebrafish gastrulation.

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Supplementary material

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/132/6/1187/DC1>

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