Friction forces position the neural anlage

Michael Smutny1, Zsuzsa Ákos2, Silvia Grigolon3, Shayan Shamipour1, Verena Ruprecht1,5, Daniel Čapek1, Martin Behrndt1, Ekaterina Papusheva1, Masazumi Tada6, Björn Hof7, Tamás Vicsek2, Guillaume Salbreux3 and Carl-Philipp Heisenberg1,7

During embryonic development, mechanical forces are essential for cellular rearrangements driving tissue morphogenesis. Here, we show that in the early zebrafish embryo, friction forces are generated at the interface between anterior axial mesoderm (prechordal plate, ppl) progenitors migrating towards the animal pole and neurectoderm progenitors moving in the opposite direction towards the vegetal pole of the embryo. These friction forces lead to global rearrangement of cells within the neurectoderm and determine the position of the neural anlage. Using a combination of experiments and simulations, we show that this process depends on hydrodynamic coupling between neurectoderm and ppl as a result of E-cadherin-mediated adhesion between those tissues. Our data thus establish the emergence of friction forces at the interface between moving tissues as a critical force-generating process shaping the embryo.

Throughout embryonic development, tissue morphogenesis depends on mechanical forces that drive cell rearrangements and global tissue shape changes1,2. In zebrafish gastrulation, epiboly, internalization, convergence and extension constitute the main cellular processes by which the embryo takes shape3. Although recent studies have unravelled key force-generating mechanisms mediating these different cellular processes4, how forces between neighbouring tissues are generated, perceived and integrated is yet poorly understood.

Development of the central nervous system in vertebrates involves extensive morphogenetic movements within the embryonic neurectoderm4. The zebrafish nervous system organization becomes first apparent at gastrulation5, and morphogenesis of the neurectoderm is accompanied by neighbouring tissues undergoing dynamic cellular reorganization6. Recent studies in zebrafish suggested that the formation of the mesoderm and endoderm (mesendoderm) germ layers is required for proper morphogenesis of the overlying neurectoderm during neural keel formation7,8. However, the mechanisms by which mesendoderm influences neurectoderm morphogenesis have only started to be unravelled.

RESULTS

Anterior axial mesendoderm (prechordal plate) collective cell migration affects neurectoderm morphogenesis

To investigate the role of mesendoderm in neurectoderm morphogenesis (for tissue organization within the gastrulating embryo, see Fig. 1), we turned to zebrafish maternal zygotic (MZ) one-eyed-pinhead (oep) mutant embryos9, which lack much of the mesendoderm germ layers due to defective Nodal/TGF-β-signalling. Interestingly, when analysing MZoep mutants at late stages of gastrulation, we found that the anterior neural anlage was positioned closer to the vegetal pole than in wild-type (wt) embryos (Fig. 2a,b,i,j and Supplementary Fig. 2k–m). This points at the intriguing possibility that mesendoderm is required for proper positioning of the anterior neural anlage. To further test this possibility, we analysed how the neurectoderm, which gives rise to the anterior neural anlage, interacts with the underlying anterior axial mesendoderm (prechordal plate, ppl) during gastrulation. Previous studies have suggested that the ppl moves as a migrating cell collective in a straight path towards the animal pole, while the neurectoderm moves in the opposite direction towards the vegetal pole (Fig. 1a–e)10. To understand how these in opposite directions moving tissues might influence each other, we first analysed the localization of molecules involved in cell–cell and cell–extracellular matrix (ECM) adhesion at the neurectoderm/ppl interface. We found that the cell–cell adhesion receptor E-cadherin accumulated at the interface between ppl and neurectoderm during gastrulation (Fig. 1f), supporting previous observations that ppl and neurectoderm cells form E-cadherin-mediated cell–cell contacts at this interface10. In contrast, ECM components, such as fibronectin, did not show any recognizable accumulations at the neurectoderm/ppl interface until late stages of gastrulation (Supplementary Fig. 1a–c),

1Institute of Science and Technology Austria, Am Campus 1, A-3400 Klosterneuburg, Austria. 2Department of Biological Physics, Eötvös University, Pázmány Péter sétány 1A, Budapest H-1117, Hungary. 3The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK. 4Centre for Genomic Regulation (CRG), The Barcelona Institute for Science and Technology, Dr. Aiguader 88, 08003 Barcelona, Spain. 5Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain. 6Department of Cell and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK.
7Correspondence should be addressed to C.-P.H. (e-mail: heisenberg@ist.ac.at)

Received 21 October 2016; accepted 17 February 2017; published online 27 March 2017; DOI: 10.1038/ncb3492

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
arguing against ECM playing an important role in mediating the interaction between ppl and neuroectoderm cells during early stages of gastrulation.\textsuperscript{11} Consistent with ppl and neuroectoderm cells forming E-cadherin-mediated cell–cell contacts, we also found interstitial fluid accumulations to be absent from places where E-cadherin accumulates at the neuroectoderm/ppl interface (Supplementary Fig. 1d). Collectively, these observations suggest that neuroectoderm and ppl constitute two directly adjacent tissues that globally move in opposite directions during gastrulation and contact each other directly at their interface via E-cadherin-mediated cell–cell adhesions.

Next we asked whether neuroectoderm movements towards the vegetal pole might be influenced by the underlying ppl migrating in the opposite direction towards the animal pole of the embryo. To this end, we compared neuroectoderm cell movements in wt embryos forming a ppl versus \textit{MZoep} mutant embryos defective in ppl formation (Supplementary Fig. 2a,i and Supplementary Videos 1 and 2). For quantifying neuroectoderm cell movements relative to the movement of ppl cells, we constructed two-dimensional (2D) cellular flow maps of velocities and directions from 3D tracking data (Fig. 2c). Consistent with previous observations\textsuperscript{10,12,13} we found that ppl progenitors migrated in a highly coordinated and directed manner towards the animal pole of the wt gastrula (Supplementary Fig. 2b–d). Remarkably, while the bulk of neuroectoderm cells in wt underwent epiboly movements directed towards the vegetal pole, cells located directly above and anterior to the leading edge of the ppl slowed down their vegetal-directed movement and reoriented their direction of motion from vegetal to animal (Fig. 2c,d and Supplementary Fig. 2e,f and Supplementary Video 3), resulting in high animal-directed movement alignment with the adjacent ppl progenitors (measured average local correlation \( C_{4/120} \) over time interval \( t \) min \( C_{4/120} = 0.37 \pm 0.03 \) (s.e.m., \( n = 6 \) embryos); Fig. 2c,f and Supplementary Fig. 2g,h and Supplementary Video 4). This local reorientation of neuroectoderm cell movements close to the leading edge of the ppl in wt was accompanied by the formation of characteristic large-scale cell flows within the neuroectoderm resembling two counter-rotating vortices, which were mirrored along the embryo midline (Fig. 2c,g and Supplementary Video 3). Notably, there was only little influence on the movements of neuroectoderm cells posterior of the ppl, probably due to posterior axial mesendoderm behind the ppl displaying much less pronounced animal-directed movements (Supplementary Fig. 1e,f). In contrast to the situation in wt embryos, neuroectoderm cells in \textit{MZoep} embryos lacking ppl progenitors (Supplementary Fig. 2i) showed none of the characteristic flow patterns found in wt and, instead, moved exclusively towards the vegetal pole (Fig. 1k–n and Supplementary Fig. 2j and Supplementary Video 5). Collectively, these observations point to the possibility that animal-directed migration of ppl cells reorient the vegetal-directed movement of adjacent neuroectoderm cells.

**Movement speed of neuroectoderm and prechordal plate (ppl) controls neuroectoderm morphogenesis**

To determine whether changing ppl cell movement would affect neuroectoderm cell rearrangements, we turned to \textit{slb}/\textit{wnt11} morphant embryos (Fig. 3a), in which ppl cells move less coordinated and slower towards the animal pole due to compromised expression of the non-canonical Wnt ligand \textit{Wnt11} (Supplementary Fig. 3e–h)\textsuperscript{14,15}. When analysing cell movements in \textit{slb} morphants, we found that neuroectoderm cells located above and ahead of the ppl displayed increased vegetal-directed movements, and that the characteristic vortex movements within the neuroectoderm were largely lost.
Continuous mesendoderm cell ingression is required for ppl cells affecting neurectoderm morphogenesis

Movement of ppl cells towards the animal pole is initiated by the synchronized ingestion of ppl progenitors at the dorsal germ ring margin. To test whether continuous ingestion of mesendoderm progenitors is required for animal-directed ppl cell migration and, consequently, their effect on neurectoderm cell movements, we analysed ppl and neurectoderm cell movements in the absence of mesendoderm cell ingestion. To this end, we transplanted 100–150 induced ppl progenitor cells below the neurectoderm close to the dorsal germ ring margin of MZoop embryos lacking endogenous mesendoderm cell ingestion and monitored their movement relative to adjacent neurectoderm cells (Fig. 4a and Supplementary Fig. 5a–c,e). Although most of the transplanted cells displayed protrusions directed towards the animal pole (Supplementary Fig. 5d), their movement coordination and directed migration were severely impaired (Supplementary Fig. 5g). This is consistent with previous suggestions that anchorage of ppl progenitors to newly internalizing cells at their rear is required for their animal-directed migration. Notably, neurectoderm cells adjacent to the transplanted ppl progenitors showed vegetal-directed movements indistinguishable from neurectoderm cells in non-transplanted MZoop mutant embryos (Fig. 4b,c and Supplementary Video 7). Moreover, as gastrulation proceeded, transplanted ppl cells started to move towards the vegetal pole in the same direction as the overlying neurectoderm cells, resulting in highly aligned vegetal-directed movements of neurectoderm and transplanted ppl cells ($C_L = 0.48 \pm 0.04$ (s.e.m. $n = 3$ embryos); Fig. 4d,e).

To test whether slowing down neurectoderm epiboly movements would restore ppl-induced redirection of neurectoderm cells, we reduced the speed of vegetal-directed neurectoderm cell movements in transplanted MZoop mutant embryos by overexpressing CA-Mypt specifically within the YSL of those embryos (Fig. 4f and Supplementary Fig. 5h–k) and monitored the interaction between transplanted ppl cells and adjacent neurectoderm cells. We found that ppl cells showed increased animal-directed movements when vegetal-directed neurectoderm cell movements were slowed down in MZoop embryos (Fig. 4g,h and Supplementary Video 8). Moreover, neurectoderm cells adjacent to the transplanted ppl cells transiently reoriented their movement towards the animal pole (Fig. 4h), resulting in temporary high animal-directed movement alignment between transplanted ppl and adjacent neurectoderm cells ($C_L = 0.53 \pm 0.04$ (s.e.m. $n = 3$ embryos); Fig. 4i,j). These observations strongly support the notion that the difference in movement speed along the AV axis between ppl and neurectoderm cells determines whether ppl influences neurectoderm cell movements or vice versa.

Friction forces between neurectoderm and ppl mediate the effect of ppl on neurectoderm morphogenesis

We hypothesized that the observed large-scale cellular rearrangements within the neurectoderm might be generated by friction forces arising at the tissue interface between ppl and neurectoderm. To test this hypothesis, we formulated a theoretical model based on the physical principles of viscous fluid motion at low Reynolds numbers (Supplementary Note). In this model, we considered the neurectoderm as a thin layer of viscous compressible fluid exposed to external friction arising at its interface with EVL and/or yolk cell and being in contact with ppl cells, which were modelled as a rectangular element exerting a friction force on the neurectoderm (Supplementary Note).

We first aimed at comparing neurectoderm velocity profiles along the animal–vegetal axis with a simplified, effectively 1D theory (Fig. 5a). To assess the effect of ppl cells on neurectoderm movement, we measured unperturbed neurectoderm epiboly movements in MZoop mutants devoid of ppl cells and subtracted those epiboly movements from the overall neurectoderm flow field in wt embryos (Supplementary Fig. 6a). Consistent with our experimental observations (Fig. 5b), we assumed that the velocity of the calculated neurectoderm flow vanishes at the ventral and dorsal margins of the neurectoderm in both the presence and absence of the ppl. In our model, the external friction force acting outside the ppl domain causes the velocity profile to decay exponentially away from ppl cells, on a length scale that decreases when the friction coefficient increases (Supplementary Note), while in the absence of such external friction, this decay is linear (Supplementary Fig. 6b). When performing a fit to experimentally obtained neurectoderm flow profiles in wt, we obtained a very close agreement between the calculated and experimentally observed flow profiles for a very low external friction force (Fig. 5b and Supplementary Fig. 6c).
Next, we extended our analysis to *slb* morphant embryos, where the effect of changing the relative velocity between neurectoderm and ppl cells was clearly detectable throughout the time frame of our analysis (6–8 hours post fertilization (hpf)). When analysing *slb* morphant embryos, we made the assumption that if the ppl cells were to exert a dynamic friction force that is linearly dependent on the relative velocities between the two tissues, we would expect the force to be decreased by the same amount as the relative velocity between neurectoderm and ppl (20% reduction). Indeed, we found that calculated neurectoderm flow profiles with a ppl friction force reduced by 20% (Supplementary Note and Supplementary Table 1) closely resembled the experimentally obtained flow profiles in *slb* morphants (Fig. 5b1). Together, our 1D analysis of neurectoderm flows in wt and 2D analysis of ppl cell movements in *slb* morphants revealed a remarkable quantitative similarity between the model predictions and experimental observations, supporting the notion that friction forces mediate the effect of ppl on neurectoderm motion.
We further asked whether our 1D description could also account for 2D neurectoderm flow patterns within the domain of observation. Since our 1D analysis revealed that external friction outside the ppl domain is very low, we neglected its contribution to neurectoderm flows in our 2D analysis. We also assumed for simplicity that the bulk and shear viscosity of the fluid are equal. We then solved the equations in the 2D rectangular domain of our experimental observations, imposing the experimentally measured...
Figure 4  Mesendoderm cell ingression is required for ppl affecting ecto cell movements. (a,f) Schematic of ppl cells (green) transplanted at 6 hpf into the dorsal side of a MZoep (a) or MZoep embryo injected with CA-Mypt mRNA into the AV axis (f). (b,g) 2D tissue flow map indicating average velocities of ecto movements along the animal–vegetal (AV) (V_{AV}) and left–right (LR) (V_{LR}) axes at the dorsal side of a transplanted MZoep mutant (b; 6.6 hpf) and transplanted MZoep embryo overexpressing CA-Mypt within the YSL (g; 6.7 hpf); local average ecto velocities indicated by arrows colour-coded ranging from 0 (blue) to 2 (red) μm min⁻¹; positions of all/leading edge transplanted ppl cells marked by black/green dots; boxed areas were used for measurements in c,h. (c,h) V_{AV} of ecto (red; boxed areas in b,g) and underlying ppl leading edge cells (green) in transplanted MZoep (c; n=3 embryos) and transplanted MZoep embryos overexpressing CA-Mypt within the YSL (h; n=3 embryos); 6–8 hpf; the vertical dashed line in h indicates the start of vegetal-directed movements of ppl cells; error bars (dashed lines), s.e.m. (d,i) 3D directional correlation between leading edge ppl and ecto cells in a transplanted MZoep (d; 6.7 hpf) and transplanted MZoep mutant embryo overexpressing CA-Mypt within the YSL (i; 6.7 hpf); colour-coded correlation ranging from 1 (red, highest) to −1 (white, lowest); red arrows indicate local averaged ecto velocities; position of all/leading edge ppl cells marked by white/green dots; blue arrowhead indicates average velocity of ppl leading edge cells; boxed areas were used for measurements in e,j. (e,j) 3D average directional correlation between leading edge ppl and adjacent ecto cells (boxed areas in d,i) in transplanted MZoep (e; n=3 embryos) and transplanted MZoep embryos overexpressing CA-Mypt within the YSL (j; n=3 embryos); 6–8 hpf; vertical dashed line (j) as in h; error bars (dashed lines), s.e.m. All scale bars, 100 μm.

velocities on the boundaries of the domain (Fig. 5c and Supplementary Note) and compared predicted to experimentally observed neurectoderm velocities. We first analysed alterations in neurectoderm movement in wt embryos displaying normal ppl cell movements. By adjusting the ratio between the force density and neurectoderm bulk viscosity {f / n_{f}} (Supplementary Note and Supplementary Table 1), we found that the predicted neurectoderm velocity profile in wt embryos matched well the magnitude and shape of the experimentally determined velocity profile (Fig. 5d,e,f–i). Assuming that the force exerted by ppl cells originates from dynamic friction between these two moving tissues with a friction coefficient ξ, we further obtained the ratio of internal viscosity to friction against ppl cells V_{AV} / ξ (Supplementary Note and Supplementary Table 1) and, given previous measurements of tissue viscosity 21, a value of the friction coefficient in the order of ξ ~ 1 pN s μm⁻³ (Supplementary Note). Applying the same logic to slb morphant embryos produced 2D neurectoderm flow patterns very closely matching the experimentally observed ones (Fig. 5d,e,l,g–j), suggesting that the friction force density at the neurectoderm/ppl interface is critical for the effect ppl cells have on neurectoderm cell movements.

Friction forces between neurectoderm and ppl depend on transient e-cadherin-mediated cell–cell contacts at the tissue interface

To understand how friction can arise at this interface, we first analysed ppl cell migration and protrusive activity by visualizing the actin cytoskeleton of migrating ppl cells (Fig. 6a,b). We found that ppl cells were organized in a shingled array along the dorsal–ventral (DV) axis of the ppl (Fig. 6a,f and Supplementary Video 9) with cells at the leading edge displaying protrusions typically adhering to the YSL surface, which served as a substrate for their migration (Fig. 6b and Supplementary Video 10). Moreover, ppl progenitors close to the interface with the neurectoderm usually trailed behind cells positioned further away from this interface along the DV extent of the ppl (Fig. 6a,b and Supplementary Video 9), consistent with the possibility that friction at this interface slows down their animal-directed migration. To further test this assumption, we plotted the velocities of ppl progenitors in wt embryos along the DV axis of the ppl (Fig. 6c). We found a linear velocity gradient along this axis with its minimum at the interface to the overlying neurectoderm (Fig. 6c), as expected for friction forces at the neurectoderm/ppl interface most strongly slowing down the movement of ppl cells directly adjacent to this interface (Fig. 6f).

We then asked which molecular players might be involved in building up friction at the neurectoderm/ppl interface. Our estimation of the friction coefficient between neurectoderm and ppl cells being in the order of ξ ~ 1 pN s μm⁻³ (Supplementary Note) argues against the possibility of interstitial fluid alone generating this friction, given that the friction coefficient for a fluid with the viscosity of water and a layer of thickness h = 100 nm (corresponding to the estimated distance between ppl and neurectoderm) would be in the order of ξ ~ 0.01 pN s μm⁻³. In contrast, our initial analysis indicated that the cell–cell adhesion receptor E-cadherin, unlike ECM components (Fig. 1f and Supplementary Fig. 1a), might be a likely candidate generating
**Figure 5** Hydrodynamic model description of the influence of ppl on ecto cell flows through friction forces at the tissue interface. (a) Illustration of 1D ecto flow description along the tissue midline axis; ppl domain exerts an animal-directed force on the ecto; the differential velocity $V$ of ecto tissue equals 0 at tissue boundaries. (b,b1) 1D analysis of ecto flow velocity ($v_i$) along the tissue midline axis in wt (b) and sib morphant (b1) embryos; predicted flow profile (red), experimentally obtained flow velocities in wt and sib morphant embryos subtracted by the flows in MZoep mutants (blue), non-subtracted flow profiles in wt and sib morphant embryos (green), and flows in MZoep mutants (purple) are shown; values of the 1D model parameters used for each experimental case are listed in Supplementary Table 1 (Supplementary Note). (c) Illustration of 2D ecto flow description within the experimental image plane; velocities at the up (U), down (D), right (R) and left (L) boundaries of the image plane are taken from experimental measurements; a uniform force density is exerted on the ecto within the ppl domain. (d-e1) 2D analysis of ecto flow velocities for wt (d,e) and sib morphant (d1,e1) embryos; upper panels (d,e) show the $v_i$ velocities along the ecto tissue midline axis and lower panels (e1) the $v_i$ velocities along the mediolateral extent of the ecto; colour labelling of curves as in b.b1; values of the 2D model parameters used for each experimental case are listed in Supplementary Table 1 (Supplementary Note). (f-g3) 2D vector density plots for the theoretical and experimental ecto flow velocity fields of wt (f-f3) and sib morphant embryos (g-g3); subtracted flow fields for wt (f1) and sib morphant (g1) embryos; non-subtracted total flow velocity fields for wt (f2,f3) and sib morphant (g2,g3) embryos generated by adding corresponding experimental MZoep velocities to the theoretical flow profiles; direction (arrows) and colour-coded velocities from 0 (white, lowest) to 2 (purple, highest). All error bars s.d.
Figure 6 E-cadherin-mediated friction forces between ppl and ecto determine ecto morphogenesis. (a,b) Confocal images of ppl donor cells expressing lifeact-GFP (actin, green) transplanted into the ppl leading edge (outlined by dashed red line in a) of a host embryo labelled with Utrophin-Cherry (actin, purple) and H2A-mCherry (nuclei, purple); asterisks, ppl cells at YSL interface (yellow), between YSL and ecto (orange), and at ecto interface (white); dorsal view as maximal z-stack projection (a); dorsal (top) and sagittal (bottom) confocal sections with ppl protrusions (arrows) and interfaces to YSL (yellow dashed lines) and ecto (white dashed lines) indicated (b). (c,d) Average instantaneous velocities of migrating ppl cells in a wt (c) and e-cadherin morphant embryo (d) along the AV and DV axis colour-coded from 0 (blue) to 2 (red) μm min⁻¹. (e) Linear regression lines of binned mean velocities of ppl cells along the normalized radial distance of the DV axis from ventral (0) to dorsal (1) for wt (green; P = 0.0006, n = 6 embryos) and e-cadherin morphant embryos (blue; P = 0.15; n = 4 embryos); P values from F-test with null hypothesis; P > 0.05, slope equals zero; error bars (dashed lines), s.e.m. (f) Schematic illustrating ppl (yellow arrow) dragging ecto cells (white arrow) and friction forces slowing down ppl cells at the ppl/ecto interface (bottom), leading to a linear velocity gradient within ppl (top); F, friction force; E-cadherin, orange line/dots. (g) 2D tissue flow map indicating velocities of ecto cell movements along the AV (Vᵥ) and left–right (LR) (Vᵥ) axis at the dorsal side of an e-cadherin morphant embryo at 6.7 hpf; local average ecto velocities indicated and colour-coded from 0 (blue) to 2 (red) μm min⁻¹; positions of all/leading edge ppl cells; black/green dots; boxed area used for measurements in h. (h) Mean velocities along the AV axis (Vᵥ) of ecto (red; boxed area in g; right y axis) and underlying ppl leading edge cells (green, left y axis) in e-cadherin morphant embryos (n = 4 embryos); 6–8 hpf; error bars (dashed lines), s.e.m. (i) 3D directional correlation between leading edge ppl and adjacent ecto cells in an e-cadherin morphant embryo at 6.7 hpf; correlation colour-coded from 1 (red, highest) to −1 (white, lowest); red arrows, local averaged ecto velocities; boxed area used for measurements in j. (j) 3D directional correlation values between leading edge ppl and adjacent ecto cells (boxed area in i) in e-cadherin morphant embryo (n = 4 embryos); 6–8 hpf; error bars (dashed lines), s.e.m. Scale bars, 20 μm (a,b) and 100 μm (g,i); arrows, AV and DV axes.

friction between these tissues. We therefore tested whether E-cadherin is needed to sustain coupling of ppl with neurectoderm cell movements by lowering the amount of E-cadherin expressed within the gastrulating embryo using e-cadherin (cdh1) morpholinos. Consistent with previous reports, we found that in e-cadherin morphant embryos neurectoderm cell movements towards the vegetal pole were...
strongly reduced whereas the EVL/YSL advanced normally in the direction of the vegetal pole (Supplementary Fig. 7a,b). Additionally we observed that under reduced E-cadherin levels, ppl progenitor cells display less correlated movements towards the animal pole with slightly diminished velocity (Supplementary Fig. 7c–f). Notably, the influence of animal-directed ppl cell migration on the overlying adjacent neurectoderm cells was drastically diminished showing nearly exclusively vegetal-directed movements of neurectoderm cells (Fig. 6g,h and Supplementary Video 11). Consequently, the directional correlation between ppl and neurectoderm cell movements was nearly completely abolished ($C_{L(120)} = -0.14 \pm 0.03$ (s.e.m., $n = 4$ embryos); Fig. 6i,j and Supplementary Video 12), and the DV velocity gradient of cells within the ppl was much less pronounced (Fig. 6d,e and Supplementary Fig. 7g). This suggests that E-cadherin is critical for building up friction at the neurectoderm/ppl interface. Notably, the loss of correlation of directional movement between ppl and neurectoderm cells in c-cadherin morphant embryos was accompanied by a more vegetal positioning of the anterior neural anlage (Supplementary Fig. 7l,m), underlining the importance of coupling neurectoderm to ppl cell movement for positioning of the neural anlage.

E-cadherin has previously been suggested to be required for radial cell intercalations within the ectoderm and ectoderm epitholy movements23-24. To exclude that the observed loss of ppl and neurectoderm tissue coupling in c-cadherin morphant embryos is merely due to a failure of E-cadherin function within the ectoderm, we transplanted e-cadherin morphant ppl cells into MZoop mutants that were beforehand injected with CA-Mypt messenger RNA into the YSL to increase the effect of ppl cells on adjacent neurectoderm...
Figure 8 Friction forces trigger tissue deformations within the ecto. (a) Ecto tissue deformations along the AV and LR axes of wt (upper panels; n = 3) and MZoep (lower panels; n = 3) embryos plotted as time-averaged strain values for each domain (50 × 50 μm); average normal strain rate is colour-coded according to amount of stretch (minimum green (0) to maximum red (10 × 10^{-3} s^{-1})) or compression (minimum green (0) to maximum blue (−10 × 10^{-3} s^{-1})); tissue flows of ecto are indicated as time-averaged velocities; the dashed line indicates ppl position and the black dot marks the ppl leading edge as reference point in wt and MZoep; the rectangle outlines the area used for defining sectors along the AV axis in b. (b) Mean normal strain rates of ecto tissue along the AV (left panels) and LR (right panels) axes of wt (upper panels; n = 3 embryos) and MZoep (lower panels; n = 3 embryos) embryos in defined sectors (100 × 200 μm) of the ecto (A1 and A2 anterior and P1 and P2 posterior of ppl leading edge; for a detailed description refer to Supplementary Fig. 1e) as a function of time during gastrulation (plotted from 6.3–7.3 in 10 min intervals); amount of stretch/compression within each sector is plotted along the y axis. (c) Ecto tissue strain rate maps derived by subtraction of AV (left panel) and LR (right panel) time-averaged strain values of wt from MZoep mutant embryos (n = 3 embryos); colour-coded as in a; tissue flows of ecto are indicated as time-averaged velocities; black dot marks ppl leading edge as reference point. (d) Illustration of kind and direction of tissue deformation in the ecto derived from normal strain; the arrows indicate the direction of stretch or compression of a tissue domain along the AV and LR axes dependent on the direction and magnitude of ecto movements.

E-cadherin-mediated friction between neurectoderm and ppl is sufficient to reorient neurectoderm cell movements

Vertical signalling from the axial mesendoderm to the overlying neurectoderm has been shown to be an important determinant of axial patterning within the forming neural keel\(^4,25\) and thus could in principle contribute to the observed influence of ppl on neurectoderm movement. To address this possibility, we thought to test whether E-cadherin-mediated friction alone might be sufficient to explain the observed effect of ppl on neurectoderm movement in the embryo. Hence, we designed an in vitro experiment where we substituted ppl cells with a layer of E-cadherin-coated beads and sheared them over a cluster of ex vivo cultured ectoderm progenitor cells to create friction between them (Fig. 7a). To mimic similar conditions as in the embryo, we moved the labelled ectoderm cell cluster (GPI-GFP membrane and H2A-mCherry nuclei) in one direction (stage movement \(\sim 0.5 \mu m \text{ min}^{-1}\)) and the E-cadherin-coated beads, mimicking the ppl, with higher velocities (top plate \(\sim 1.5 \mu m \text{ min}^{-1}\)) in the opposite direction of the ectoderm cell cluster (Fig. 7a,b and Supplementary Fig. 8a–c). Remarkably, we observed that this movement of E-cadherin-coated beads was sufficient to reorient cell movements within the ectoderm cell cluster (Fig. 7d,f), leading to high local correlation between bead and ectoderm cell movements (Fig. 7h,j) and to generate double vortex flows within the ectoderm cluster reminiscent of the situation in vivo (Fig. 7k). In contrast, no such effects were obtained when using uncoated control beads (Fig. 7c,e,g,i), suggesting that E-cadherin-mediated contact between beads and ectoderm cells is critical for the beads to reorient ectoderm cell movements. Collectively, these findings support the view that E-cadherin-mediated friction between ppl and neurectoderm cells is sufficient to explain the observed effect of ppl on neurectoderm movement in the embryo.

Friction forces lead to distinct tissue deformations within the neurectoderm

To further corroborate the role of frictional forces on neurectoderm morphogenesis, we asked whether neurectoderm cells become compressed in front of the ppl cells and stretched behind, as one would expect if the ppl cells would be pushing the overlying neurectoderm towards the animal pole. To this end, we compared neurectoderm tissue deformation in wt versus MZoep mutant embryos by calculating normal tissue domain strain rates along the AV and left–right (LR) axes (Fig. 8d; normal strain) and shear strain rates (Supplementary Fig. 8h) from measured neurectoderm cell velocities. We observed that both normal and shear strain rates appeared considerably different between wt and MZoep mutant embryos during the course of

ARTICLES
gastrointestinal tract, and other tissues, has previously been speculated to be required for the mesendoderm in gastrulation. Friction forces emerging at the interface between fluids and the plasma membrane have previously been implicated in vascular development and tissue deformation during Drosophila gastrulation. Our findings demonstrate that the generation of friction forces between forming tissues sliding against each other constitutes a key regulatory mechanism of embryo morphogenesis in development.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

Note: Supplementary Information is available in the online version of the paper.

ACKNOWLEDGEMENTS

We would like to thank R. Hauschild for technical assistance and the scientific service units at the IST Austria for continuous support. This work was supported by grants from the OMAA Ernst Mach Predoctoral Scholarship to Z.A., Cancer Research UK to M.T., the Spanish Ministry of Economy and Competitiveness ‘Centro de Excelencia Severo Ochoa 2013–2017’ and CERCA Programme/Generalitat de Catalunya to V.R., the European Union’s Seventh Framework Program/ERC Grant Agreement to B.H. (306589) and to T.V. (COLLMOT project; 227878), the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001317), the UK Medical Research Council (FC001317) and the Wellcome Trust (FC001317) to S.G. and G.S., and the Austrian Science Foundation (FWF): I930-B20 to C.-P.H.

AUTHOR CONTRIBUTIONS

M.S. and C.-P.H. conceived the project and designed the experiments. M.S. performed the experiments and data analysis with support from D.C. for in-situ hybridizations and analysis, M.B. for CM-Mpt injections and EVL/YSL analysis and E.P. for immunohistochemistry; Z.A. developed computational tools for data analysis; S.S. and V.R. wrote scripts for data analysis; S.G. and G.S. developed the theory and S.G., S.S. and G.S. performed quantitative modelling; B.H. contributed to model interpretation; M.T. developed reagents and T.V. contributed to collective motion analysis and interpretation; M.S., G.S. and C.-P.H. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://dx.doi.org/10.1038/ncb3402

Reprints and permissions information is available online at www.nature.com/reprints

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Lecuit, T., Lenne, P.-F. & Munro, E. Force generation, transmission, and integration during cell and tissue morphogenesis. *Annu. Rev. Cell Dev. Biol.* **27**, 157–184 (2011).
2. Heisenberg, C.-P. & Bellaihe, Y. Forces in tissue morphogenesis and patterning. *Cell* **153**, 948–962 (2013).
3. Solnica-Krezel, L. & Sepich, D. Gastrointestinal tube development: making and shaping germ layers. *Annu. Rev. Cell Dev. Biol.* **28**, 687–717 (2012).
4. Wilson, S. W. & Houart, C. Early steps in the development of the forebrain. *Dev. Cell* **6**, 167–181 (2004).
5. Apell, B. Zebrafish neural induction and patterning. *Dev. Dynam.* **219**, 155–168 (2000).
6. Wu, X. & Fraser, S. E. Order and coherence in the fate map of the zebrafish nervous system. *Development* **121**, 2595–2609 (1995).
7. Araya, C. et al. Mesoderm is required for coordinated cell movements within zebrafish neural plate in vivo. *Neural Dev.* **9**, 9 (2014).
8. Araya, C., Carmona-Fontaine, C. & Clarke, J. D. W. Extracellular matrix couples the convergence movements of mesoderm and neural plate during the early stages of neurulation. *Dev. Dynam.* **245**, 580–589 (2016).
9. Gritsman, K. et al. The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. *Cell* **97**, 121–132 (1999).
10. Montero, J.-A. et al. Shield formation at the onset of zebrafish gastrulation. *Development* **132**, 1187–1198 (2005).
11. Latimer, A. & Jessen, J. R. Extracellular matrix assembly and organization during zebrafish gastrulation. *Matrix Biol.* **29**, 89–96 (2010).
12. Diaz-Muñoz, A. et al. Control of directed cell migration in vivo by membrane-to-cortex attachment. *PLoS Biol.* **8**, e1000544 (2010).
13. Dumortier, J. G., Martin, S., Meyer, D., Rosa, F. M. & David, N. B. Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. *Proc. Natl Acad. Sci. USA* **109**, 16945–16950 (2012).

14. Heisenberg, C. P. et al. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76–81 (2000).

15. Ulrich, F. et al. Slb/Wnt11 controls hypoblast cell migration and morphogenesis at the onset of zebrafish gastrulation. *Development* **130**, 5375–5384 (2003).

16. Hatta, K., Kimmel, C. B., Ho, R. K. & Walker, C. The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350**, 339–341 (1991).

17. Thirue, C., Thirue, B., Halpern, M. E. & Postlethwait, J. H. Goosecoid expression in neuroderm and mesendoderm is disrupted in zebrafish cyclops gastrulas. *Development* **164**, 420–429 (1994).

18. Behrndt, M. et al. Forces driving epithelial spreading in zebrafish gastrulation. *Science* **338**, 257–260 (2012).

19. Krieg, M. et al. Tensile forces govern germ-layer organization in zebrafish. *Nat. Cell Biol.* **10**, 429–436 (2008).

20. Weber, G. F., Bjerke, M. A. & DeSimone, D. W. A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev. Cell* **22**, 104–115 (2012).

21. Babb, S. G. & Marrs, J. A. E-cadherin regulates cell movements and tissue formation in early zebrafish embryos. *Dev. Dynam.* **230**, 263–277 (2004).

22. Kane, D. A., McFarland, K. N. & Warga, R. M. Mutations in half baked/E-cadherin block cell behaviors that are necessary for teleost epiboly. *Development* **132**, 1105–1116 (2005).

23. Shimizu, T. et al. E-cadherin is required for gastrulation cell movements in zebrafish. *Mech. Dev.* **122**, 747–763 (2005).

24. Wessely, O. & De Robertis, E. M. Neural plate patterning by secreted signals. *Neuron* **33**, 489–491 (2002).

25. Cui, G., Yang, X., Chuai, M., Glazier, J. A. & Weijer, C. J. Analysis of tissue flow patterns during primitive streak formation in the chick embryo. *Dev. Biol.* **284**, 37–47 (2005).

26. Fleury, V. Clarifying tetrapod embryogenesis by a dorso-ventral analysis of the tissue flows during early stages of chicken development. *BioSystems* **109**, 460–474 (2012).

27. Rozbicki, E. et al. Myosin-II-mediated cell shape changes and cell intercalation contribute to primitive streak formation. *Nat. Cell Biol.* **17**, 397–408 (2015).

28. Voiculescu, G., Bodenstein, L., Lau, I.-I. & Stern, C. D. Local cell interactions and self-amplifying individual cell ingression drive amniote gastrulation. *eLife* **3**, e01817 (2014).

29. Tzima, E. et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* **437**, 426–431 (2005).

30. He, B., Dubrovinski, K., Polyakov, O. & Wieschaus, E. Apical constriction drives tissue-scale hydrodynamic flow to mediate cell elongation. *Nature* **508**, 392–396 (2014).
METHODS

Embryo staging and fish line maintenance. Zebrafish (Danio rerio) were maintained as described previously\(^2\). Embryos were raised at 28–31 °C in E3 buffer and staged according to morphological criteria\(^2\). Zebrafish lines (female and male) between the age of 0.5–2.5 yr used for obtaining embryos: wild-type (wt) strains TL and AB and the following transgenic and mutant lines were used: MZoept\(^{-}\)\()\)\(^{-}\)(ref. 9), Tg(dharmacoRFP)\(^{24}\), MZoept;Tg(dharmacoRFP), Tg(gsc:GFP)\(^{29}\), Tg(gsc:GFP-CAAX), Tg(actb2-Cherry-UtrCh) and Tg(actb2:Lifeact-FP)\(^{40}\). No cell lines were used in this study. All animal experiments were carried out along the guidelines of the Ethics and Animal Welfare Committee (ETK) in Austria.

Microinjections of mRNA morpholin antisense oligonucleotides and dextran. Capped mRNA for injection was synthesized using the SP6 Message Machine Kit (Ambion). For ubiquitous mRNA overexpression, 100 pg Tg\(^{-}\)MET\(\text{H}\)O\(S\) in 1-μl volume was injected into one-cell-stage embryos. To generate mesoderm progenitors, one-cell-stage Tg\((\text{gsc:GFP})\), Tg\((\text{gsc:GFP-CAAX})\) or Tg\((\text{actb2:Lifeact-FP})\) embryos were injected with 100 pg cyclop (cyc) mRNA\(^{31}\) and 2 ng casanova (cas) morpholin (MO; GeneTools) oligomers\(^{31}\). To downregulate myosin-II activity specifically within the YSL, 50–75 pg of mRNA encoding for a constitutively active myosin-II phosphatase 1 (CA-Mpt) consisting of the N terminus (amino acids 1–300) of the human myosin phosphatase targeting subunit 1 lacking the inhibitory domain\(^{32}\), together with 100 pg of h2afya-mCherry mRNA, was directly injected into the YSL of embryos between 512K and high stage (2.75–3.3 hpf)\(^{33}\). To interfere with prechordal plate (ppl) progenitor cell number, 0.5–1 ng of cyc MO (GeneTools) targeted against the ATG start codon of the cyc cDNA was injected into one-cell-stage embryos\(^{34}\). To reduce E-cadherin and Wnt11 expression levels, 34 ng Tg\((\text{actb2:Cherry-UtrCH})\) or 6 ng of MZoep;Tg\((\text{gsc:GFP})\) morpholino (MO; GeneTools)\(^{29}\) mRNA, was directly injected into the YSL of embryos between 512K and high stage (2.75–3.3 hpf)\(^{33}\). To interfere with prechordal plate (ppl) progenitor cell number, 0.5–1 ng of cyc MO (GeneTools) targeted against the ATG start codon of the cyc cDNA was injected into one-cell-stage embryos\(^{34}\). Interstitial fluid was labelled by injection of dextran Alexa Fluor 647 (10,000 MW; ThermoFisher Scientific) at high stage (3.3 hpf) into the extracellular space of embryos. Interstitial fluid was labelled by injection of dextran Alexa Fluor 647 (10,000 MW; ThermoFisher Scientific) at high stage (3.3 hpf) into the extracellular space of embryos.

Whole-mount imaging and antibodies. For whole-mount imaging, embryos were injected with Tg\((\text{actb2:Cherry-UtrCH})\) and subsequently imaged on an upright fluorescence microscope (Olympus SXZ 12) equipped with a QImaging MicroPublisher 5.0 camera. Live cell imaging of whole embryos was performed by confocal microscopy and optical clearing. To remove the host embryo at 60% epiboly (6 hpf). For detecting the dorsal side of the host embryo, Tg\((\text{dharmacoRFP})\) embryos were used expressing Dharma:EGFP at their dorsal side. Transplanted embryos were mounted for imaging as described above.

Embryo fixation and cryosectioning. Embryos were fixated at 60%, 80% and 90% epiboly and fibronectin was detected with a polyclonal antibody (anti-fibronectin), incubated with secondary antibody (anti-rabbit Alexa Fluor 568; Invitrogen). Embryos were washed three times with 1× PBS containing 0.05% Tween (PBSTT) and pre-blocked with 5% purified bovine serum albumin (BSA, Sigma-Aldrich, A9418) and 0.5% Triton-X in PBSTT. Embryos were incubated with a primary antibody for 16 h at 4 °C before washing with PBST and incubating overnight with secondary antibody (Alexa 488-conjugated goat anti-rabbit, ThermoFisher Scientific, A-11008; 1:5000 dilution) and rhodamine-phalloidin for F-actin staining (ThermoFisher Scientific, R415; 1:200 dilution). Embryos were washed 4× for 30 min with PBST and nuclei were stained with DAPI nuclei acid stain (ThermoFisher Scientific, D1306).

Immunohistochemistry. For whole-mount immunohistochemistry, embryos were fixed for 2 h with 2% paraformaldehyde in 1× PBS, washed twice after fixation with 50 mM glycine in 1× PBS, 0.05% Triton-X, 0.9% NaCl, 0.1% BSA before being mounted on a glass slide using a poly-l-lysine coated glass slide (Sigma-Aldrich, A8834; 1× PBS). Primary antibodies were performed overnight in PBST with 2% purified BSA at 4 °C. Embryos were subsequently washed with PBST 4× for 30 min and incubated overnight with secondary antibody (Alexa 488-conjugated goat anti-rabbit, ThermoFisher Scientific, A-11008) and rhodamine-phalloidin for F-actin staining (ThermoFisher Scientific, R415; 1:200 dilution). Embryos were washed 4× for 30 min with PBST and nuclei were stained with DAPI nuclei acid stain (ThermoFisher Scientific, D1306).

Whole-mount confocal imaging. Whole-mount confocal imaging was performed using a Leica SP5 confocal microscope equipped with a Tissue-Tek O.C.T. Compound (Sakura Finetek; 0%10%30%50%70%90%100% OCT solution) containing 1.3 mM CaCl\(_2\) (ThermoFisher Scientific, D1306).

Whole-mount immunohistochemistry and antibodies. For whole-mount immunohistochemistry, embryos were fixed for 4 h in 1% paraformaldehyde in 1× PBS, washed twice after fixation with 50 mM glycine in 1× PBS, 0.05% Triton-X, 0.9% NaCl, 0.1% BSA before being mounted on a glass slide using a poly-l-lysine coated glass slide (Sigma-Aldrich, A8834; 1× PBS). Primary antibodies were performed overnight in PBST with 2% purified BSA at 4 °C. Embryos were subsequently washed with PBST 4× for 30 min and incubated overnight with secondary antibody (Alexa 488-conjugated goat anti-rabbit, ThermoFisher Scientific, A-11008) and rhodamine-phalloidin for F-actin staining (ThermoFisher Scientific, R415; 1:200 dilution). Embryos were washed 4× for 30 min with PBST and nuclei were stained with DAPI nuclei acid stain (ThermoFisher Scientific, D1306).

Whole-mount immunohistochemistry and antibodies. For whole-mount immunohistochemistry, embryos were fixed for 2 h with 2% paraformaldehyde in 1× PBS, washed twice after fixation with 50 mM glycine in 1× PBS, 0.05% Triton-X, 0.9% NaCl, 0.1% BSA before being mounted on a glass slide using a poly-l-lysine coated glass slide (Sigma-Aldrich, A8834; 1× PBS). Primary antibodies were performed overnight in PBST with 2% purified BSA at 4 °C. Embryos were subsequently washed with PBST 4× for 30 min and incubated overnight with secondary antibody (Alexa 488-conjugated goat anti-rabbit, ThermoFisher Scientific, A-11008) and rhodamine-phalloidin for F-actin staining (ThermoFisher Scientific, R415; 1:200 dilution). Embryos were washed 4× for 30 min with PBST and nuclei were stained with DAPI nuclei acid stain (ThermoFisher Scientific, D1306).

Preparation of histological sections. For tissue sectioning, pre-stained embryos were re-fixed in 2% PFA, embedded in increasing concentrations of OCT medium (Tissue-Tek O.C.T. Compound, Sakura Finetek; 0%–10%–30%–50%–70%–90% diluted in 30% sucrose/PBS), shock-frozen in 90% OCT solution on dry ice, and cryo-sectioned at a thickness of 20 μm before embedding in ProLong Gold antifade mounting (ThermoFisher Scientific, P36930).

Calculations of tissue strain rates. Strain rates were calculated within the neuroectoderm close to the animal–vegetal axis in the wt and MZoept mutant embryos.
To calculate tissue strain rates, the neurectoderm tissue was subdivided into small domains of 50 µm^2 boxes in xy (~50 cells per domain per time point) and the velocity of cells within each box was averaged to calculate its instantaneous average ensemble. The strain rates were then calculated similar to a previous approach, by using spatial derivatives of the velocities within the neighbouring boxes along the animal–vegetal (AV) and lateral (LR) axes of the embryo, considering the adjacent domains. To distinguish direction and kind of strain, we calculated normal strain rates along the AV, LR axes and shear strain rates. Normal strain rates determine the stretch (positive value) or compression (negative value) across the tissue domain along a specific direction (AV or LR) and shear strain rates capture the change of angle when the tissue deforms, whereby the angle of the domain (unit of the tissue) can shrink (positive value) or enlarge (negative value) during deformation. Strain rates were calculated as follows:

\[ \varepsilon_{xx} = \frac{\partial v}{\partial y} \]
\[ \varepsilon_{yy} = \frac{\partial v}{\partial y} \]
\[ \varepsilon_{xy} = \frac{\partial u}{\partial x} \]
\[ \varepsilon_{xy} = \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \]

where \( x \) and \( y \) are mathematical representations of the LR and AV axes, \( u \) and \( v \) are the velocities in these directions respectively and \( \varepsilon \) resembles strain rate.

Image and data processing for flow and correlation analysis. Images acquired from multiplanar live cell imaging were initially processed with Imaris software (LaVision Bio Tec) to compile channels from imaging data, and the exported files were further processed using ImageJ software (Bitplane) to visualize the recorded channels in three dimensions. Full data sets containing all of the channels from live cell imaging were used for identifying embryo landmarks needed to align all embryos in the same way for comparison of different experiments. Each imaged embryo was rotated and aligned along the AV axis at the dorsal side of the embryo using the gc-GFP, or in the case of MZoop mutant embryos, the dIsharaEGFP signal as dorsal marker for correct embryo orientation. Ppl progenitor nuclei were extracted by surface masking of the gc-GFP signal within prechordal plate progenitors. Neurectoderm cell nuclei were calculated from non-surface masked areas, and nuclei of remaining deep cells including paraxial mesoderm, endoderm and YSN were identified by their characteristic positions/movements and then manually subtracted. Nuclei positions of ppl progenitors and neurectoderm cells in xyz-dimensions were extracted for each time point and used for further quantitative analysis.

Cell tracking data containing nuclei positions over time were analysed with custom-made Perl scripts. From the 3D cell positions instantaneous velocity of a cell \( i \) at time \( t \) was calculated as follows:

\[ v(t) = \frac{r(t + \Delta t) - r(t)}{\Delta t} \]

where \( v(t) \) is the velocity vector of cell \( i \) at time \( t \) and \( r(t) \) is the position of cell \( i \) at time \( t \) and \( \Delta t \) is the elapsed time between two consecutive 3D image sets in the time-lapse videos.

Analysis and visualization of ppl progenitor cell movements. In this section we delineate different analysis types that are based on methods generally used to quantify collective motion behaviour\(^{44,45}\) to characterize collective cell migration.

To visualize how individual cell movements correlate with the average movement of the ppl cell collective, we calculated correlation values between the direction of movement of each individual cell and the average movement direction of the collective as follows:

\[ C(t) = \psi(t) \cdot \overrightarrow{v}_{\text{avg}}(t) \]

where \( \psi(t) \) is the normalized 3D velocity vector of cell \( i \) and \( \overrightarrow{v}_{\text{avg}}(t) = \frac{1}{N(t)} \sum_{i=1}^{N} \psi(t) \)

is the average normalized 3D velocity vector of the group at time \( t \) and \( N(t) \) is the number of ppl cells at time \( t \). We calculated \( C(t) \) for every time point for each cell. \( C(t) \) can take values between 1 (if a cell movement is perfectly aligned with the average movement of the ppl collective) and −1 (if a cell is moving in the opposite direction of the collective). Correlation values of individual cells \( C(t) \) are indicated in images for a representative time point by the colour of the velocity vectors (Supplementary Fig. 2b).

To quantify the alignment of cell movements, we defined the ‘order parameter’ as the absolute value of the averaged normalized velocity as follows:\(^6\)

\[ \psi(t) = \overrightarrow{v}_{\text{avg}}(t) = \frac{1}{N(t)} \sum_{i=1}^{N} \psi(t) \]

where \( \psi(t) \) is the normalized velocity of a cell and \( N(t) \) is the total number of cells at time \( t \). \( \psi(t) \) can take values between 0 (if cells move randomly, disordered movement) and 1 (if cells move uniformly in the same direction, highly ordered movement; Supplementary Fig. 2c).

Calculations and visualization of neurectoderm flows. To quantify and visualize neurectoderm cell motion without ppl progenitor cells, we calculated velocity flow maps in spatially defined areas of the embryo. Three-dimensional cell velocity vectors were averaged in 50 by 50 µm^2 sectors in xy planes and over the full z direction for every time point to create a grid covering the whole area of the visualized embryo. For visualization we plotted xy projections of the 3D velocity vectors for every time point and reconstructed a velocity flow map. Magnitude of the average velocity, namely

\[ 1 \sum_{i=1}^{N} v_i(t) \]

where \( N \) is the number of cells in a sector at time \( t \), was calculated and is indicated by the colour of the arrow in each sector. The centre point of the grid was determined as the middle point of the imaged area.

To quantify and visualize neurectoderm cell motion with underlying ppl progenitor cells, we determined the centre 0,0 point of the above-mentioned grid as the average position of the first 20 leading cells. We used this 0,0 grid position as a reference point to compare the cell movements from different embryos. For each time point of image acquisition, the 0,0 grid position was superimposed to keep the reference point fixed for every time frame and the 50 x 50 µm^2 sectors, containing the average instantaneous cell velocities, were back projected around this reference point (Fig. 2c and Supplementary Fig. 2e,f). This method allowed us to quantify cell velocities in the adjacent neurectoderm in a relative position to the leading ppl progenitor cells and to directly compare different data sets with each other.

Averaged neurectoderm velocity fields were calculated by making both time and ensemble averages for each experimental case. Time averages were taken over the whole duration of image acquisition (typically 120 min) for wt, cmyc morphant, sib morphant, CA-Mypt and MZoop mutant embryos and visualized as time-averaged neurectoderm flow velocity fields (Fig. 2g).

Directional correlation analysis of neurectoderm and prechordal plate progenitor cell movements. To quantify the correlation between the movement of neurectoderm (‘ecto’ in equations) and underlying ppl progenitor cells (‘pp’ in equations), we calculated their directional correlation in every sector of the grid (grids were positioned the same way as described above) for each time point as follows:

\[ C_{\text{ecto-pp}}(t) = \overrightarrow{v}_{\text{ecto}}(t) \cdot \overrightarrow{v}_{\text{pp}}(t) \]

where \( \overrightarrow{v}_{\text{ecto}}(t) \) and \( \overrightarrow{v}_{\text{pp}}(t) \) are the normalized averaged 3D velocity vectors in a grid at time \( t \) of neurectoderm and ppl progenitor cells, respectively (Supplementary Fig. 2g). \( C_{\text{ecto-pp}}(t) \) can take values between 1 (ectoderm and ppl progenitor cells move in the same direction) and −1 (they move in opposite directions). The \( C_{\text{ecto-pp}}(t) \) value of each grid sector is indicated in images and videos by the colour of the sector (Fig. 2e and Supplementary Fig. 2g,h).

To investigate the effect of ppl movement on neurectoderm cells that are positioned anterior to the leading edge of the ppl, we calculated the directional correlation between \( \overrightarrow{v}_{\text{ecto}}(t) \) and the average normalized velocity vector of the first 20 leading ppl cells as follows:

\[ C_{\text{ecto-leading}}(t) = \overrightarrow{v}_{\text{ecto}}(t) \cdot \overrightarrow{v}_{\text{leading}}(t) \]

The \( C_{\text{ecto-leading}}(t) \) value of each grid sector is indicated in images and videos by the colour of the sector (Fig. 1k).

Instantaneous speed, directionality and polarity analyses of ppl cells. Cell speed \( v_{\text{ct}} \) was calculated from single cell trajectories with positions

\[ r(t) = (x(t), y(t), z(t)) \]
Data were selected every frame to obtain instantaneous velocities

\[ v = |\text{d}r(n \cdot \text{lag})|/(n \cdot \text{lag}) \]

with

\[ \text{d}r(n \cdot \text{lag}) = r(t + n \cdot \text{lag}) - r(t) \]

and \( \text{lag} \) being the time interval between successive frames. Instantaneous velocity values \( v \) calculated from single trajectories were averaged to obtain the cell speed \( v_{\text{cell}} \) (Supplementary Fig. 2d).

To calculate directionality values, single cell trajectories were split into segments of equal length (5 frames). Directionality indices for single trajectories were calculated via a sliding window as the ratio of start-to-end distance versus the summed distance between successive frames in a segment. Obtained directionality values were averaged over all segments in a single trajectory. This analysis yielded values between [0,1], with higher movement directionality closer to 1 (Supplementary Fig. 2d).

For the calculation of speed gradients along the DV axis bright-field images of embryos were obtained to measure the embryo radius \( R \). The value \( R \) was used as an input parameter to fit \( (x, y, z) \) coordinates of ppl cells to a sphere with radius \( R \) to obtain the centre of the embryo \( (x_0, y_0, z_0) \). Ppl cell coordinates were shifted to the origin by linear translation

\[ (x^* = x - x_0, y^* = y - y_0, z^* = z - z_0) \]

and transformed to spherical coordinates \( (r, \theta, \phi) \). In this reference frame the DV axis is represented by the \( R \) coordinate (the AP axis and LR axis by \( \theta \) and \( \phi \) coordinates respectively). Two-dimensional velocity maps along the DV and AV axis were generated from instantaneous velocity values \( v(R, \theta, \phi) \) calculated as described above and plotted at interpolated cell positions between consecutive frames (Fig. 6c,d). Speed gradients along the DV axis were obtained from instantaneous velocities \( v(R) \) and were binned and averaged to calculate mean instantaneous velocity values (Fig. 6e).

Direction of protrusion formation of transplanted ppl progenitor cells was analysed for each video in steps of 15–20 min with Fiji software using the angle measurement tool. Angle between detected protrusion and the AV axis was measured, where an angle of 0° corresponds to the animal and an angle of 180° to the vegetal pole, and a 90° and 270° angle for the right and left axis respectively. Angles were plotted in polar plots using IgorPro software (WaveMetrics) (Supplementary Fig. 5d).

Quantification of neural plate positioning. After whole-mount in situ hybridization, the embryos were imaged using a dissecting stereoscope with the Leica Imaging Application. Fiji software was utilized to fit a circle around the embryo and to measure the angle between the vegetal pole and the anterior border of the otsc2 expression domain by using the built-in angle tool. Box-whisker plots were generated to compare angles from different developmental stages (Supplementary Fig. 2m).

Statistical analysis and reproducibility. Statistical analysis was performed using software Prism 5 (GraphPad) and R (Bell Laboratories). To compare the mean values, unpaired Student’s \( t \)-tests were used to calculate two-tailed \( P \) values for two-group comparison and Student’s \( t \)-tests with Benjamini–Hochberg correction for multiple-group testing correction were used to calculate the false discovery rate for each \( P \) value. The data meet the assumption of the tests and the variance is estimated to be similar between groups that are compared. We used a non-parametric unpaired test because we assume that the data follow a Gaussian distribution and that in the compared groups the individual values were not paired or matched with one another. All \( P \) values are reported within the figure legends. All \( n \)-values report biological replicates (embryos) if not stated otherwise. No statistical method was used to predetermine the sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. For interpreting linear regression slopes we performed an \( F \)-test to report the \( P \) value (two-tailed) testing the null hypothesis that the overall slope is zero \( (P > 0.05) \). Where whisker–box plots were used, the centre of the box shows the median (the additional red dot shows the mean), and the whiskers show the minimum and maximum measured value, respectively (capped by 1.5 interquartile range; values measured outside are shown as outliers). Representative stereoscope/confocal/multiphoton images of zebrafish embryos, or ex vivo isolated cells, or western blot in Figs 1a,c,f, 2a,b,i,j, 3a,g,m,s, 6a,b and 7b and Supplementary Figs 1a–n, 2a,i,k,l, 3a,e,i,j, 4b,d,i, 5b–d,i,j, 7a,b,l and 8a–c have been successfully repeated between 3 and 6 times.

**Code availability.** Relevant computational codes used for data processing are available from the authors on request.

**Data availability.** All data that support the conclusions in the study are available from the authors on reasonable request.

---

32. Westerfield, M. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (Danio Rerio) 4th edn (Univ. Oregon Press, 2000).
33. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. Stages of embryonic development of the zebrafish. *Dev. Dyn.* 203, 253–310 (1995).
34. Ryu, S. L. et al. Regulation of dharma/bozozok by the Wnt pathway. *Dev. Biol.* 231, 397–409 (2001).
35. Doitsidou, M. et al. Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell* 111, 647–659 (2002).
36. Compagnon, J. et al. The notochord breaks bilateral symmetry by controlling cell shapes in the zebrafish laterality organ. *Dev. Cell* 31, 774–783 (2014).
37. Rebagliati, M. R., Toyama, R., Haffter, P. & Dawid, I. B. cyclops encodes a nodal-related factor involved in midline signaling. *Proc. Natl Acad. Sci. USA* 95, 9932–9937 (1998).
38. Weiser, D. C., Row, R. H. & Kimelman, D. Rho-regulated myosin phosphorylate establishes the level of protrusive activity required for cell movements during zebrafish gastrulation. *Development* 136, 2375–2384 (2009).
39. D’Amico, L. A. & Cooper, M. S. Morphogenetic domains in the yolk syncytial layer of axiating zebrafish embryos. *Dev. Dynam.* 222, 611–624 (2001).
40. Karlen, S. & Rebagliati, M. A morpholino phenocopy of the cyclops mutation. *Genesis* 30, 126–128 (2001).
41. Lele, Z., Bakkers, J. & Hammerschmidt, M. Morpholino phenocopies of the swirl, snailhouse, somitabun, minifin, silberblick, and pipetail mutations. *Genesis* 30, 190–194 (2001).
42. Blanchard, G. B. et al. Tissue tectonics: morphogenetic strain rates, cell shape change and intercalation. *Nat. Methods* 6, 458–464 (2009).
43. Vicsek, T. & Zafeiris, A. Collective motion. *Phys. Rep.* 517, 71–140 (2012).
44. Méhes, E. & Vicsek, T. Collective motion of cells: from experiments to models. *Integr. Biol.* 6, 831–854 (2014).
45. Szabó, B. et al. Phase transition in the collective migration of tissue cells: experiment and model. *Phys. Rev. E. Stat. Nonlin. Soft. Matter Phys.* 74, 061908 (2006).
Supplementary Figure 1 Fibronectin and interstitial fluid localization at the neurectoderm-to-prechordal plate interface during zebrafish gastrulation. (a, b, c) Immunofluorescence confocal images of the neurectoderm (ecto)-to-prechordal plate (ppl) interface (white dashed line) in a wild type (wt) embryos at 6 (a), 8 (b), and 9 (c) hpf showing Fibronectin staining (pseudo-colored with Fire LUT) in maximum intensity projections of dorsal views (top panels) and sagittal sections (middle panels); red dashed line outlines position of ppl leading edge cells; blue dashed line indicates ecto-to-EVL interface, and yellow dashed line shows YSL interface to ppl and ecto; bottom panels are sagittal sections of the ecto-to-ppl interface stained for F-actin (phalloidin) to mark this interface; double-sided arrows indicate animal (A) to vegetal (V) and dorsal (V) to ventral (V) embryo axes; asterisk labels ppl leading edge cell; scale bar, 20 µm. (d) Multiphoton live cell images showing interstitial fluid (IF) accumulation (dextran-Alexa Fluor 647, left panel), F-actin localization (Tg(actb1:lifeact-GFP), middle panel) and a combination of those different labels (right panel) at the ecto-to-ppl interface (white dashed line) at 7 hpf; red arrows indicate extracellular cavities filled with IF at the ecto-to-ppl and ecto-to-YSL interfaces; white arrows indicate ecto-to-ppl cell-cell contacts devoid of IF accumulations; blue dashed line indicates ecto-to-EVL interface, and yellow dashed line shows YSL interface to ppl and ecto; double-sided arrows indicate AV and dorsal DV embryo axes; asterisk labels ppl leading edge cell; scale bar, 20 µm. (e) Multiphoton live cell image of Tg(gsc:GFP) embryo (t = 120 min, 8 hpf) with pseudo-colored spots marking positions of nuclei within the axial mesendoderm (green); dorsal view with double-sided arrows indicating AP to VP and left (L) to right (R) embryo axes; color-code indicates mean total cell speeds of axial mesendoderm cells moving to the animal pole after internalization (cyan, 0-2 and yellow/magenta >2 µm/min); position of anterior (ppl) and posterior mesendoderm marked; scale bar, 50 µm. (f) Average instantaneous cell speeds in µm/min of internalized axial mesendoderm cells in wt embryos (n=6 embryos) plotted along the normalized distance along the AV axis from anterior (0) to posterior (1); green dashed line marks position of transition from anterior (ppl) to posterior axial mesendoderm, error bars, s.e.m.
Supplementary Figure 2
Supplementary Figure 2: Prechordal plate and neurectoderm cell movements and neural plate positioning in wild type and MZoep mutant embryos. (a) Fluorescent images of a wild type (wt) Tg(gsc:GFP) embryo showing neurectoderm nuclei (H2A-BFP, cyan) and gsc-expressing GFP-labeled prechordal plate (ppl) cells at a representative time point during gastrulation (t = 65 min, 7.1 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); animal (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100 µm. (b) Correlation of ppl cell movements in a wt embryo at a representative time point during gastrulation (t = 111.7 min, 7.9 hpf); ppl cells are visualized as arrows in a 2D plot and color-coded corresponding to their 3D correlation values between 1 (red, maximum correlation) and -1 (blue, minimum correlation); every 3rd cell is plotted; AP, animal pole; VP, vegetal pole; scale bar, 50 µm. (c) Average degree of alignment of ppl cell movements in wt embryos (n=5 embryos) calculated from 6 to 8 hpf (120 min); the order parameter corresponds to the degree of alignment ranging from 0 (disordered movement) to 1 (highly ordered movement); error bars, s.e.m. (d) Mean instantaneous cell speed and directionality of ppl cells at a representative time point during gastrulation (t = 65 min, 7.1 hpf); the neurectoderm domain was measured for embryos at different stages during gastrulation (k, l) and plotted as box-whisker graphs; n, embryos analyzed from 4 independent experiments; student’s t-test (*P value indicated) for all graphs comparing same stages; ***, P <0.001, (ns) non significant, P>0.05; n (wt, bud) = 36, n (wt, 90%) = 36, n (wt, 80%) = 34, n (wt, 70%) = 29, n (MZoep, bud; P <0.0001) = 24, n (MZoep, 90%; P<0.0001) = 36, n (MZoep, 80%; P<0.0001) = 20, n (MZoep, 70%; P<0.358) = 18; box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker, minimum.

For 3D correlation calculations, 3D movement correlation analysis between neurectoderm and ppl cells in defined sectors along the AV axis of the embryo. For 3D correlation calculations, neurectoderm cell velocities along the AV (V_{AV}), left-right (LR) (V_{LR}, see (e)) and dorsal-ventral (DV) axis (V_{DV} in sectors of 130x100 µm) were measured; sectors were positioned and color-coded relative to the ppl leading edge (yellow dot); A1-3 and P1-3, sector anterior and posterior of the ppl leading edge (yellow dot), respectively. (h) 3D movement correlation between leading edge ppl and adjacent neurectoderm cells in defined sectors along the AV axis of wt embryos (n=6 embryos) plotted from 6 to 8 hpf (120 min); colors of curves correspond to respective sectors in (e) and (g); error bars, s.e.m. (i) Fluorescent images of a MZoep:Tg(dharma:EGFP) mutant embryo showing neurectoderm nuclei (H2A-BFP, cyan) and Dharma (dharma:EGFP, green, marked with asterisk) expression at the dorsal blastoderm margin at a representative time point during gastrulation (t = 74.22 min, 7.2 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); animal (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100 µm. (j) Mean movement velocities (µm/min) of neurectoderm cells along the AV axis (V_{AV}) in MZoep mutant embryos (n=4 embryos) plotted over from 6 to 8 hpf (120 min); colors of curves correspond to sectors outlined in (e); error bars, s.e.m. (k, l) Anterior neural anlage in wt (k) and MZoep mutant (l) embryos marked by whole-mount in situ hybridization of otx2 mRNA expression at consecutive stages of gastrulation from 70% epiboly to bud stage (7 - 10 hpf); posterior axial mesoderm was detected by no tail (ntl) mRNA expression (arrows); animal pole (dorsal down), dorsal (animal pole up) and lateral (dorsal right) views are shown; arrowheads mark the anterior most edge of the neural plate; scale bars 200 µm. (m) Quantitative analysis of neural plate position during gastrulation in MZoep versus wt embryos. The angle (*) between the vegetal pole and the anterior border of the otx2 expression domain was measured for embryos at different stages during gastrulation (k, l) and plotted as box-whisker graphs; n, embryos analyzed from 4 independent experiments; student’s t-test (P value indicated) for all graphs comparing same stages; ***, P <0.001, (ns) non significant, P>0.05; n (wt, bud) = 36, n (wt, 90%) = 36, n (wt, 80%) = 34, n (wt, 70%) = 29, n (MZoep, bud; P <0.0001) = 24, n (MZoep, 90%; P<0.0001) = 36, n (MZoep, 80%; P<0.0001) = 20, n (MZoep, 70%; P<0.358) = 18; box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker, minimum.
**SUPPLEMENTARY INFORMATION**

Supplementary Figure 3 Prechordal plate cell movements and neural plate positioning in cyc and slb morphant embryos. (a, e) Fluorescence images of a Tg(gsc:GFP) cyclops (cyc) (a) and silberblick (slb) morphant (e) embryo showing H2A-BFP expression (cyan) in all nuclei and GFP (green, white outline) expression in gsc-expressing prechordal plate (ppl) cells at a representative time point during gastrulation (a; t = 71.40 min, 7.2 hpf and e; t = 74.22 min, 7.2 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); animal (AP) and vegetal pole (VP) indicated by arrows; red line in (e) indicates widened ppl internalization zone; scale bar, 100µm. (b, f) Number of internalized ppl cells in Tg(gsc:GFP) cyc (b; blue curve, n = 3 embryos) and slb (f; blue curve, n = 3 embryos) morphant embryos (blue curve, n = 3 embryos) versus wt (green curve, n = 6 embryos) embryos plotted between 6 and 8 hpf (120 min); error bars, s.e.m. (c, g) Average degree of alignment of ppl cell movements in cyc (c) magenta curve/squares, n = 3 embryos) and slb morphant (g; magenta curve/dots, n=3 embryos) versus wt (green curve/dots, see Supplementary Fig. 2c) embryos plotted from 6 to 8 hpf (120 min); error bars, s.e.m. (d, h) Mean instantaneous ppl cell speed and directionality of cyc (d; gray bar graph, n = 4 embryos; P(speed) = 0.0061, P(dir) = 0.033) and slb morphant (gray bar graphs, n = 3 embryos, P(speed) = 0.0025, P(dir) < 0.0001) versus wt (white bar graph, see Supplementary Fig. 2d) embryos plotted as bar graphs; error bars, s.e.m.; student’s t-test for all graphs; ***, p < 0.001, ** p < 0.01; *, p < 0.05. (i, j) Anterior neural plate anlage in cyc and slb morphant embryos marked by whole-mount in situ hybridization of otx2 mRNA expression at consecutive stages of gastrulation from 70% epiboly to bud stage (7 - 10hpf); posterior axial mesoderm was detected by no tail (ntl) mRNA expression (arrows); animal pole (dorsal down), dorsal (animal pole up) and lateral (dorsal right) views are shown; arrowheads mark the most anterior edge of the neural plate; scale bar 200 µm. (k) Quantitative analysis of neural plate position in cyc and slb morphant versus wt embryos during gastrulation. The angle (°) between the vegetal pole and the anterior border of the otx2 expression domain was measured for embryos at different stages during gastrulation (i, j) and plotted as box-whisker graphs; n, embryos analyzed from 4 independent experiments; student’s t-test (P value indicated) for all graphs comparing same stages; ***, P < 0.001, ns (non significant), P > 0.05; n (wt, bud) = 36, n (wt, 90%) = 36, n (wt, 70%) = 29, n (slb, bud; P < 0.0001) = 23, n (slb, 90%; P < 0.0001) = 17, n (slb, 80%; P < 0.0001) = 20, n (slb, 70%; P = 0.134) = 16, n (cyc, bud; P < 0.0001) = 40, n (cyc, 90%; P < 0.0001) = 39, n (cyc, 80%; P < 0.0001) = 32, n (cyc, 70%; P = 0.851) = 27, red dots mark mean values; box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker, minimum.
Supplementary Figure 4 Prechordal plate cell movements and neural plate positioning in wild type embryos overexpressing CA-Mypt within the yolk syncytial layer. (a) Schematic illustration of CA-Mypt and H2A-mCherry mRNA injection into the yolk syncytial layer (YSL) of an embryo at high stage (3.3 hpf). (b) Confocal images of the enveloping layer (EVL)/YSL epiboly progression in F-actin labeled Tg(actb1::GFP-UtrCH) wild type (wt) control (lower panel) and embryos injected with constitutively active myosin II phosphatase mRNA into the YSL (CA-Mypt, upper panel) at 8 hpf; both embryos were co-injected with H2A-mCherry mRNA into the YSL to mark YSL nuclei. (c) Quantification of the average advancement (µm/min) of the EVL margin of wt control and CA-Mypt injected embryos between 7 and 9 hpf; student’s t-test; ***, P < 0.001; n=4 embryos; error bars, s.e.m. (d) Fluorescence images of a Tg(gsc::GFP) embryo overexpressing CA-Mypt and H2A-mCherry (magenta, arrows) within the YSL, also showing H2A-BFP expression within all nuclei and GFP-expression in gsc-expressing prechordal plate (ppl) progenitors (green, white outline) at a representative time point during gastrulation (t = 75 min, 6.25 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); animal (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100µm. (e) Number of internalized ppl cells in Tg(gsc::GFP) embryos overexpressing CA-Mypt within the YSL (blue curve, n = 4 embryos) versus wt embryos (green curve) plotted from 6 to 8 hpf (120 min); error bars, s.e.m. (f) Directional correlation of ppl cell movements in a wt embryo overexpressing CA-Mypt within the YSL at a representative time point during gastrulation (t = 77.4 min, 6.8 hpf); ppl cells are visualized as arrows in a 2D plot and color-coded according to their 3D correlation values between 1 (red, maximum correlation) and -1 (blue, minimum correlation); every 3rd cell is plotted; AP, animal pole; VP, vegetal pole; scale bar, 50 µm. (g) Average degree of alignment of ppl movements in embryos overexpressing CA-Mypt within the YSL (magenta curve/squares, n = 3 embryos) versus wt embryos (gray curve/dots, see Supplementary Fig. 1c) plotted from 6 to 8 hpf (120 min); the order parameter corresponds to the degree of alignment ranging from 0 (disordered movement) to 1 (highly ordered movement); error bars, s.e.m. (h) Mean ppl cell instantaneous speed and directionality in CA-Mypt injected (gray bar graphs, n = 4 embryos; P(speed) = 0.323, P(dir) = 0.702) versus wt (white bar graphs, see Supplementary Fig. 2d) embryos plotted over 120min (6 to 8 hpf) as bar graphs; error bars, s.e.m.; student’s t-test for all graphs; ns (not-significant), P > 0.05. (i) Anterior neural anlage in embryos overexpressing CA-Mypt within the YSL marked by whole-mount in situ hybridization of otx2 mRNA expression at consecutive stages of gastrulation from 70% epiboly to bud stage (7 - 10hpf); posterior axial mesoderm was detected by no tail (ntl) mRNA expression (yellow arrows); animal pole (dorsal down), dorsal (animal pole up) and lateral (dorsal right) views are shown; red arrowhead marks the most anterior edge of the neural plate; 200 µm. (j) Quantitative analysis of neural plate position during gastrulation in embryos overexpressing CA-Mypt in the YSL versus wt embryos. The angle (°) between the vegetal pole and the anterior border of the otx2 expression domain was measured for embryos at different stages during gastrulation (i) and plotted as box-whisker graphs; n, embryos analyzed from 4 independent experiments; student’s t-test (P value indicated) for all graphs comparing same stages; ***, P < 0.01; **, P < 0.05, (ns) non significant, P >0.05; n (wt, bud) = 36, n (wt, 90%) = 36, n (wt, 70%) = 29, n (CA-Mypt, bud: P = 0.49) = 16, n (CA-Mypt, 90%: P = 0.0259) = 22, n (CA-Mypt, 80%: P = 0.0016) = 34, n (CA-Mypt, 70%: P = 0.0016) = 12; red dots mark mean values; box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker, minimum.
Supplementary Figure 5 Movement of transplanted prechordal plate cells in MZoep mutant embryos. (a, h) Schematic illustration of a MZoep; Tg(dharma:EGFP) (a) and a MZoep; Tg(dharma:EGFP) mutant embryo that was injected with CA-Mypt mRNA into the YSL at high stage (h; 3.3 hpf) transplanted with prechordal plate (ppl) cells (green) into the dorsal side at 60% epiboly (6 hpf); asterisk marks position of dorsal marker Dharma; orange arrows indicate reduced vegetal-directed movement of EVL margin (h); AP, animal pole; VP, vegetal pole; L, left; R, right. (b, i) Bright-field/fluorescence image of a MZoep; Tg(dharma:EGFP) mutant embryo at 90% epiboly (9 hpf) and a MZoep; Tg(dharma:EGFP) mutant embryo at 80% epiboly (8 hpf) that overexpresses CA-Mypt and the nuclei marker H2A-mCherry (red) within the YSL containing transplanted GFP-labeled ppl cells from Tg(gsc:GFP) donor (b) and Tg(gsc:GFP-CAAX) donor (i) embryos; ppl cell nuclei are marked by H2A-mCherry (i; red, co-localizes with green ppl cells); dashed white line indicates position of transplanted ppl progenitors; arrowhead points at anterior edge of ppl cells; asterisk marks dharma:EGFP expression (green, asterisk); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); animal pole (AP) and vegetal pole (VP) indicated by arrows; scale bar, 200 µm. (c, j) Fluorescence images of representative time points during gastrulation (c; t = 47.19 min, 6.8 hpf and j; t = 56.39 min, 6.9 hpf) showing a MZoep; Tg(dharma:EGFP) (c) and a MZoep; Tg(dharma:EGFP) mutant embryo which overexpresses CA-Mypt and the nuclei marker H2A-mCherry (magenta) within the YSL (j) containing transplanted gsc-expressing GFP-labeled ppl cells (white outline) from Tg(gsc:GFP) donor (c) and Tg(gsc:GFP-CAAX) donor (j) embryos; all nuclei are marked by H2A-mCherry (c; magenta) and H2A-BFP (j; cyan) expression, and the dorsal side of the embryos is marked by dharma:EGFP expression (green, asterisk); dorsal sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); animal pole (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100 µm. (d) Protrusion orientation of ppl cells transplanted into MZoep mutants: top panel, fluorescence image of ppl cells with cytoplasm in green (gsc:GFP) and nuclei in cyan (H2A-BFP); animal pole up; scale bar, 20 µm. Bottom panel, polar plot or protrusion orientation of ppl cells (n = 48 cells from 2 embryos) with 0° = animal pole, 180° = vegetal pole. (e) Number of ppl cells transplanted into MZoep mutant embryos (n=3 embryos) plotted from 6 to 8 hpf (120 min); error bars, s.e.m. (f) Directional correlation of transplanted ppl cell movements in a MZoep mutant embryo at a representative time point during gastrulation (t = 83.5 min, 7.4 hpf); ppl cells are visualized as arrows in a 2D plot and color-coded according to their 3D correlation values between 1 (red, maximum correlation) and -1 (blue, minimum correlation); every 5th cell is plotted; AP, animal pole; VP, vegetal pole; scale bar, 50 µm. (g) Average degree of alignment of transplanted ppl cell movements in MZoep mutant embryos (magenta curve/squares, n=3 embryos) versus endogenous ppl cell movements in wt embryos (green curve/dots, see Supplementary Fig. 2c) from 6 to 8 hpf (120 min); the order parameter corresponds to the degree of alignment ranging from 0 (disordered movement) to 1 (highly ordered movement); error bars, s.e.m. (h) Mean neur ectoderm cell velocities along the animal-vegetal (AV) axis (V_{AV}) (measurement area indicated by black box in Supplementary Fig. 2e) in MZoep mutant embryos (red curve, n=3 embryos) and MZoep mutant embryos overexpressing CA-Mypt in the YSL (black curve, n=3 embryos); error bars, s.e.m.
Supplementary Figure 6 Effect of external friction on one-dimensional neurectoderm flow profile. (a) For capturing the flow profile induced solely by prechordal plate (ppl) cells, MZoep mutants devoid of ppl cells were used to measure unperturbed epiboly movements, and those movements were subtracted from the overall neurectoderm flow field in wt embryos. This allowed decomposing the neurectoderm flow field and obtaining the ppl-induced movement alterations only. In the 2D description, neurectoderm flows exclusively within the experimental image plane (red square) were taken into account. (b) Theoretical 1D flow profile when the external friction coefficient $\xi_0$ between neurectoderm and tissues other than the ppl, such as the yolk cell and/or EVL, is varied. In case the external friction coefficient is increased, the range of flow triggered by ppl cells is decreased (blue: $\xi_0/\eta = 10^{-11}\,\mu m^{-2}$, orange: $\xi_0/\eta = 10^{-9}\,\mu m^{-2}$, green: $\xi_0/\eta = 10^{-6}\,\mu m^{-2}$, all curves: $f/\eta = -4.3\times10^{-5}\,\mu m^{-1}\cdot min^{-1}$). (c) Experimental velocities in wt embryos (blue dots) compared to theoretical flow profiles for the parameter settings as used in Fig. 5 (red dotted line, $f/\eta = -4.3\times10^{-5}\,\mu m^{-1}\cdot min^{-1}$, $\xi_0/\eta = 2.4\times10^{-6}\,\mu m^{-2}$), and for zero external friction (green line, $f/\eta = -3.5\times10^{-5}\,\mu m^{-1}\cdot min^{-1}$, $\xi_0/\eta = 0$). The experimental velocity profile in wt embryos is well explained by either a small ($\xi_0 < \eta/L_{E}^2$) or vanishing ($\xi_0 = 0$) external friction coefficient.
Supplementary Figure 7
**Supplementary Information**

**Supplementary Figure 7** Prechordal plate cell movements and neural plate positioning in e-cadherin morphant embryos. (a) Brightfield/fluorescence image of a wild type (wt) Tg(gsc:GFP) (top panel) and e-cadherin (e-cad) morphant embryo (bottom panel) with gsc-expressing GFP-labeled prechordal plate progenitor (ppl) cells (green, white outline) at 80% epiboly; dorsal views, animal pole up; the increasing distance between the margins of the enveloping layer (EVL; red dashed line) shows neur/ectoderm epiboly delay in e-cad morphant embryos; scale bar, 200 µm. (b) Fluorescence images of a Tg(gsc:GFP) e-cad morphant embryo showing H2A-mCherry (magenta) expression in all nuclei and GFP (green) expression in ppl cells (white outline) at a representative time point during gastrulation (t = 80.30 min, 7.3 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper panel mark sagittal section plane in lower panel); red and blue dashed lines as in (A); animal pole (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100 µm. (c) Number of internalized ppl cells in Tg(gsc:GFP) e-cad morphant (blue curve, n=4 embryos) versus wt (green curve) embryos plotted from 6 to 8 hpf (120 min); error bars, s.e.m. (d) Correlation of ppl cell movements in a e-cad morphant embryo at a representative time point during gastrulation (t = 80 min, 7.3 hpf); ppl cells are visualized as arrows in a 2D plot and color-coded according to their 3D correlation values between 1 (red, maximum correlation) and -1 (blue, minimum correlation); every 3rd cell is plotted; AP, animal pole; VP, vegetal pole; scale bar, 50 µm. (e) Average degree of alignment of ppl movements in e-cad morphant (magenta curve/squares, n=3) versus wt (green curve/dots, see Supplementary Fig. 2c) embryos from 6 to 8 hpf (120 min); the order parameter corresponds to the degree of alignment, ranging from 0 (disordered movement) to 1 (highly ordered movement); error bars, s.e.m. (f) Mean ppl instantaneous speed and directionality in e-cad morphant [gray bar graphs, n=4 embryos; P(speed) = 0.0362; P(dir) = 0.222] versus wt (white bar graphs, see Supplementary Fig. 2d) embryos plotted as bar graphs; error bars, s.e.m.; student’s t-test for all graphs; *, P < 0.05; (ns) non significant, P > 0.05. (g) Model of friction generation under E-cadherin reduced conditions (compare with wt in Fig. 6f) in e-cadherin morphant embryo leads to decreased friction at the ppl-to-neurectoderm (ectoderm) interface and to non-graded velocities within the ppl (left panel); Ff, friction force; orange dashes indicate remaining cadherin; reduced E-cadherin-mediated adhesion between ppl and neurectoderm leads to loss of frictional drag and vegetal-directed movements (red arrow) of neurectoderm cells (right panel; yellow arrows indicate ppl movement); double-sided arrows indicate embryonic axes, animal (A) to vegetal (V), dorsal (D) to ventral (V). (h) 2D tissue flow map indicating velocities (µm/min) of neur/ectoderm (ectoderm) cell movements along the AV (VAV) and left-right (LR) (VLR) axes at the dorsal side of a MZoep embryo overexpressing CA-Mypt within the YSL and transplanted with e-cad morphant ppl cells (t = 41.40 min, 6.7 hpf) at a representative time point; average velocity vector for each defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) µm/min; positions of all/leading edge ppl cells are marked by black/green dots; black boxed area was used for mean velocity measurements in (i); scale bar, 100 µm. (i) Mean movement velocities (µm/min) along the AV axis (VAV) of ppl leading edge progenitor cells (green curve, left y-axis) and neurectoderm (ecto) cells positioned above the ppl leading edge (black boxed area in h; red curve, right y-axis) in MZoep embryos overexpressing CA-Mypt within the YSL and transplanted with e-cad morphant ppl cells (n=4 embryos) plotted from 6 to 8 hpf; vertical dashed line indicates start of vegetal-directed movements of ppl cells; error bars, s.e.m. (j) 3D directional correlation values between leading edge ppl and adjacent neurectoderm (ecto) cells in MZoep embryo overexpressing CA-Mypt within the YSL and transplanted with e-cad morphant ppl cells (t = 41.40 min, 6.7 hpf) at a representative time point during gastrulation; degree of correlation is color-coded ranging from 1 (red, highest) to -1 (white, lowest); average neurectoderm velocities for each defined area are marked; black boxed area was used for local correlation measurements in (k); scale bar, 100 µm. (k) 3D directional correlation values between leading edge ppl and adjacent neurectoderm (ecto) cells (black boxed area in j) in MZoep embryos overexpressing CA-Mypt within the YSL and transplanted with e-cad morphant ppl cells (n=4 embryos) plotted from 6 to 8 hpf; error bars, s.e.m. (l) Anterior neural anlage in e-cad morphant embryos marked by whole-mount in situ hybridization of otx2 mRNA expression at consecutive stage during gastrulation from 70% to 90% epiboly (7 - 9hpf); posterior axial mesoderm was detected by no tail (ntl) mRNA expression (yellow arrows), animal pole (dorsal down), dorsal (animal pole up) and lateral (dorsal right) views are shown; red arrowheads mark the most anterior edge of the neural plate; scale bar, 200 µm. (m) Quantitative analysis of neural plate position during gastrulation in e-cad morphant versus wt embryos; the angle (°) between the vegetal pole and the anterior border of the otx2 expression domain was measured for embryos at different stages (l) and plotted as box-whisker graphs; n, embryos analyzed from 4 independent experiments; student’s t-test (P value indicated) for all graphs comparing same stages; ***, P <0.001, (ns) non significant, P >0.05; n (wt, 90%) = 36, n (wt, 80%) = 34, n (wt, 70%) = 29, n (e-cad, 90%; P<0.0001) = 30, n (e-cad, 80%; P<0.0001) = 37, n (e-cad, 70%; P = 0.00036) = 41; box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker, minimum.
Supplementary Figure 8 Alterations in ectoderm movements upon application of E-cadherin mediated friction ex vivo and shear-strain-induced neurectoderm tissue deformation in vivo. (a) Bright-field/fluorescence image showing setup of magnetic polystyrene beads (20 µm diameter) and fluorescent reference beads (red, 4 µm diameter) attached to a glass plate used to apply friction onto ectoderm cells; dashed line outlines shape of polystyrene cluster; scale bars, 100 µm and 20 µm for magnified area. (b) Western Blot analysis showing detection of E-cadherin ectodomain (80 kDa) eluted from magnetic polystyrene beads coupled to E-cadherin-Fc Chimera (E-Fc) or uncoated control beads; molecular weight markers, 100 and 200 kDa. (c) Section of maximum projection confocal image (see Fig. 7a; t = 19.33 min) showing top plate with fluorescent reference beads and selected beads are highlighted (red arrows in xy and yz cross-section); cross-section (yz; red rectangle) shows the position of E-Fc-coated beads (outlined in orange) at the ectoderm cell interface (yellow dashed line); direction of beads movement (top plate; - y; velocity ~ 1.5 µm/min) is indicated; scale bars, 100µm in xy and 20 µm in yz. (d) Shear strain-induced neurectoderm tissue deformations of wild type (wt, upper panels; n=3 embryos) and MZoe (lower panels; n=3 embryos) embryos plotted as time-averaged strain values for each domain (50 x 50 µm); average shear strain rate is color-coded according to amount of plane distortion [minimum green (0) to maximum red (5 x10^{-3}s^{-1})]; tissue flows of neurectoderm are indicated as time-averaged velocities; dashed line indicates ppl and black dot marks ppl leading edge as reference point in wt and MZoe; rectangle outlines area used for defining sectors along the animal-vegetal (AV) axis in (e). (e) Mean shear strain rates of neurectoderm tissue of wt (upper panels; n=3 embryos) and MZoe (lower panels; n=3 embryos) in defined sectors (100 x 200 µm) are plotted along the AV axis over time of gastrulation (plotted from 6.3 to 7.3 hpf in 10 min intervals); sectors were positioned and color-coded relative to the ppl leading edge (anterior A1-2 and posterior P1-2 of the ppl leading edge; for detailed description refer to Supplementary Fig. 1e); amount of plane distortion [minimum green (0) to maximum red (10 x10^{-3}s^{-1})] is plotted along the y-axis; (f) Neurectoderm tissue strain rate maps derived by subtraction of time-averaged shear strain values of wt from MZoe embryos (n=3 embryos); color-code as in (f); tissue flows of neurectoderm are indicated as time-averaged velocities; dashed line indicates ppl and black dot marks ppl leading edge as reference point. (g) Illustration of shear strain tissue deformation in the neurectoderm; arrows indicate direction of plane distortion of a tissue domain along the AV and left-right (LR) axis dependent on the direction and magnitude of neurectoderm movements; shear strain-induced domain angle of plane distortion can shrink (positive value) or enlarge (negative value).
Supplementary Video Legends

Supplementary Video 1  Live cell imaging of cell movements in wt embryo. Multiphoton time-lapse imaging of a wild type (wt) Tg(gsc:GFP) embryo with gsc-expressing GFP-labeled prechordal plate progenitor (ppl) cells (green) and neurectoderm cells at the dorsal side of the embryo from 6 to 8 hpf (123 min); all nuclei were labeled with histone H2A-BFP; animal/vegetal pole, up/down.

Supplementary Video 2  Life cell imaging of cell movements in MZoep mutant embryo. Multiphoton time-lapse imaging of a MZoep;Tg(dharma:EGFP) mutant embryo (Dharma:EGFP signal green) showing neurectoderm cells at the dorsal side of the embryo from 6 to 8.1 hpf (129 min); all nuclei were labeled with histone H2A-BFP; animal/vegetal pole, up/down.

Supplementary Video 3  2D velocities of neurectoderm cells in wt embryo. Tissue flow map indicating velocity vectors of neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the dorsal side of a wild type (wt) embryo between 6 to 8 hpf (117 min); average velocity vector for each defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) µm/min; position of all/leading edge neurectoderm (ppl) cells are indicated as black/green dots; xy-axes in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 4  3D correlation of neurectoderm and prechordal plate (ppl) cell movements in wt embryo. Movement correlation between neurectoderm and underlying (ppl) cells at the dorsal side of a wild type (wt) embryo between 6 to 8 hpf (118 min); degree of correlation is color-coded ranging from 1 (red, highest correlation) to -1 (white, lowest correlation); average neurectoderm movement velocities and direction for each defined area are indicated by arrows; position of all/leading edge ppl cells are indicated as white/green dots; blue arrow marks movement direction of ppl leading edge cells; xy-axes in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 5  2D velocities of neurectoderm cells in MZoep mutant embryo. Tissue flow map indicating velocity vectors of neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the dorsal side of a MZoep mutant embryo between 6 to 8 hpf (121 min); average velocity vector for each defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) µm/min; xy-axes in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 6  3D correlation of neurectoderm and prechordal plate (ppl) cell movements in CA-Mypt injected embryo. Movement correlation between neurectoderm and underlying prechordal plate (ppl) cells at the dorsal side of a wt embryo overexpressing CA-Mypt in the YSL between 6 to 8 hpf (118 min); degree of correlation is color-coded ranging from 1 (red, highest correlation) to -1 (white, lowest correlation); average neurectoderm movement velocities and direction for each defined area are indicated by arrows; position of all/leading edge ppl cells are indicated as white/green dots; blue arrow marks movement direction of ppl leading edge cells; xy-axes in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 7  2D velocities of neurectoderm cells in ppl-transplanted MZoep mutant embryo. Tissue flow map indicating velocity vectors of neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the dorsal side of a transplanted MZoep mutant embryo between 6 to 7.5 hpf (91 min); average velocity vector for each defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) µm/min; xy-axes in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 8  2D velocities of neurectoderm cells in ppl-transplanted and CA-Mypt injected MZoep mutant embryo. Tissue flow map indicating velocity vectors of neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the dorsal side of a MZoep mutant embryo overexpressing CA-Mypt within the YSL between 6 to 8 hpf (120 min); average velocity vector for each defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) µm/min; position of all/leading edge ppl cells are indicated as black/green dots; xy-axis in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 9  Arrangement of leading and trailing prechordal plate (ppl) cells in wild type (wt) embryo. Consecutive z-sections of a fluorescent imaging stack showing lifeact-GFP (F-actin) expressing ppl cells transplanted into the ppl leading edge of a wt embryo expressing Utrophin-mCherry (F-actin) and H2A-mCherry (nuclei); section starts at the ppl-neurectoderm interface and progresses through the leading edge ppl to the ppl-YSL interface; animal pole to the left; z-section taken from movie 16 at t = 12.36 min; scale bar, 20 µm.

Supplementary Video 10  Life cell imaging of leading and trailing prechordal plate (ppl) cells in wild type (wt) embryo. Fluorescence time-lapse imaging of lifeact-GFP (F-actin) expressing ppl cells transplanted into the ppl leading edge of a wt embryo expressing Utrophin-mCherry (F-actin) and H2A-mCherry (nuclei) starting at 70% epiboly (7 hpf); dorsal (top, animal pole left) and sagittal (bottom, animal pole left) sections through the embryo with dual (left side) and single (right side) color label; time in mins; scale bar, 20 µm.

Supplementary Video 11  2D velocities of neurectoderm cells in e-cadherin morphant embryo. Tissue flow map indicating velocity vectors of neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the dorsal side of a e-cadherin morphant embryo between 6 to 8 hpf (120 min); average velocity vector for each defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) µm/min; position of all/leading edge prechordal plate (ppl) cells are indicated as black/green dots; xy-axis in µm; time in mins; animal/vegetal pole, up/down.

Supplementary Video 12  3D correlation of neurectoderm and prechordal plate (ppl) cell movements in e-cadherin morphant embryo. 3D movement correlation between neurectoderm and underlying prechordal plate (ppl) cells at the dorsal side of e-cadherin morphant embryo 6 to 8 hpf (120 min); degree of correlation is color-coded ranging from 1 (red, highest correlation) to -1 (white, lowest correlation); average neurectoderm movement velocities and direction for each defined area are indicated by arrows; position of all/leading edge ppl cells are indicated as white/green dots; blue arrow marks movement direction of ppl leading edge cells; xy-axes in µm; time in mins; animal/vegetal pole, up/down.
SUPPLEMENTARY NOTE

In these supplementary informations we propose a theoretical description of flows arising in the neurectoderm as a result of the underlying motion of ppl cells. In order to analyze the specific effect of ppl cells on neurectoderm flows, we use experimental measurements of neuroectoderm velocity field in the presence of ppl cells, denoted \( v^{\text{tot}} \), and experimental measurements of neuroectoderm velocity field in the absence of ppl cells in MZoeo mutants (denoted \( v^{\text{MZ}} \)). We then calculate the difference between these velocity fields:

\[ v = v^{\text{tot}} - v^{\text{MZ}}. \]

Our theoretical description aims at reproducing this velocity field.

1. Simplified one dimensional description of neurectoderm flows

We describe the neurectoderm as a two-dimensional viscous compressible fluid, flowing with velocity vector \( \mathbf{v} \). We assume that the effect of ppl cells is to exert an external force on the neuroectoderm, and we analyze the flow profile induced by this external force.

1.1. Main equation of one-dimensional viscous flow. We first discuss a simplified description of the flow in one dimension. We aim here at understanding flow profiles measured along the animal-vegetal axis of the embryo, with \( y \) denoting the coordinate along the animal-vegetal axis, going positively towards the vegetal axis (Fig. 5a). We consider the velocity towards the vegetal pole \( v_y \), and the tension within the tissue is denoted \( \sigma_y \). We consider the tissue to be fluid with effective one-dimensional viscosity \( \bar{\eta} \), such that the stress within the tissue reads

\[ \sigma_y = \bar{\eta} \partial_y v_y \]

We assume that neurectoderm cells flow with a friction coefficient \( \xi_0 \) relative to the surrounding tissues other than ppl cells. For instance, \( \xi_0 \) could be caused by relative friction between the neurectoderm and the EVL or by friction between the neurectoderm and the yolk. In addition, we assume that within the ppl domain, delimited by the region \( y^{\text{ppl}} - L_y^{\text{ppl}} < y < y^{\text{ppl}} + L_y^{\text{ppl}} \),
ppl cells exert a uniform force density \( f \). Force balance at low Reynolds number then reads:

\[
\partial_y \sigma_y = \xi_0 v_y \quad \text{outside of ppl domain}
\]

\[
\partial_y \sigma_y = \xi_0 v_y - f \quad \text{inside the ppl domain}
\]

Finally, we denote \( L^E \) the distance from the animal pole to the neurectoderm margin, such that \( y = 0 \) denotes the animal pole and \( y = -L^E \) and \( y = L^E \) denote the neurectoderm margin on the ventral and dorsal side. We impose that the flow vanishes at the margin, \( v_y(-L^E) = v_y(L^E) = 0 \). The solution for the flow reads:

\[
v_y = C_1 \left( e^{\frac{y}{l_0}} - e^{-\frac{2L^E+y}{l_0}} \right), \quad y < y^{ppl} - L_y^{ppl}
\]

\[
v_y = C_2 e^{\frac{y}{l_0}} + C_3 e^{-\frac{y}{l_0}} + \frac{f}{\xi_0}, \quad y^{ppl} - L_y^{ppl} < y < y^{ppl} + L_y^{ppl}
\]

\[
v_y = C_4 \left( e^{\frac{y}{l_0}} - e^{-\frac{2L^E+y}{l_0}} \right), \quad y > y^{ppl} + L_y^{ppl}
\]

with \( l_0 = \sqrt{\eta/\xi_0} \) an hydrodynamic length, and \( C_i, i = 1, \ldots, 4 \) are constants which can be determined from the conditions of continuity of the velocity and its derivative at the boundaries of the ppl domain. The flow profile induced by a localized external force decays exponentially away from the ppl region exponentially on the length \( l_0 \) (Supplementary Fig. 6b).

In the limit where friction acting on the tissue from other sources than ppl cells can be neglected, \( (l_0 \gg L^E) \), the solution for the flow profile reads:

\[
v_y = \frac{fL_y^{ppl}}{\eta} \left( 1 - \frac{y^{ppl}}{L^E} \right) (L^E + y), \quad y < y^{ppl} - L_y^{ppl}
\]

\[
v_y = -\frac{f}{2\eta} \left( (y - y^{ppl})^2 + \frac{2L_y^{ppl}y^{ppl}}{L^E} (y - y^{ppl}) + \frac{L_y^{ppl}}{L^E} \left( 2y^{ppl}^2 - 2L^2 + L^E L_y^{ppl} \right) \right), \quad y^{ppl} - L_y^{ppl} < y < y^{ppl} + L_y^{ppl}
\]

\[
v_y = \frac{fL_y^{ppl}}{\eta} \left( 1 + \frac{y^{ppl}}{L^E} \right) (L^E - y), \quad y > y^{ppl} + L_y^{ppl}
\]

In that case the flow profile decays linearly towards the EVL margin.

1.2. Comparison to experimental flow profile along the dorsal midline.
1.2.1. Wild-type velocity profiles. To compare to experiment, we first aimed at matching theoretical profiles with wt velocity profiles along the animal-vegetal axis, on the dorsal line. Estimates of the lengths $L_{\text{ppl}}, L_E$ and $y_{\text{ppl}}$ used for comparison are discussed in section 3 and reported in Table 2.

A fitting procedure then gave $f/\tilde{\eta} = -4.2 \cdot 10^{-5} \mu \text{m}^{-1} \cdot \text{min}^{-1}$ and $\tilde{\eta}/\xi_0 = 6.3 \cdot 10^5 \mu \text{m}^2$ (Table 1), corresponding to an hydrodynamic length $l_0 = 793 \mu \text{m}$. Because $l_0$ is close to the system size $L_E$, we conclude that external friction arising from other tissues than ppl cells has a small effect on the flow profile in wt. Predicted profiles without external friction from surrounding tissues ($\xi_0 = 0$) indeed also match experimental profiles closely (Supplementary Fig. 6c).

Assuming that the force density $f$ exerted by ppl cells on the neuroectoderm can be described by a dynamic friction force, we then have the following relation:

$$f = \xi (v_{\text{ppl}}^y - v_{\text{tot}}^y),$$

where $v_{\text{ppl}}^y$ is the velocity of ppl cells, $v_{\text{tot}}^y$ is the wild-type neuroectoderm total velocity at the same point and $\xi$ is the friction coefficient. To determine the friction coefficient, we average Eq. 11 in the domain of ppl cells, assuming here for simplicity that the force density $f$ is homogeneous within the ppl domain:

$$f = \xi \langle v_{\text{ppl}}^y - v_{\text{tot}}^y \rangle.$$

We then estimate the experimental values of $\langle v_{\text{ppl}}^y \rangle$ and $\langle v_{\text{tot}}^y \rangle$. The corresponding values are reported in Table 1. We then obtain an estimate of $f/\xi$, and using the value of $f/\tilde{\eta}$ obtained by comparison with experimental profiles, we then can estimate the ratio $\tilde{\eta}/\xi$ (Table 1). A characteristic hydrodynamic length can be defined from this ratio by:

$$l_1 = \sqrt{\tilde{\eta}/\xi}.$$  

Note that $l_1 \neq l_0$ since $\xi$ is associated to friction acting in between the neurectoderm and ppl cells, while $\xi_0$ is associated to friction in between the neurectoderm and other tissues. We find $l_1 \simeq 288 \mu \text{m}$, corresponding to a friction coefficient about 8 times larger in between neurectoderm and ppl cells than in between neurectoderm and other tissues.
**SUPPLEMENTARY NOTE**

### Table 1. Values of parameters obtained by comparison of the one dimensional simplified theory and two-dimensional theory with experiments.

| Exp | \( f/\bar{\eta} \) | \( \bar{\eta}/\xi_0 \) | \( \langle v_{y}^{\text{ppl}} \rangle \) | \( \langle v_{y}^{\text{tot}} \rangle \) | \( f/\xi \) | \( \bar{\eta}/\xi \) | \( l_1 \) |
|-----|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| **Unit** | \( \mu m^{-1} \cdot \text{min}^{-1} \) | \( \mu m^2 \) | \( \mu m/\text{min} \) | \( \mu m/\text{min} \) | \( \mu m/\text{min} \) | \( \mu m^2 \) | \( \mu m \) |
| **wt** | \(-4.2 \cdot 10^{-5}\) | 6.3 \( \cdot 10^5\) | \(-4\) | \(-0.5\) | \(-3.5\) | 8.4 \( \cdot 10^4\) | 290 |
| **slb** | \(-3.4 \cdot 10^{-5}\) | 6.3 \( \cdot 10^5\) | \(-2.5\) | \(0.4\) | \(-2.9\) | 8.4 \( \cdot 10^4\) | 290 |

### Two-dimensional description

| Exp | \( f/\eta_b \) | \( \eta_b/\eta \) | \( \langle v_{y}^{\text{ppl}} \rangle \) | \( \langle v_{y}^{\text{tot}} \rangle \) | \( f/\xi \) | \( \eta_b/\xi \) | \( l_2 \) |
|-----|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| **Unit** | \( \mu m^{-1} \cdot \text{min}^{-1} \) | \( \mu m/\text{min} \) | \( \mu m/\text{min} \) | \( \mu m/\text{min} \) | \( \mu m^2 \) | \( \mu m \) |
| **wt** | \(-2 \cdot 10^{-4}\) | 1 | \(-4\) | \(-0.5\) | \(-3.5\) | 1.8 \( \cdot 10^4\) | 133 |
| **slb** | \(-1.6 \cdot 10^{-4}\) | 1 | \(-2.5\) | \(0.4\) | \(-2.9\) | 1.8 \( \cdot 10^4\) | 133 |

1.3. *slb mutant velocity profiles.* ppl cells have a reduced velocity in slb mutants. We therefore estimate the reduction in the magnitude of ppl exerted force density \( f \) from Eq. 12, using experimentally measured average velocities in the ppl region (Table 1). We then obtain the corresponding predicted theory profile, keeping mechanical parameters other than \( f \) as in wt, and using geometrical parameters reported in Table 2. This yields a very good agreement with experimental profiles (Fig. 5-b1).

### 2. TWO-DIMENSIONAL FLOW

2.1. **Main equations.** We discuss here the two-dimensional flow profiles obtained in the region of experimental measurement, a square domain of size \( 2L \). For simplicity, we ignore the curvature of the embryo and use 2D cartesian coordinates \( x, y \). For comparison with flows observed in the zebrafish embryo, the \( x \) axis is going on the surface of the embryo along the left-right direction, away from the dorsal midline of the embryo, and the \( y \) axis as going along the animal-vegetal direction, away from the animal pole. The corresponding geometry is represented in Fig. 5a. As before, we assume that the effect of ppl cells is to exert an external force on the neuroectoderm. We solve for the theoretical flow within the region of observation, and impose experimentally measured velocities at the boundary of the region.
Force balance on the neuroectoderm in two dimensions now reads

$$\partial_t \sigma_{ij} = -f_{ij}^{\text{ppl}},$$

where $\sigma_{ij}$ is the two-dimensional stress tensor within the neurectoderm, and $f_{ij}^{\text{ppl}}$ is the two-dimensional force density exerted by ppl cells on the neurectoderm.

The stress tensor $\sigma_{ij}$ in the neuroectoderm tissue reads:

$$\sigma_{ij} = 2\eta \left[ v_{ij} - \frac{1}{2} v_{kk} \delta_{ij} \right] + \eta_b v_{kk} \delta_{ij},$$

where $\eta$ is the neurectoderm shear viscosity, $\eta_b$ is the neurectoderm bulk viscosity, and $v_{ij} = (\partial_i v_j + \partial_j v_i)/2$ is the symmetric part of the gradient of flow.

We further choose the following form for the force density exerted by ppl cells:

$$f_{ij}^{\text{ppl}} = f \left( \mathcal{H}[x + L_x^{\text{ppl}}] - \mathcal{H}[x - L_x^{\text{ppl}}] \right) \times \left( \mathcal{H}[y - y_y^{\text{ppl}} + L_y^{\text{ppl}}] - \mathcal{H}[y - y_y^{\text{ppl}} - L_y^{\text{ppl}}] \right) \delta_{ij}.$$  

where $\mathcal{H}$ is the Heaviside function. This choice corresponds to a uniform force density with magnitude $f$, acting along the $y$ direction, and exerted on a rectangular domain of size $2L_x^{\text{ppl}} \times 2L_y^{\text{ppl}}$, centred around $x = 0$ and $y = y_y^{\text{ppl}}$.

By using the constitutive equation 15, the force balance equation 14, and the force density 16, we obtain the following equation for the flow field:

$$\begin{cases}
\eta \Delta v_x + \eta_b (\partial_x^2 v_x + \partial_y \partial_x v_y) = 0, \\
\eta \Delta v_y + \eta_b (\partial_y^2 v_y + \partial_y \partial_x v_x) = -f \left( \mathcal{H}[x + L_x^{\text{ppl}}] - \mathcal{H}[x - L_x^{\text{ppl}}] \right) \times \left( \mathcal{H}[y - y_y^{\text{ppl}} + L_y^{\text{ppl}}] - \mathcal{H}[y - y_y^{\text{ppl}} - L_y^{\text{ppl}}] \right). 
\end{cases}$$

Eqs. 17 are then solved on a rectangular domain of size $2L \times 2L$. The boundary conditions are set by imposing experimentally measured velocity profiles

$$\begin{align*}
v_x(L, y) &= v_x^{R,\text{exp}}(y), & v_y(L, y) &= v_y^{R,\text{exp}}(y) \\
v_x(-L, y) &= v_x^{L,\text{exp}}(y), & v_y(-L, y) &= v_y^{L,\text{exp}}(y) \\
v_x(x, L) &= v_x^{U,\text{exp}}(x), & v_y(x, L) &= v_y^{U,\text{exp}}(x) \\
v_x(x, -L) &= v_x^{D,\text{exp}}(x), & v_y(x, -L) &= v_y^{D,\text{exp}}(x). \end{align*}$$
2.2. Calculation of flow fields. To solve for the velocity field and get explicit expressions for \( v_x \) and \( v_y \), we used the method of superposition. For convenience, we used the following decomposition of the velocity field:

\[
v_x(x, y) = v_{x, \text{per}}(x, y) + \sum_{n=1}^{N} \alpha_n(y) \sin \left( \frac{n\pi(x - L)}{2L} \right) + \sum_{n=1}^{N} \beta_n(x) \cos \left( \frac{n\pi(y - L)}{2L} \right) + \beta_0(x)
\]

\[
v_y(x, y) = v_{y, \text{per}}(x, y) + \sum_{n=1}^{N} \gamma_n(y) \cos \left( \frac{n\pi(x - L)}{2L} \right) + \sum_{n=1}^{N} \delta_n(x) \sin \left( \frac{n\pi(y - L)}{2L} \right) + \gamma_0(y)
\]

In the equation above, \( v_{x, \text{per}}(x, y) \) and \( v_{y, \text{per}}(x, y) \) are solution of Eq. 17 which are periodic along the \( x \) axis and have vanishing velocities at the boundaries \( y = -L \) and \( y = L \). The additional truncated Fourier sums are introduced to verify the remaining boundary conditions.

2.2.1. Determination of the periodic solution. To obtain the periodic solution, we introduce the Fourier transforms of the velocity field along the \( x \) direction:

\[
\begin{align*}
\hat{v}_x(k_x, y) &= \frac{1}{\sqrt{2\pi}} \int dx e^{-ik_x x} v_{x, \text{per}}(x, y) \\
\hat{v}_y(k_x, y) &= \frac{1}{\sqrt{2\pi}} \int dx e^{-ik_x x} v_{y, \text{per}}(x, y)
\end{align*}
\]

where \( k_x = \pi n / L \) with \( n \in \mathbb{Z} \). We then obtain from Eq. 17:

\[
\begin{align*}
\eta \partial_y^2 \hat{v}_x + \eta_b ik_x \partial_y \hat{v}_y - (\eta + \eta_b) k_x^2 \hat{v}_x &= 0 \\
(\eta + \eta_b) \partial_y^2 \hat{v}_y + \eta_b ik_x \partial_y \hat{v}_x - \eta k_x^2 \hat{v}_y &= C(k_x) \mathcal{H}_y,
\end{align*}
\]

where we have introduced \( C(k_x) = -f \sqrt{2 \sin(k_x L_{\text{ppl}})} / k_x \) for \( k_x \neq 0 \) and \( C(0) = -f \sqrt{2 / \pi L_{\text{ppl}}^2} \), and \( \mathcal{H}_y = \mathcal{H}[y - y_{\text{ppl}} + L_{y_{\text{ppl}}}] - \mathcal{H}[y - y_{\text{ppl}} - L_{y_{\text{ppl}}}] \). These equations can be rewritten after rearrangement

\[
\begin{align*}
\partial_y^4 \hat{v}_x - 2k_x^2 \partial_y^2 \hat{v}_x + k_x^4 \hat{v}_x &= -\frac{C(k_x) \eta_b k_x \mathcal{H}_y}{\eta(\eta + \eta_b)} \\
\partial_y^4 \hat{v}_y - 2k_x^2 \partial_y^2 \hat{v}_y + k_x^4 \hat{v}_y &= \frac{C(k_x) \mathcal{H}_y}{\eta(\eta + \eta_b)} - \frac{Ck_x^2 \mathcal{H}_y}{\eta}.
\end{align*}
\]
SUPPLEMENTARY NOTE

We solve separately these equations in the following three domains (1), \(-L^\text{ppl}_y < y < L^\text{ppl}_y\); (2), \(y > L^\text{ppl}_y\), (3), \(y < -L^\text{ppl}_y\), and the corresponding solutions read for \(k_x \neq 0\):

\[
\begin{aligned}
\tilde{v}_x^1 &= (D_1 + D_2 y)e^{k_x y} + (D_3 + D_4 y)e^{-k_x y} \\
\tilde{v}_y^1 &= \left[\frac{i}{\eta_0 k_x} (2\eta + \eta_0) D_2 - iD_1 - iD_2 y\right] e^{k_x y} + \left[\frac{i}{\eta_0 k_x} (2\eta + \eta_0) D_4 + iD_3 + iD_4 y\right] e^{-k_x y} - \frac{C(k_x)}{\eta k_x^2} \\
\tilde{v}_x^2 &= (G_1 + G_2 y)e^{k_x y} + (G_3 + G_4 y)e^{-k_x y} \\
\tilde{v}_y^2 &= \left[\frac{i}{\eta_0 k_x} (2\eta + \eta_0) G_2 - iG_1 - iG_2 y\right] e^{k_x y} + \left[\frac{i}{\eta_0 k_x} (2\eta + \eta_0) G_4 + iG_3 + iG_4 y\right] e^{-k_x y} \\
\tilde{v}_x^3 &= (M_1 + M_2 y)e^{k_x y} + (M_3 + M_4 y)e^{-k_x y} \\
\tilde{v}_y^3 &= \left[\frac{i}{\eta_0 k_x} (2\eta + \eta_0) M_2 - iM_1 - iM_2 y\right] e^{k_x y} + \left[\frac{i}{\eta_0 k_x} (2\eta + \eta_0) M_4 + iM_3 + iM_4 y\right] e^{-k_x y}.
\end{aligned}
\]

(27)

where we have introduced 12 constants \(D_i, G_i, M_i\), that can be determined from boundary conditions. Using the boundary conditions of vanishing velocity at the boundaries \(y = -L\) and \(y = L\), together with the continuity of the velocity field \(v_x, v_y\) and of the stress components \(\sigma_{xy}\) and \(\sigma_{yy}\) at the interfaces \(y = L^\text{ppl}_y\) and \(y = -L^\text{ppl}_y\), we obtain a system of equations that allow to determine all the constants. For \(k_x = 0\), the solution reads

\[
\begin{aligned}
\tilde{v}_x &= 0 \\
\tilde{v}_y^1 &= -\sqrt{2 \pi \frac{f u^\text{ppl}_y}{2(\eta + \eta_0)}} y^2 + K_1 y + K_2 \\
\tilde{v}_y^2 &= W_1 y + W_2 \\
\tilde{v}_y^3 &= Z_1 y + Z_2.
\end{aligned}
\]

(28)

The 6 constants \(K_i, W_i\) and \(Z_i\) are determined from the boundary conditions imposing the continuity of the velocity at the ppl boundary, the continuity of the stress \(\sigma_{yy}\) at the ppl boundary, and zero \(v_y\) velocity at the domain boundary.

From the solution in Fourier space, the real-space solution is then obtained from

\[
\begin{aligned}
v_{x}^{\text{per}}(x, y) &= \frac{1}{\sqrt{2\pi}} \frac{\pi}{L} \sum_{k_x} e^{ik_x x} \tilde{v}_x(k_x, y), \\
v_{y}^{\text{per}}(x, y) &= \frac{1}{\sqrt{2\pi}} \frac{\pi}{L} \sum_{k_x} e^{ik_x x} \tilde{v}_y(k_x, y).
\end{aligned}
\]

(29)

where the sum is numerically performed in practice for \(0 < n < N_{\text{max}}\). In the results reported here, we chose \(N_{\text{max}} = 400\).
2.2.2. Determination of the remaining solution of the homogeneous equation. The remaining part of the solution \( \bar{v}_x = v_x - v_x^{\text{per}} \) and \( \bar{v}_y = v_y - v_y^{\text{per}} \) verifies the homogeneous equation

\[
\eta \Delta \bar{v}_x + \eta_b (\partial_x^2 \bar{v}_x + \partial_y \partial_x \bar{v}_y) = 0, \quad (30)
\]

\[
\eta \Delta \bar{v}_y + \eta_b (\partial_y^2 \bar{v}_y + \partial_y \partial_x \bar{v}_x) = 0. \quad (31)
\]

We find that these equations are satisfied using the following form for the undetermined functions in Eq. 22:

\[
\alpha_n(y) = (A_1^n + A_2^ny)e^{-\frac{ny}{L}} + (A_3^n + A_4^ny)e^{\frac{ny}{L}} \quad (32)
\]

\[
\gamma_n(y) = (a_n A_1^n + A_1^n + A_2^ny)e^{-\frac{ny}{L}} + (a_n A_3^n - A_3^n - A_4^ny)e^{\frac{ny}{L}} \quad (33)
\]

\[
\beta_n(x) = (a_n D_2^n + D_1^n + D_2^n x)e^{-\frac{nD_1}{L}} + (a_n D_3^n - D_3^n - D_4^n x)e^{\frac{nD_1}{L}} \quad (34)
\]

\[
\delta_n(x) = (D_1^n + D_2^n x)e^{-\frac{nD_1}{L}} + (D_3^n + D_4^n x)e^{\frac{nD_1}{L}} \quad (35)
\]

\[
\beta_0(x) = D_1^0 + D_2^0 x \quad (36)
\]

\[
\gamma_0(y) = A_1^0 + A_2^0 y \quad (37)
\]

with \( a_n = (2L(2\eta + \eta_b)/(n\pi \eta_b)). \)

We then impose the boundary conditions for \( \bar{v}_x, \bar{v}_y: \)

\[
\bar{v}_x(L, y) = \bar{v}_x^{R, \text{exp}}(y) = v_x^{R, \text{exp}}(y) - v_x^{\text{per}}(L, y) \quad (38)
\]

\[
\bar{v}_y(L, y) = \bar{v}_y^{R, \text{exp}}(y) = v_y^{R, \text{exp}}(y) - v_y^{\text{per}}(L, y) \quad (39)
\]

\[
\bar{v}_x(-L, y) = \bar{v}_x^{L, \text{exp}}(y) = v_x^{L, \text{exp}}(y) - v_x^{\text{per}}(-L, y) \quad (40)
\]

\[
\bar{v}_y(-L, y) = \bar{v}_y^{L, \text{exp}}(y) = v_y^{L, \text{exp}}(y) - v_y^{\text{per}}(-L, y) \quad (41)
\]

\[
\bar{v}_x(x, L) = \bar{v}_x^{U, \text{exp}}(x) = v_x^{U, \text{exp}}(x) \quad (42)
\]

\[
\bar{v}_y(x, L) = \bar{v}_y^{U, \text{exp}}(x) = v_y^{U, \text{exp}}(x) \quad (43)
\]

\[
\bar{v}_x(x, -L) = \bar{v}_x^{D, \text{exp}}(x) = v_x^{D, \text{exp}}(x) \quad (44)
\]

\[
\bar{v}_y(x, -L) = \bar{v}_y^{D, \text{exp}}(x) = v_y^{D, \text{exp}}(x). \quad (45)
\]
A set of equations is then obtained by projecting the boundary conditions in Fourier sine and cosine series for $n > 0$ (Einstein convention is used for repetition of indices):

\[
\begin{align*}
\bar{v}_m^{x,R} &= \left(D_1^n + (a_n + L)D_2^n\right)e^{-\frac{mx}{L}} + \left(-D_3^n + (a_n - L)D_4^n\right)e^{\frac{mx}{L}} \\
\bar{v}_m^{x,L} &= \left(D_1^n + (a_n - L)D_2^n\right)e^{\frac{mx}{L}} + \left(-D_3^n + (a_n + L)D_4^n\right)e^{-\frac{mx}{L}} \\
\bar{v}_m^{y,U} &= \left(A_1^n + (a_n + L)A_2^n\right)e^{-\frac{ny}{L}} + \left(-A_3^n + (a_n - L)A_4^n\right)e^{\frac{ny}{L}} \\
\bar{v}_m^{y,D} &= \left(A_1^n + (a_n - L)A_2^n\right)e^{\frac{ny}{L}} + \left(-A_3^n + (a_n + L)A_4^n\right)e^{-\frac{ny}{L}} \\
\bar{v}_m^{c} &= (a_nA_1^n + A_1^n)d_{nm} + A_2^n c_{nm} + \left(a_nA_4^n - A_4^n\right)f_{nm} - A_4^n g_{nm} + A_0^n b_m + \left[(D_1^n + D_2^n L)e^{-\frac{mx}{L}} + (D_3^n + D_4^n L)e^{\frac{mx}{L}}\right]c_{nm} \\
\bar{v}_m^{d} &= (-1)^n\left[(a_nA_2^n + A_2^n)d_{nm} + A_3^n e_{nm} + \left(a_nA_4^n - A_4^n\right)f_{nm} - A_4^n g_{nm}\right] + A_0^n b_m + \left[(D_1^n - D_2^n L)e^{\frac{mx}{L}} + (D_3^n - D_4^n L)e^{-\frac{mx}{L}}\right]c_{nm} \\
\bar{v}_m^{e} &= \left[(A_1^n + A_2^n L)e^{-\frac{ny}{L}} + (A_3^n + A_4^n L)e^{\frac{ny}{L}}\right]c_{nm} + \left[(D_1^n + a_nD_2^n)d_{nm} + D_2^n e_{nm} + (a_nD_3^n - D_4^n)\right]f_{nm} - (D_4^n g_{nm} + D_2^n b_m \\
\bar{v}_m^{f} &= \left[(A_1^n - A_2^n L)e^{\frac{ny}{L}} + (A_3^n - A_4^n L)e^{-\frac{ny}{L}}\right]c_{nm} + (-1)^n\left[(D_1^n + a_nD_2^n)d_{nm} + D_2^n e_{nm} + (a_nD_3^n - D_4^n)\right]f_{nm} - (D_4^n g_{nm} + D_2^n b_m,
\end{align*}
\]

and for $n = 0$:

\[
\begin{align*}
\bar{v}_0^{x,R} &= 2D_1^0 + 2D_2^0 L \\
\bar{v}_0^{x,L} &= 2D_1^0 - 2D_2^0 L \\
\bar{v}_0^{y,U} &= 2A_1^0 + 2A_2^0 L \\
\bar{v}_0^{y,D} &= 2A_1^0 - 2A_2^0 L,
\end{align*}
\]

and the 4 additional conditions for $\bar{v}_0^{x,U}$, $\bar{v}_0^{x,D}$, $\bar{v}_0^{y,R}$, $\bar{v}_0^{y,L}$ are approximately satisfied by continuity of the velocity at the corners of the rectangle. In the equations 46 to 53, we have introduced
the coefficients

\[ c_{nm} = \frac{2n[-1 + (-1)^{m+n}]}{\pi(n^2 - m^2)} \text{ for } m \neq n, c_{nn} = 0 \] (58)

\[ d_{nm} = \frac{2n[-e^{-\frac{nm\pi}{2}} + (-1)^m e^{\frac{nm\pi}{2}}]}{(n^2 + m^2)\pi} \] (59)

\[ e_{nm} = 2L\frac{[m^2(2 - n\pi) - n^2(2 + n\pi)]e^{-\frac{nm\pi}{2}} - (-1)^m[n^2(n\pi - 2) + m^2(2 + n\pi)]e^{\frac{nm\pi}{2}}}{[(n^2 + m^2)\pi]^2} \] (60)

\[ f_{nm} = \frac{2n[-(-1)^{(1+m)}e^{-\frac{nm\pi}{2}} + e^{\frac{nm\pi}{2}}]}{(m^2 + n^2)\pi} \] (61)

\[ g_{nm} = \frac{2L[n^2(n\pi - 2) + m^2(2 + n\pi)]e^{\frac{nm\pi}{2}} - (-1)^m[m^2(n\pi - 2) + n^2(2 + n\pi)]e^{-\frac{nm\pi}{2}}}{[(n^2 + m^2)\pi]^2} \] (62)

\[ b_n = \frac{4L[1 - (-1)^n]}{(n\pi)^2}, \] (63)

and the Fourier coefficients of the boundary conditions

\[ v_{nx}^{x,R/L} = \frac{1}{L} \int_{-L}^{L} dy \tilde{v}_{R/L,\exp}(y) \cos \frac{n\pi(y - L)}{2L} \] (64)

\[ v_{ny}^{x,R/L} = \frac{1}{L} \int_{-L}^{L} dy \tilde{v}_{R/L,\exp}(y) \cos \frac{n\pi(y - L)}{2L} \] (65)

\[ v_{nx}^{x,U/D} = \frac{1}{L} \int_{-L}^{L} dx \tilde{v}_{U/D,\exp}(x) \cos \frac{n\pi(x - L)}{2L} \] (66)

\[ v_{ny}^{x,U/D} = \frac{1}{L} \int_{-L}^{L} dx \tilde{v}_{U/D,\exp}(x) \cos \frac{n\pi(x - L)}{2L} \] (67)

To obtain the unknown constants \( A^n, D^n \) the system of linear equations 46 to 57 is solved numerically for \( 0 \leq n \leq N \). In practice we choose \( N = 100 \).

2.3. Comparison to two-dimensional experimental flow profiles. To compare theoretical flow profiles with experimentally measured velocity profiles, we assumed \( \eta/\eta_b = 1 \). We then adjusted the normalised force exerted by ppl cells, \( f/\eta_b \). We found that values of \( f/\eta_b \) reported in Table 1 accounted well for 2D experimental profiles obtained in wild-type and in slb mutants. In slb mutants, the force exerted by ppl was decreased by 20%, in accordance to the decrease in relative velocity between ppl cells and neurectoderm (Table 1).
As in section 1.2, we then can estimate the ratio $\eta_b/\xi$ from the value of $f/\eta_b$ and the value of $f/\xi$. A characteristic hydrodynamic length can be defined from this ratio by:

$$l_2 = \sqrt{\eta_b/\xi}.$$  \hfill (68)

Note that although we expect $l_1$ and $l_2$ to have similar order of magnitude, these two lengths are not equal in general as $l_2$ is defined from the 2D bulk viscosity of the fluid, while $l_1$ is defined from an effective one-dimensional viscosity. We report in Table 1 a value of $l_2 \simeq 133\mu m$.

To estimate a corresponding physical value of friction against the ppl cells, one would need an estimate of the neurectoderm viscosity $\eta_b$. Since we are not aware of direct measurements, we estimate it from $\eta_b \sim \eta_{3D} h$ with $\eta_{3D}$ the tissue 3D shear viscosity and $h$ the width of the neurectoderm. Using measurements of zebrafish deep-cell viscosities $\eta_{3D} \simeq 10^3 Pa.s$ [1] and $h \simeq 20\mu m$, we arrive at $\eta_b \simeq 2 \cdot 10^4 Pa.s.\mu m$ and $\xi \simeq 1 Pa.s.\mu m^{-1}$.

3. DETERMINATION OF GEOMETRICAL PARAMETERS

3.1. Determination of geometrical parameters. We discuss here the determination of geometrical parameters of the theoretical description. Geometrical parameters were measured at 70\% epiboly.

- The initial position of the leading edge of ppl cells relative the margin, along the dorsal midline of the embryo, was measured in each experiment. In addition, ppl cells velocity was acquired over time by cell tracking. By integrating the ppl cell AV velocity over one hour time frame, we calculated the overall displacement of ppl cells over this period. By using their initial position, we were able to pinpoint the location of the leading edge at the end of the considered time frame. From this average position we then extracted the value of $y^{ppl}$.
- The length $L^{E}$ was set according to experimental measurements of the distance from the animal pole to the EVL boundary measured at 70\% epiboly. Its value in each experiment is given in Table 2.
- The lengths $L_{x}^{ppl}$ and $L_{y}^{ppl}$ are obtained from measurements of the size of the domain covered by ppl cells and are reported in Table 2.
Table 2. Values of the geometrical parameters used for the plots in Fig.5. Lengths are reported at 70% epiboly.

- We also report in Table 2 the size of the measurement domain $L$ used for comparison between theory and experiment.

REFERENCES

[1] H. Morita, S. Grigolon, M. Bock, G. S. Krens, G. Salbreux & C.-P. Heisenberg, Developmental Cell 40, no. 4 (2017): 354-366.