Adhesion Functions in Cell Sorting by Mechanically Coupling the Cortices of Adhering Cells

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Differential cell adhesion and cortex tension are thought to drive cell sorting by controlling cell-cell contact formation. Here, we show that cell adhesion and cortex tension have different mechanical functions in controlling progenitor cell-cell contact formation and sorting during zebrafish gastrulation. Cortex tension controls cell-cell contact expansion by modulating interfacial tension at the contact. By contrast, adhesion has little direct function in contact expansion, but instead is needed to mechanically couple the cortices of adhering cells at their contacts, allowing cortex tension to control contact expansion. The coupling function of adhesion is mediated by E-cadherin and limited by the mechanical anchoring of E-cadherin to the cortex. Thus, cell adhesion provides the mechanical scaffold for cell cortex tension to drive cell sorting during gastrulation.

Cell adhesion and cortex tension are commonly assumed to function in cell sorting by controlling cell-cell contact formation (1–5), with adhesion increasing the contact size and cortex tension decreasing it (3–7). To clarify how cell adhesion and cortex tension function in progenitor cell-cell contact formation and sorting during zebrafish gastrulation, we first developed a mechanical description of two progenitor cells in contact, on the basis of previous models of cell-cell adhesion and sorting (4, 5). The cells are described as fluid objects with a viscoelastic cortex under tension and adhesive bonds maintaining the cell-cell contact. The size of the cell-cell contact is determined by the balance of forces at the contact boundary:

$$\cos(\theta) = \frac{\gamma_i}{2\gamma_{cm}} = \frac{2\gamma_{cc} - \omega}{2\gamma_{cm}} \quad (1)$$

where $\theta$ is the contact angle of the two adhering cells (Fig. 1A) (8, 9). The tension $\gamma_i$ at the cell-cell interface has a positive contribution arising from the cortex tension $\gamma_{cm}$ of the two cells at the contact and a negative contribution arising from adhesion (adhesion tension) of magnitude $\omega$. Outside of the contact, the tension at the cell-medium interface is equal to the cortex tension $\gamma_{cm}$ at this interface.

To characterize the mechanical parameters that control progenitor cell-cell contact formation, we first determined the ratio of the

Fig. 1. Surface tensions and contact shape in progenitor cell doublets. (A) Surface tensions controlling cell doublet shape at steady state. The contact angle $\theta$ results from the balance between the adhesion tension $\omega$ and the cortex tensions at the cell-medium $\gamma_{cm}$ and cell-cell interfaces $\gamma_{cc}$ (9). (B) Homotypic ectoderm (ecto), mesoderm (meso), and endoderm (endo) doublets during contact formation (movies S1 to S3). Scale bar, 10 $\mu$m. Measured contact angles $\theta$ are plotted over time as mean ± SEM binned over 100 s for ecto (red, n = 39), meso (green, n = 20), and endo (blue, n = 26) doublets (table S1). (C) Sketch of homotypic triplets before and after separation (9). Bulge volume $V_c$ is measured at the former cell-cell contact after separation and normalized to the cell body volume $V_i$ for ecto (n = 11), meso (n = 13), and endo (n = 5) triplets (movies S4 to S6 and table S2). (D) Tension ratios $\gamma_{cc}/\gamma_{cm}$ and $\omega/2\gamma_{cm}$ computed from homotypic triplet and doublet shapes plotted for ecto (n = 11), meso (n = 13), and endo (n = 5). Mean ± SEM (table S3) (9). (E) Sketch of myosin [Myl12.1-eGFP (enhanced green fluorescent protein)] localization in homotypic doublets of pTol2-β-actin:myl12.1-eGFP transgenic zebrafish. Measured mean fluorescence intensity at the cell-cell interface $I_{cc}$ is normalized to the mean intensities at the cell-medium interfaces $I_{cm}$ of both cells for ecto (n = 25), meso (n = 33), and endo (n = 17) doublets. Scale bar, 5 $\mu$m.
Fig. 2. Contact strength and structure in progenitor cell doublets. (A) Separation force $F_s$ of ectoderm (red; $n = 104/41/18$), mesoderm (green; $n = 30/16/11$), and endoderm (blue; $n = 44/23/16$) homotypic doublets is plotted as mean ± SEM at 1-, 5-, and 10-min contact times (movie S7 and table S4) (9). (B) Ratio of separation force $F_s$ to contact radius $R_c$ of ecto ($n = 37$), meso ($n = 15$), and endo ($n = 25$) homotypic doublets after 5-min contact time (table S5). (C) Sketch of a cell doublet showing cadherin (purple), actin (cyan), and myosin (orange). Optical sections through cell-cell contacts of homotypic doublets stained with antibodies against Cdh1, Ctnnb1, and Ctnna; phalloidin for F-actin; or expressing Myl12.1-mCherry (fig. S3). Scale bar, 5 µm.

Fig. 3. Cytoskeletal anchoring of Cdh2 in progenitor cell-cell contact formation. (A) Ectoderm homotypic doublets expressing Cdh2-eGFP plus LifeAct-RFP (red fluorescent protein) ($n = 15$), eGFP-Ctnnb1 ($n = 20$), or Ctnna-eGFP ($n = 30$) at 5 min contact time before and after separation (movies S8 to S11). Fluorescence intensity at the contact and contact size measured during the separation process (purple and/or cyan on diagram) are plotted as mean ± SEM relative to the value before the separation (table S6). Scale bars, 10 µm. (B and C) Ratio of separation force to contact radius $F_s/R_c$ (B) or contact angle $\theta$ (C) measurements of ectoderm (red) or endoderm (blue) homotypic doublets expressing Cdh2-eGFP ($n = 17/20$) or Cdh2Δcyto-eGFP ($n = 20/14$) at 5-min contact time. Values are plotted as mean ± SEM and normalized to Cdh2 expression level at the measured contact and at the contact of ecto doublets (movies S12 to S15 and tables S7 and S8).
interfacial tensions at the cell-cell and cell-medium interfaces by measuring the contact angle $\theta$ of freely adhering cell doublets ex vivo and using Eq. 1. Homotypic ectoderm doublets showed a larger contact angle $\theta$ and consequently a lower ratio of cell-cell to cell-medium interfacial tensions $\frac{\gamma_{cc}}{2\gamma_{cm}}$ than mesoderm and endoderm doublets (Fig. 1B).

To derive the relative contribution of the cortex tension $\gamma_c$ and the adhesion tension $\pi$ to the ratio of cell-cell to cell-medium interfacial tensions $\frac{2\gamma_{cc} - \pi}{2\gamma_{cm}}$, we measured the ratio of cortex tensions at the cell-cell to cell-medium interfaces $\frac{\gamma_{cc}}{\gamma_{cm}}$. To this end, we probed cortex tension at the cell-cell interface by separating contacting progenitor cells ex vivo, using a dual pipette aspiration assay (DPA) and analyzing resulting shape changes directly after separation (Fig. 1C). We observed a rapid increase in curvature in the region of the dissolved cell-cell contact, indicative of reduced cortex tension $\gamma_{cc}$ at this location compared to cortex tension $\gamma_{cm}$ at the cell-medium interface. To calculate the ratio of cortex tensions $\frac{\gamma_{cc}}{\gamma_{cm}}$ from the curvature change at the dissolved cell-cell contact in the different progenitor cell types, we modeled the cell-cell and cell-medium interfaces as elastic shells under tension, consistent with the short time scales of our experiment (9). Using this model, we found $\frac{\gamma_{cc}}{\gamma_{cm}}$ to be lower in contacting ectoderm cells compared to mesoderm and endoderm cells (Fig. 1D). Consistent with this, we observed that in progenitor cell doublets, non-muscle myosin-2 (Myl12.1) was reduced at cell-cell compared to the cell-medium interfaces, and that this reduction was more pronounced in ectoderm compared to mesoderm and endoderm doublets (Fig. 1E).

Having determined the ratio of tensions at the cell-cell and cell-medium interfaces, we computed the adhesion tension $\pi$ using Eq. 1. We found that for all three progenitor cell types, the magnitude of the adhesion tension $\pi$ was considerably smaller than the cortex tension $\gamma_{cc}$ at the cell-cell interface (Fig. 1D). This indicates that the cell-cell interfacial tension $\gamma_i$ is dominated by the cortex tension $\gamma_{cc}$ at this interface. It further suggests that the cell-cell contact angle $\theta$, and thus the contact size, is predominantly controlled by the ratio of cortex tensions $\frac{\gamma_{cc}}{\gamma_{cm}}$ between these interfaces and that adhesion tension $\pi$, contrary to previous suggestions (8), has only little function in contact expansion.

Although these findings argue against a critical function of adhesion tension in cell-cell contact expansion, formation of adhesive bonds is still essential to mechanically couple the contractile...
We found that ectoderm doublets exhibit higher pulling forces, previously implicated in control of mechanical resistance of adhesion bonds to cell-cell separation. Before separation, Cdh1, or to the cortical cytoskeleton limits their mechanical strength of cadherins across the cell-cell contact end, we first determined whether the binding extent, mesoderm and endoderm doublets (Fig. 2B and figs. S5 and S6). This suggests that proper cytoskeletal anchoring of cadherins is an essential factor determining the difference in separation force and cell-cell contact size between the progenitor cell types.

We next asked how far the observed effect of cadherin cytoskeletal anchoring on $F_c/R_c$ and $\theta$ influences progenitor cell sorting. Consistent with a critical function of cell-cell contact formation in cell sorting, replacing endogenous Cdh1 with either full-length or truncated Cdh2 in homotypic ectoderm or endoderm progenitor cell aggregates led to cells expressing Cdh2 being sorted into the middle, surrounded by cells expressing Cdh2Delta cyto (Fig. 4A). These marked similarities between the situations in cell doublets ex vivo and in vivo suggest that progenitor cell sort in vivo is driven by the same mechanisms of cell-cell contact formation as observed ex vivo.

The mechanical coupling function of cadherins at the cell-cell contact critically requires sufficient anchoring of cadherins to the cortical cytoskeleton. Considering that mechanical load on cadherin adhesion complexes has previously been suggested to modify its cytoskeletal anchoring strength (15, 16), it is intriguing to speculate that this anchoring strength in the different progenitor cell types is determined by the cortex tension of these cells pulling on the mechanosensitive adhesion complexes, thereby setting their anchoring strength.

References and Notes
9. Information on materials and methods is available on Science Online.

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Supplementary Materials
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Supplementary Text and Methods
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Movies S1 to S23
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cortices of the adhering cells at the contact and to support stresses normal to the adhesion zone arising from cortical tension, intracellular pressure, or external forces (10, 11). Notably, this mechanical coupling function of adhesion is distinct from the role adhesion plays in providing the adhesion tension $\alpha$, where it acts in the direction tangential to the contact zone, thereby expanding it. To characterize the function of mechanical coupling due to adhesion in progenitor cell-cell contact formation and sorting, we asked how its strength is controlled in the different progenitor cell types, and whether interfering with its strength affects progenitor cell-cell contact formation and cell sorting.

To determine the strength of mechanical coupling due to adhesion, we used the DPA assay to mechanically separate homotypic progenitor cell doublets ex vivo and measure the corresponding separation force $F_s$ (12). Considering that cell-cell separation was achieved rapidly ($<1$ s), our experiments can be described as the separation of two elastic solids, where the separation force $F_s$ depends on different factors, including the number and dissociation rate of adhesion bonds (9, 13). We found that ectoderm doublets exhibit higher $F_s$ at contact times varying from 1 to 10 min than mesoderm and endoderm doublets (Fig. 2A). This difference in $F_s$ between the progenitor cell types is not just a consequence of differences in cell-cell contact size, because normalizing $F_s$ by the contact radius ($F_s/R_c$) (9) still yielded higher values for ectoderm compared to mesoderm and endoderm doublets (Fig. 2B and fig. S1). Differences in adhesion molecule density at the contact area are also unlikely to account for the different $F_s$, as the expression levels of E-cadherin (Cdh1) and associated proteins, previously shown to mediate adhesion in all three progenitor cell types (1), did not correlate with the different $F_s$ in these cells (Fig. S2).

We next asked whether differences in the mechanical resistance of adhesion bonds to pulling forces, previously implicated in controlling $F_s$ (12), are responsible for the differences in $F_s$ between the progenitor cell types. To this end, we first determined whether the binding strength of cadherins across the cell-cell contact or to the cortical cytoskeleton limits their mechanical resistance by analyzing the segregation of adhesion complex components during cell-cell separation. Before separation, Cdh1, $\beta$-Catenin (Ctnmb1), $\alpha$-Catenin (Ctnna), and actin, but not Myl12.1, were found to accumulate in a dense ring-like structure at the margin of the cell-cell contact in ectoderm and, to a lesser extent, mesoderm and endoderm doublets (Fig. 2C and figs. S3 and S4). This suggests that higher $F_s$ values coincide with adhesion molecule clusters accumulating at the contact edge. Upon separation by means of the DPA, progenitor cells remained connected via long plasma membrane tethers, and N-cadherin (Cdh2; see below for using Cdh2 as a proxy for Cdh1) together with Ctnmb1, which directly binds to Cdh2 (10), accumulated in these tethers (Fig. 3A). By contrast, Ctnna, which directly or indirectly couples Ctnmb1 to the actin cytoskeleton (14, 15), did not colocalize with Cdh2 and Ctnmb1 in the tethers, but instead disassembled from the dissolving cell-cell contact together with actin (Fig. 3A). These findings indicate that cadherins dissociate from the cytoskeleton during progenitor cell separation, suggesting that cytoskeletal anchoring of cadherins limits the mechanical resistance of adhesion bonds to pulling forces.

To explore whether modifying cytoskeletal anchoring of cadherins, and thus the mechanical resistance of adhesion bonds to pulling forces, affects $F_s$ and cell-cell contact formation, we expressed either a full-length version of Cdh2 or a truncated version of Cdh2 that cannot bind to the cortical cytoskeleton (Cdh2Delta cyto) (12) in progenitor cells deprived of endogenous Cdh1 expression (cdh1 morphant cells). Expressing similar levels of Cdh2 in homotypic ectoderm and endoderm cell doublets changed neither their endogenous relative difference in $F_s$ nor their contact angle $\theta$. However, expressing Cdh2Delta cyto strongly reduced $F_s/R_c$ and $\theta$ to similar low levels in both cell types (Fig. 3, B and C, and figs. S5 and S6). This suggests that proper cytoskeletal anchoring of cadherins is an essential factor determining the difference in separation force and cell-cell contact size between the progenitor cell types.

We next asked how far the observed effect of cadherin cytoskeletal anchoring on $F_s/R_c$ and $\theta$ influences progenitor cell sorting. Consistent with a critical function of cell-cell contact formation in cell sorting, replacing endogenous Cdh1 with either full-length or truncated Cdh2 in homotypic ectoderm or endoderm progenitor cell aggregates led to cells expressing Cdh2 being sorted into the middle, surrounded by cells expressing Cdh2Delta cyto (Fig. 4A). These observations suggest that proper cytoskeletal anchoring of cadherins is essential for progenitor cell sorting.

To address how our observations of cell doublets ex vivo relate to the situation within the gastrulating embryo, we asked whether progenitor cells segregate in vivo by using the same cell-cell contact formation strategies as their counterparts ex vivo. Analysis of the cell cortex in germ layer progenitor cells in vivo showed that similar to what we observed ex vivo, cortical Myl12.1 was preferentially localized at cell-interstitial space/extracellular matrix interfaces (Fig. 4B). This suggests that cortex tension is reduced at cell-cell interfaces both ex vivo and in vivo. Moreover, analysis of progenitor cell-cell contacts in vivo revealed a ring-like localization of adhesion molecules reminiscent of the situation in cell doublets ex vivo (Fig. 4C). Finally, progenitor cell cells separating in vivo often formed membrane tethers at their dissolving contacts containing Cdh2 (Fig. 4D), similar to cell doublets ex vivo that were separated by means of the DPA. These marked similarities between the situations ex vivo and in vivo suggest that progenitor cell sorting in vivo is driven by the same mechanisms of cell-cell contact formation as observed ex vivo.

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