

# Adherens junction function and regulation during zebrafish gastrulation

Antonino Schepis<sup>1,\*†</sup> and W. James Nelson<sup>1,2</sup>

<sup>1</sup>Department of Biology; Stanford University; Stanford, CA USA; <sup>2</sup>Department of Molecular and Cellular Physiology; Stanford University; Stanford, CA USA

<sup>†</sup>Current affiliation: MRC Centre for Developmental Neurobiology; King's College London; London, UK

**T**he adherens junction (AJ) comprises multi-protein complexes required for cell-cell adhesion in embryonic development and adult tissue homeostasis. Mutations in key proteins and misregulation of AJ adhesive properties can lead to pathologies such as cancer. In recent years, the zebrafish has become an excellent model organism to integrate cell biology in the context of a multicellular organization. The combination of classical genetic approaches with new tools for live imaging and biophysical approaches has revealed new aspects of AJ biology, particularly during zebrafish gastrulation. These studies have resulted in progress in understanding the relationship between cell-cell adhesion, cell migration and plasma membrane blebbing.

opposing cells to form the adherens junction (AJ).<sup>2</sup> The cytoplasmic domain binds proteins that belong to the catenin family that includes p120-catenin,  $\beta$ -catenin and  $\alpha$ -catenin.<sup>3</sup> p120-catenin is necessary for the stabilization of the cadherin complex at the plasma membrane, and together with  $\beta$ -catenin associates with cadherins during their intracellular transport to the plasma membrane.<sup>4</sup>  $\beta$ -catenin binds  $\alpha$ -catenin, and  $\alpha$ -catenin mediates interactions with the actin cytoskeleton.<sup>5</sup> Recent studies have reevaluated the role of mammalian  $\alpha$ -catenin from a simple static molecular bridge between AJs and the actin cytoskeleton, to an active role in regulating actin cytoskeleton and membrane dynamics;<sup>6–9</sup> it is unknown whether zebrafish  $\alpha$ -catenin has properties similar to the mammalian isoform. Additional functions for  $\alpha$ -catenin may include mechanical transduction and cell polarization.<sup>10,11</sup> The catenin proteins are also involved in signal transduction pathways where they work as a sensor between the adhesive property of the plasma membrane and gene expression.<sup>12–14</sup>

In order to gain insights about the role and regulation of the AJs it is important to explore in vivo models. In this respect, *Danio rerio* (zebrafish) gastrulation has become an excellent model system.<sup>15</sup> Gastrulation is a critical stage of animal development at the end of which the embryo acquires the future body plan including the anterior-posterior and dorsal-ventral axes, and the separation of the three germ layers (ectoderm, mesoderm and endoderm).<sup>16,17</sup> Gastrulation involves cell migration, cell sorting and tissue remodeling all of which require very fine regulation of cell-cell adhesion.<sup>17,18</sup>

## Introduction

Cell-cell adhesion plays a critical role in many processes in embryonic development, adult homeostasis and diseases. Defining the molecular mechanisms involved in cell-cell adhesion is, therefore, critical to understanding many fundamental problems in tissue organization, dynamics and function. While there are many different cell-cell adhesion proteins, the cadherin family of cell-cell adhesion proteins is thought to play a central role in initiating cell-cell adhesion and controlling cellular dynamics and fate.<sup>1</sup> The cadherin family comprises trans-membrane proteins that share multiple copies of an external domain called the EC domain.<sup>1</sup> Classical cadherins have five EC domains that mediate trans-interactions with the extracellular domain of cadherins on

**Keywords:** adherens junction, gastrulation, zebrafish, cell-cell adhesion, cell migration, morphogenesis

Submitted: 04/03/12

Revised: 04/17/12; 04/23/12

Accepted: 05/01/12

<http://dx.doi.org/10.4161/cam.20583>

\*Correspondence to: Antonino Schepis;  
Email: [antonino.schepis@kcl.ac.uk](mailto:antonino.schepis@kcl.ac.uk)

Commentary to: Schepis A, Sepich D, Nelson WJ.  $\alpha$ E-catenin regulates cell-cell adhesion and membrane blebbing during zebrafish epiboly. *Development* 2012; 139:537–46; PMID:22190637; <http://dx.doi.org/10.1242/dev.073932>

Zebrafish gastrulation consists of four morphogenetic processes: epiboly, internalization, convergence and extension.<sup>19</sup> Epiboly is defined as the spreading of a one tissue over another that causes the thinning of the initial tissue.<sup>20</sup> Epiboly in zebrafish involves two processes: spreading of the most external cell layer (the enveloping layer, EVL) and, independently, spreading of deep cells over the yolk cell until the yolk is completely covered by the two cell layers.<sup>20</sup> Spreading of the EVL cell layer may be driven by the changing morphology of yolk syncytial layer (YSL).<sup>21,22</sup> The YSL is a cytosolic, yolk-free region of the yolk cell found immediately under the blastoderm and connected to the EVL through tight junctions.<sup>23,24</sup> The YSL may provide a pulling force on the EVL margin through an actin ring located just below the EVL margin.<sup>21</sup> The main process that drives deep cell epiboly is radial intercalation, which involves the movement of deep cells from deeper layers to most superficial layers (Fig. 1).<sup>25</sup> This causes thinning of the presumptive ectoderm and, as a consequence, the presumptive ectoderm spreads over the yolk.<sup>25</sup>

Internalization occurs when the mesoendoderm moves through the blastoderm beneath the prospective ectoderm.<sup>25,26</sup> Around mid-gastrulation, convergence and extension movements take place. Convergence narrows the tissue mediolaterally while extension elongates the embryo antero-posteriorly.<sup>19</sup> The different

subtypes of mesodermal precursor undergo different cell movements that contribute to the final convergence and extension.<sup>15</sup>

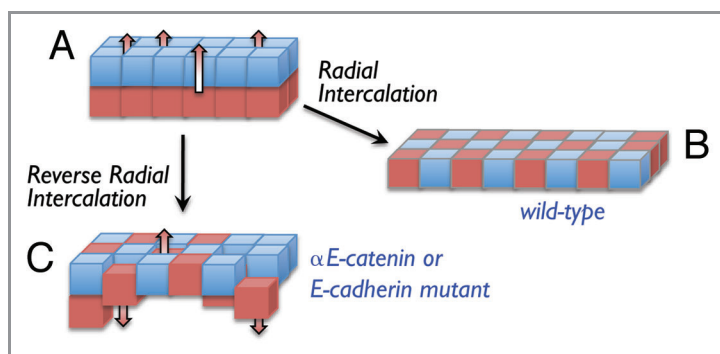
### Adherence Junction Function during Zebrafish Gastrulation

The role of E-cadherin in gastrulation has been examined in mutant embryos, and in morphant embryos depleted of the protein by morpholino (an antisense oligo used to block translation or splicing of the targeted mRNA). Strong mutant alleles and a high concentration of morpholino cause epiboly arrest or delay, and strong defects in gastrulation.<sup>27-29</sup> Epiboly arrest is due to defects in radial intercalation. E-cadherin mutant and morphant cells undergo radial intercalation but they are not able to establish stable cell-cell contacts with the upper layer cells, and move back in the lower layer (reverse radial intercalation or de-intercalation; Fig. 1).<sup>27,30</sup> The deep cells in the most external layer establish cell-cell contacts with the opposing basal membrane of the EVL cells.<sup>29</sup> In E-cadherin mutant/morphant embryos, the EVL/deep cells interactions are impaired, which might contribute to the overall defect in radial intercalation of the deep cells.<sup>29,30</sup>

A low concentration of morpholino is useful to dissect the role of E-cadherin in mesoderm migration. E-cadherin is required for the collective migration of the pre-chordal plate progenitor.<sup>26</sup> During their migration, the pre-chordal plate

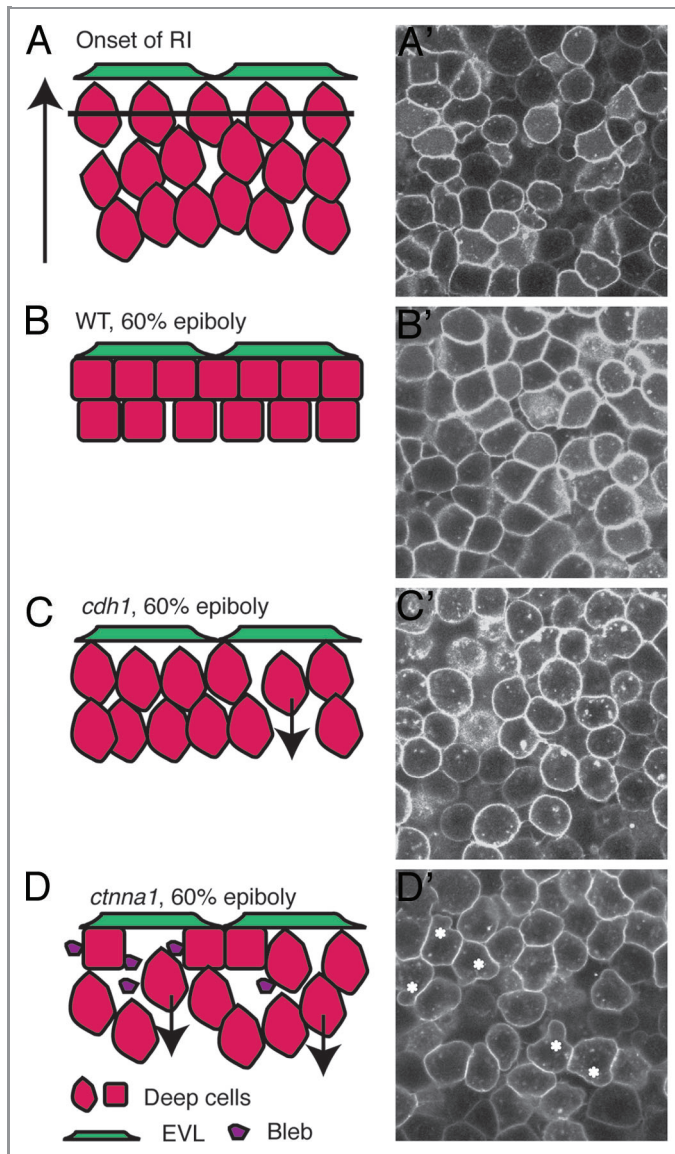
progenitors need to establish contact with the prospective ectoderm located over them and E-cadherin is required to mediate this interaction. Moreover, morphant cells fail to properly elongate and decrease their rate of migration.<sup>26</sup> The progenitors of the pre-chordal plate are surrounded by another subpopulation of mesodermal cells that have a lower level of E-cadherin.<sup>31</sup> Increasing the level of E-cadherin in the latter cells affects the correct cell migration of the pre-chordal plate progenitors indicating that differentially expression of E-cadherin may finely modulate the correct migration of mesodermal precursors as a whole tissue.<sup>31</sup> Finally, E-cadherin plays a role in coordinating the convergence and extension movement of the YSL nuclei with the movement of the mesodermal precursors.<sup>23,32</sup> N-cadherin is required for lateral mesoderm migration and a gradient of bone morphogenic protein (BMP) that is present in the gastrula might regulate N-cadherin mediated cell migration.<sup>33,34</sup>

Depletion of  $\alpha$ E-catenin by morpholino causes a delay in epiboly.<sup>35</sup> The delay is caused by defects in both radial intercalation and EVL epiboly.  $\alpha$ E-Catenin morphant cells, like E-cadherin morphant cells, undergo radial intercalation but move back into the lower layer (Fig. 1). In spite of similar defects in cell-cell adhesion, the structure of the deep cells layers appears different in the E-cadherin morphant and  $\alpha$ E-catenin morphant (Fig. 2). These differences could reflect different cell behaviors and the presence of E-cadherin in the  $\alpha$ E-catenin morphant cells. Additionally,  $\alpha$ E-catenin depleted cells exhibit protracted plasma membrane blebbing (see below for further discussion). The delay in EVL epiboly correlates with the failure of the EVL cells at the margin to properly elongate (changing in cell morphology that correlates with epiboly progression). The fact that  $\alpha$ E-catenin causes an epiboly defect in the EVL could be due to: (1)  $\alpha$ E-catenin plays some role in regulating the actin cytoskeleton similar to what described in tissue culture cells<sup>6</sup> or (2)  $\alpha$ E-catenin depletion affects the functionality of other adhesion complex such as tight junction.<sup>24</sup> This additional role of  $\alpha$ -catenin might occur in the EVL and YSL.



**Figure 1.** Radial intercalation. (A) Deep cells from the lower layer (red) move upwards and intercalate between the deep cells in the upper layer.<sup>25</sup> (B) Radial intercalation drives expansion of the upper layer and triggers the overall epiboly of the deep cell tissue.<sup>25</sup> (C) In  $\alpha$ E-catenin morphants and E-cadherin mutant/morphant embryos, the deep cells are able to undergo radial intercalation but some cells migrate back in the lower layer (revRI). The revRI affects the proper expansion of the upper layer, resulting in a delay or block in epiboly of the deep cell tissue.<sup>27,35</sup>

## Adherens Junction Regulation during Zebrafish Gastrulation



**Figure 2.** Tissue morphogenesis during radial intercalation. (A–D) Structure of the deep cell layers at the onset of epiboly (A) and at 60% epiboly in wild-type (B), and E-cadherin (C) and  $\alpha$ E-catenin morphants (D). (A) Deep cells are organized in multiple layers with weak cell-cell contacts and have a rounded morphology;<sup>57</sup> the deep cells of the deeper layer migrate toward the upper layer (the arrow indicates the direction of migration); the horizontal line defines the focal plane for the images shown in (A'–D') (see below). (B) At 60% epiboly in a wild-type background, deep cells stabilize cell-cell contacts and form one or two compact layer.<sup>25,27</sup> (C) At 60% epiboly in E-cadherin (*cdh1*) morphant/mutant embryos, the deep cells fail to stabilize cell-cell contacts and some cells undergo *revRI*<sup>27,35</sup> (direction of arrow). (D) At 60% epiboly in  $\alpha$ E-catenin (*ctnna1*) morphant embryos, the deep cells undergo *revRI* (direction of arrow) but also exhibit extensive membrane blebbing.<sup>35</sup> (A'–D') Confocal images of the most external deep layers below the EVL. The horizontal bar in (A) indicates the focal plane. Cells express a membrane bound GFP to highlight the plasma membrane, and images are shown in reverse contrast. (A') Wild-type external layer at the onset of epiboly; some deep cells are rounded and have not yet engaged in stable cell-cell contacts. (B') Wild-type external layer at 60% epiboly; the deep cell form a compacted layer of cells. (C') The external cell layer in an E-cadherin morphant at 60% epiboly; the deep cells are rounded and fail to form an external compacted layer of cells. (D') The external cell layer in an  $\alpha$ E-catenin morphant at 60% epiboly; the deep cells are more engaged in cell-cell contacts than the E-cadherin morphants, but they also fail to form a compacted layer of cells and have extensive plasma membrane blebbing (cells marked by an asterisk).

An example of E-cadherin regulation is the repression of gene expression by Snail during epithelial-to-mesenchyme transition (EMT).<sup>36–38</sup> Two studies highlight this type of regulation of cell-cell adhesion in zebrafish.<sup>31,39</sup> The first study focused on the role of the two zebrafish Snail isoforms, each of which regulates the repression of E-cadherin in different regions of the mesoderm.<sup>31</sup> The second study highlighted E-cadherin repression by a pathway activated by prostaglandin. Interfering with prostaglandin signaling by depleting its receptor results in broad overexpression of E-cadherin, which caused defects in gastrulation. The increased E-cadherin expression is due to snail degradation by the proteasome.<sup>39</sup>

An increasing number of reports highlight the importance of post-translational regulation of proteins during morphogenesis to enable the adhesive properties of cells to be changed rapidly.<sup>18</sup> Regulation of cadherin by intracellular trafficking is an efficient pathway to quickly change cell-cell adhesion.<sup>40</sup> For example, pre-chordal plate progenitor cells respond to a migratory cue from Wnt11<sup>26,41</sup> that in turn regulates the E-cadherin intracellular localization through Rab5.<sup>42</sup> Depletion of zebrafish prion protein also causes defects in radial intercalation similar to E-cadherin mutants due to the relocation of E-cadherin to intracellular Rab11-positive vesicles.<sup>43</sup>

Another way to influence cell-cell function is to functionally block the interaction between E-cadherin and the cytoplasmic catenin complex. In zebrafish, overexpression of  $G\alpha_{12/13}$  negatively regulates E-cadherin-dependent cell-cell adhesion, and in vitro inhibits binding of  $\beta$ -catenin to E-cadherin.<sup>44,45</sup> Moreover, Wnt signaling is necessary to stabilize  $\beta$ -catenin and in turn  $\beta$ -catenin stabilized E-cadherin at the plasma membrane during the onset of epiboly.<sup>46</sup> Finally influencing the plasma membrane domain to which the AJ is localized can influence cell polarity. For example, the adhesion molecule epCAM is necessary for the enrichment of E-cadherin at the basal membrane of the EVL.<sup>30</sup>



## Does the Adherens Junction Play a Role in Plasma Membrane Blebbing?

Blebbing is a type of plasma membrane protrusion driven by detachment of the plasma membrane from the cell cortex and cytoplasmic hydrostatic pressure, which cause the plasma membrane to transiently protrude outwards.<sup>47</sup> Plasma membrane blebbing, unlike lamellipodial formation, does not involve actin polymerization in the initial protruding phase. Once the membrane bleb is formed, ERM proteins (ezrin or moesin) are recruited to the plasma membrane to reconnect the membrane to the actin cytoskeleton in the cell cortex (membrane-to-cortex attachment, MCA).<sup>48-50</sup> Finally, activation of Rho signaling, myosin II activity and F-actin assembly cause the retraction of the membrane bleb.<sup>48,50,51</sup>

The zebrafish has been particularly useful in gaining new insight into the mechanisms underlying plasma membrane blebbing.<sup>49,51-53</sup> The regulation of blebbing plays a critical role in tuning the timing and direction of the migration of cells during gastrulation;<sup>49,53-55</sup> blebbing is also used by the primordial germ cells (PGC) to reach the future gonad.<sup>51,52</sup> The role of cell-cell adhesion during plasma membrane blebbing is poorly understood. Depletion of E-cadherin leads to defects in the migration of PGC, and a high concentration of morpholino impairs blebbing.<sup>51</sup> In contrast, increased E-cadherin levels, and hence cell-cell adhesion, correlates with increased plasma membrane blebbing.<sup>39</sup>

Depletion of  $\alpha$ E-catenin causes protracted membrane blebbing by deep cells undergoing radial intercalation.<sup>35</sup> Depletion of E-cadherin does not cause blebbing, whereas co-depletion of E-cadherin and  $\alpha$ E-catenin inhibits membrane blebbing. To understand the relationship between  $\alpha$ E-catenin and E-cadherin in plasma membrane blebbing, transplantation experiments have been performed in

which a group of cells are transferred between embryos of different genetic backgrounds to test if a phenotype is cell autonomous. Morphant cells depleted of  $\alpha$ E-catenin have protracted membrane blebbing in a wild-type background. In contrast, when these cells are transferred into an E-cadherin morphant embryo they do not exhibit membrane blebbing.<sup>35</sup> These results indicate that membrane blebbing requires E-cadherin-mediated regulation of cortical tension and, perhaps, cell-cell contact. Significantly, E-cadherin depletion also inhibited increased blebbing caused by ezrin depletion, which is required for MCA.<sup>35,49</sup>

Based on this result,  $\alpha$ E-catenin may have a role in regulating the dynamic connection between the plasma membrane and the cell cortex. During radial intercalation, deep cells migrate in the upper deep cell layer and establish cell-cell contact/adhesion with neighboring cells. The plasma membrane of deep cells is very dynamic and possible under tension. Under those conditions, membrane blebbing can still be triggered but with low frequency. Depletion of  $\alpha$ E-catenin, or ezrin, destabilizes the connection between the plasma membrane and the cell cortex and, therefore, relieves the suppression of membrane dynamics that is not matched by opposing forces at the cell cortex. Reducing the level of E-cadherin decreases plasma membrane activity/tension, therefore reducing the requirement for factors that normally suppress blebbing.

Two recent reports appear to support this model. The first report showed that deep cells mutant for *eomesodermin*, a transcription factor needed to trigger the onset of epiboly, displayed more blebbing than control cells.<sup>56</sup> Interestingly the increased blebbing in the *eomesodermin* mutant did not correlate with increased E-cadherin levels (tested by western blotting) although the morphology of the most external deep cell layer resembled cells with increased cell-cell adhesion. The

second report showed that mesodermal cells after internalization undergo a phase in which they bleb followed by a transition to a mesenchymal morphology and behavior.<sup>55</sup> Mesodermal cells mutant for *spade-tail* (a transcription factor) remain much longer in the blebbing phase than wild-type cells. Again, these cells do not show any increase of cadherins at the membrane, however they are more adhesive than control cells. These observations suggest that inactivation of MCA factors it is not matched by decreased adhesive property of involuting mesodermal cells. Interestingly, the adhesive properties are not dictated by the amount of cadherins present in the cell but most likely by post-translation modifications (see above). Taken together these reports indicate that blebbing might be regulated by tuning the interaction between the cell cortex and cell-cell adhesion complexes.

## Perspective

In past 10 years, many studies have highlighted the function and regulation of AJs during zebrafish gastrulation. These studies started with the characterization of mutants and morphants to dissect AJ functions in complex morphogenetic process. The advance of new methods allowed the study of cellular behaviors in parallel with studies of the biophysical properties of cells. These advances have led to a new appreciation of the role of cell-cell adhesion complexes and their link to the actin cytoskeleton in plasma membrane dynamics and cortical tension during complex developmental processes such as collective cell migration and cell sorting. In this respect, the study of zebrafish gastrulation has much to offer the cell biologist in the future.

## Acknowledgments

This work was supported by a grant from the NIH (GM35527) to W.J.N. and by a Marie Curie OIF (PIOF-GA-2009-236027) to A.S.

## References

- Hallebleib JM, Nelson WJ. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev* 2006; 20:3199-214; PMID:17158740; <http://dx.doi.org/10.1101/gad.1486806>
- Harrison OJ, Bahna F, Katsamba PS, Jin X, Brasch J, Vendome J, et al. Two-step adhesive binding by classical cadherins. *Nat Struct Mol Biol* 2010; 17:348-57; PMID:20190754; <http://dx.doi.org/10.1038/nsmb.1784>
- Shapiro L, Weis WI. Structure and biochemistry of cadherins and catenins. *Cold Spring Harb Perspect Biol* 2009; 1:a003053; PMID:20066110; <http://dx.doi.org/10.1101/cshperspect.a003053>
- Stemmler MP. Cadherins in development and cancer. *Mol Biosyst* 2008; 4:835-50; PMID:18633485; <http://dx.doi.org/10.1039/b719215k>
- Benjamin JM, Nelson WJ. Bench to bedside and back again: molecular mechanisms of alpha-catenin function and roles in tumorigenesis. *Semin Cancer Biol* 2008; 18:53-64; PMID:17945508; <http://dx.doi.org/10.1016/j.semcancer.2007.08.003>
- Benjamin JM, Kwiatkowski AV, Yang C, Korobova F, Pokutta S, Svitkina T, et al. AlphaE-catenin regulates actin dynamics independently of cadherin-mediated cell-cell adhesion. *J Cell Biol* 2010; 189:339-52; PMID:20404114; <http://dx.doi.org/10.1083/jcb.200910041>
- Borghini N, Lowndes M, Maruthamuthu V, Gardel ML, Nelson WJ. Regulation of cell motile behavior by crosstalk between cadherin- and integrin-mediated adhesions. *Proc Natl Acad Sci U S A* 2010; 107:13324-9; PMID:20566866; <http://dx.doi.org/10.1073/pnas.1002662107>
- Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI. Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* 2005; 123:903-15; PMID:16325583; <http://dx.doi.org/10.1016/j.cell.2005.09.021>
- Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ. Deconstructing the cadherin-catenin-actin complex. *Cell* 2005; 123:889-901; PMID:16325582; <http://dx.doi.org/10.1016/j.cell.2005.09.020>
- Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M. alpha-Catenin as a tension transducer that induces adherens junction development. *Nat Cell Biol* 2010; 12:533-42; PMID:20453849; <http://dx.doi.org/10.1038/ncb2055>
- Gladden AB, Hebert AM, Schneeberger EE, McClatchey AI. The NF2 tumor suppressor, Merlin, regulates epidermal development through the establishment of a junctional polarity complex. *Dev Cell* 2010; 19:727-39; PMID:21074722; <http://dx.doi.org/10.1016/j.devcel.2010.10.008>
- Schlegelmilch K, Mohseni M, Kirak O, Pruszk J, Rodriguez JR, Zhou D, et al. Yap1 acts downstream of alpha-catenin to control epidermal proliferation. *Cell* 2011; 144:782-95; PMID:21376238; <http://dx.doi.org/10.1016/j.cell.2011.02.031>
- Kim SW, Park JI, Spring CM, Sater AK, Ji H, Otchere AA, et al. Non-canonical Wnt signals are modulated by the Kaiso transcriptional repressor and p120-catenin. *Nat Cell Biol* 2004; 6:1212-20; PMID:15543138; <http://dx.doi.org/10.1038/ncb1191>
- Heuberger J, Birchmeier W. Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling. *Cold Spring Harb Perspect Biol* 2010; 2:a002915; PMID:20182623; <http://dx.doi.org/10.1101/cshperspect.a002915>
- Solnica-Krezel L. Gastrulation in zebrafish – all just about adhesion. *Curr Opin Genet Dev* 2006; 16:433-41; PMID:16797963; <http://dx.doi.org/10.1016/j.gde.2006.06.009>
- Gilbert SF. *Developmental biology*. Sunderland, Mass.: Sinauer Associates, 2010.
- Solnica-Krezel L. Conserved patterns of cell movements during vertebrate gastrulation. *Curr Biol* 2005; 15: R213-28; PMID:15797016; <http://dx.doi.org/10.1016/j.cub.2005.03.016>
- Hammerschmidt M, Wedlich D. Regulated adhesion as a driving force of gastrulation movements. *Development* 2008; 135:3625-41; PMID:18952908; <http://dx.doi.org/10.1242/dev.015701>
- Rohde LA, Heisenberg CP. Zebrafish gastrulation: cell movements, signals, and mechanisms. *Int Rev Cytol* 2007; 261:159-92; PMID:17560282; [http://dx.doi.org/10.1016/S0074-7696\(07\)61004-3](http://dx.doi.org/10.1016/S0074-7696(07)61004-3)
- Lepage SE, Bruce AEE. Zebrafish epiboly: mechanics and mechanisms. *Int J Dev Biol* 2010; 54:1213-28; PMID:20712002; <http://dx.doi.org/10.1387/ijdb.093028sl>
- Köppen M, Fernández BG, Carvalho L, Jacinto A, Heisenberg CP. Coordinated cell-shape changes control epithelial movement in zebrafish and *Drosophila*. *Development* 2006; 133:2671-81; PMID:16794032; <http://dx.doi.org/10.1242/dev.02439>
- Cheng JC, Miller AL, Webb SE. Organization and function of microfilaments during late epiboly in zebrafish embryos. *Dev Dyn* 2004; 231:313-23; PMID:15366008; <http://dx.doi.org/10.1002/dvdy.20144>
- Carvalho L, Heisenberg CP. The yolk syncytial layer in early zebrafish development. *Trends Cell Biol* 2010; 20:586-92; PMID:20674361; <http://dx.doi.org/10.1016/j.tcb.2010.06.009>
- Siddiqui M, Sheikh H, Tran C, Bruce AE. The tight junction component Claudin E is required for zebrafish epiboly. *Dev Dyn* 2010; 239:715-22; PMID:20014098; <http://dx.doi.org/10.1002/dvdy.22172>
- Warga RM, Kimmel CB. Cell movements during epiboly and gastrulation in zebrafish. *Development* 1990; 108:569-80; PMID:2387236
- Montero JA, Carvalho L, Wilsch-Bräuninger M, Kilian B, Mustafa C, Heisenberg CP. Shield formation at the onset of zebrafish gastrulation. *Development* 2005; 132:1187-98; PMID:15703282; <http://dx.doi.org/10.1242/dev.01667>
- Kane DA, McFarland KN, Warga RM. Mutations in half baked/E-cadherin block cell behaviors that are necessary for teleost epiboly. *Development* 2005; 132:1105-16; PMID:15689372; <http://dx.doi.org/10.1242/dev.01668>
- Babb SG, Marrs JA. E-cadherin regulates cell movements and tissue formation in early zebrafish embryos. *Dev Dyn* 2004; 230:263-77; PMID:15162505; <http://dx.doi.org/10.1002/dvdy.20057>
- Shimizu T, Yabe T, Muraoka O, Yonemura S, Aramaki S, Hatta K, et al. E-cadherin is required for gastrulation cell movements in zebrafish. *Mech Dev* 2005; 122:747-63; PMID:15905076; <http://dx.doi.org/10.1016/j.mod.2005.03.008>
- Slanchev K, Carney TJ, Stemmler MP, Koschorz B, Amsterdam A, Schwarz H, et al. The epithelial cell adhesion molecule EpcAM is required for epithelial morphogenesis and integrity during zebrafish epiboly and skin development. *PLoS Genet* 2009; 5:e1000563; PMID:19609345; <http://dx.doi.org/10.1371/journal.pgen.1000563>
- Blanco MJ, Barrallo-Gimeno A, Acloque H, Reyes AE, Tada M, Allende ML, et al. Snail1a and Snail1b cooperate in the anterior migration of the axial mesendoderm in the zebrafish embryo. *Development* 2007; 134:4073-81; PMID:17965052; <http://dx.doi.org/10.1242/dev.006858>
- Carvalho L, Stühmer J, Bois JS, Kalaidzidis Y, Lecaudey V, Heisenberg CP. Control of convergent yolk syncytial layer nuclear movement in zebrafish. *Development* 2009; 136:1305-15; PMID:19279138; <http://dx.doi.org/10.1242/dev.026922>
- Warga RM, Kane DA. A role for N-cadherin in mesodermal morphogenesis during gastrulation. *Dev Biol* 2007; 310:211-25; PMID:17826762; <http://dx.doi.org/10.1016/j.ydbio.2007.06.023>
- von der Hardt S, Bakkers J, Inbal A, Carvalho L, Solnica-Krezel L, Heisenberg CP, et al. The Bmp gradient of the zebrafish gastrula guides migrating lateral cells by regulating cell-cell adhesion. *Curr Biol* 2007; 17:475-87; PMID:17331724; <http://dx.doi.org/10.1016/j.cub.2007.02.013>
- Schepis A, Sepich D, Nelson WJ. alphaE-catenin regulates cell-cell adhesion and membrane blebbing during zebrafish epiboly. *Development* 2012; 139:537-46; PMID:22190637; <http://dx.doi.org/10.1242/dev.073932>
- Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2000; 2:76-83; PMID:10655586; <http://dx.doi.org/10.1038/35000025>
- Batlle E, Sancho E, Francí C, Domínguez D, Monfar M, Baulida J, et al. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* 2000; 2:84-9; PMID:10655587; <http://dx.doi.org/10.1038/35000034>
- Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; 139:871-90; PMID:19945376; <http://dx.doi.org/10.1016/j.cell.2009.11.007>
- Speirs CK, Jernigan KK, Kim SH, Cha YI, Lin F, Sepich DS, et al. Prostaglandin Gbetagamma signaling stimulates gastrulation movements by limiting cell adhesion through Snail1a stabilization. *Development* 2010; 137:1327-37; PMID:20332150; <http://dx.doi.org/10.1242/dev.045971>
- Ulrich F, Heisenberg CP. Trafficking and cell migration. *Traffic* 2009; 10:811-8; PMID:19490534; <http://dx.doi.org/10.1111/j.1600-0854.2009.00929.x>
- Heisenberg CP, Tada M, Rauch GJ, Saúde L, Concha ML, Geisler R, et al. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 2000; 405:76-81; PMID:10811221; <http://dx.doi.org/10.1038/35011068>
- Ulrich F, Krieg M, Schötz EM, Link V, Castanon I, Schnabel V, et al. Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. *Dev Cell* 2005; 9:555-64; PMID:16198297; <http://dx.doi.org/10.1016/j.devcel.2005.08.011>
- Málaga-Trillo E, Solís GP, Schrock Y, Geiss C, Luncz L, Thomanetz V, et al. Regulation of embryonic cell adhesion by the prion protein. *PLoS Biol* 2009; 7:e55; PMID:19278297; <http://dx.doi.org/10.1371/journal.pbio.1000055>
- Lin F, Chen S, Sepich DS, Panizzi JR, Clendenon SG, Marrs JA, et al. Galpha12/13 regulate epiboly by inhibiting E-cadherin activity and modulating the actin cytoskeleton. *J Cell Biol* 2009; 184:909-21; PMID:19307601; <http://dx.doi.org/10.1083/jcb.200805148>
- Meigs TE, Fields TA, McKee DD, Casey PJ. Interaction of Galpha 12 and Galpha 13 with the cytoplasmic domain of cadherin provides a mechanism for beta-catenin release. *Proc Natl Acad Sci U S A* 2001; 98: 519-24; PMID:11136230; <http://dx.doi.org/10.1073/pnas.021350998>
- Cadwalader EL, Condic ML, Yost HJ. 2-O-sulfotransferase regulates Wnt signaling, cell adhesion and cell cycle during zebrafish epiboly. *Development* 2012; 139:1296-305; PMID:22357927; <http://dx.doi.org/10.1242/dev.078238>
- Charras G, Paluch E. Blebs lead the way: how to migrate without lamellipodia. *Nat Rev Mol Cell Biol* 2008; 9:730-6; PMID:18628785; <http://dx.doi.org/10.1038/nrm2453>

48. Charras GT, Hu CK, Coughlin M, Mitchison TJ. Reassembly of contractile actin cortex in cell blebs. *J Cell Biol* 2006; 175:477-90; PMID:17088428; <http://dx.doi.org/10.1083/jcb.200602085>
49. Diz-Muñoz A, Krieg M, Bergert M, Ibarlucea-Benitez I, Muller DJ, Paluch E, et al. Control of directed cell migration in vivo by membrane-to-cortex attachment. *PLoS Biol* 2010; 8:e1000544; PMID:21151339; <http://dx.doi.org/10.1371/journal.pbio.1000544>
50. Sahai E, Marshall CJ. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat Cell Biol* 2003; 5:711-9; PMID:12844144; <http://dx.doi.org/10.1038/ncb1019>
51. Kardash E, Reichman-Fried M, Maitre JL, Boldajipour B, Pampusheva E, Messerschmidt EM, et al. A role for Rho GTPases and cell-cell adhesion in single-cell motility in vivo. *Nat Cell Biol* 2010; 12:47-53; sup pp 1-11.
52. Blaser H, Reichman-Fried M, Castanon I, Dumstrei K, Marlow FL, Kawakami K, et al. Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. *Dev Cell* 2006; 11:613-27; PMID:17084355; <http://dx.doi.org/10.1016/j.devcel.2006.09.023>
53. Weiser DC, Row RH, Kimelman D. Rho-regulated myosin phosphatase establishes the level of protrusive activity required for cell movements during zebrafish gastrulation. *Development* 2009; 136:2375-84; PMID:19515695; <http://dx.doi.org/10.1242/dev.034892>
54. Weiser DC, Pyati UJ, Kimelman D. Gravin regulates mesodermal cell behavior changes required for axis elongation during zebrafish gastrulation. *Genes Dev* 2007; 21:1559-71; PMID:17575056; <http://dx.doi.org/10.1101/gad.1535007>
55. Row RH, Maitre JL, Martin BL, Stockinger P, Heisenberg CP, Kimelman D. Completion of the epithelial to mesenchymal transition in zebrafish mesoderm requires Spadetail. *Dev Biol* 2011; 354:102-10; PMID:21463614; <http://dx.doi.org/10.1016/j.ydbio.2011.03.025>
56. Du S, Draper BW, Mione M, Moens CB, Bruce A. Differential regulation of epiboly initiation and progression by zebrafish Eomesodermin A. *Dev Biol* 2012; 362:11-23; PMID:22142964; <http://dx.doi.org/10.1016/j.ydbio.2011.10.036>
57. Concha ML, Adams RJ. Oriented cell divisions and cellular morphogenesis in the zebrafish gastrula and neurula: a time-lapse analysis. *Development* 1998; 125:983-94; PMID:9463345

© 2012 Landes Bioscience.

Do not distribute.