2. Morphogenetic cellular rearrangements during early zebrafish development

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Shortly after fertilization the vertebrate embryo starts an intensive cycle of cell division that often do not imply growth in size. This process originates a mass of cells known as blastoderm, where all the cells named blastomeres are identical to their neighbors and at least morphologically, the whole embryo does not give any clue of organization within the three axes of the space. Morphogenetic movements during vertebrate gastrulation guide to the organization of a trilaminar embryo from this initial mass of blastodermal cells. We know much about vertebrate gastrulation thanks to the use of animal models with ex-uterus development including frog or fish embryos. In fact zebrafish embryos have been revealed as a powerful tool to understand vertebrate gastrulation due to the embryo accessibility and the easiness in application of optical and genetic technology for investigation. In these organisms ectoderm, mesoderm and endoderm are formed after the coordination of basically three different processes occurring during vertebrate gastrulation: epiboly, cell internalization and convergence and extension movements. In concert with cellular rearrangements cell determination mechanisms are operating and each progenitor is brought to its appropriate position within the embryo accomplishing the establishment of the body plan in the gastrula.

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Cleavage

Zebrafish eggs consist on a rounded cell of around 500µm diameter made out of yolk and cytoplasm. The newly fertilized egg present a small thickening free of yolk named ooplasm that contain the maternal pronucleus at the animal pole, while the rest of the ooplasm is intermingled with yolk granules thorough the rest of the egg [1]. Preparation of the egg for development requires the separation of organelles and maternal components (i.e. mRNAs or proteins) from the yolk. Rapidly in a mechanism known as ooplasmic segregation cytoplasm separates from the yolk being preferentially accumulated at the prospective animal pole of the embryo [2] to form the blastodisc. Separation of ooplasm from vitelloplasm usually is favored by activation of the egg upon sperm penetration. The cortex of normal eggs contains a meshwork of F-actin based microfilaments associated with the plasma membrane, whose contractility is totally required for ooplasmic segregation to occur [3, 4]. Striking work has been performed in zebrafish embryos by monitoring Ca\textsuperscript{2+} accumulation within the cells. Sperm penetration into the oocyte cytoplasm causes free calcium transients that move from the forming blastodisc into the peripheral cortex of the animal hemisphere correlating with ooplasmic segregation [5, 6]. It has been proposed that these elevated transient of free calcium modulate F-actin microfilaments contractility squeezing the non-yolky cytoplasm. Additionally it is known that microtubules efficiently transport foreign microinjected particles as well as cytoplasmic determinants including maternal mRNA [7-10]. Thus, the egg has at this point a central bulk of yolk surrounded by non-yolky cytoplasm (yolk cytoplasmic layer) preferentially accumulated at the animal pole of the embryo (see Figure 1).

Shortly after fertilization embryonic cells begin to divide in a developmental process known as cleavage (Figure 1). This process is characterized by a series of cell divisions without an increase in cell mass controlled by mechanisms still to be characterized. Zebrafish embryos posses a meroblastic cleavage where divisions are incomplete during the first rounds, thus the big yolk cell remains indivisible and the blastomeres located on top of the big yolky cell undergo cleavage. In zebrafish cleavage proceed symmetrically with a periodicity of around 15 minutes during the first divisions where cell cycle of the blastomeres consists on mitosis and a short interphase [11]. The division pattern can be predicted only for the first set of synchronic division. Thus, the first cleavage furrow (2 blastomeres) is followed by a second perpendicular one (4 blastomeres). Afterwards, three rounds of division take place in the daughter cells where the cleavage furrow align on the same orientation and parallel respect to the first one (8-16-32 blastomeres).
Morphogenetic cellular rearrangements during early zebrafish development

Figure 1. Ooplasmic segregation and cleavage. Shortly after laid, the zebrafish egg accumulates most of the non-yolky cytoplasm at one edge of the oocyte in a mechanism known as ooplamic segregation. After ooplasmic segregation the zebrafish egg consists on a big mass of yolk located on the vegetal pole, with most of the non-yolky cytoplasm located on top, the animal pole. After fertilization meroblastic cleavage begin displaying synchronized cell division for almost 10th cycles in the cell at the animal pole. Yolk cell do not divide but keep cytoplasmic contact with most vegetal blastomeres of the blastoderm.

Next, equatorial divisions undergo (64 blastomeres), but at this point they are much difficult to predict into the blastocist. During the first 3 rounds of division all blastomeres remain communicated with the yolk cell. Staging of the embryo at this early period is based on the number of cells [11, 12].

Ca²⁺ release from internal stores within the cell seems to be involved in modulation of the early events of development. Ca²⁺ accumulation at the forming cytokinetic furrow is totally necessary for cell division to occur and predict the division plane within the blastomeres during cleavage [13-16]. As for ooplasmic segregation, intracellular Ca²⁺ appears to be modulating the F-actin based cytoskeleton controlling the contractile machinery and vesicle trafficking facilitating cell division and providing a source of cell membrane for daughter cells after cytokinesis [17].

In addition it is known that at these early stages of cleavage the molecular mechanisms that are controlling embryo dorsalization are already functioning [18, 19]. It is thought that around the third round of cleavage dorsal specification has been already activated. Thus, dorsal determinants that are initially located at the vegetal pole of the yolk are lately transported animalwards by a special microtubule array. In this regard, ablation of
vegetal regions of the yolk cell causes alterations in the dorsal organization of the embryo when performed during early cleavage stages and not later [7, 20-22].

Concerning dorsal specification, in an embryo as young as at two cell stage there is an asymmetric distribution in the pattern of activation of the MAP Kinase P38 within the blastomeres. This asymmetric activation seems to be induced by the same mechanisms that dorsal specification but it is related with the maintenance of the proper and synchronic cleavage in the prospective dorsal blastomeres with respect to the others. Blocking P38 activation does not cause lost of dorsal fate acquisition but dramatically alters and impairs cytokinesis in cells of the dorsal region. In this regard it has been proposed that synchronic cleavage is an advance acquired in vertebrates, which requires being stimulated. Thus invertebrates usually display asynchronic cleavage and asymmetric P38 activation would keep cells in the dorsal region dividing in the same way than at ventral positions [23].

Along the cleavage period the blastodisc is a mass of non-motile blastomeres on top of a big yolk cell [24, 25]. However around the 512 cells stage, which corresponds to the 10\textsuperscript{th} cell division cycle, a mechanism known as midblastula transition (MBT) takes place and the three major cell lineages of the early embryo start to develop. In general at this point cells slow down the cell cycle and activate their transcriptional machinery [24]. The outer layer of cells behave distinctly from the rest, thus they lose synchronicity of the cell cycle and dramatically slow down cell divisions and acquire pseudoepithelial characters forming a monostratified layer. In this layer, cells are rich in tight junctions and a posses a specialized cortical actin cytoskeleton. This tissue is known as the enveloping layer (EVL) that will become the periderm, an extraembryonic protective covering that is eliminated during late development.

By 3 hours after fertilization, when the embryo is composed of around 1000 cells (1k-cell stage), cells of the blastoderm located at most vegetal positions (marginal blastomeres) that have remained with cytoplasmic communication with the yolk cell, collapse into the yolk cell forming a multinucleate layer (yolk syncytial layer; YSL) in the non-yolky cytoplasm underlaying the blastoderm [26]. Firstly the YSL form a narrow ring along the edge of the blastoderm called external YSL (E-YSL) but rapidly it spreads bellow the blastoderm organizing a complete internal yolk syncytial layer (I-YSL). Nuclei here will maintain cariokinesis only for two to three more cycles and will undergo morphogenetic movements [12]. Between the EVL and the YSL, which are firmly bounded by tight junction along the confluence border, the third lineage of cells form the deep cell layer (DEL) that will later originates during gastrulation all three embryonic germ layers:
morphogenetic cellular rearrangements during early zebrafish development. In these cells the general efficiency of synchrony decreases.

Basically overall morphological changes within the egg provide the clues to establish some transitional stages after MBT and before epiboly. Thus 1k cell stage is followed by high blastula stage and shortly after that the big yolky cell and the overlying blastoderm have reorganized its overall appearance close to a sphere in the so called sphere stage. Around one hour after MBT, following sphere stage, doming of the yolk cell initiates epiboly, a mechanism by which the blastoderm spreads vegetally over the yolk cell increasing its surface area by decreasing its thickness. Morphologic characteristics of the different stages of zebrafish embryonic development have been carefully described by Kimmel et al., 1995 [27].

**Epiboly**

As mentioned, initiation of epiboly characterizes the dome stage of zebrafish development, a zebrafish stage in which blastodermal cells become increasingly motile. All three early lineages, EVL, YSL, and DEL, participate in this morphogenetic process [28]. The consequence of epiboly movements is that blastoderm overgrows the yolk cell surface, but importantly, accompanied by the EVL and the YSL (see Figure 2). Doming of the yolk helps in cell mixing during epiboly and a mechanism known as radial intercalation is one of the most important pushing forces. This process consists on cells from deeper regions moving outwardly to intercalate between more superficial cells. Attempts to establish cell fates on blastoderm cells have failed until shortly before gastrulation as epibolizing morphogenetic movements cause unpredictable cell mixing [29, 30]. However, probabilistic fields of fate have been established [31, 32].

Blastoderm epiboly and, in general, gastrulation movements seems to be partially independent of YSL movements. Epibolic expansion of the YSL is critically dependent of a microtubules array in the yolk cell. Two different arrays of microtubules exist in the cortical cytoplasm, thus the anuclear yolk cytoplasmic layer (YCL) possesses microtubules aligned in the direction of epiboly, extending toward the vegetal pole, and the YSL display the organization of intercrossing interphase or mitotic microtubules. The oriented microtubule array is visible in the yolk cell within the embryo as early as at two cell stage [7]. As epiboly progresses the YCL array of microtubules reorganize to the YSL configuration. Importantly, external disruption of microtubules using UV light or nocodazole considerably alters YSL epiboly but only partially inhibits epiboly in the EVL and DEL [33, 34]. During epiboly it seems that YSL is promoting the stabilization and organization of
Figure 2. Epiboly. Shortly after midblastula transition the doming of the yolk into the blastoderm causes the initiation of epiboly. Epiboly consists on the covering of the yolk cell by the blastoderm, which spread on its surface. Radial intercalation is a morphodynamic process based on cell movements that cause blasnomeres of the most vegetal layers of the blastoderm to intercalate between the most animal layers of cells. This movement cause the lost of thickness of the blastoderm as it moves vegetalward “engulfing” the yolk cell. Schematic drawings in this figure illustrate the mechanism of radial intercalation representing a portion of the deep cell layer (DEL) under the enveloping layer (EVL).

the microtubules in the yolk cell. Thus the YSL produces pregnelonone from cholesterol through the expression and activation of the esteroidogenic enzyme cholesterol side-chain cleavage enzyme, P450scc (Cyp11a1), what stimulates microtubule stabilization [35].

Massive endocytosis occurs in the external YSL in the proximity of the region where EVL is firmly bounded to the YSL via tight junctions. This area is the invaded by I-YSL what seems to favor EVL movement. However there is also an important mechanism that seems to modulate EVL cell morphology and epiboly. Around 30% epiboly F-actin starts to accumulate at the YSL at the proximity but vegetally to the EVL anchorage area. Actin accumulation is modulated by phosphorilated myosin light chain 2 that organizes a contractile actin ring in this region of the YSL [36]. The MAP kinase 4 Misshapen, which is required for epiboly and gastrulation movements, is modulating the formation of this actin/myosin2 ring. Alterations in the organization of this actin/myosin2 ring formation cause epiboly abrogation affecting exclusively to the EVL, although this alteration secondarily alters deep cell epiboly. All these findings have lead to a model in which local constriction mediated by
the actin ring guide the “engulfment” of the yolk cell by the EVL. Intriguingly it seems to be an evolutionary conserved mechanism as this process based on an actin ring extraordinarily resembles what happens in dorsal closure during Drosophila development [36].

There are however more evidences that indicate that although coordinated and somehow interrelated, DEL epiboly is independent of EVL and YSL. Thus there are several mutants with arrested epiboly in the DEL that present normal EVL and YSL movements [37, 38]. One of the most characteristic epiboly mutants is defective on the gene that encodes E-cadherin [39]. E-cadherin is a cell-membrane protein involved in homotypic cell adhesion. E-cadherin mRNA is provided maternally and it has been shown to be necessary for blastomeres adhesion during cleavage, and at later stages for aspects of morphogenesis during gastrulation and epiboly [40, 41]. Indeed in the developing epiblast E-cadherin is expressed displaying a radial gradient with blastodermal cells on the external layers having higher expression that in the deeper layers. This is translated in differences in cell morphologies being able to distinguish two different layers of cells within the blastoderm with a progressively softer border in between as radial intercalation progresses. Thus, cells of the external layer with high expression of E-cadherin are organized as epithelial-like tissue with polyhedral shapes as they spread below the EVL. However cells of the internal layer with lower expression of E-cadherin are organized more loosely associated and with much rounder shapes. In the E-cadherin mutant half baked (hab) these two differently organized cell layers are not found [39]. During normal epiboly radial intercalations favor that cells from the internal layer intercalate and integrate within the external layer. However in hab mutant these cell intercalations are reduced in number and much slower than in wt, in fact the cells often are not maintained in the external layer of the epiblast and delaminate [39, 42].

During epiboly dorsal specification can be easily detected morphologically and molecularly in the embryo prior to gastrulation. Morphological criteria have been proposed to distinguish the dorsal side of the early embryo. Thus there are reports supporting that when looking at the circumferential boundary separating the blastoderm and the yolk cell, exactly at the point where the dorsal territory is determined, the angle of the separation boundary tends to be equal to 180°. However in the opposite region these surfaces have a different curvature, always lower than 180° [43].

There is also a cluster of deep cells below the EVL and at the margin of the blastoderm named as forerunner cells that are detectable by fluorescent labeling at late blastula and can predict the site of shield formation at the dorsal side of the embryo. At gastrulating stages when the shield forms, these cells are displaced to the leading edge of the blastoderm epiboly movements.
They are the precursors of the Kuppfer's Vesicle and extraordinarily relevant in the organization of the left/right axis of the embryo. It is known that forerunner cells organize a Ca$^{2+}$ based flux responsible of bilateral asymmetry in gene expression [44].

Finally, early during development, microtubular mechanisms maternally orchestrated within the egg guides the accumulation at the prospective dorsal side of the embryo of high β catenin activity and enrichment in the nodal related protein Squint [45, 46]. This occurs previously to shield formation, which is the equivalent structure of the dorsal lip of the blastopore in amphibians.

**Gastrulation**

Zebrafish gastrulation consists on a stereotypical set of cellular movements of the blastodermal cells leading to the formation of the three germ layers: ectoderm, mesoderm and endoderm. Gastrulation movements organize the embryo in two transitory layers of cells that will latter generate the trilaminar embryo. Thus, as epiboly reach 50% of the yolk cell, the morphogenetic process of gastrulation starts and the blastoderm begins to lose cells underneath organizing a second cell layer below. At this point upper layer of epibolizing cells is named epiblast, precursor of the ectoderm, while the cellular layer that is being organized bellow the epiblast is known as hypoblast and contains the mesendodermal precursors (that will latter segregate into the mesodermal and endodermal layer). Gastrulation imply three different type of cellular movements which are epiboly as it continues and contributes to germ layer formation, cell internalization where cells delaminate from the epiblast to organize the hypoblast beneath; and convergence and extension movement consisting on mediolateral intercalations that cause accumulation of cells at the dorsal side of the embryo and at the same time contribute to its elongation in the anterior-posterior axis. Lately its being accepted than extension is more complex than only mediolateral intercalation and it seems that once internalized cells can also migrate toward the animal pole in a directed manner as they converge and intercalate. Thus, we could talk also about a fourth movement that undergo concomitantly to convergence and extension. In zebrafish embryos, at contrary to what happen in Xenopus, all movements seem to be quite independent from each other [47, 48].

**Germ ring formation and internalization**

As mentioned, when blastoderm has covered around 50% of the yolk cell epibolic migration toward the vegetal pole slow down for a short period of
time. This phenomenon responds to the initiation of internalization, what means the initiation of germ layer formation. Cells at the leading edge of blastoderm stop to move vegetalward and change their direction to move firstly inward toward the yolk cell and rapidly start anterior migration toward the animal pole. Cell internalization begins at the dorsal side of the embryo and expands laterally all along the border of the blastoderm [49]. This causes cell accumulation at the marginal region of the blastoderm originating a thickened region just at this level known as the germ ring (see Figure. 3). First cells to internalize at the dorsal side of the embryo will form the embryonic “shield” (see Figure 3). The shield is easily recognizable within the dorsal side of the blastoderm margin as a group of mesendodermal cells that associate after internalization. First cells to internalize constitute the prechordal plate that will become the most anterior mesoderm and endoderm, namely the precursors of the endodermal pharynx and mesodermal derivatives such as the hatching gland [50].

Figure 3. Germ ring formation and anterior migration. Around 50% epiboly, epibolic movements transitorily slow down and cells of the leading edge of the blastoderm margin accumulate generating a thickening all along such margin named the germ ring. Shortly after germ ring formation cells become motile and migrate animalward organizing the hypoblast layer below the epiblast layer. The hypoblast contains the mesendodermal precursor while the epiblast contains the ectodermal precursors. Schematic drawings in this figure illustrate these processes representing a lateral view on a section focusing on the shield forming region.
In amniota embryos cell internalization from the epiblast during gastrulation is mediated by epithelial mesenchyme transition (EMT). EMT involves a mechanism sharing multiple cellular and molecular aspects of tumor metastasis and basically consists on loss of epithelial characteristics of a tissue, including tight cellular junctions, to form a disperse tissue containing motile cells able to spread out and invade new regions [51]. Cell internalization in zebrafish occurs by single cell delamination at the germ ring [42, 52]. However, EMT has not been observed during gastrulation in anamniote embryos [42, 53]. In zebrafish neither the epiblast nor the hypoblast cells exhibit clear epithelial features and both cell types are highly motile showing dramatic changes in their cellular morphology, as they are rich in cellular protrusions [54]. It seems that internalizing mesendodermal progenitor cells may change their general state of adhesiveness, which allows them then to segregate from the epiblast and take on a more mesenchymal appearance [42, 53]. Recently it has been shown that not only differential cell adhesiveness but also distinct tensile forces at the cell cortex, which are modulated by the actomyosin contractile cytoskeleton, are crucial in the segregation in the different tissue progenitors during gastrulation [55]. In conjunction with cell adhesion it seems that segregation of two different tissues depend of the different tensile forces established at the cell cortex at the level of cell to cell or cell to medium interface [55].

Once internalized mesendodermal precursors migrate anteriorly underneath the epibolizing epiblast toward the animal pole (see figure 3). Shortly after internalization mesendodermal cells move upward seeking the epiblast inner surface that they will use as substrate for migration [42]. The differential adhesive properties and cell cortex tensile forces generate a border between both tissues that, in spite of being in contact, avoid cell population intermixing [55]. At this point opposite migration are taking place, thus while the epiblast continues its migration vegetalwards, hypoblast migrates underneath toward the animal pole of the embryo. Axial mesendodermal cells move as a tightly packed group of cells giving rise to axial structures such as the prechordal plate and notochord, while paraxial mesendodermal progenitor cells migrate as more loosely associated mesenchymal cells [25, 56].

Nodal related signals including Cyclops (Cyc) and Squint (Sqt), their cofactor the EGF-CFC protein One eyed pinhead (Oep) and the nodal antagonist Lefty are responsible of mesendodermal cell specification during gastrulation [57, 58]. Maternal-zygotic oep (MZoep) embryos lack response to nodal signals and are not able to induce mesendoderm from the epiblast lacking hypoblast formation. However it seems that even in absence of mesendodermal induction these cells are able to internalize. Thus, on one
hand, when a MZoep mutant cell is transplanted into the blastoderm margin of a wild type host, such cell is able to internalize with the forming hypoblast. Interestingly, this cell is not able to move anteriorly with its wild type partners and move vegetalwards with the vegetal movement of the margin, not contributing to the hypoblast formation. On the other hand if a WT cell is implanted into the blastoderm of a MZoep embryo, it internalizes independently of the absence of partner cells, being able to differentiate into mesendoderm and move toward the animal pole of the embryo [52]. In sum all these suggest that mesendodermal induction is independent of cell internalization but is required for anterior migration.

**Anterior migration of the mesendodermal cells**

A group of transcription factors regulating anterior migration in the hypoblast is the SNAIL family of transcription factors. Snail1a and Snail1b are expressed in the migrating hypoblast cells in an overlapping manner except at the level of the precordial plate. Loss of function of any of them causes dramatic shortening of the anterior-posterior axis what seem not to be due to cell specifications alterations. However cell migration appears to be dramatically disturbed even in the precordial plate, which is affected non-cell autonomously. It seems that in absence of these transcription factors cells may express cadherin1 (E-cadherin), a feature that does not allow cells to migrate in a dissociated manner. Indeed in zebrafish embryos has been reported that E-cadhein is required for proper anterior cell migration of axial mesendoderm [41]. In wild type embryos absence of Snail1a or Snail1b expression would allow the high level of expression of E-caherin, which is needed for proper anterior migration of this cells, however alteration in the surrounding hypoblast would cause the morphogenetic disturbances due to abnormal cell migration in a non-cell autonomous manner of the precordial plate mesendoderm [53].

STAT3 is a member of the group of signal transducers and activator of transcription that seems to promote cell motility in the mesendodermal precursors of the precordial plate in a cell autonomous manner and in the neighbouring cells in a non-cell autonomous manner. STAT3 has been shown to upregulate the Zn2C transporter LIV1 and it has been proposed that LIV1 promotes hypoblast cell migration. Phenotypes caused by knocking down either STAT3 or LIV1 reflect deficient anterior migration with shortened anterior-posterior axis [59].

Polarized anterior migration toward the animal pole is mediated by the polarized formation of cellular protrusions at the leading edge of mesendodermal cells [54, 60]. Phosphoinositide 3 kinase (PI3K) pathway has...
been involved in this process [60]. PI3K is an enzyme involved in phospholipids metabolism regulating the phosphorylation of Phosphatidylinositol (Ptdlns) on the third carbon of the inositol ring. These types of lipids phosphorylated at the position 3 can be recognized at the membrane surface by enzymes containing the plekstrin homology domain (PH), being collected and activated at the cell surface where they activate their effectors. In zebrafish it has been shown that class IA PI3K is activated at the leading edge of the migrating mesendodermal cells stimulating the production of Ptdlns-3,4,5-P and causing in consequence the recruitment of the kinase PKB(Akt) that posses the PH domain. PKB is activated at the cell surface of such leading edge where it regulates the polarized polymerization of the actin complex required to organize cellular protrusions. Absence of PI3K activity causes loss of cellular protrusions generating much less efficient motility in these rounded mesendodermal cells, which now move much slower with the consequent morphogenetic alterations in the embryo. Platelet derived growth factor (PDGF) has been proposed as the upstream extracellular signal that would activate PI3K upon binding and activation of its tyrosine kinase receptor [60].

**Convergence and extension movements**

After internalization and as mesendodermal cells start to migrate animalwards, both ectodermal and mesendodermal progenitors undergo Convergence and Extension (CE; see figure 4). In CE cells move towards the dorsal side by mediolateral cell intercalations that cause mediolateral narrowing and anterior-posterior extension of the developing body axis at the dorsal side of the embryo. Molecular mechanisms controlling CE movements in zebrafish are being elucidated on the basis of the analysis of multiple mutants that display typical phenotypes of deficient CE movements, including shorter anterior-posterior axis and wider embryonic structures at the end of gastrulation. Importantly many of these mutants affect the non-canonical WNT signaling pathway, which is independent of β catenin and shares homologue proteins with the Planar Cell Polarity (PCP) pathway that guide epithelial polarization during drosophila wing development [48]. Mutants deficient in non canonical WNT pathway as pipetail (ppt)/wnt5a [61], knypek (kny)/glipican4/6 [62]; trilobite (tri)/strabismus (stbm)/Van gohl-like 2 (Vangl2) [63]; or silberblick (slb)/wnt11 [64] or studies on factors involved in this pathway as Prickle (PK1) [65] or frizzled 7 (Fz7) [66] have demonstrated that this pathway is responsible of the polarized migration and mediolateral intercalation than take place during CE. Interestingly it has been shown that radial intercalation, in a similar fashion that occur in the epiblast
during epiboly, is also involved in convergence and extension movements especially at the level of the paraxial mesoderm [67]. Importantly, the non canonical WNT pathway it is also involved in regulating the polarized behaviour of these cells during such movements [67].

Much advance has been done in the analysis of the slb mutant, which shows one of the most dramatic phenotype in CE [64]. By analyzing this mutant at cellular level it has been demonstrated that Wnt11 modulates proper cell morphology and protrusive activity during CE to achieve proper mediolateral intercalations and extension [54]. Presumably this effect on the control of proper cell shape in order to perform appropriate cell movements is modulated through the activation of small GTPases of the rho family including Rac, Cdc42 or RhoA [68-70]. These proteins are key effectors in modulating cytoskeletal rearrangements and organizing fillopodia, lamellipodia and pseudopodia, which are basic for proper cell migration. However via another small GTPase known as Rab5, largely involved in modulation of the endocytic pathway, Wnt11 regulates the adhesiveness of

![Figure 4](image_url)

**Figure 4.** Convergence and extension. As the germ ring forms and anterior migration starts, the cells of the epiblast and hypoblast germ layers begin to converge towards the dorsal side of the gastrula and extend along the forming anterior–posterior body axis. Convergence movements first become apparent in the germ ring by a local thickening at the prospective dorsal side becoming the zebrafish embryonic organizer named ‘shield’. While converging, mesendodermal and ectodermal progenitors undergo medio–lateral cell intercalations, leading to a thinning of the forming body axis along its medio–lateral extent and consequently elongating along the anterior–posterior axis. Schematic drawings in this figure illustrate the mechanism of medio-lateral cell intercalation and subsequent antero-posterior elongation.
mesendodermal cells to control CE movements. Slb mutant cells present weakened adhesive properties in comparison with WT cells, thus both adhesion to extracellular matrix molecules and adhesiveness to the neighboring cells is altered [71, 72]. Wnt11 modulates cellular cohesion by controlling E-cadherin activity via endocytosis. This is modulated through Rab5c that strikingly can rescue the slb phenotype when overexpressed in mutant embryos [72]. It has been them proposed that Wnt11 through Rab5c promotes the ability of mesendodermal cells to dynamically assemble and disassemble E-cadherin-based cell-cell junctions required for effective cell cohesion and migration during gastrulation. Additionally it has also been shown that Wnt11 modulates cell contact persistence by interacting at the cell membrane with its receptor Frizzled 7 (Fz7) and the atypical cadherin Flamingo [66]. In consistence with all these results morphant embryos for E-cadherin, Flamingo (fmi) or Rab5c have been shown to display CE defects during gastrulation [41, 66, 73].

Acknowledgements

We are thankful to Prof. Juan Hurlé and Dr. Jesús Chimal-Monroy for critically reading earlier versions of this manuscript. JAM work is supported by the Ramón y Cajal program and grant BFU2005-04393/BMC from the Spanish Sciences and Innovation Ministry. NT-P work is supported by IDICAN and I Plan Regional de I+D+i de Cantabria.

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