

## The role of *Sib/Wnt11* at the onset of zebrafish gastrulation

During vertebrate gastrulation, coordinated cellular rearrangements lead to the formation of a gastrula containing three germ layers, ectoderm, mesoderm and endoderm. In this study, researchers analyzed the role of a *slb/wnt11* mutant in regulating cell movements and morphology during zebrafish gastrulation.

Researchers used confocal timelapse imaging to follow the movement of epiblast (ectoderm, red – rhodamine) and hypoblast (prechordal plate, green – GFP) cells in wild type and *slb* mutant embryos at the onset of gastrulation. Z-stacks were obtained with 1.5  $\mu\text{m}$  steps over a total vertical distance of 66  $\mu\text{m}$ . For each experiment, 12-20 image stacks were acquired in 4-minute time intervals. The z-stacks were then volume rendered in 3D using Volocity® Visualization. Using Volocity, they showed that most hypoblast and epiblast cells in wild type embryos move parallel to the surface of the yolk sac in a straight path towards the animal pole and germ ring, respectively (Figure 1 A-E). In contrast, hypoblast cells of *slb* mutants showed less directed movements (Figure 1 F-J). This is also shown in the supplementary movies, made using Volocity.

To quantify the differences in cell movements in wild type and *slb* mutant embryos, Volocity Quantitation was used to calculate the average persistence (the shortest distance between the start and end points of the movement divided by the total distance moved, as a percentage) as an expression of how ‘straight’ or ‘direct’ a cell moves, and the velocity (in  $\mu\text{m}/\text{min}^{-1}$ ) of these movements in 3D. To do this, Volocity was used to measure the positions of the geometric centre of the cell (the centroid) in 3D at 4-minute intervals. The results were plotted as single dots in the track diagrams in Figure 1, E and J. Both the average velocity and persistence of cell movements within the hypoblast (but not the epiblast) were reduced significantly in *slb* mutants.

Researchers went on to show that the *slb* hypoblast cells also exhibited defects in the orientation of their cellular processes. They concluded that *slb/wnt11*-mediated orientation of cellular processes plays a key role in facilitating and stabilizing movements of hypoblast cells in the germ ring. This study provided the first direct evidence that the *slb/wnt11* signaling pathway is involved in regulating process orientation and migratory cell movements at early stages of zebrafish gastrulation.

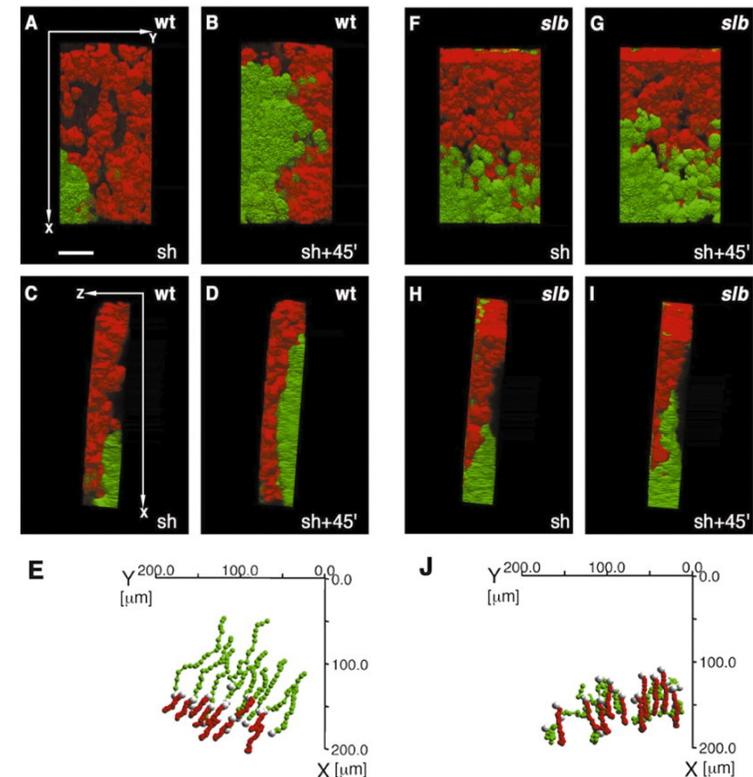


Figure 1: Prechordal plate precursor cells (green) and overlying epiblast cells (red) in wild type (A-D) and *slb* (F-I) *gscGFP* embryos at shield stage (sh) and 45 minutes later (sh+45'). Ventral (A,B,F,G) and lateral (C,D,H,I) views are shown. Scale bar in A: 50  $\mu\text{m}$ . Parts E and J show track diagrams to show the movements of prechordal plate precursor cells (green) and epiblast cells (red) in wild type (E) and *slb* (J) *gscGFP* embryos along the x (anterior-posterior) and y (medio-lateral) axes. Each line represents the track of one cell, with the first timepoint shown in white.