Self-organised symmetry breaking in zebrafish reveals feedback from morphogenesis to pattern formation

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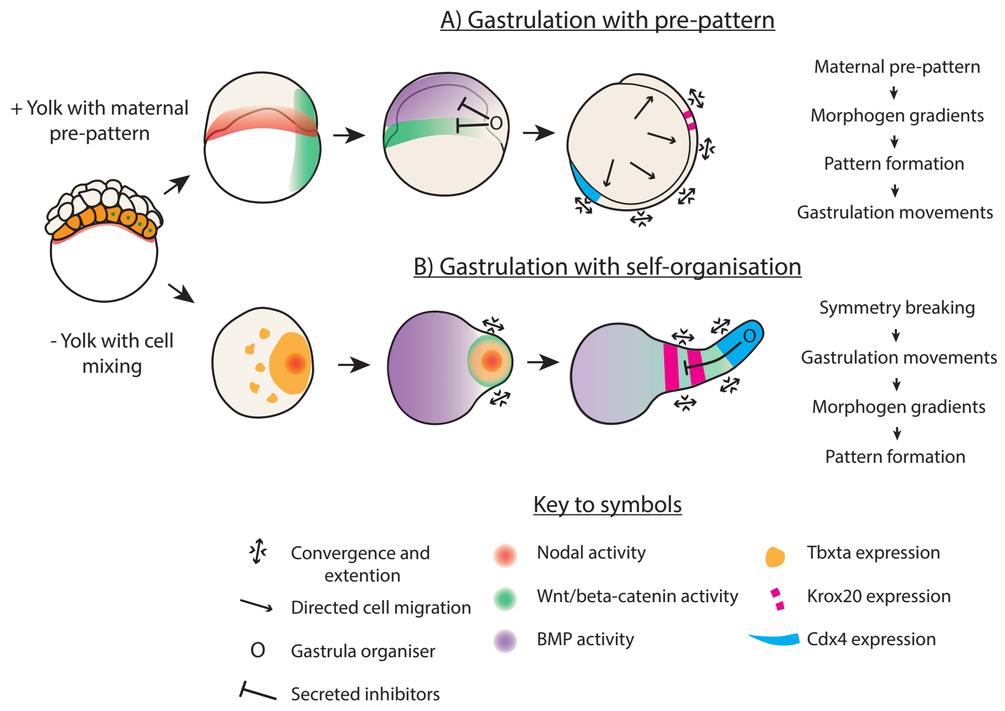
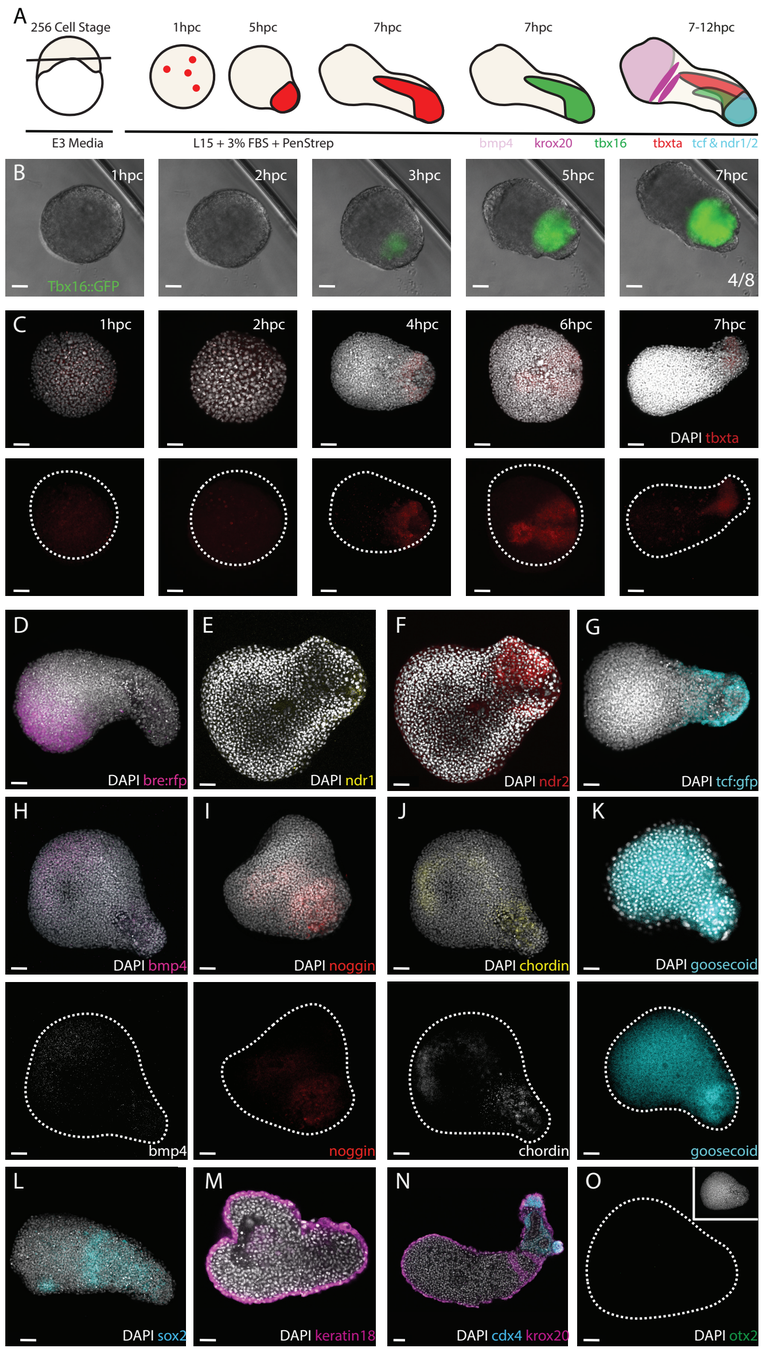
A fundamental question in developmental biology is how the early embryo breaks initial symmetry to establish the spatial coordinate system later important for the organisation of the embryonic body plan. In zebrafish, this is thought to depend on the inheritance of maternal mRNAs, cortical rotation to generate a dorsal pole of beta-catenin activity and the release of Nodal signals from the yolk syncytial layer (YSL). Recent work aggregating mouse embryonic stem cells has shown that symmetry breaking can occur in the absence of extra-embryonic tissue. To test whether this is also true in zebrafish, we separated embryonic cells from the yolk and allowed them to develop as aggregates. These aggregates break symmetry autonomously to form elongated structures with an anterior-posterior pattern. Extensive cell mixing shows that any pre-existing asymmetry is lost prior to the breaking morphological symmetry, revealing that the maternal pre-pattern is not strictly required for early embryo patterning. Following early signalling events after isolation of embryonic cells reveals that a pole of Nodal activity precedes and is required for elongation. The blocking of PCP-dependent convergence and extension movements disrupts the establishment of opposing poles of BMP and Wnt/TCF activity and the patterning of anterior-posterior neural tissue. These results lead us to suggest that convergence and extension plays a causal role in the establishment of morphogen gradients and pattern formation during zebrafish gastrulation.



Symmetry breaking and axial patterning can occur in the absence of extra-embryonic signals.



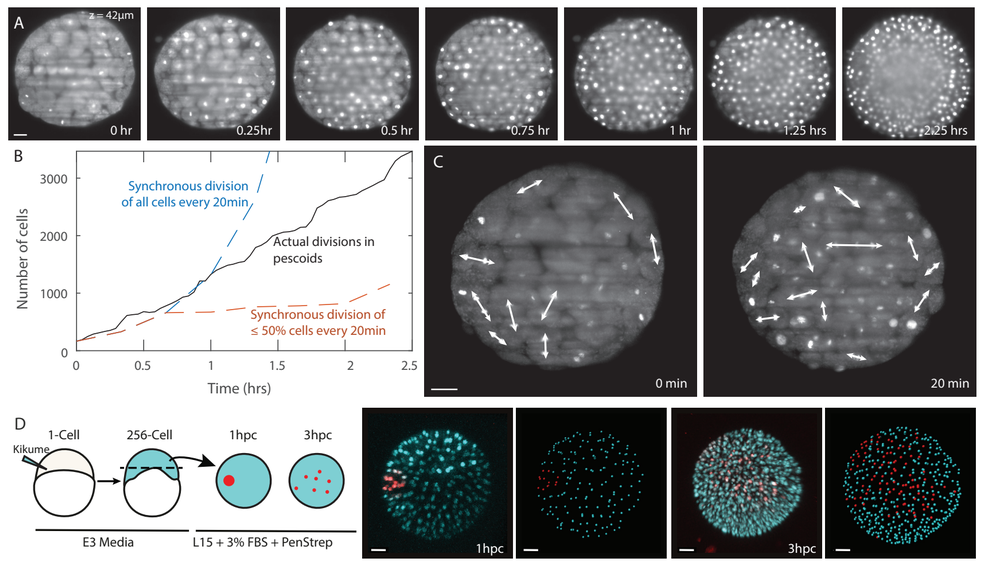
A-C.
Explants at the 246 cell stage can self-organise a tbxta and tbx16 poissitive pole of expression as they elongate

D-G.
By 7 hours post culture (hpc) they have a high region of BMP activity directly opposite to a Nodal and Wnt/beta catenin activity domain in the elongated end

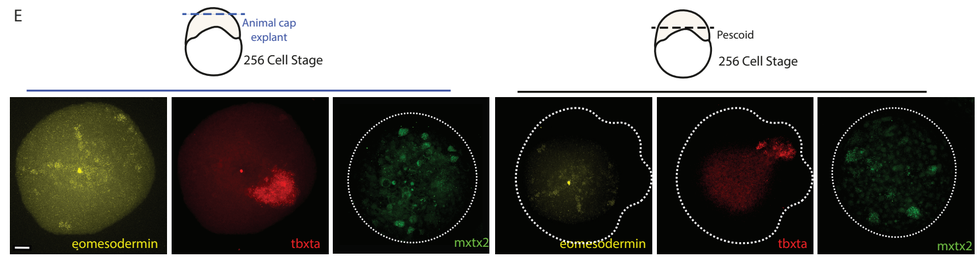
H-K.
The elongated end also expresses markers of the embryonic organiser such as goosecoid, and the BMP inhibitors noggin and chordin

L-O.
By 10hpc they have an anterior-posterior patterned neural tissue with two hindbrain krox20 stripes and cdx4 expression in the post posterior tip

Spatial pre-patterns are lost due to extensive cell divisions and cell mixing.

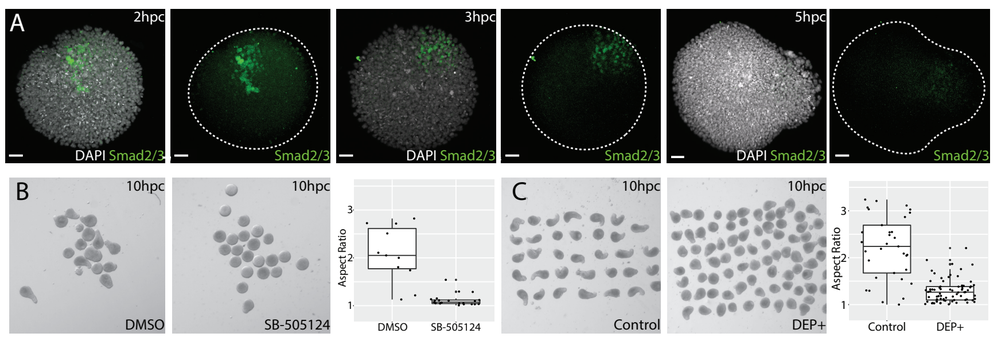


A-C. Early cell divisions act to mix cells suggesting this alone might be sufficient to remove any pre-pattern within the explants.
D. Labels on one side of the explant rapidly disperse within 3hpc.

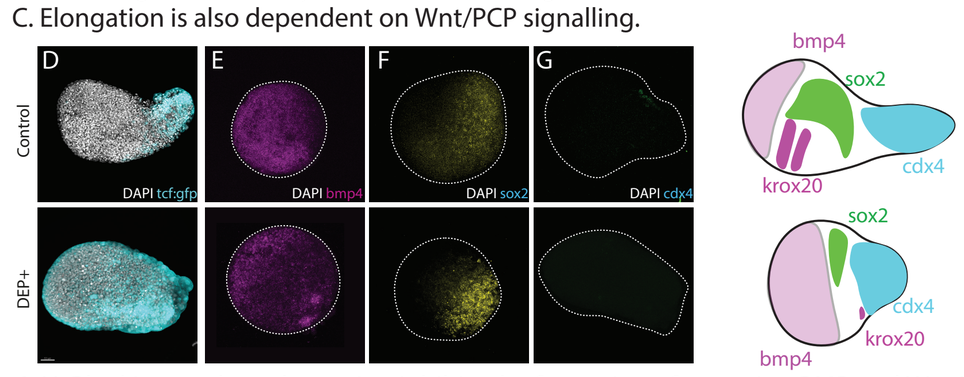


E. Cell mixing prior to the 256 cell stages results in maternally inherited mRNAs being localised to both animal caps and complete embryonic explants.

Convergence and extension is upstream of anterior-posterior patterning.

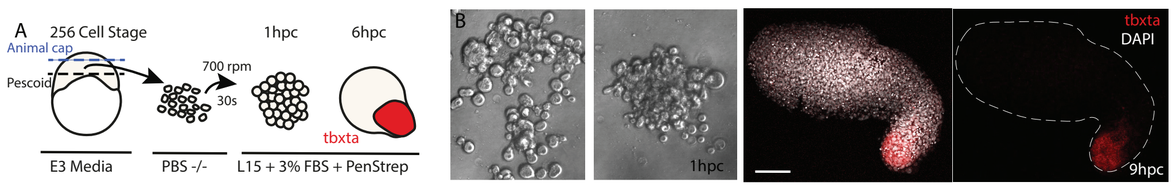


A. The earliest polorized signal we observed prior to elongation is phospho-smad2/3, indicative of Nodal pathway activation.
B. Inhibiting Nodal activity blocks explant elongation.
C. Elongation is also dependent on Wnt/PCP signalling.

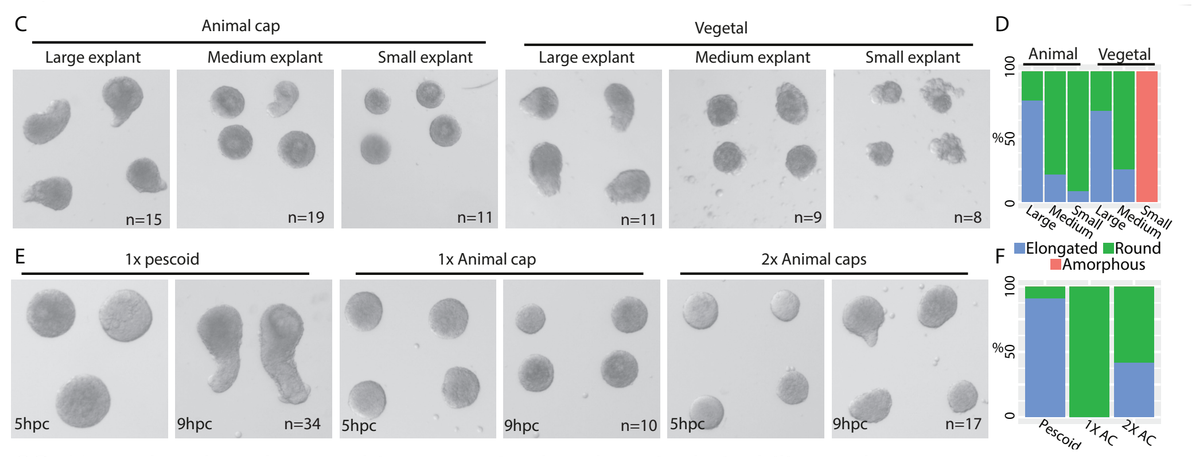


A-H. Blocking explant elongation inhibits the formation of opposing BMP and Wnt/beta-catenin gradients and the establishment of anterior-posterior neural patterning

Symmetry breaking is robust to experimental cell mixing.



A. Dissociation and reggregation of explants does not impact their ability to elongate and polarize tbxta expression



C,D. Vegetal explants have no bias over animal explants in their ability to elongate.
E, F. Animal caps can generate protrusions when combined as doublets

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#Equal contribution.

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