RESEARCH ARTICLE | DEVELOPMENT

Pitx2 patterns an accelerator-brake mechanical feedback through latent TGFβ to rotate the gut

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Authors Info & Affiliations

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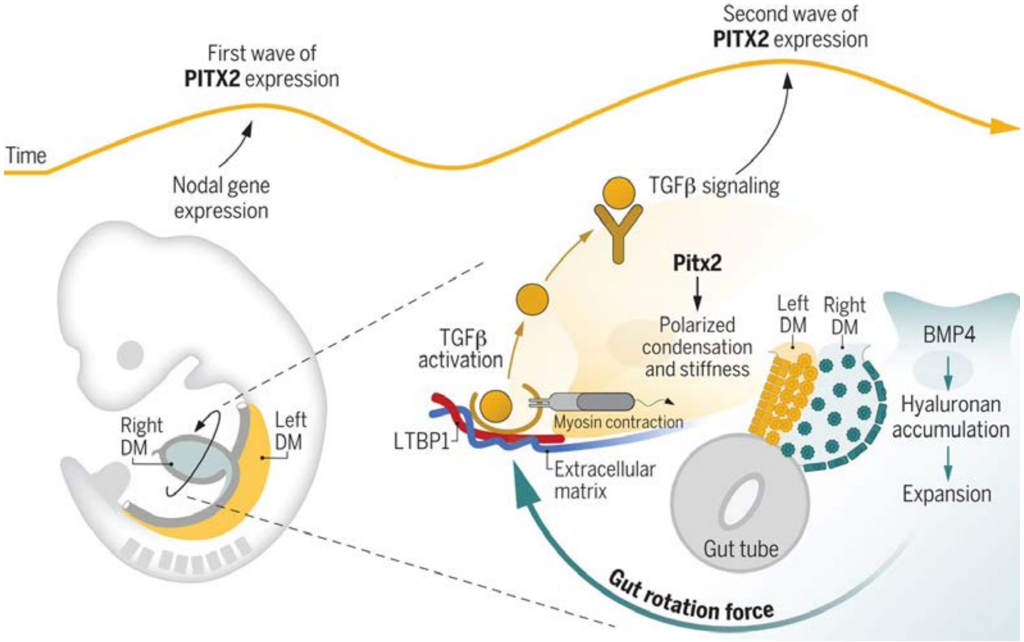
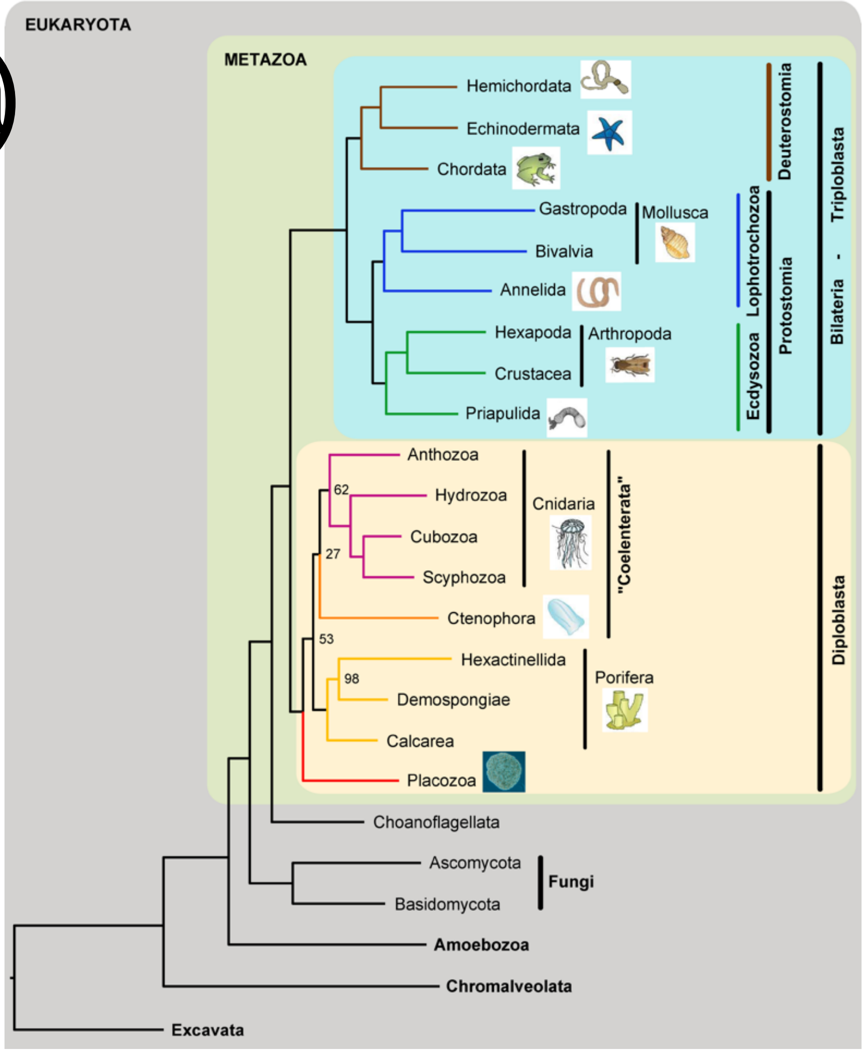


The right to remain symmetrical: Mechanobiology of the Sea Urchin Rudiment Morphogenesis

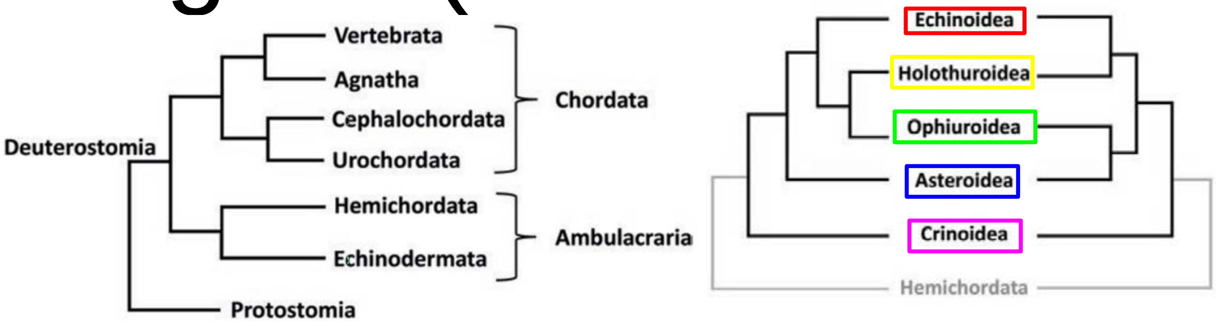
Elizabeth Rodriguez
Ignacio Leal
Juan Pablo Venegas
Mariana Tovar

Background (Echinodermata)

Why are they considered a good model for Evo-Devo studies?



Background (Echinodermata)

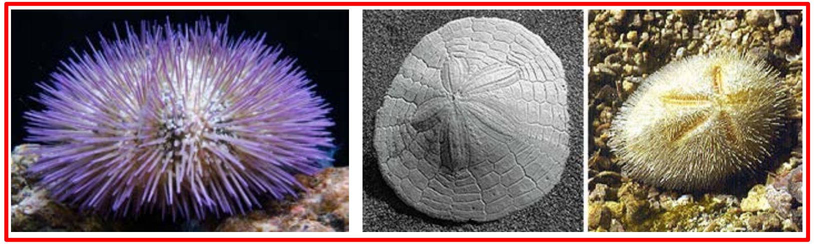


Smith, L.C. *et al.* (2018). Echinodermata: The Complex Immune System in Echinoderms. In: Cooper, E. (eds) Advances in Comparative Immunology. Springer, Cham. https://doi.org/10.1007/978-3-319-76768-0_13

Sharing characteristics

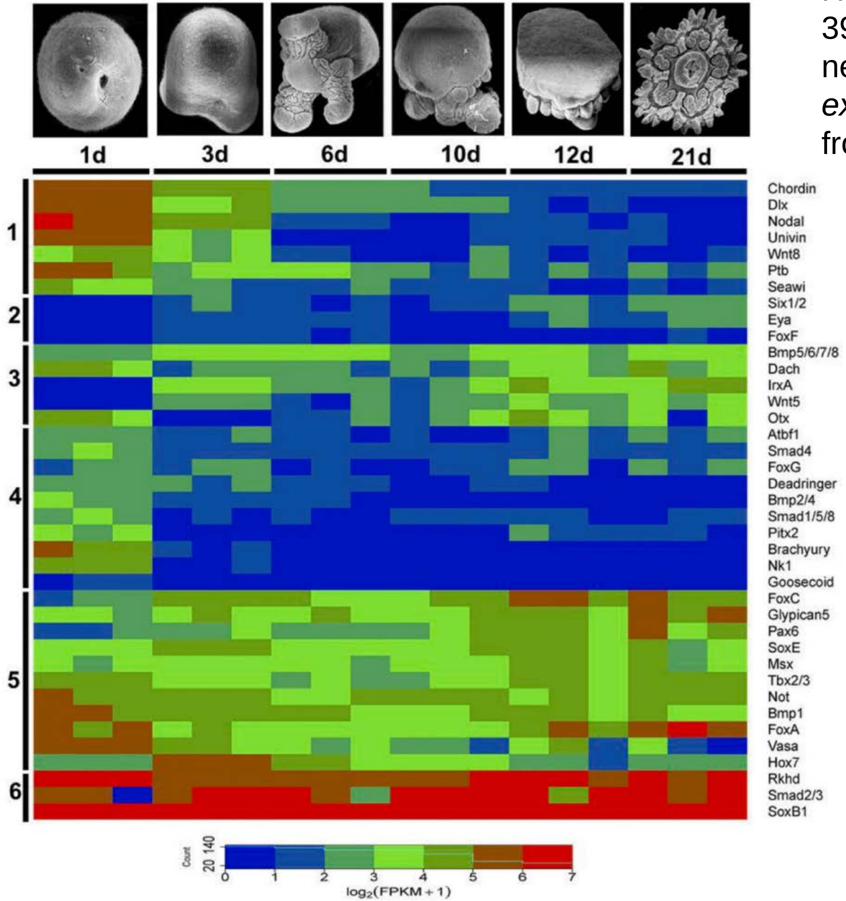
Bilateral larvae form

Pentarradial body plan



Background (Echinodermata)

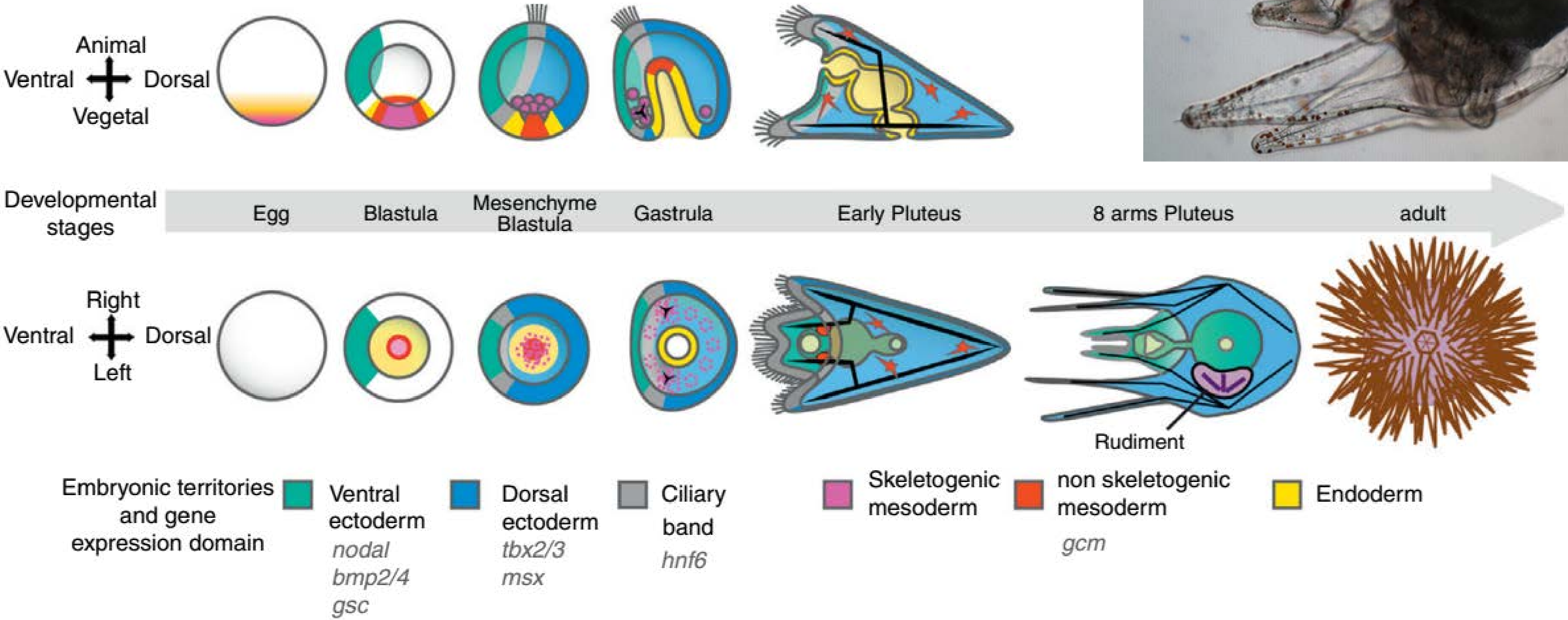
A few genes have been identified as related to symmetry



Heatmap analysis showing expression of 39 genes of the Nodal and BMP regulatory network in the transcriptome of *Parvulastra exigua* at six developmental time point from the gastrula to the juvenile.

Byrne M, Koop D, Strbenac D, et al. Transcriptomic analysis of Nodal - and BMP- associated genes during development to the juvenile seastar in Parvulastra exigua (Asterinidae). Marine Genomics. 2021 Oct;59:100857. DOI: 10.1016/j.margen.2021.100857. PMID: 33676872; PMCID: PMC8922652.

Background (Echinoidea: sea urchins)

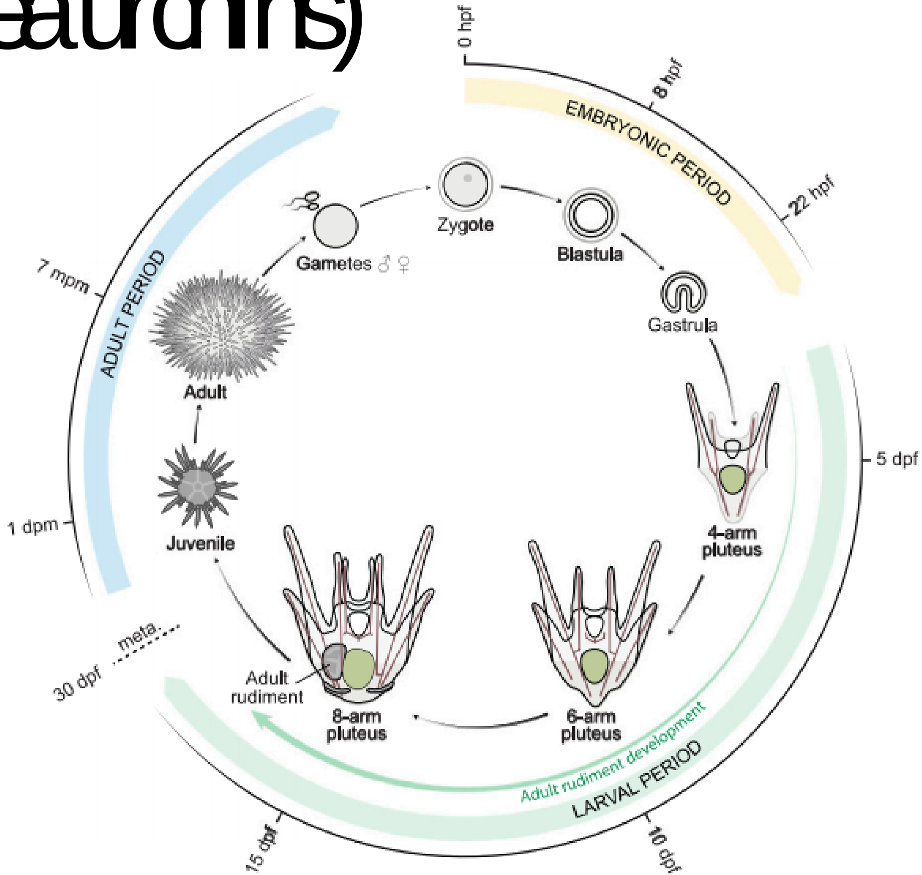


Molina MD, de Crozé N, Haillot E, Lepage T. Nodal: master and commander of the dorsal-ventral and left-right axes in the sea urchin embryo. Curr Opin Genet Dev. 2013 Aug;23(4):445-53. doi: 10.1016/j.gde.2013.04.010. Epub 2013 Jun 14. PMID: 23769944.

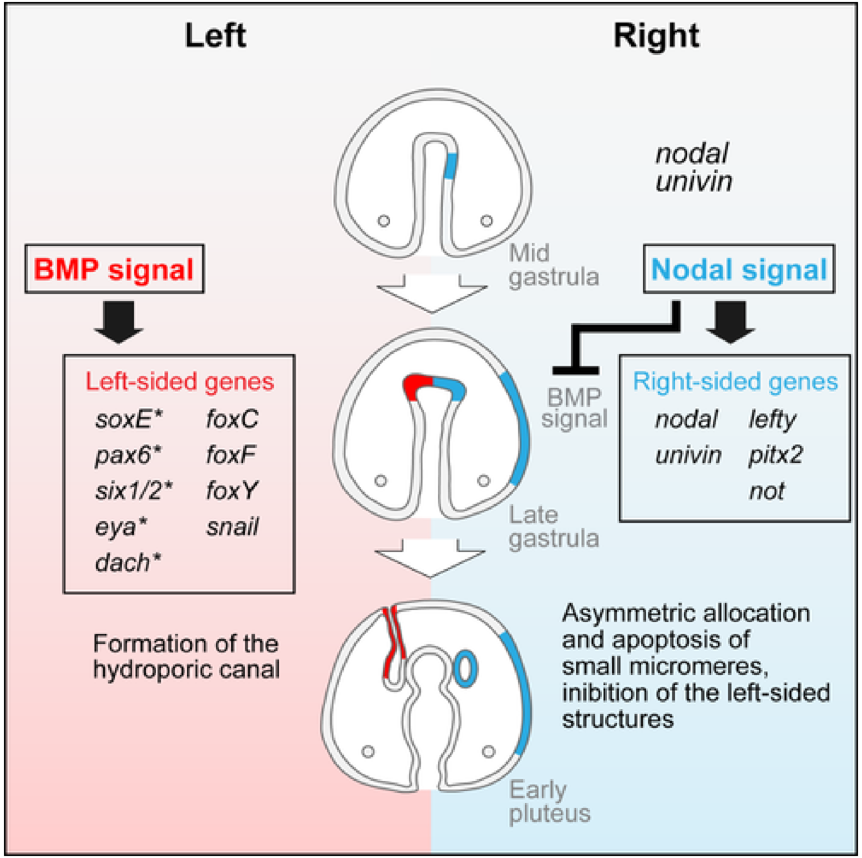
Background (Echinoidea: sea urchins)

Paracentrotus lividus

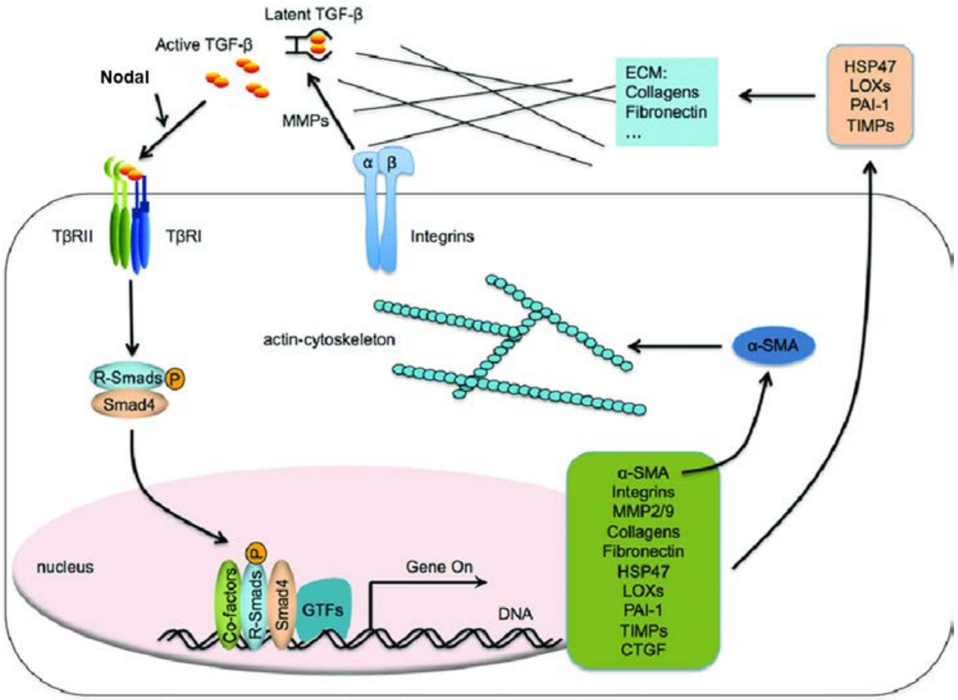
Described 33 stages
Light microscopy and confocal microscopy on fixed animals.



Formery L, Wakefield A, Gesson M, Toisoul L, Lhomond G, Gilletta L, Lasbleiz R, Schubert M and Croce JC (2022), Developmental atlas of the indirect-developing sea urchin Paracentrotus lividus: From fertilization to juvenile stages. Front. Cell Dev. Biol. 10:966408. doi: 10.3389/fcell.2022.966408



Su, Y. H. (2014). Telling left from right: Left-right asymmetric controls in sea urchins. *Genesis*.



Yan, X., Xiong, X., & Chen, Y. G. (2018). Feedback regulation of TGF-β signaling. *Acta biochimica et biophysica Sinica*.

How does Nodal signaling promote rudiment formation on the left side?

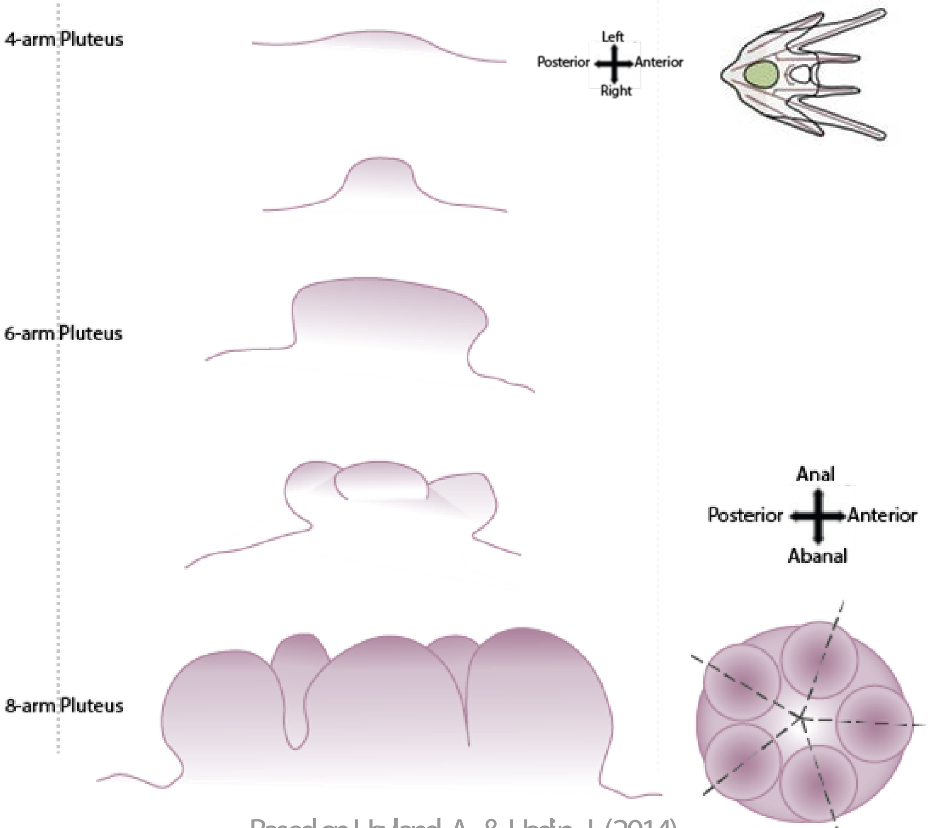
Hypothesis

The sea urchin rudiment morphogenesis is initiated and driven by differential stiffness across the tissue fue to asymmetric gene expression

General Aim

Characterize the mechanicals properties and asymmetric gene expression of the sea urchin rudiment throughout morphogenesis to isolate the driving factors of the process

The Morphogenesis of the Sea Urchin rudiment

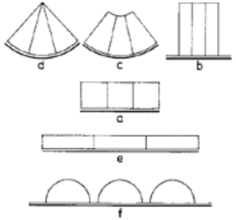


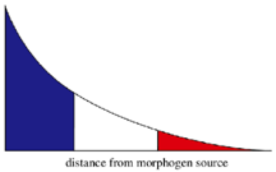
Based on Heyland, A., & Hodin, J. (2014).

2 fundamental events:

- ★ Asymmetry in rudiment initiation
- ★ Pentaradial symmetry axes formation

Many of such changes in sea urchin development may be regarded as arising from changes in curvature, which in turn are brought about by changes in adhesion and tension in the cell membrane, and hence contact between the cells.



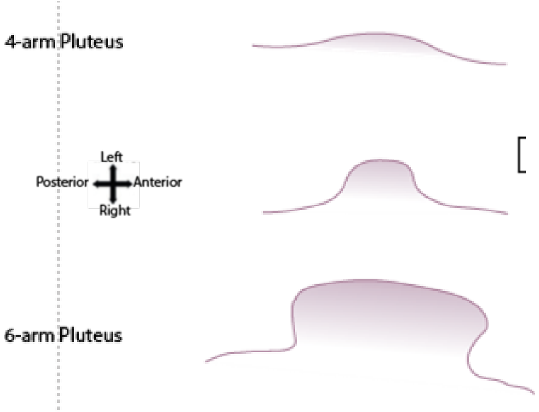


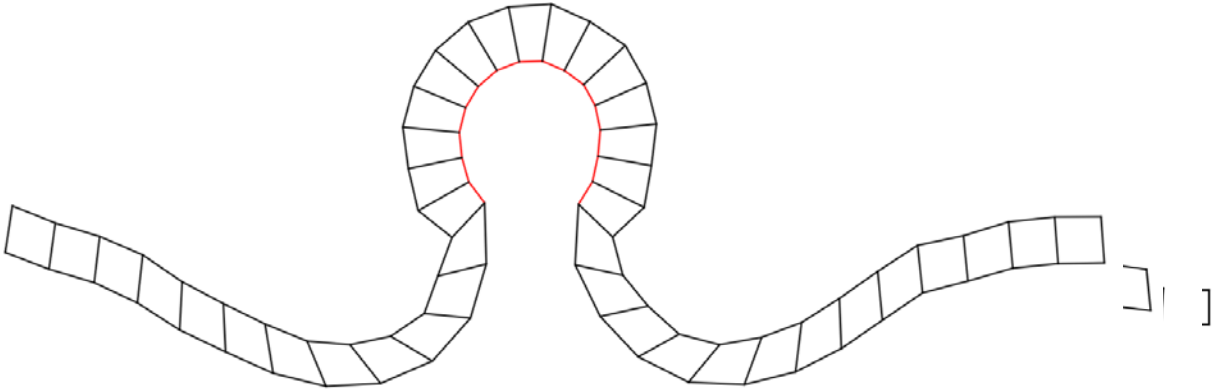
Gustafson, T., & **Wolpert, L.** (1963). The Cellular Basis of Morphogenesis and Sea Urchin Development

Minimal Simulations: Rudiment Initiation

Epithelial monolayers can describe rudiment evagination from the left coelom

★ Only varying cell stiffness

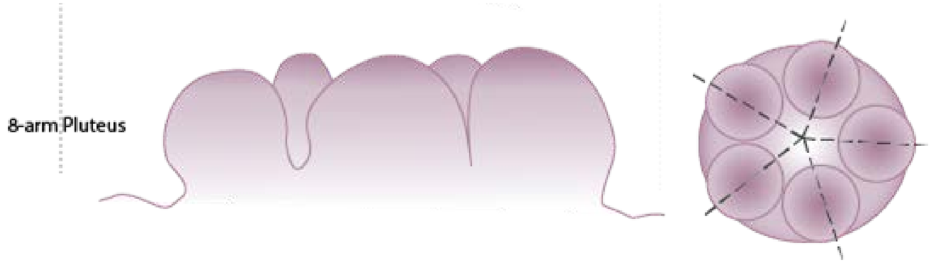


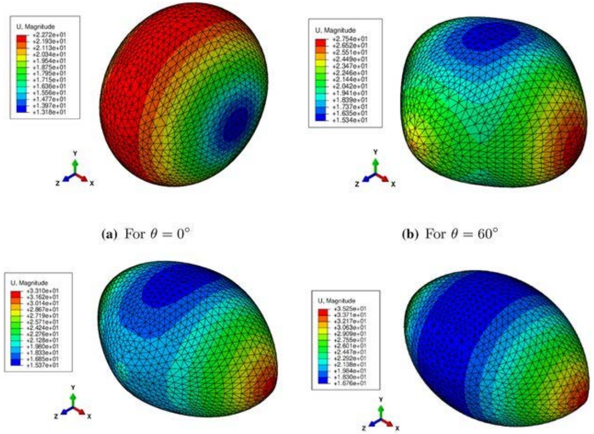


Minimal Simulations: Rudiment Axes formation

More complex physical simulations could give insights into the mechanisms behind establishment of symmetry

- ★ Inflating balloons in constraints
- ★ Deforming spheres

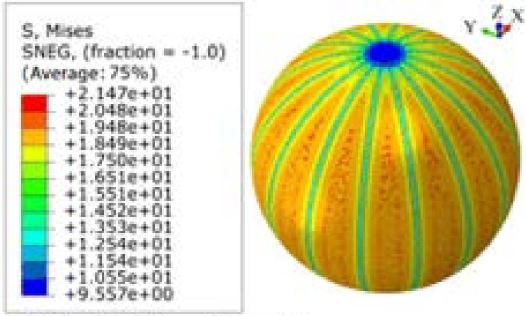




(a) For $\theta = 0°$ (b) For $\theta = 60°$

(c) For $\theta = 75°$ (d) For $\theta = 90°$

Friderikos, O., Baranger, E., Olive, M et al. On the stability of POD basis interpolation on Grassmann manifolds for parametric model order reduction (2022)

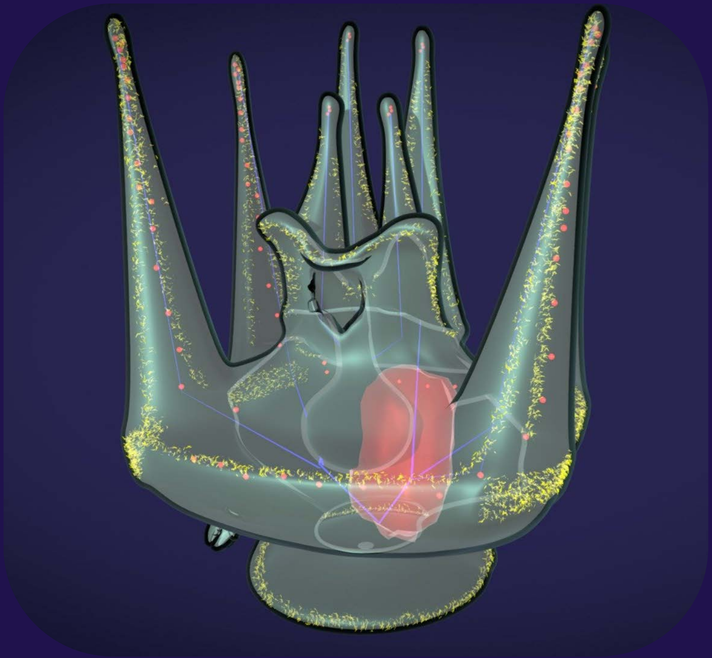


a) FEA results of the welding model

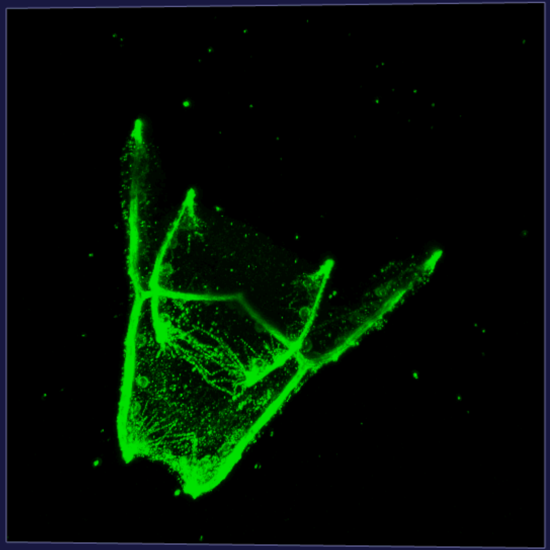
Xie, W.-C, Wang, X.-L., Duan, D.-P., & Tang, J.-W. Finite Element Simulation of the Microstructure of Stratospheric Airship Envelopes (2020)

Refining the Models: Embryo Imaging

Confocal or Light Sheet Microscopy can capture tissue topology and suggest better starting conditions

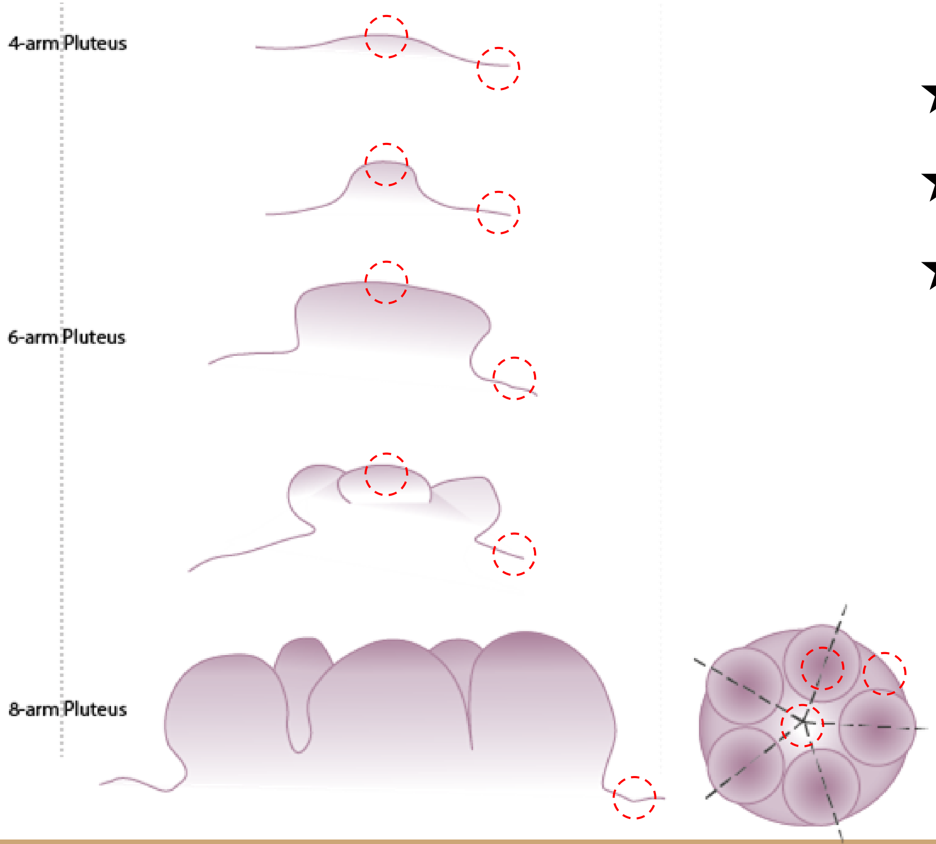


UC San Diego. Sketchfab

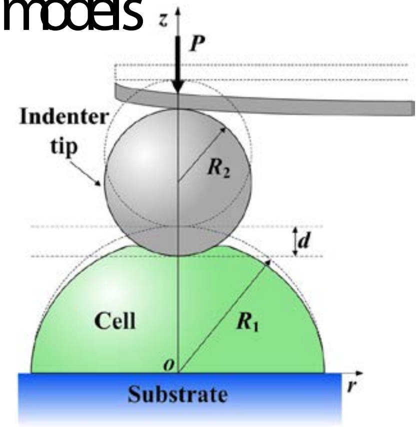


6a9 immunostain
Ettensohn Lab. Carnegie Mellon University

Testing the model: *In vivo* Stiffness measurements



- ★ Nano indenter
- ★ Several points in space and time
- ★ Measurement of stiffness as predicted by the models

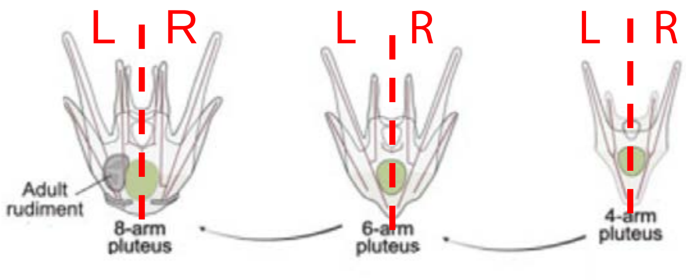


$$E = \frac{\sigma}{\varepsilon}$$

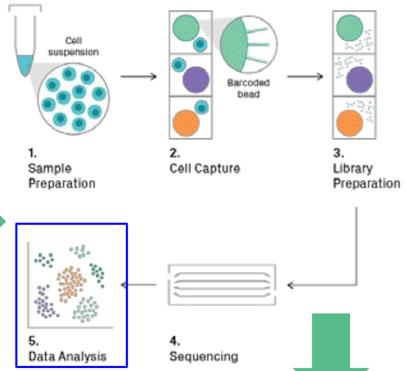
Ding, Y., Xu, GK & Wang, GF. On the determination of elastic moduli of cells by AFM based indentation (2017)

Analyze the expression levels of genes related to Nodal/BMP signaling, as well as those associated with stiffness, during the developmental stage of the adult rudiment

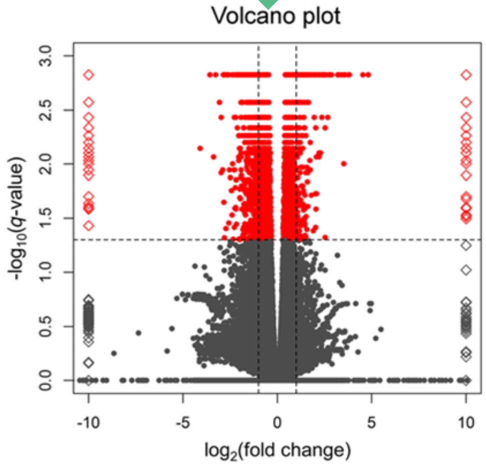
Larval Period



Bisectioning

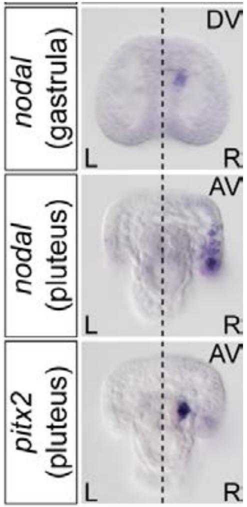


Single-cell RNAseq



Differential expression analysis

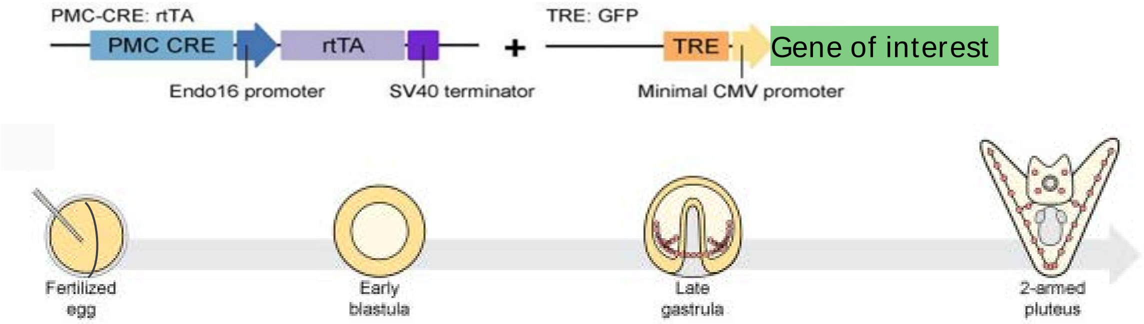
In situ hybridization

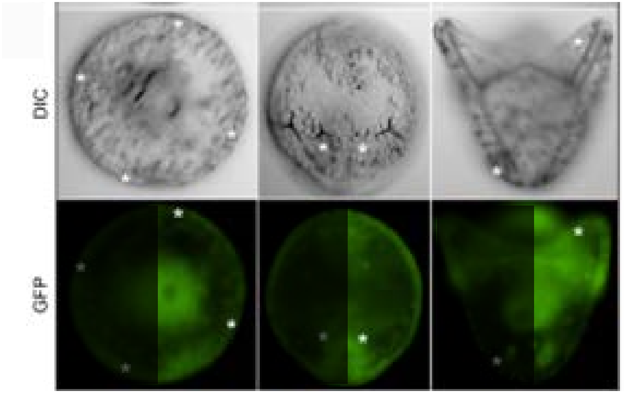


Genes used as markers to annotate the cell clusters of the adult rudiment.

pax6
soxE

The Influence of Gene Expression on Mechanical Asymmetry



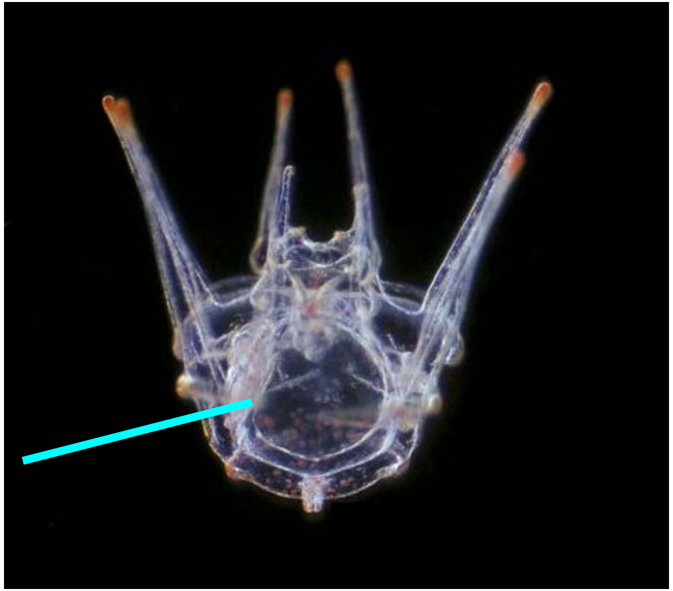


Examine silencing or overexpression through *in situ* hybridization and rudiment formation.

Analyze variations in stiffness

J.Khor and C.Ettensohn (2023) Development

Studying and Manipulating Tissue Stiffness during Rudiment Formation



Softer:

Laser ablation.

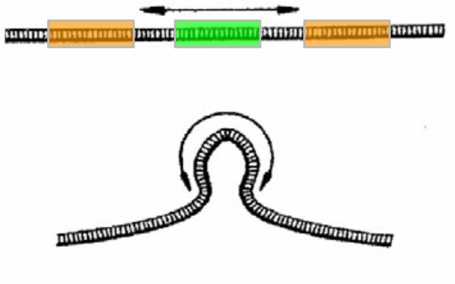
Blebbistatin.

Stiffer:

Nanoindenter(*).

Injection of DD-MLC.

Optical tweezers.



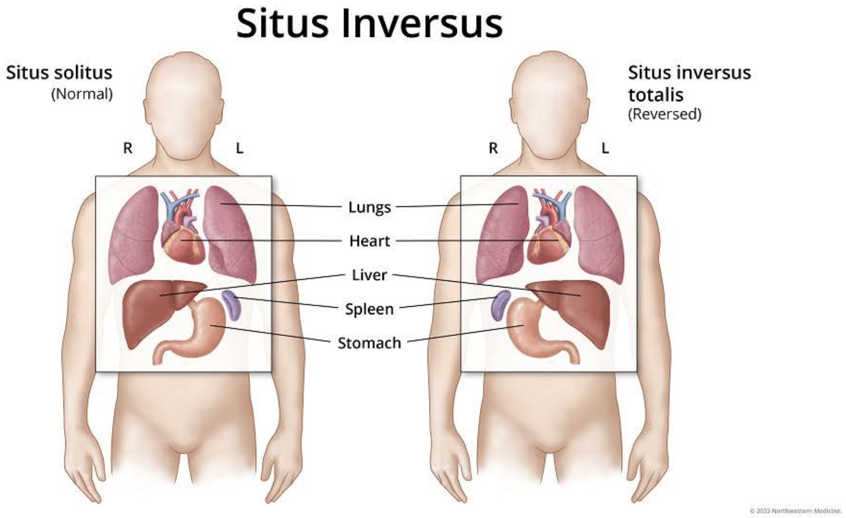
Inhibit Rudiment formation (L)

Induce Rudiment formation (R)

Recue inhibition of genes (Sox9, BMP,etc) by manipulating tissue stiffness

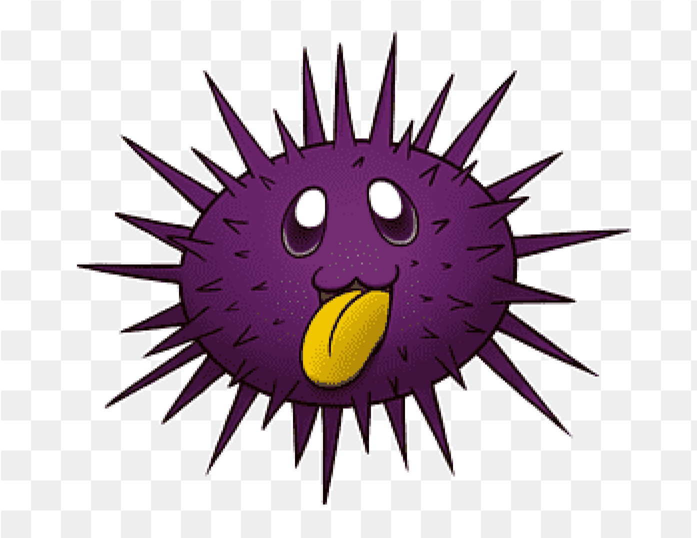
Projections and Significance of the Research

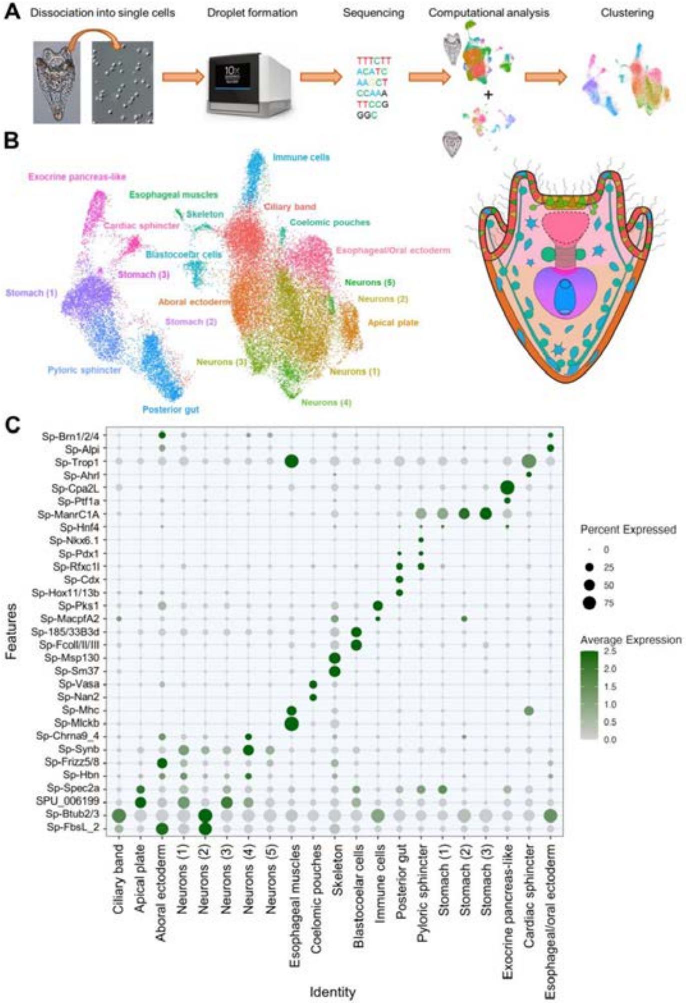
Investigate alterations in stiffness and their correlation with gene expression during the formation of asymmetry in embryonic development.

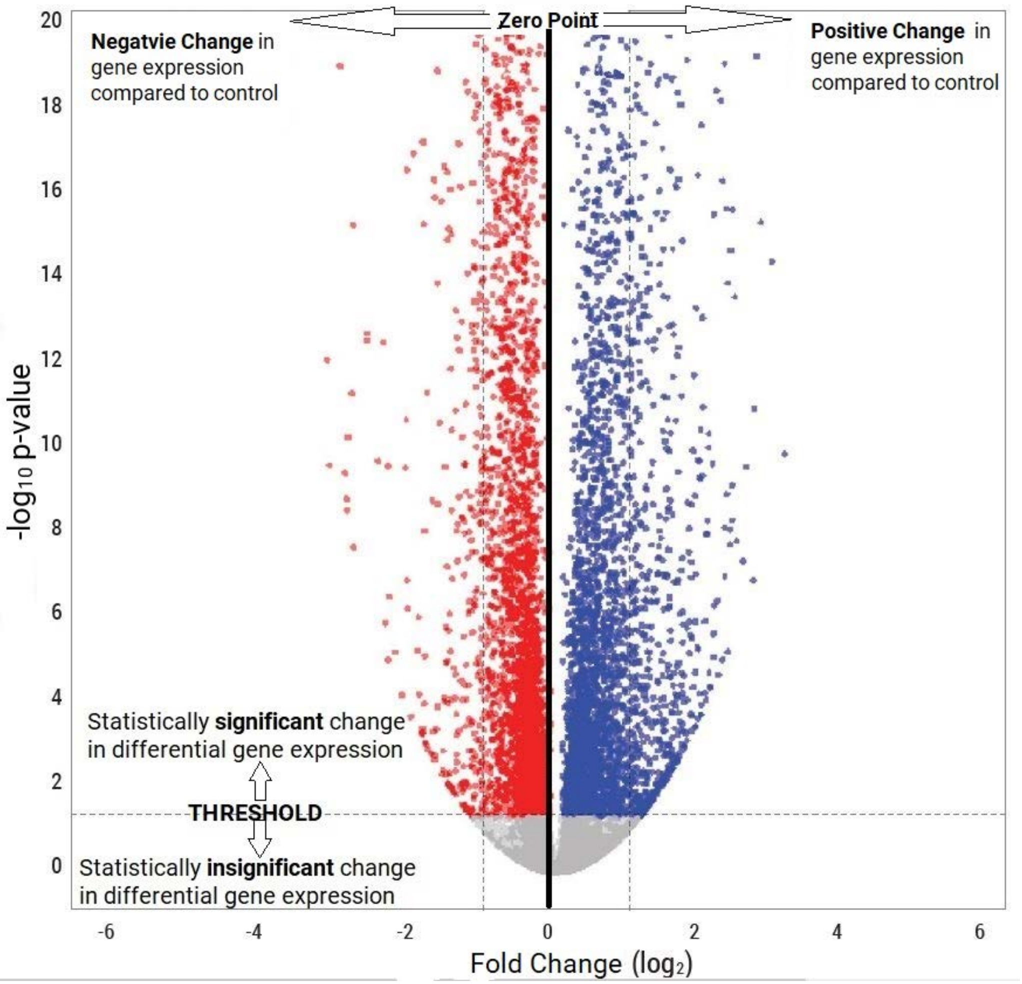


Northwestern Medicine University webpage

Thank you




A
Dissociation into single cells
Droplet formation
Sequencing
Computational analysis
Clustering
TTTCTT
ACATC
AAGCT
CCAAA
TTCCG
GGC
B
Immune cells
Exocrine pancreas-like
Esophageal muscles
Ciliary band
Skeleton
Coelomic pouches
Cardiac sphincter
Esophageal/Oral ectoderm
Blastocoelar cells
Stomach (3)
Neurons (5)
Stomach (1)
Aboral ectoderm
Neurons (2)
Stomach (2)
Apical plate
Pyloric sphincter
Neurons (3)
Neurons (1)
Neurons (4)
Posterior gut
C
Sp-Brn1/2/4
Sp-Alpi
Sp-Trop1
Sp-Ahrl
Sp-Cpa2L
Sp-Ptf1a
Sp-ManrC1A
Sp-Hnf4
Sp-Nkx6.1
Sp-Pdx1
Sp-Rfxc1l
Sp-Cdx
Sp-Hox11/13b
Sp-Pks1
Sp-MacpfA2
Sp-185/33B3d
Sp-FcolI/II/III
Sp-Msp130
Sp-Sm37
Sp-Vasa
Sp-Nan2
Sp-Mhc
Sp-Mlckb
Sp-Chrna9_4
Sp-Synb
Sp-Frizz5/8
Sp-Hbn
Sp-Spec2a
SPU_006199
Sp-Btub2/3
Sp-FbsL_2
Features
Ciliary band
Apical plate
Aboral ectoderm
Neurons (1)
Neurons (2)
Neurons (3)
Neurons (4)
Neurons (5)
Esophageal muscles
Coelomic pouches
Skeleton
Blastocoelar cells
Immune cells
Posterior gut
Pyloric sphincter
Stomach (1)
Stomach (2)
Stomach (3)
Exocrine pancreas-like
Cardiac sphincter
Esophageal/oral ectoderm
Identity
Percent Expressed
0
25
50
75
Average Expression
2.5
2.0
1.5
1.0
0.5
0.0



Zero Point
Negatvie Change in gene expression compared to control
Positive Change in gene expression compared to control
Statistically significant change in differential gene expression
THRESHOLD
Statistically insignificant change in differential gene expression
-log10 p-value
Fold Change (log2)
20
18
16
14
12
10
8
6
4
2
0
-6
-4
-2
0
2
4
6