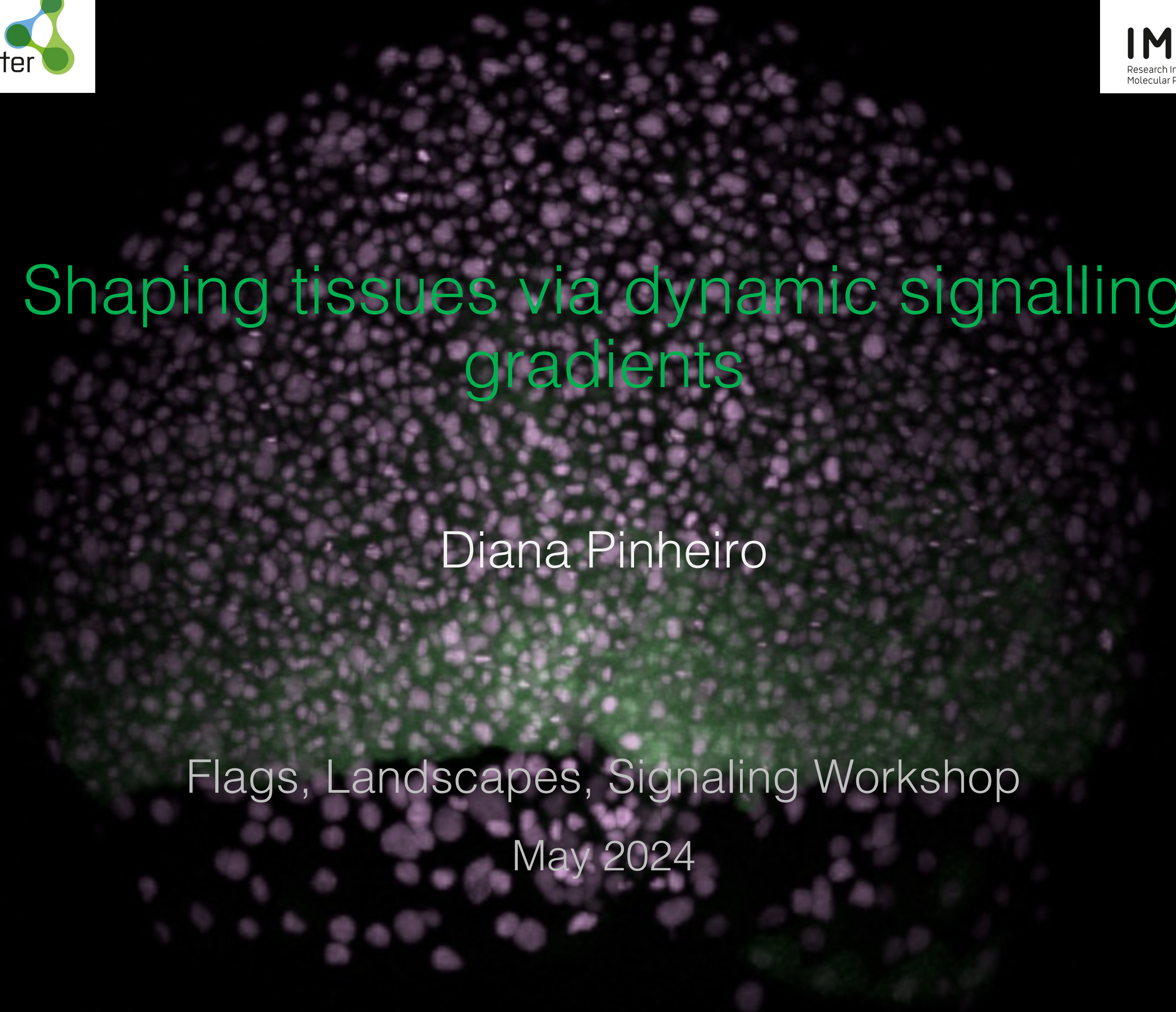


Shaping tissues via dynamic signalling gradients

Diana Pinheiro

Flags, Landscapes, Signaling Workshop

May 2024

Embryonic development

Zygote

It is a fundamentally multiscale process!

Information stored in the nucleus of a single fertilized cell, bridging 10 orders of magnitude.

Despite this, embryonic development is:

Highly reproducible!

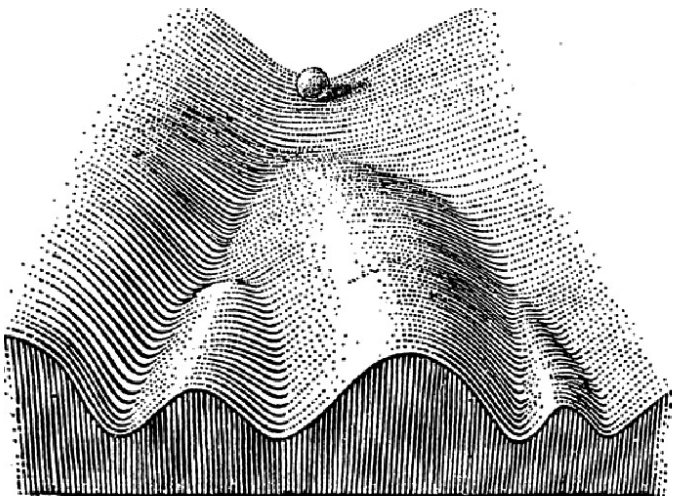
Self-organized!

The Shining (1980); www.redballoonlearner.org.

“Building” embryos requires:

Tissue patterning

Progressive increase in cell specialization.

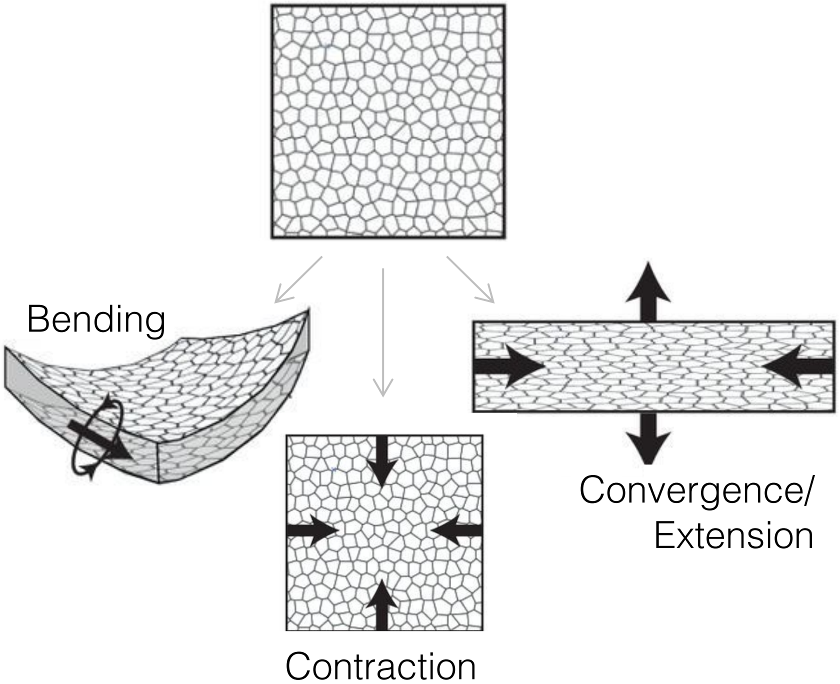


+

Morphogenesis

Complex 3D changes in tissue/embryo shape.

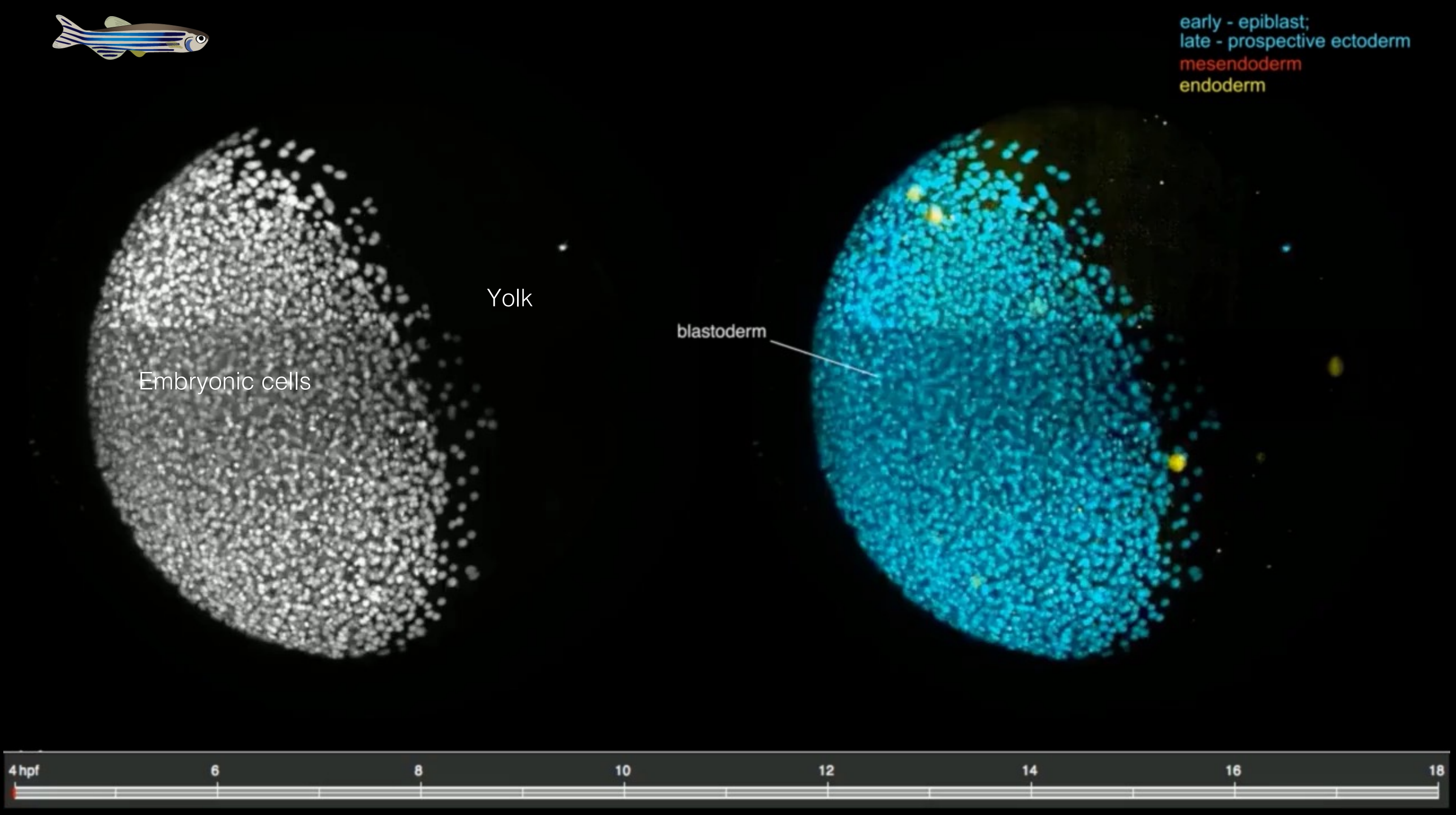
(requires cells to exert force!)



Waddington. 1957; Alt, et al. 2017.

Embryo development = Patterning + Morphogenesis

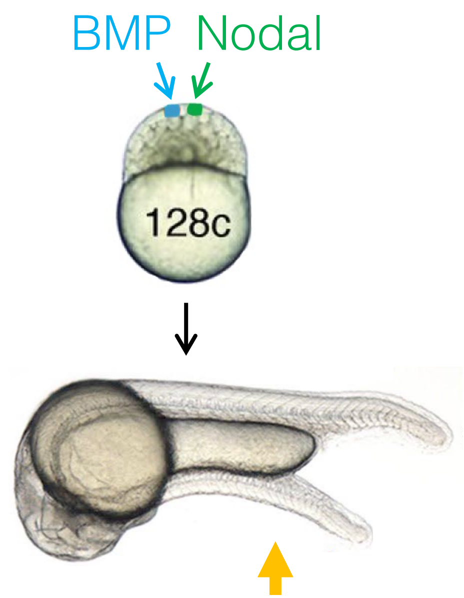
Zebrafish



Shah G, et al. 2019.

A handful of morphogens triggers patterning and morphogenesis

Gastrulation

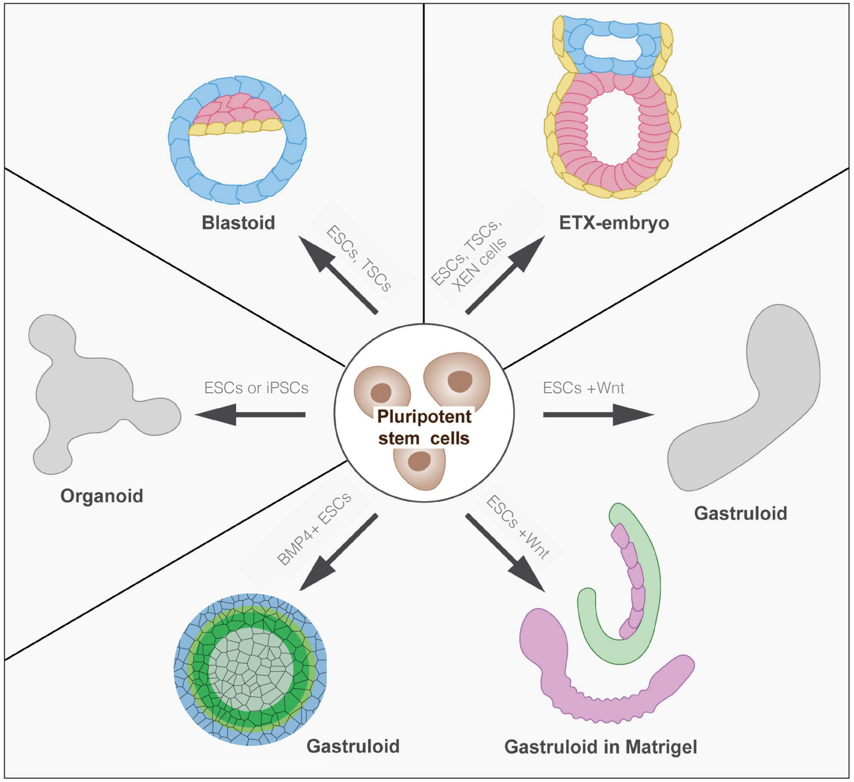


Second body axis

with correct cell types + right shape!

Xu et al. 2014.

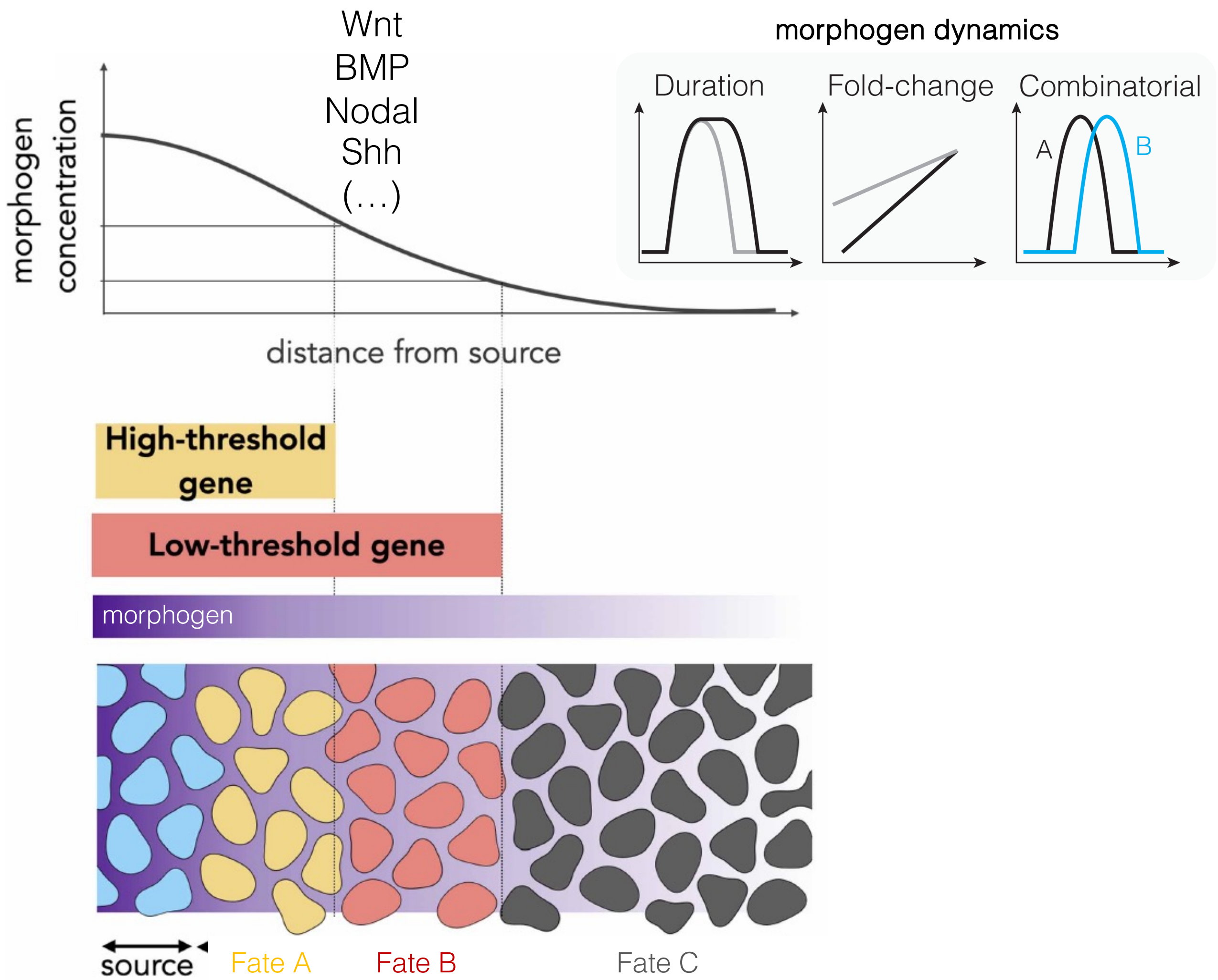
A handful of morphogens triggers patterning and morphogenesis



How is this mechanistically achieved?

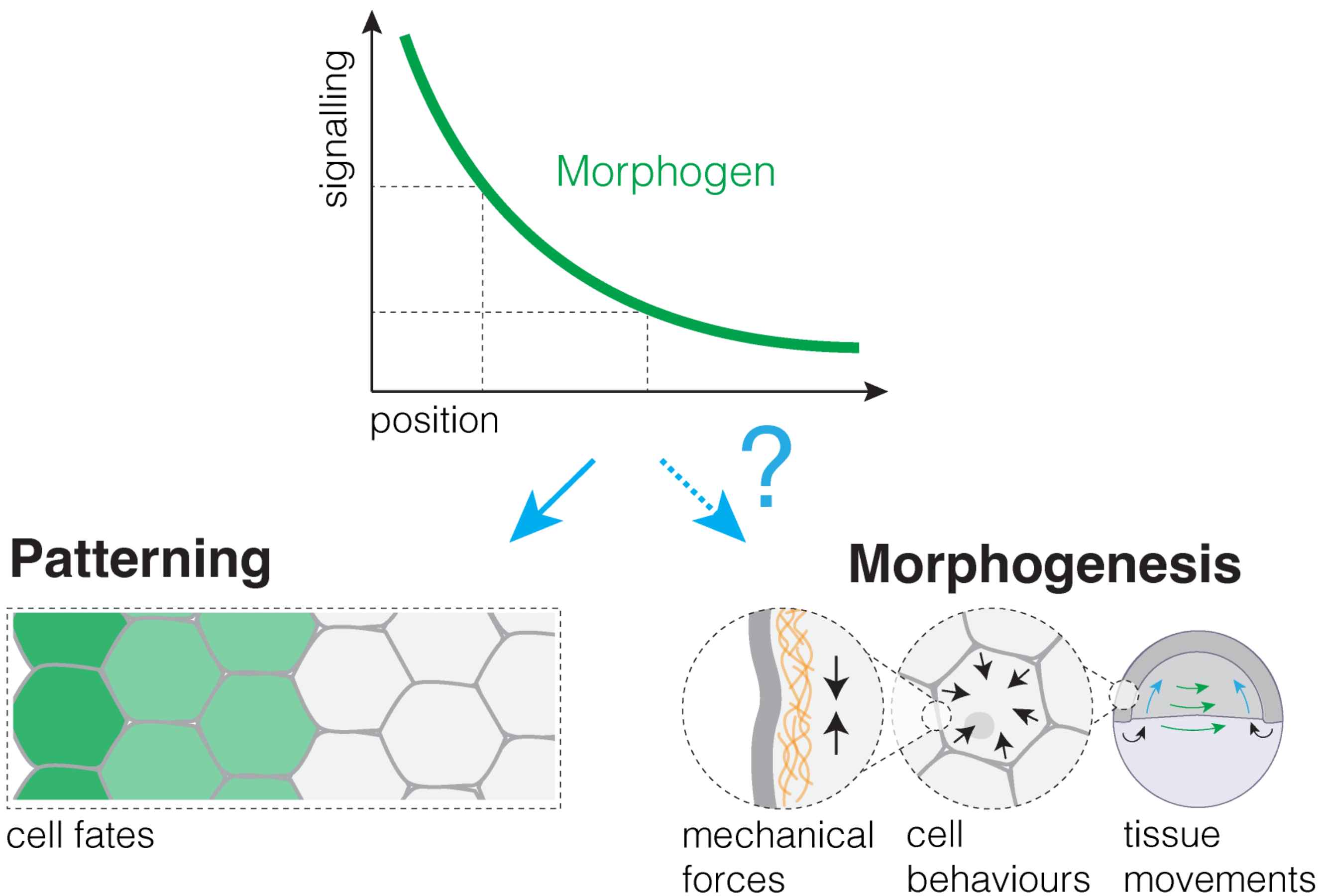
Azhar, et al. 2021.

From morphogens to complex tissue patterning



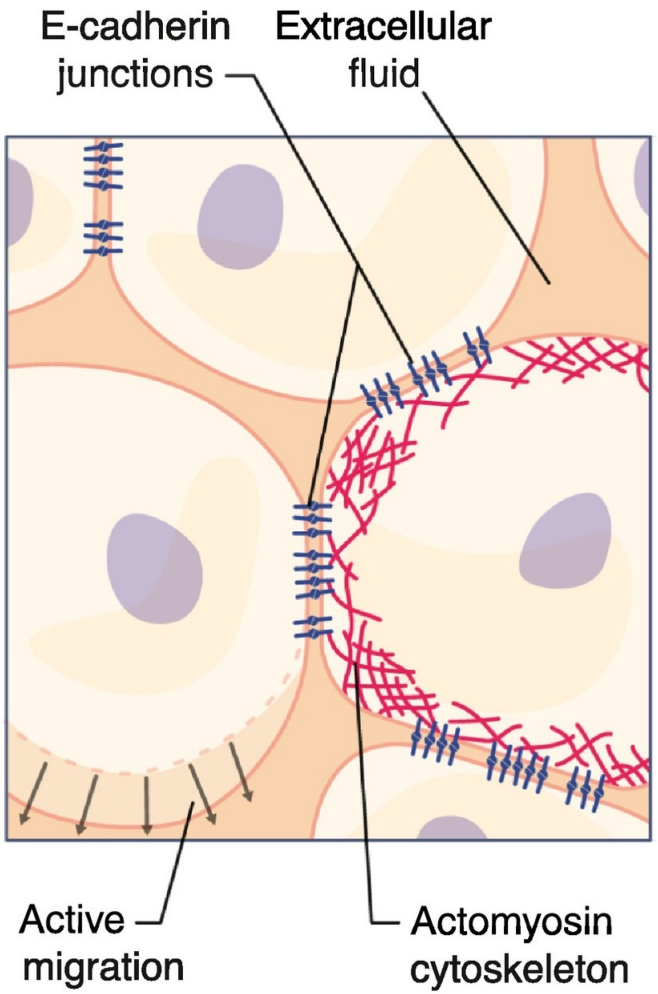
Adapted from York HM, et al. 2020.

From morphogens to morphogenesis: a gap in knowledge!



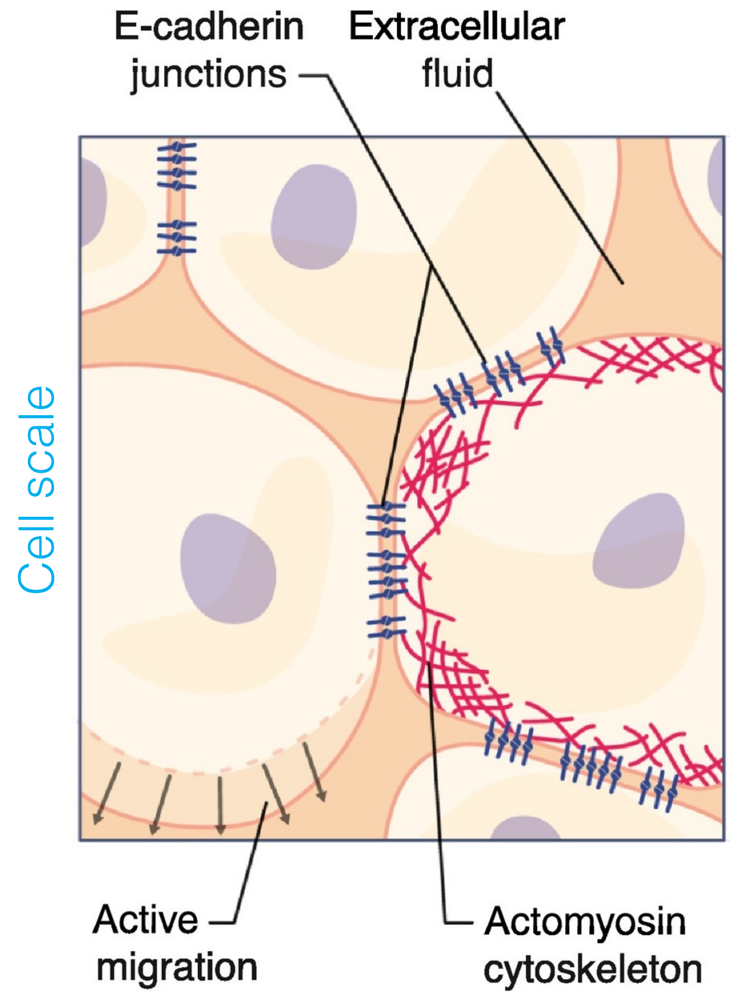
Mechanical forces shaping developing embryos

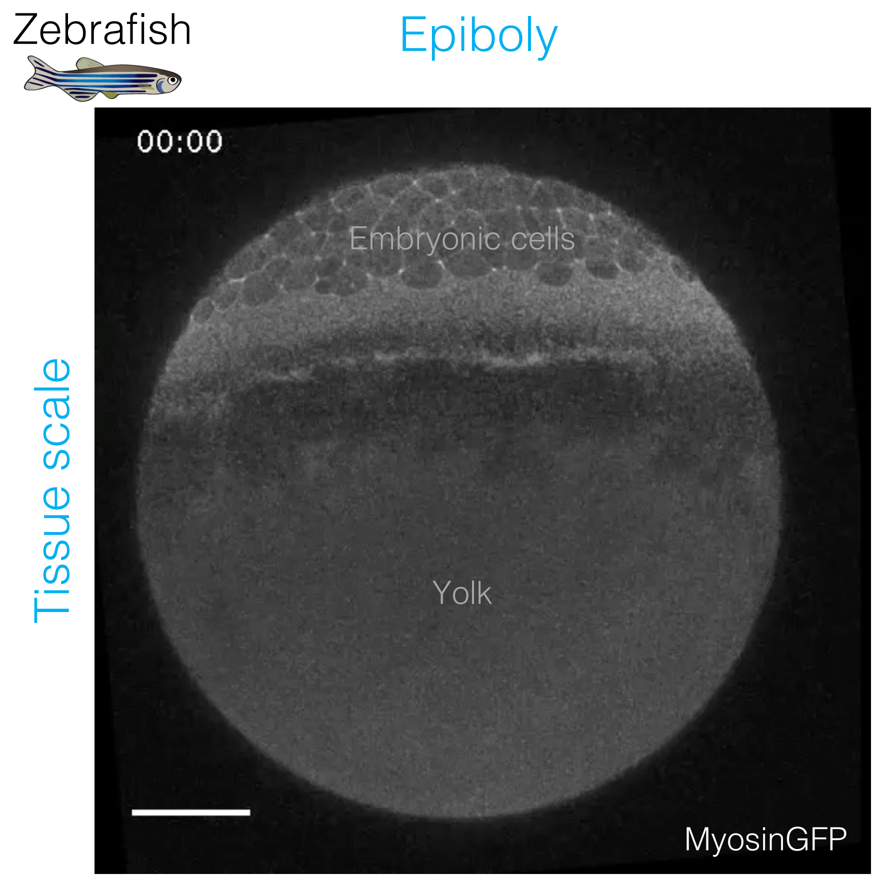
Cell scale



Adapted from Hannezo and Heisenberg. 2022

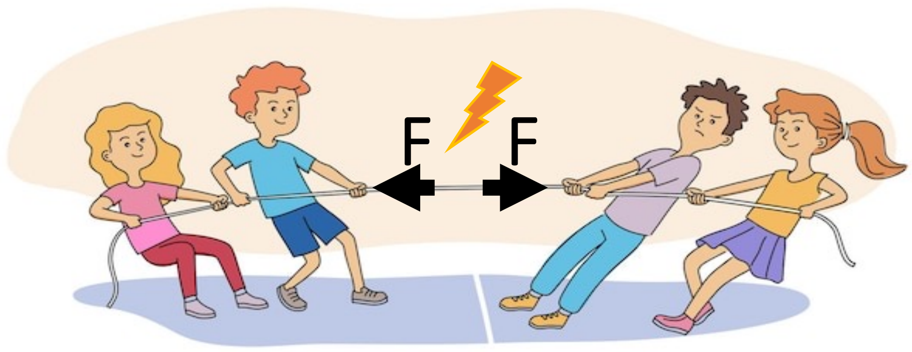
Mechanical forces shaping developing embryos





Adapted from Hannezo and Heisenberg. 2022; Bellaiche and Heisenberg. 2017.

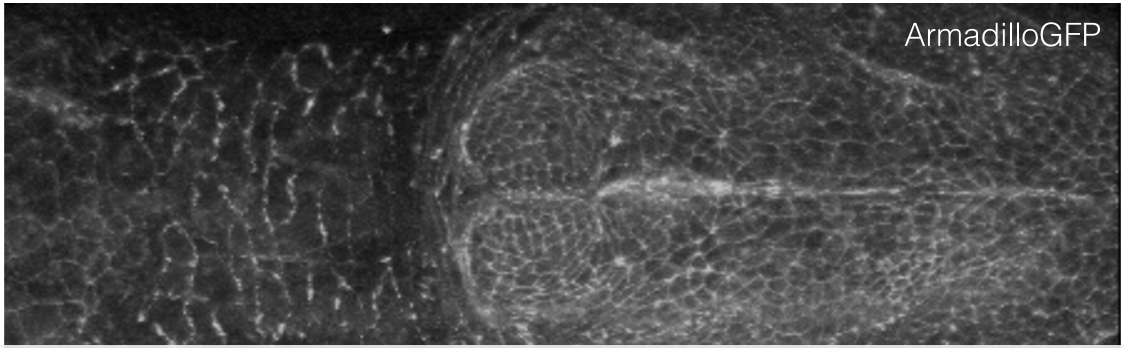
How can we test whether and where forces are being exerted?



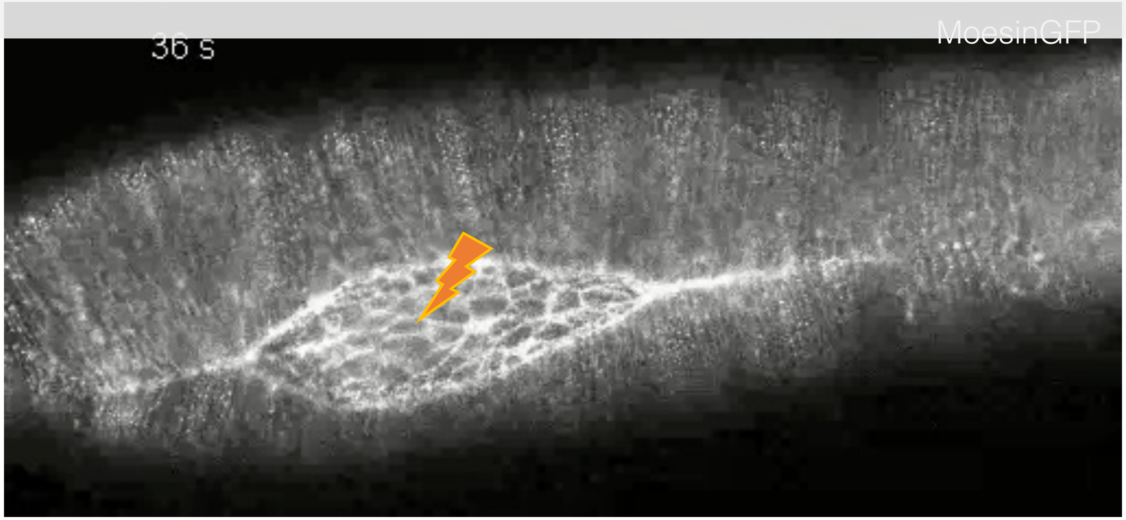
How can we test whether and where forces are being exerted?

Drosophila

Dorsal closure

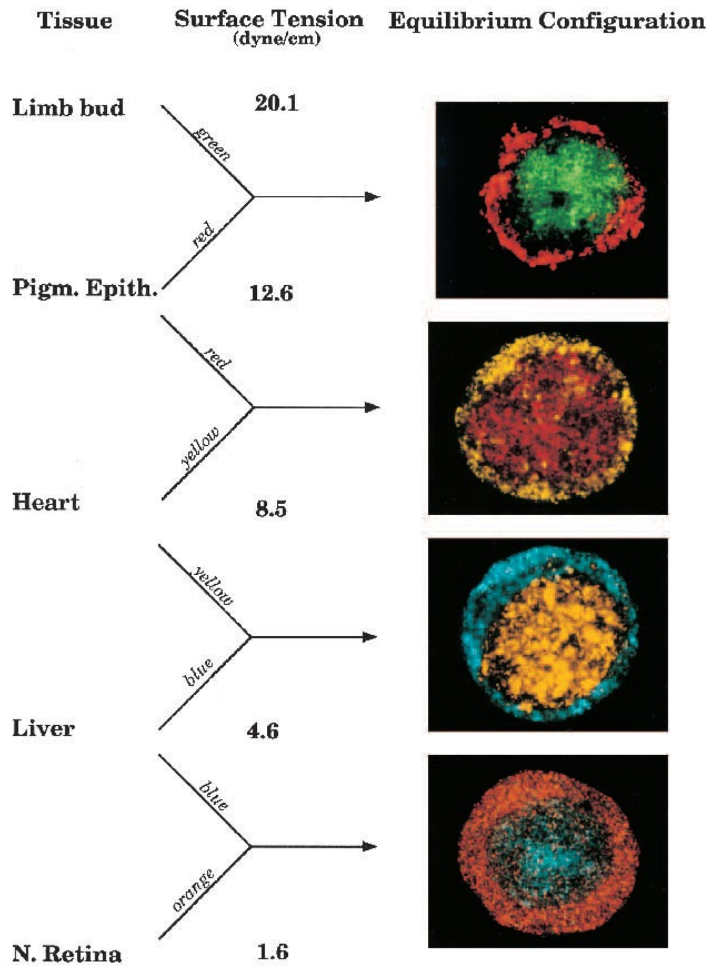


How do morphogens organize morphogenesis?



Bellaiche and Heisenberg. 2017.

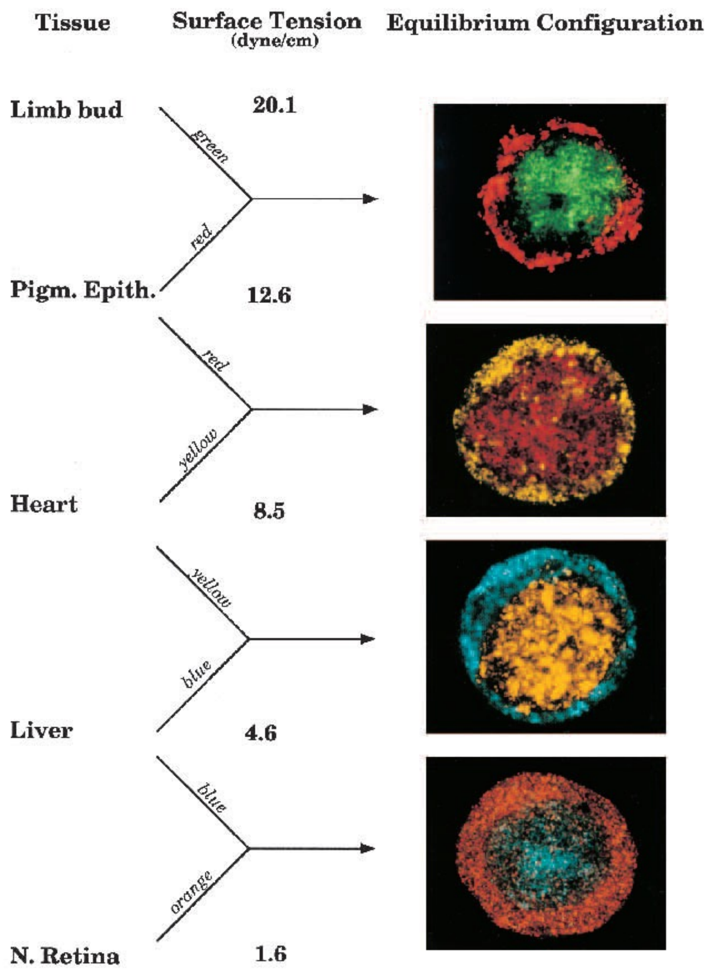
Cells of different fates exhibit distinct mechanical properties



Differences in surface tension drive differential cell positioning, via sorting!

Foty RA, et al. 1996.

Cells of different fates exhibit distinct mechanical properties



Programmed

Morphogen signaling

Cell fates/
Tissue patterning

Mechanical forces/
Tissue morphogenesis

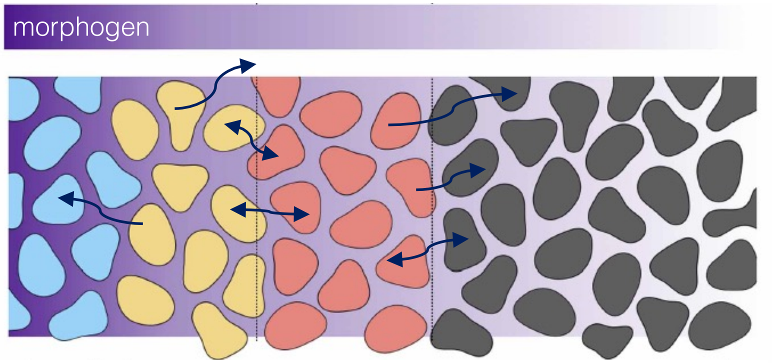
While this model is attractive…

Foty RA, et al. 1996.

Most developing tissues are not static during nor after tissue patterning

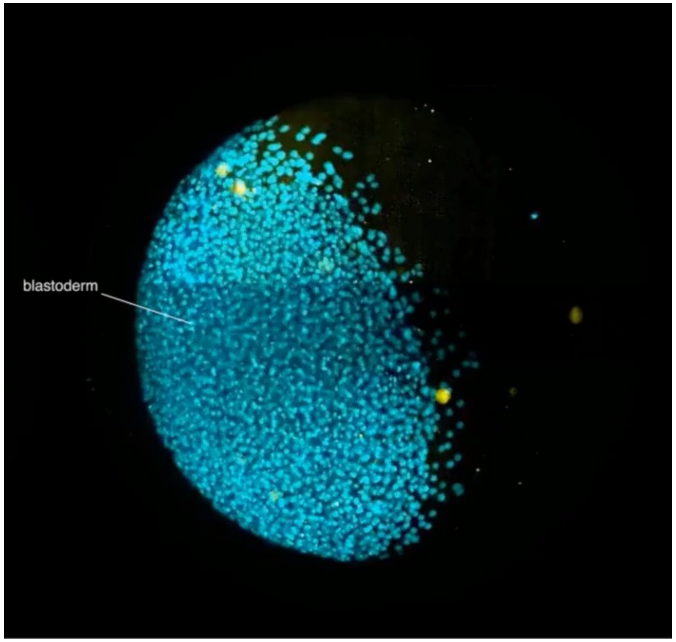
Local cell displacements

Cell division, cell migration, cell-cell rearrangements…



Tissue-scale morphogenesis

Collective migration, convergence & extension, internalization…

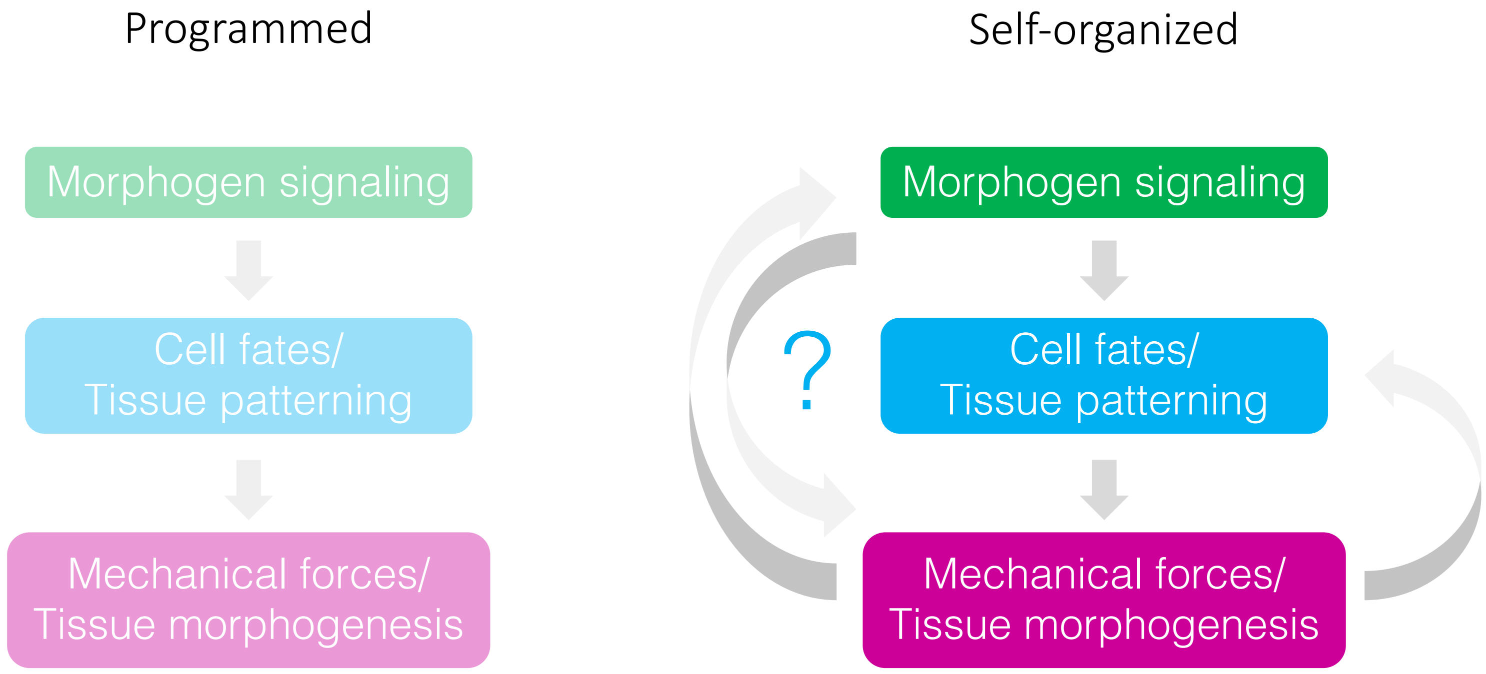


Prospective ectoderm Mesoderm Endoderm

Suggests the need for feedbacks coupling patterning and morphogenesis…

Adapted from Shah G, et al. 2019; York HM, et al. 2020.

Logic of developmental programs



How morphogenetic programs are encoded by morphogen signaling and/or emerging cell fates?

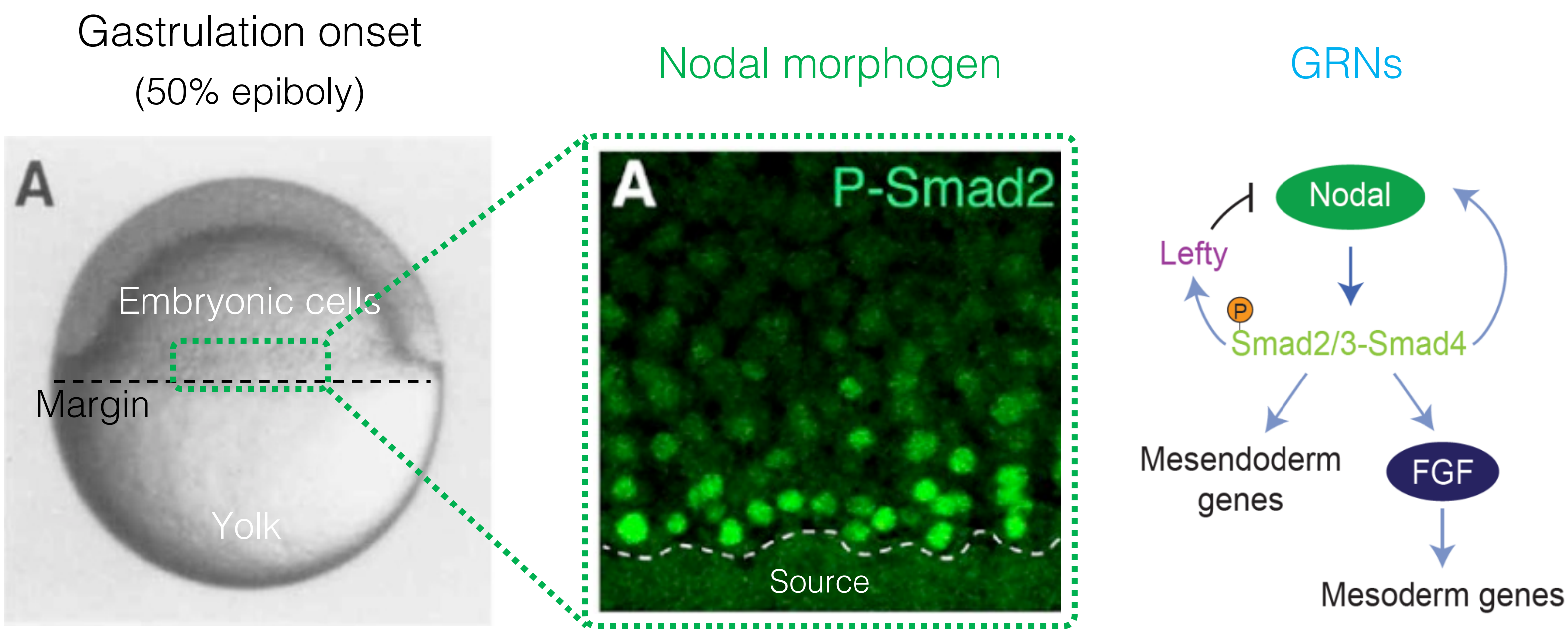
Zebrafish

Model system + Developmental stage

Gastrulation

”It is not birth, marriage or death, but gastrulation which is truly the most important time in your life…” (Lewis Wolpert)

Patterning + Morphogenesis of the germ layers



Adapted from Kimmel C, et al. 1995; Van Boxtel M, et al. 2017.

”It is not birth, marriage or death, but gastrulation which is truly the most important time in your life…” (Lewis Wolpert)

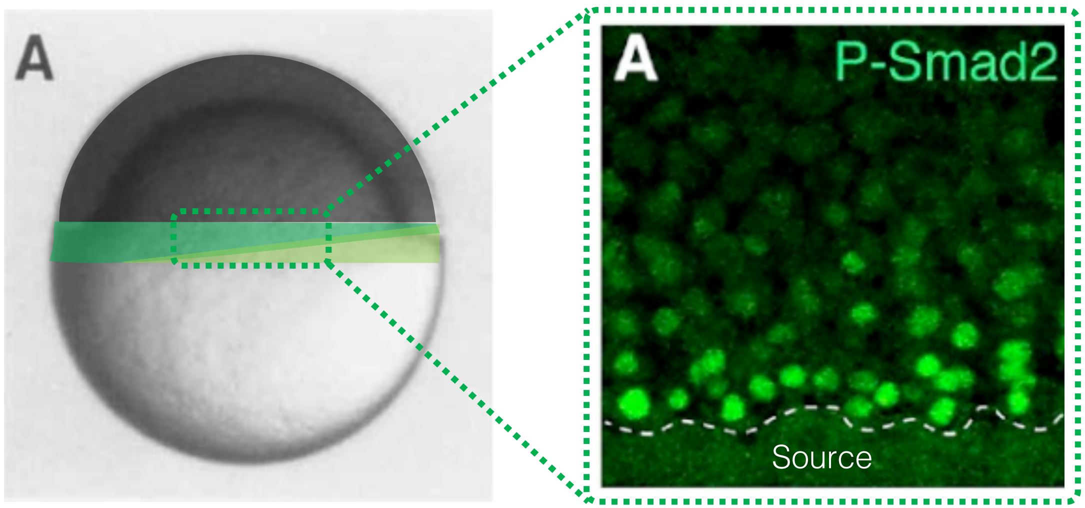
Patterning + Morphogenesis of the germ layers

Gastrulation onset

(50% epiboly)

Nodal morphogen

Tissue patterning



Ectoderm

Mesoderm

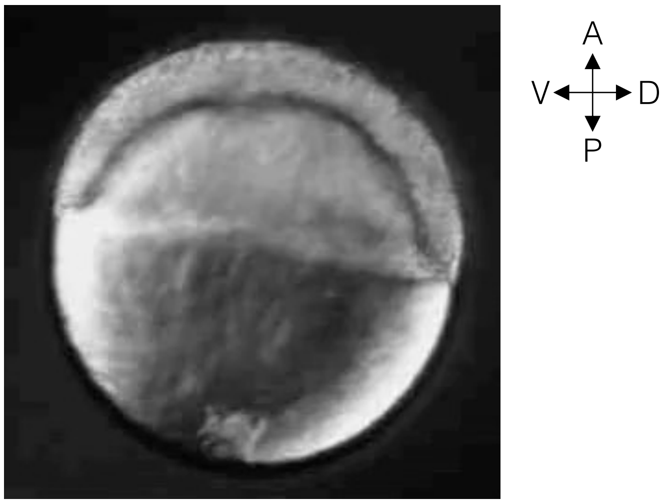
Endoderm

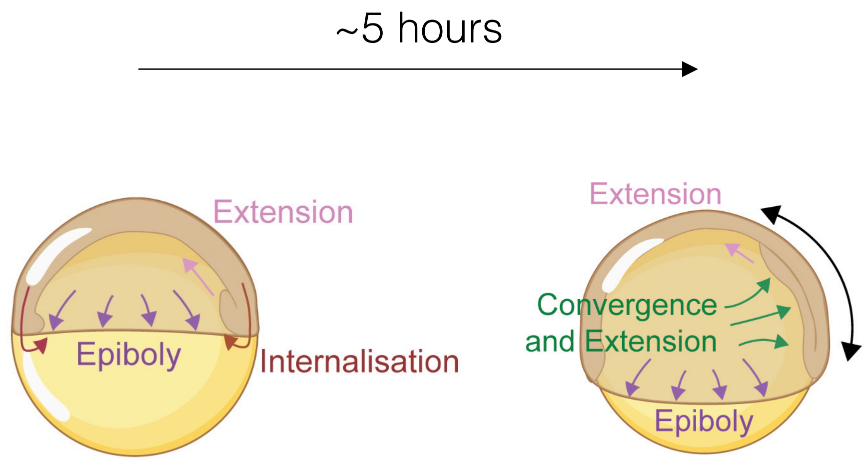
Adapted from Kimmel C, et al. 1995; Van Boxtel M, et al. 2017.

"It is not birth, marriage or death, but gastrulation which is truly the most important time in your life…" (Lewis Wolpert)

Patterning + Morphogenesis of the germ layers

Bright field



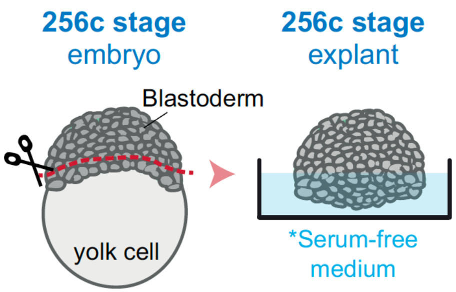


How are signaling gradients, emerging cell fate patterns and morphogenetic outputs integrated in space and time?

Adapted from Kane DA, et al. 1996; Narayanan R, et al. 2021.

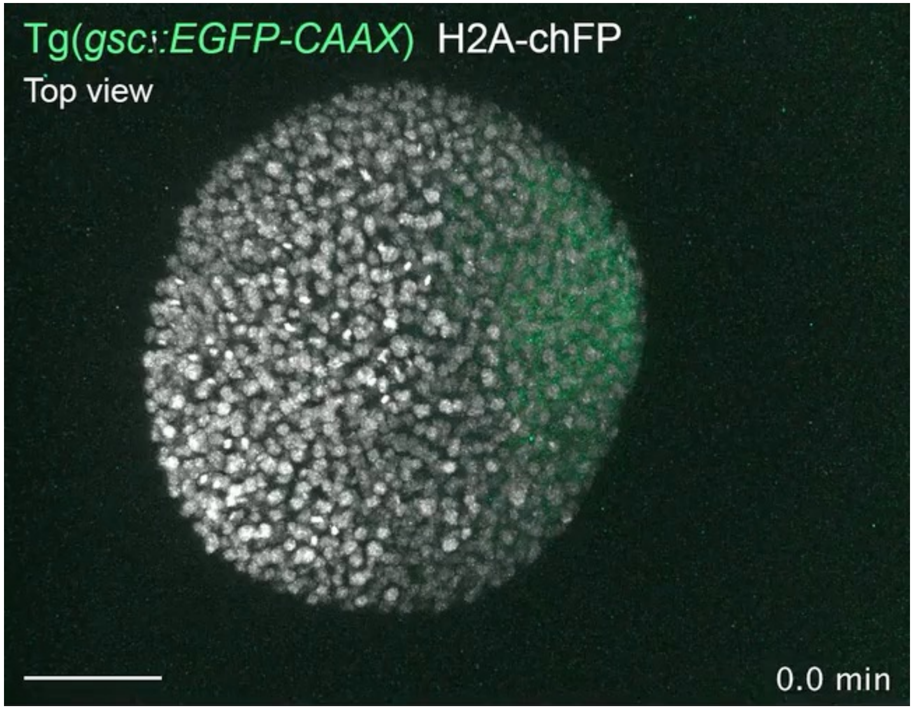
Deconstructing to understand: Zebrafish explants ("pescoids")

Blastoderm explants ("pescoids")



Main source of patterning signals

Anterior mesendoderm marker Nuclei

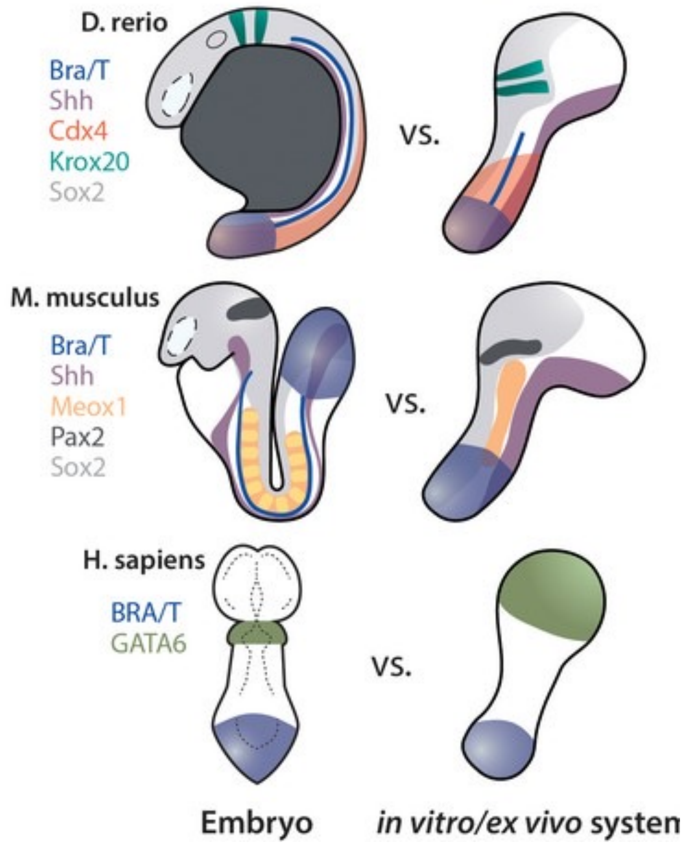


Anterior-Posterior patterning...

...but also robust axis extension by the end of gastrulation.

Schauer A*, Pinheiro D*, et al. 2020 (see also Fulton, T, et al. 2020 and Williams, M, et al. 2020).

Zebrafish ”gastruloids” recapitulate the patterning and morphogenetic programs of intact embryos

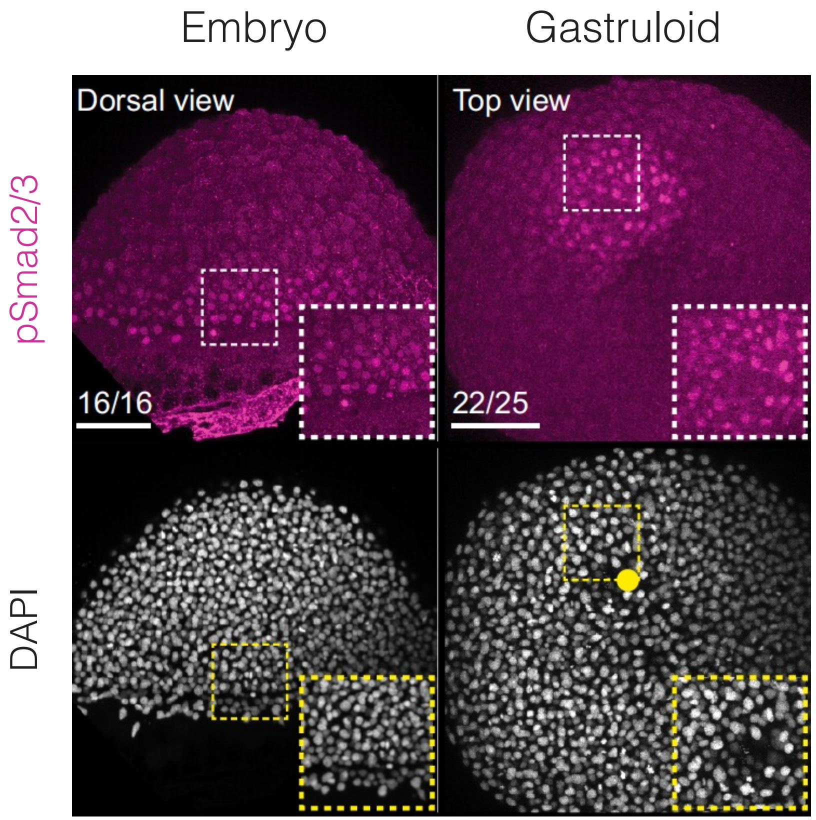


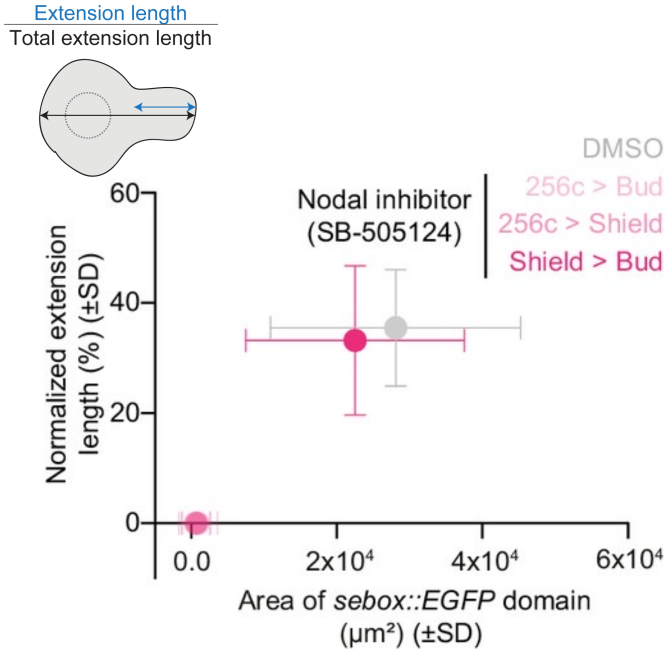
Also shows remarkable similarities with other **PSC-derived gastruloid systems**!

How is this achieved?

Adapted from Anlas, K and Trivedi, V. 2021.
Schauer, A*, Pinheiro, D*, et al. 2020 (see also Fulton, T, et al. 2020, Williams, M, et al. 2020).

Nodal morphogens are essential for both mesendoderm induction and morphogenesis in the “gastruloids”

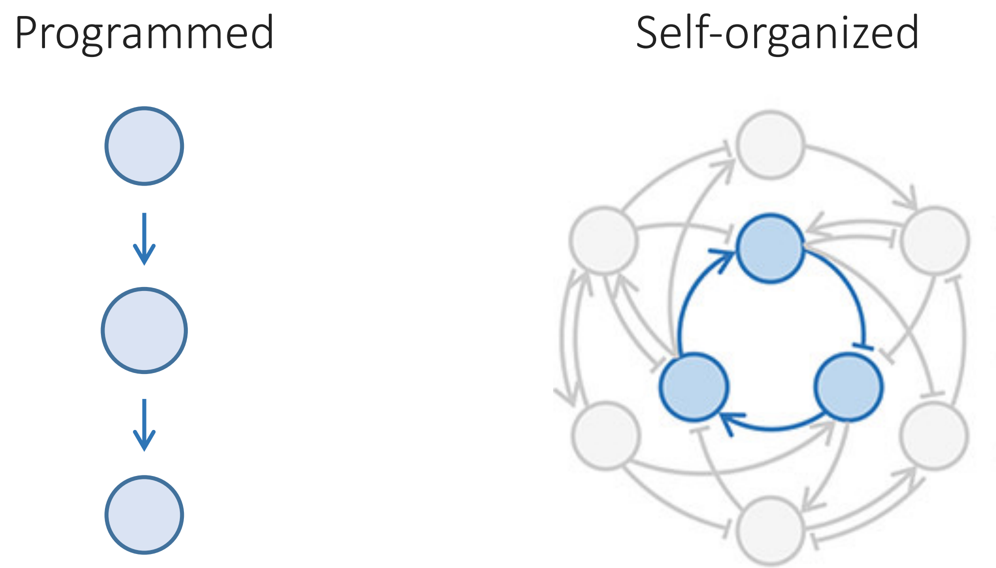




Proxy for mesendoderm specification!

Summary I:

Maternal signals are necessary to initiate Nodal signaling in zebrafish gastruloids

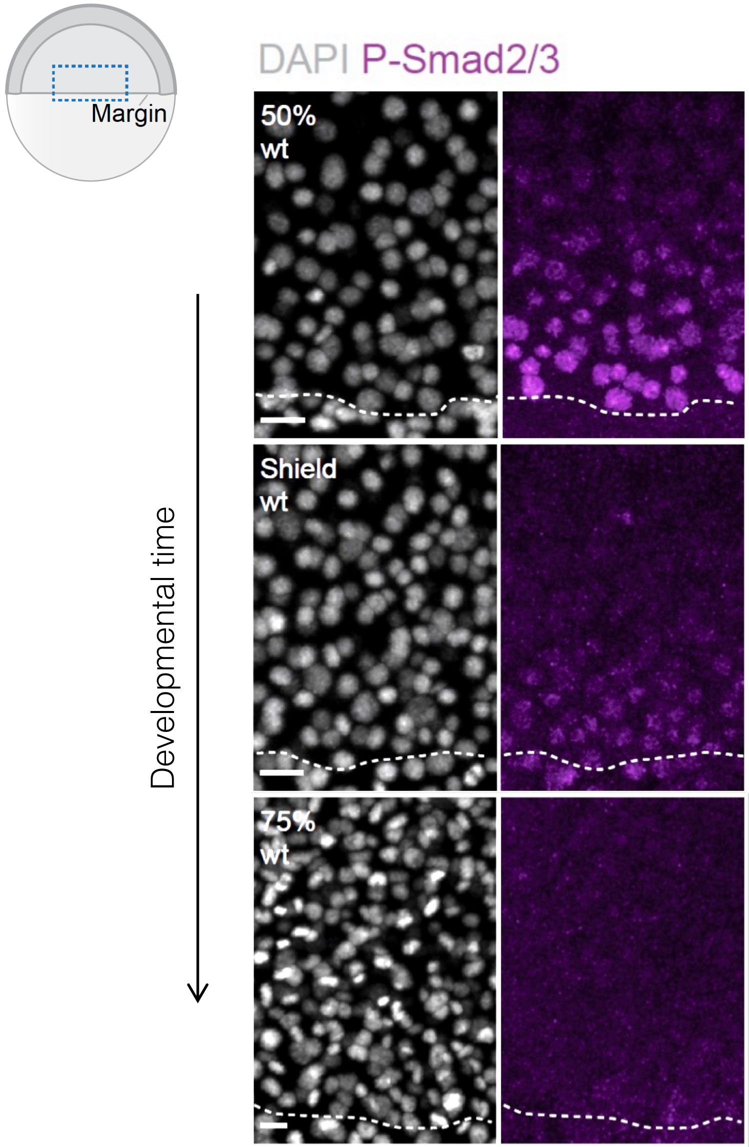


Genetically-encoded self-assembly!

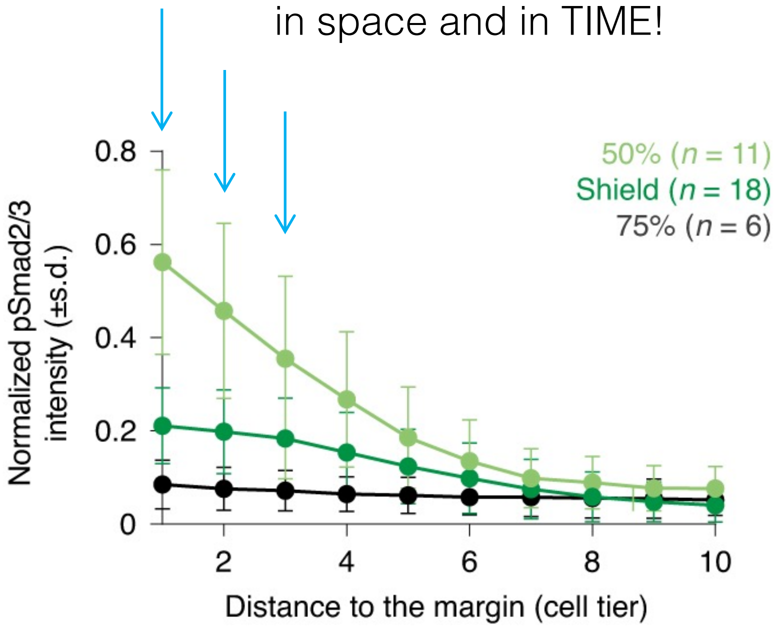
Decouple the role of Nodal vs. cell fate in organizing morphogenesis!

How are patterning and morphogenesis coordinated *in vivo*?

Nodal signaling steadily decreases during gastrulation

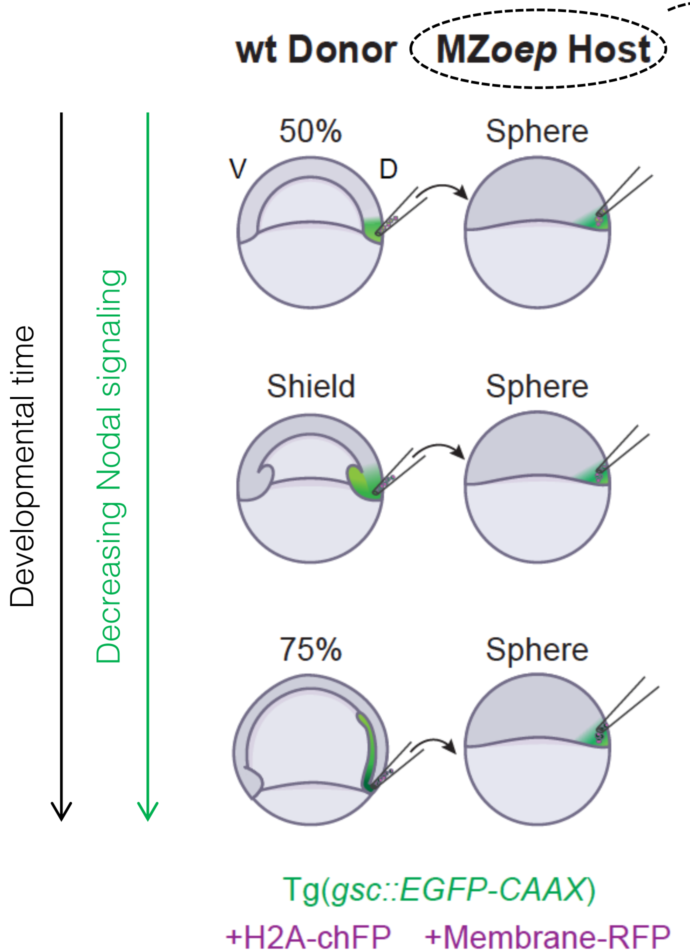


Nodal signaling is dynamic
in space and in TIME!



Pinheiro D, et al. 2022.

Deconstruct to understand:
Link between Nodal signaling and morphogenetic potential?



Lacks both mesendoderm
&
internalization movements…

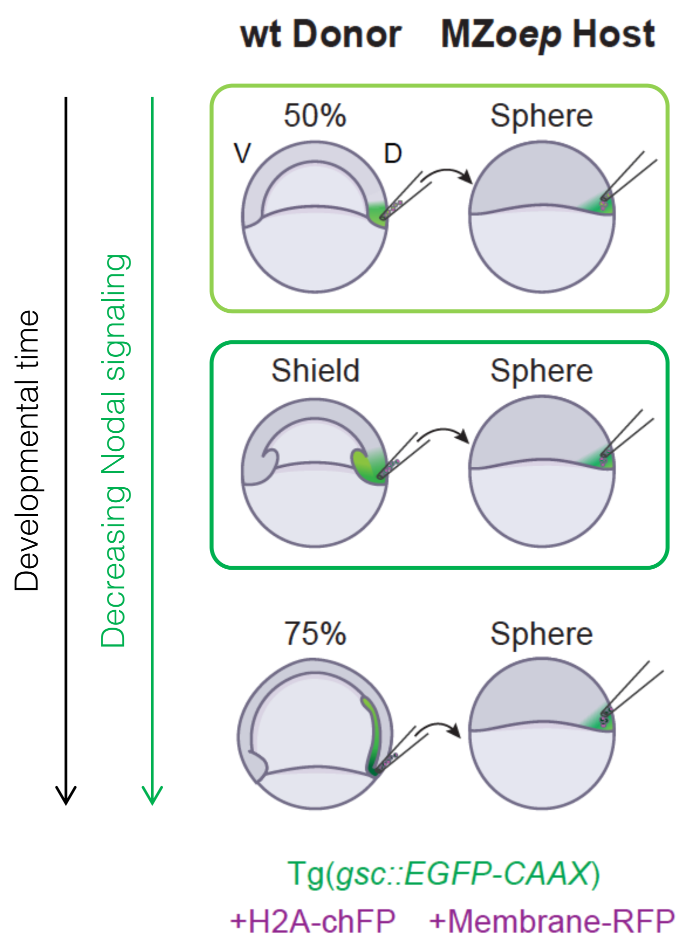
Autonomous migratory capacity of the donor cells…

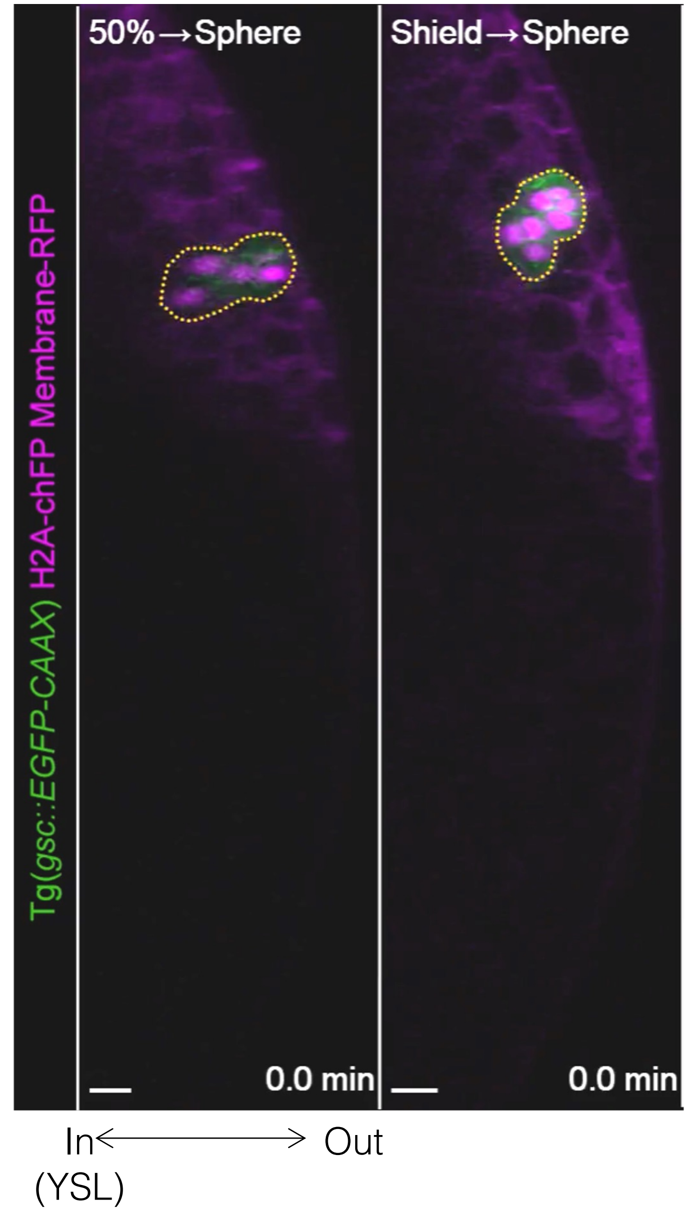
vs.

…collective effects arising during normal gastrulation movements.

*Inspired by classical experiments from: Carmany-Rampey, Schier. 2001; David, Rosa. 2001.

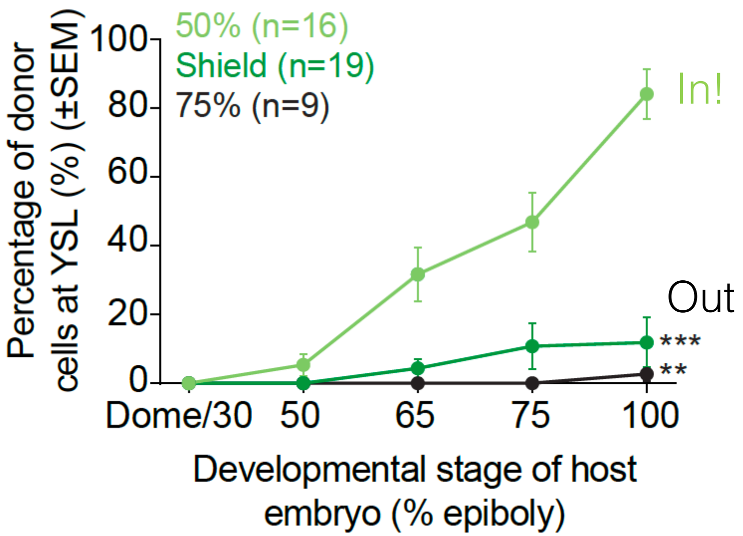
Internalization capacity of mesendoderm cells decays abruptly during gastrulation





*YSL = Yolk Synctictial Layer (In!)

Internalization capacity of mesendoderm cells decays abruptly during gastrulation



Key implications…

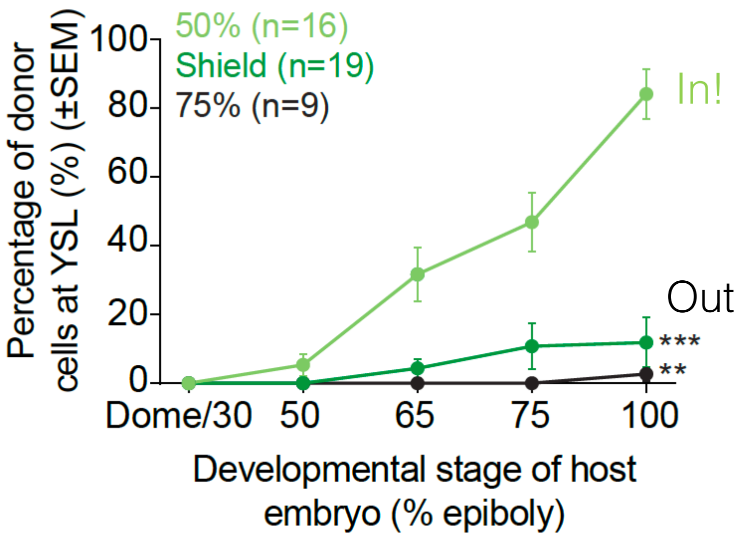
Later mesendoderm cells (w/decreasing Nodal signaling) are unable to internalize autonomously.

Nodal, and not mesendoderm commitment *per se*, is sufficient to regulate cell internalization.

*YSL = Yolk Synctidial Layer (In!)

See also Liu et al. 2018.

Internalization capacity of mesendoderm cells decays abruptly during gastrulation



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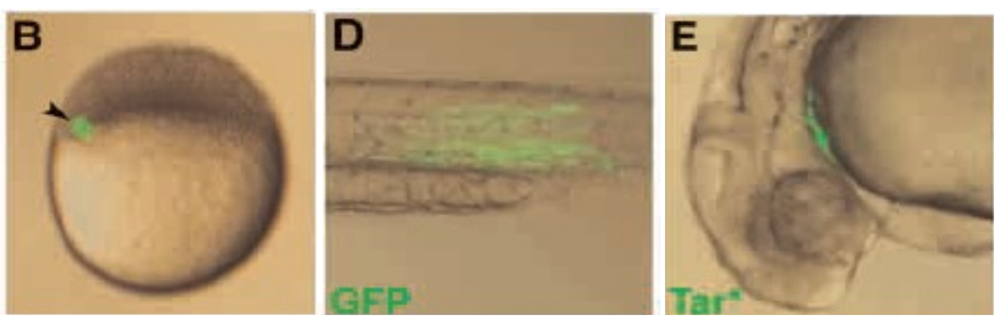
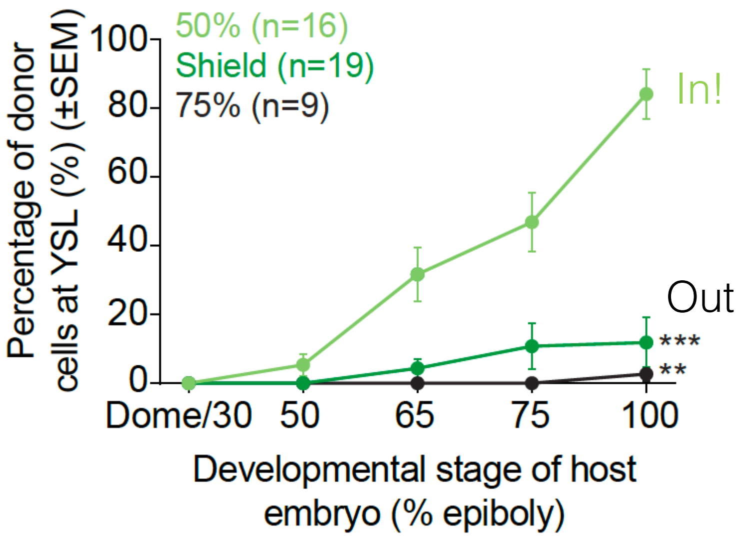


Even a single mesendoderm cell can internalize autonomously!

*YSL = Yolk Synctícial Layer (In!)

See also Liu et al. 2018; David, Rosa. 2001.

Internalization capacity of mesendoderm cells decays abruptly during gastrulation



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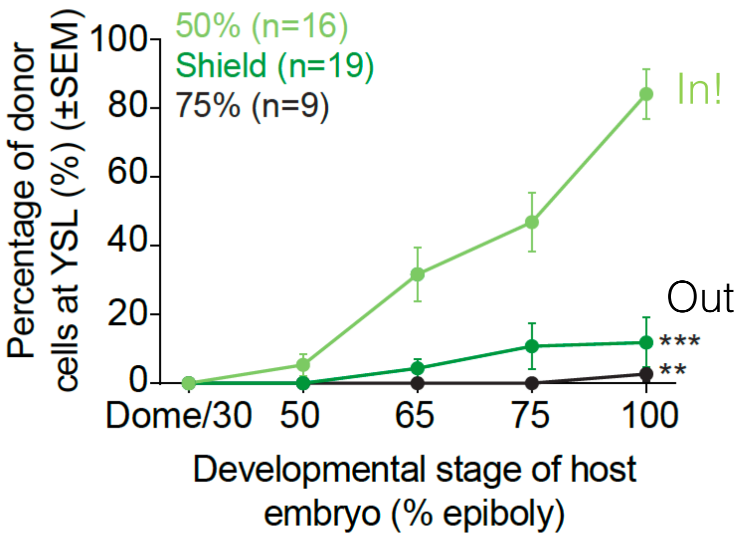
How do later mesendoderm cells eventually internalize in vivo?

>1500 cells internalizing (in 2h!)

*YSL = Yolk Syncticial Layer (In!)

See also Liu et al. 2018.

Internalization capacity of mesendoderm cells decays abruptly during gastrulation



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Biophysical mechanism by which Nodal controls mesendoderm internalization?

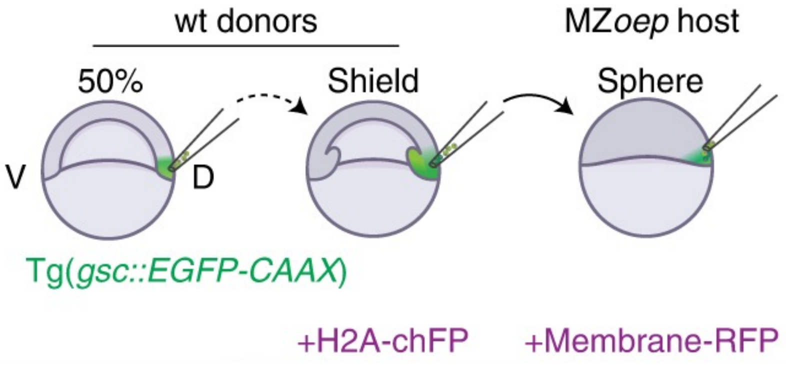
Can this dual role of Nodal couple patterning and morphogenesis?

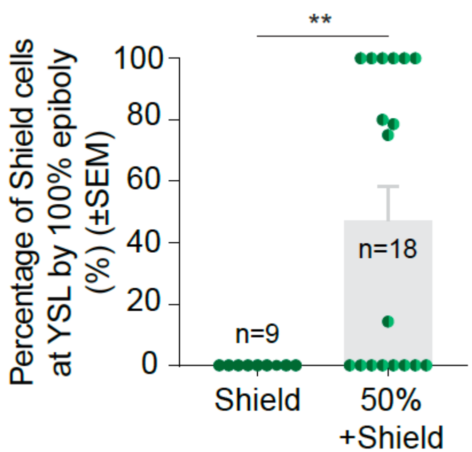
*YSL = Yolk Syncticial Layer (In!)

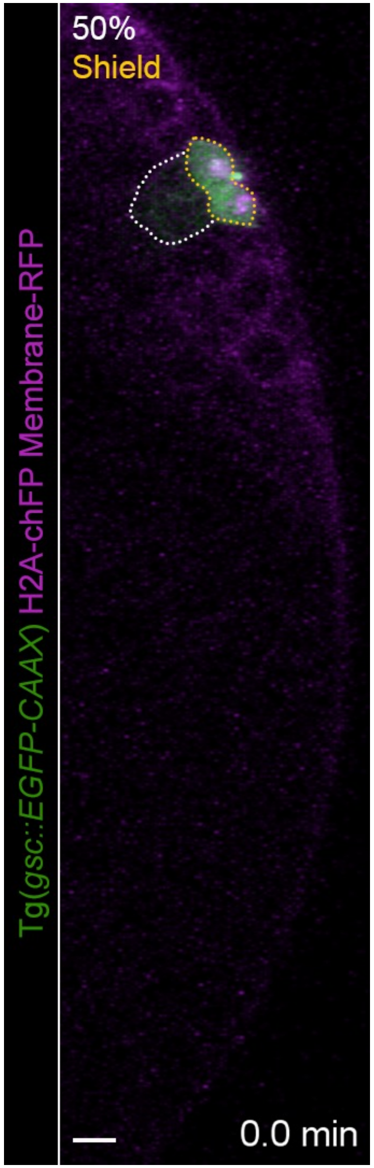
See also Liu et al. 2018.

Contact between early mesendoderm cells with is sufficient to pull later cells towards the inside of the embryo

Collective process?







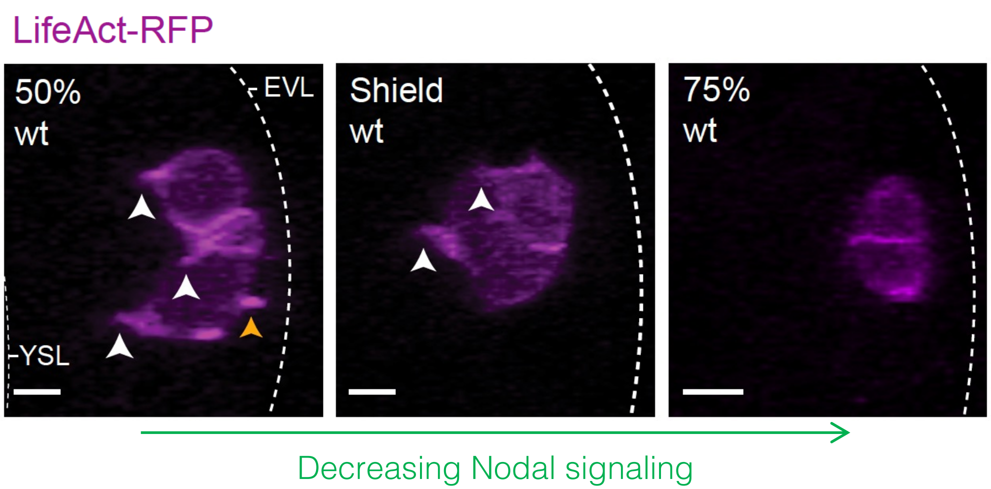
System to recapitulate the minimal morphogenetic unit!

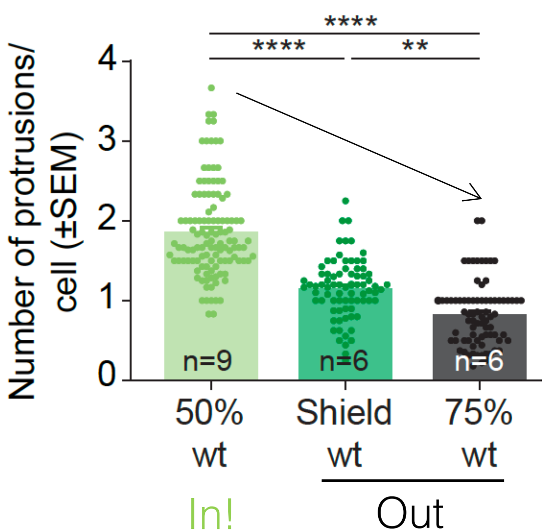
Biophysical basis of leaders vs. followers?

*YSL = Yolk Synctician Layer (In!)

Mesendoderm protrusiveness decays during gastrulation

Active migration forces are involved in mesendoderm internalization. (Krens et al, 2017 and Giger et al. 2017)





No differences in protrusion orientation!

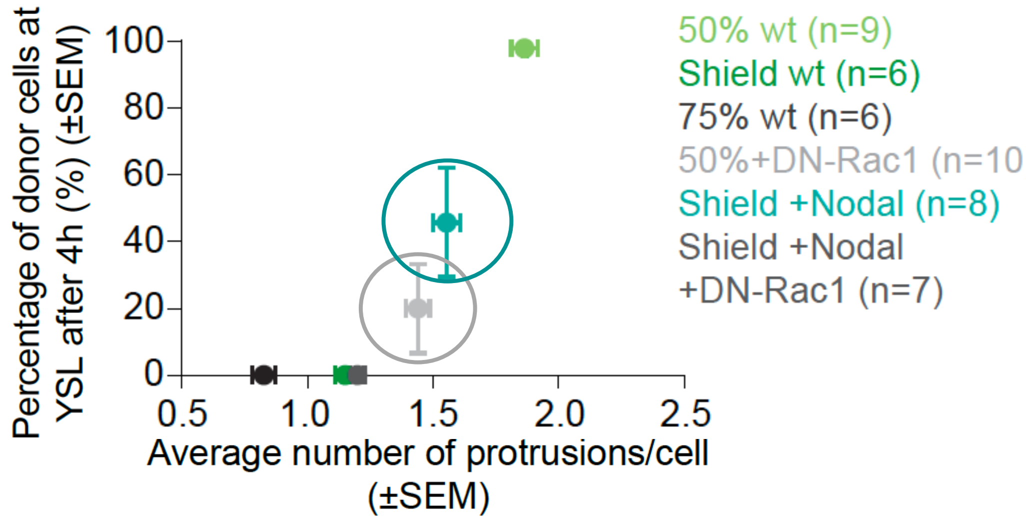
Is it sufficient?

*YSL = Yolk Syncticial Layer (In!) EVL = Enveloping Layer (Out!)

By modulating cell protrusiveness or Nodal signaling, we can convert leaders into followers and vice-versa…

Drastic changes in internalization outcomes

Output displacement



Small changes in protrusion number

Input force

Highly non-linear relationship!

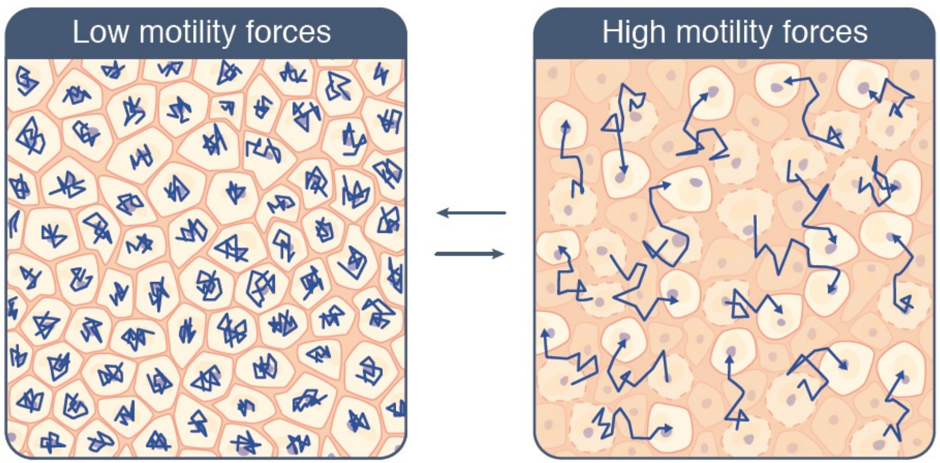
*YSL = Yolk Syncticial Layer (In!)

Exploring the non-linear relationship between mesendoderm protrusiveness and internalization capacity

Motility-driven (un)jamming

(Bi and Manning, 2016; Mitchel JA, et al. 2020)

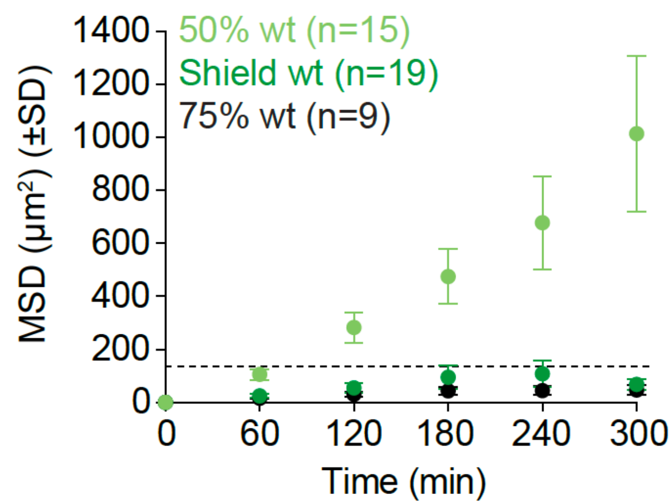
Followers? Leaders?



Jammed **Unjammed**

↓ cell rearrangement rate
Caged motion

↑ cell rearrangement rate
↑ MSD



Can we quantitatively predict internalization outcomes and dynamics?

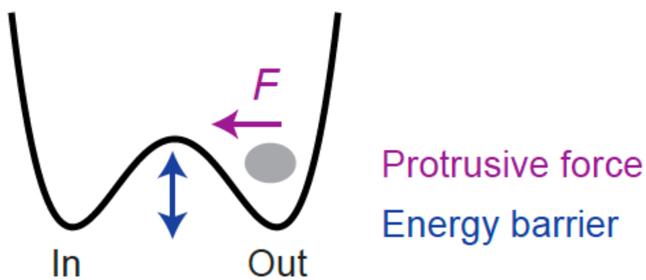
Adapted from Hannezo and Heisenberg. 2022.
See also Stooke-Vaughan, Campàs. 2019; Petridou, et al. 2019; Atia et al. 2021; Lawson-Keister, Manning. 2021.

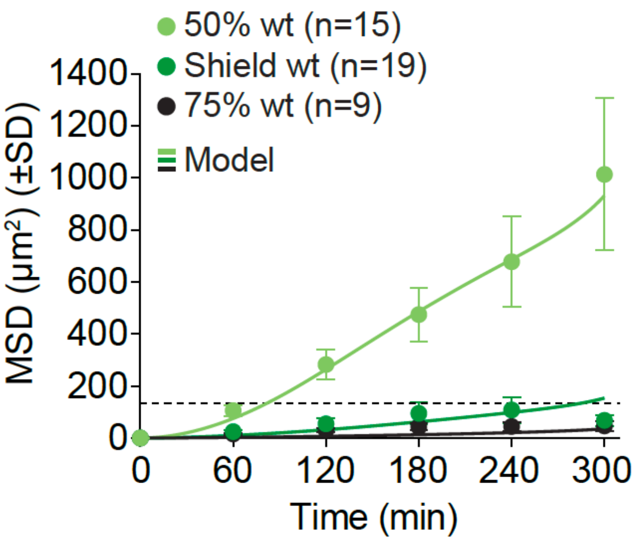
Exploring the non-linear relationship between mesendoderm protrusiveness and internalization capacity

Motility-driven (un)jamming

(Bi and Manning, 2016; Mitchel JA, et al. 2020)

Modeled as a bi-stable energy landscape.





In collaboration with Édouard Hannezo (IST Austria).

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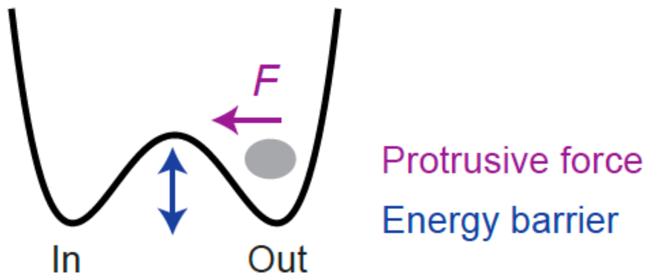

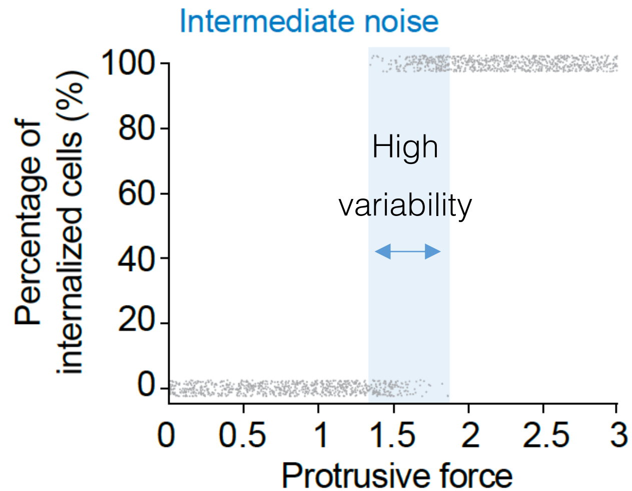

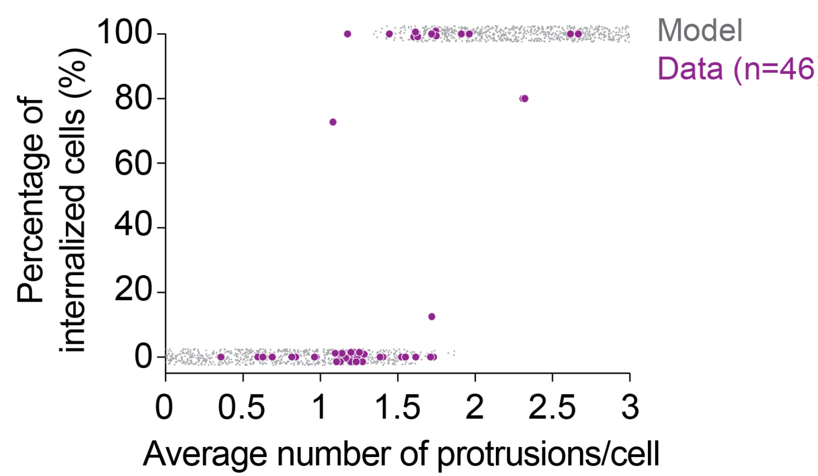

In collaboration with Édouard Hannezo (IST Austria).

Nodal signaling regulates mesendoderm cell protrusiveness and internalization capacity via a motility-driven unjamming transition

Motility-driven (un)jamming

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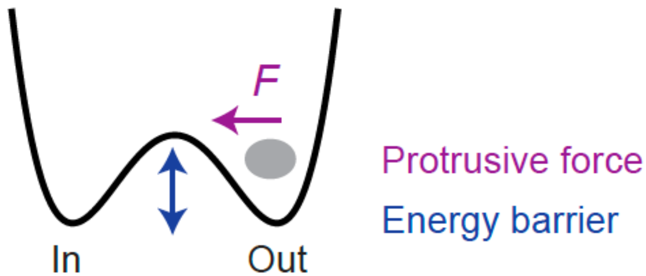
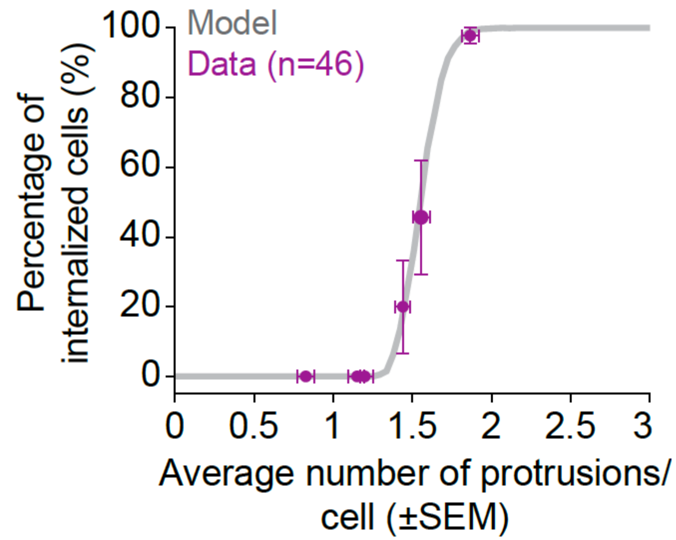


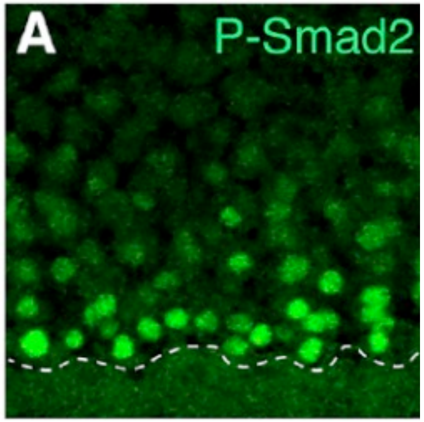


In collaboration with Édouard Hannezo (IST Austria).

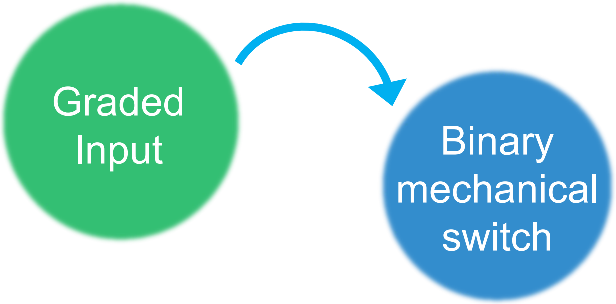
Nodal signaling regulates mesendoderm cell protrusiveness and internalization capacity via a motility-driven unjamming transition

Motility-driven (un)jamming

(Bi and Manning, 2016; Mitchel JA, et al. 2020)



Van Boxtel M, *et al*. 2017.

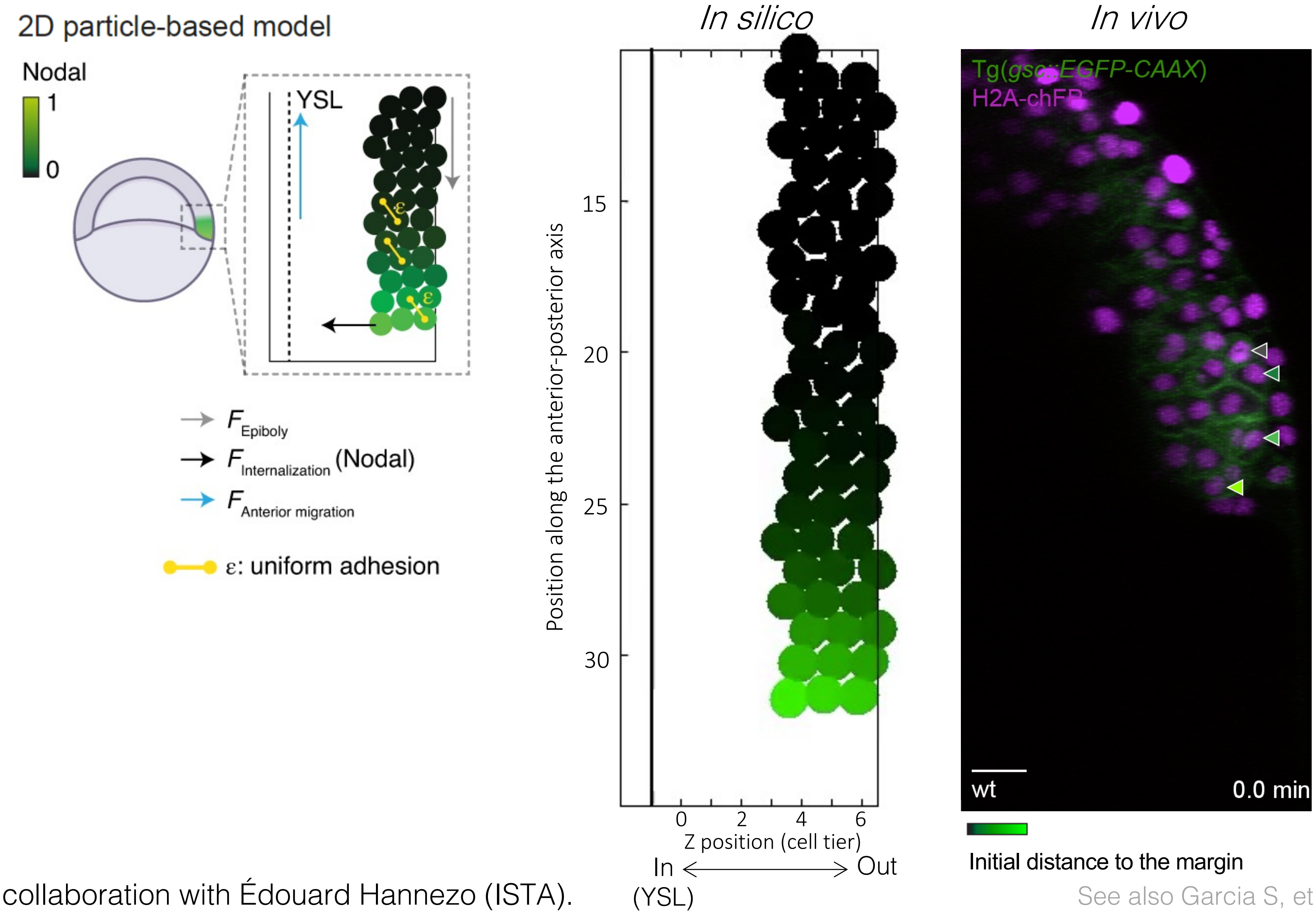


Beyond the transplants: What about the intact embryo??

Can this dual role of Nodal couple patterning and morphogenesis?

In collaboration with Édouard Hannezo (IST Austria).

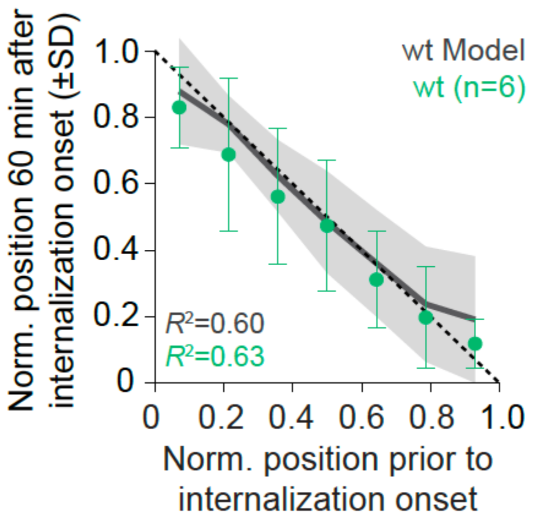
A small proportion of unjammed leaders is sufficient to trigger tissue-scale internalization movements



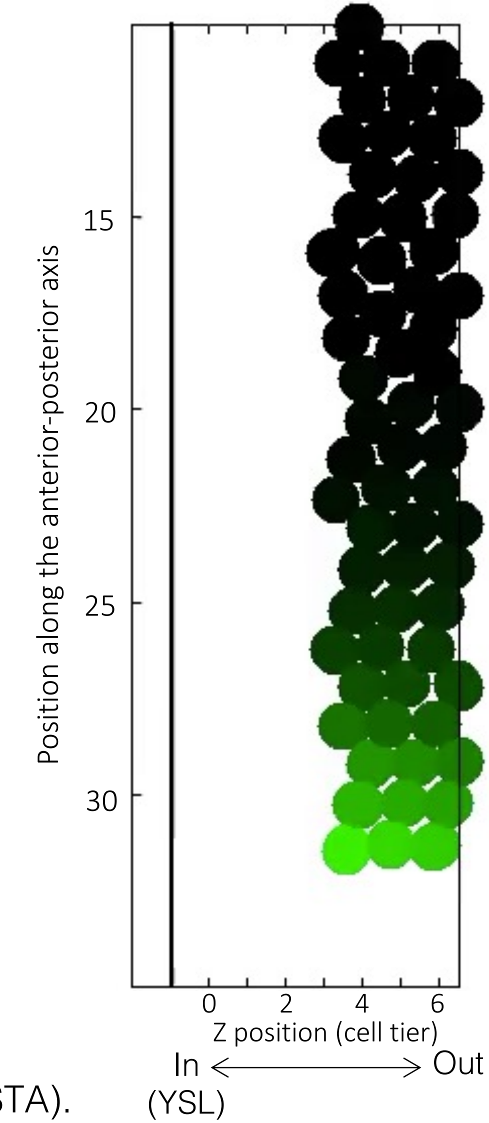
In collaboration with Édouard Hannezo (ISTA).

See also Garcia S, et al. 2015.

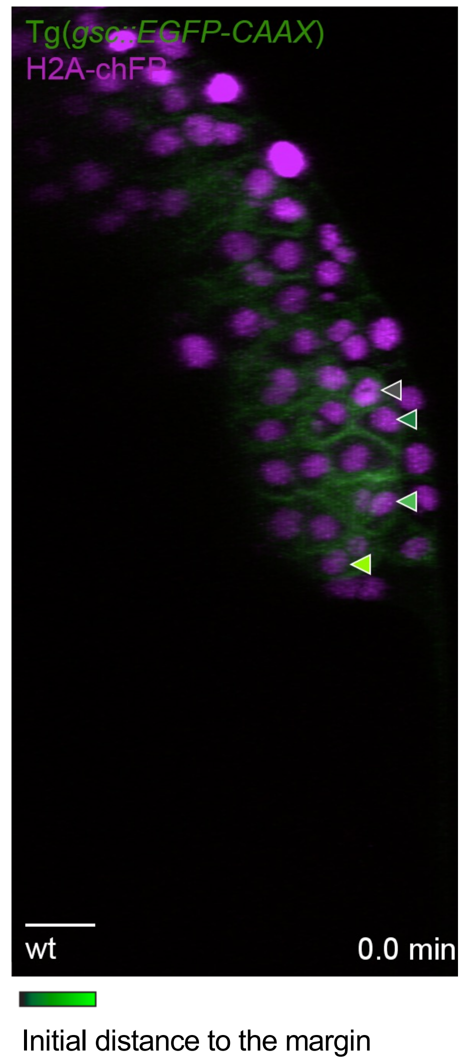
Nodal not only encodes positional information, but also ensures it is preserved despite complex morphogenesis



In silico



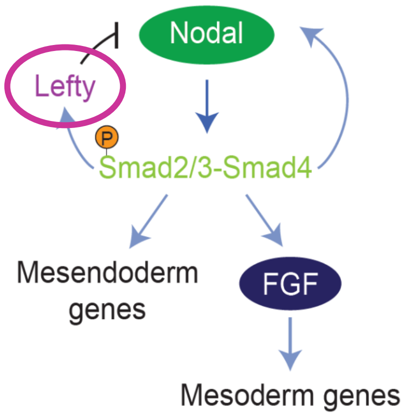
In vivo

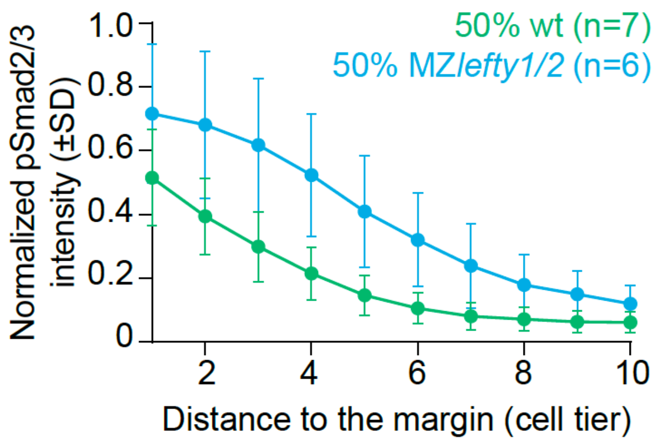


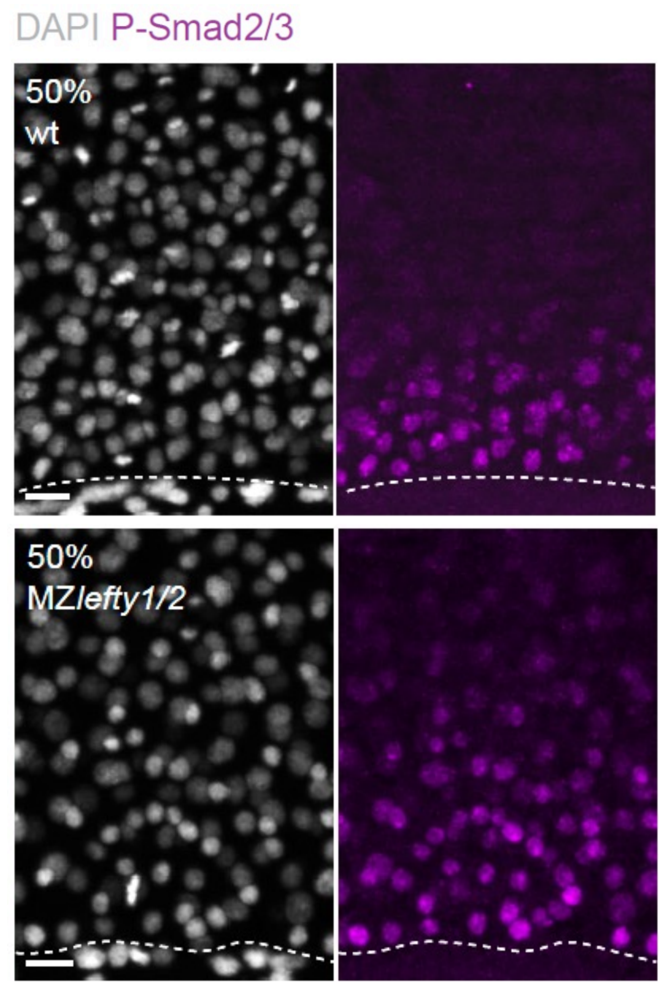
In collaboration with Édouard Hannezo (ISTA).

See also Garcia S, et al. 2015.

Challenging the model: manipulating the Nodal signaling gradient *in vivo*







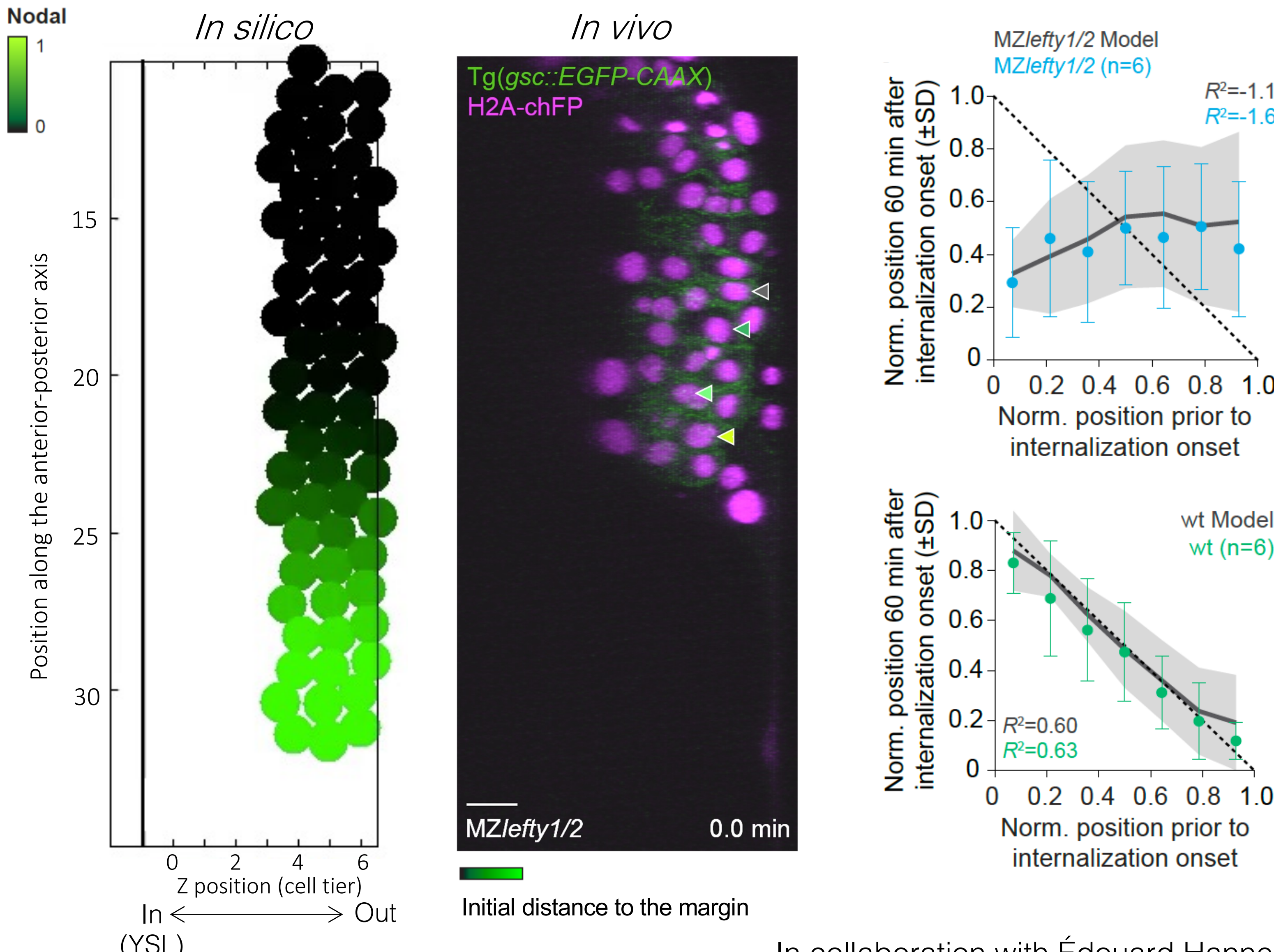
Prediction:

Expanded Nodal signaling gradient → Higher number of leader cells.

*Dashed lines: Deep cell margin.

Also in Rogers, KW et al. 2017.

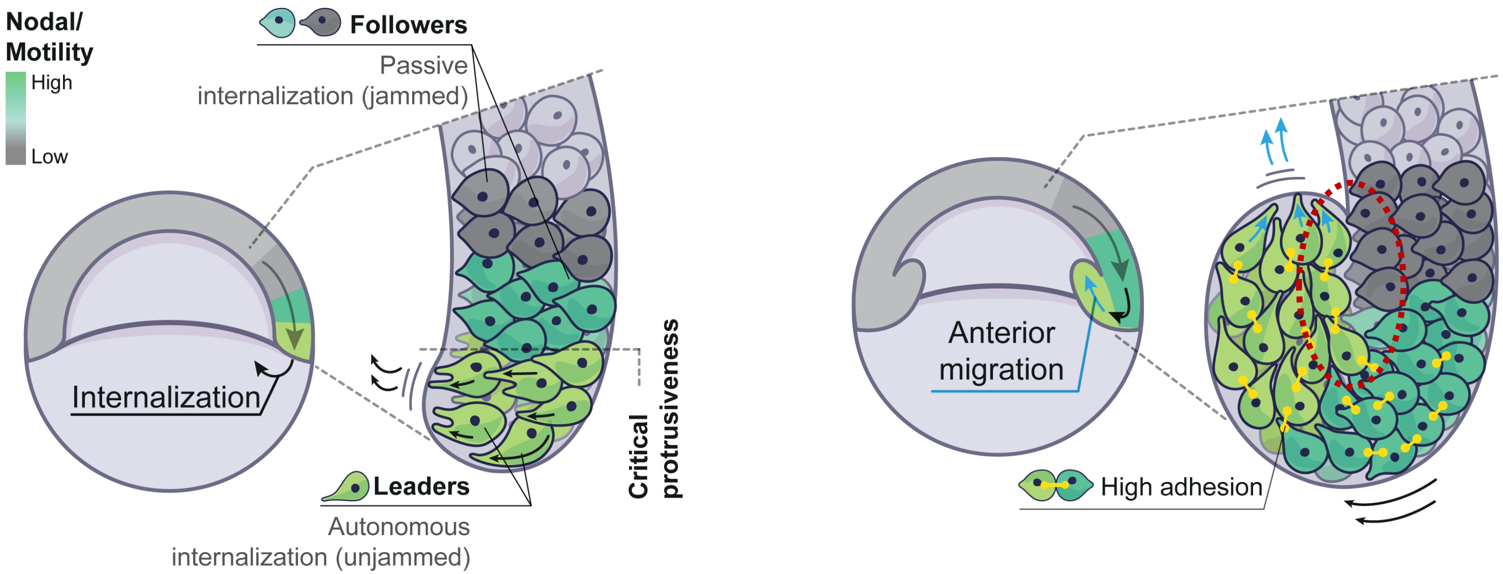
The shape of the Nodal gradient determines the proportion of leader/follower cells



In collaboration with Édouard Hannezo (ISTA).

Summary II:

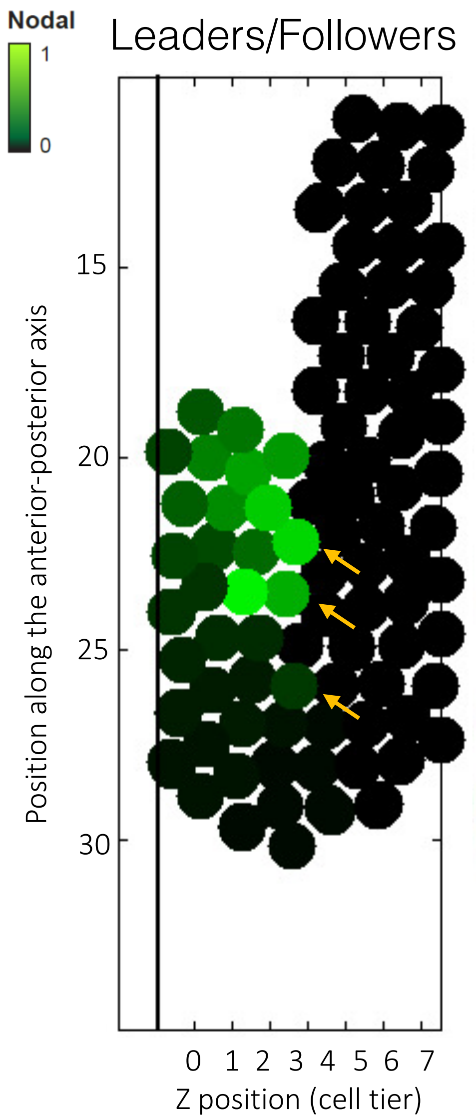
Nodal signaling triggers ordered mesendoderm internalization, via motility-driven unjamming

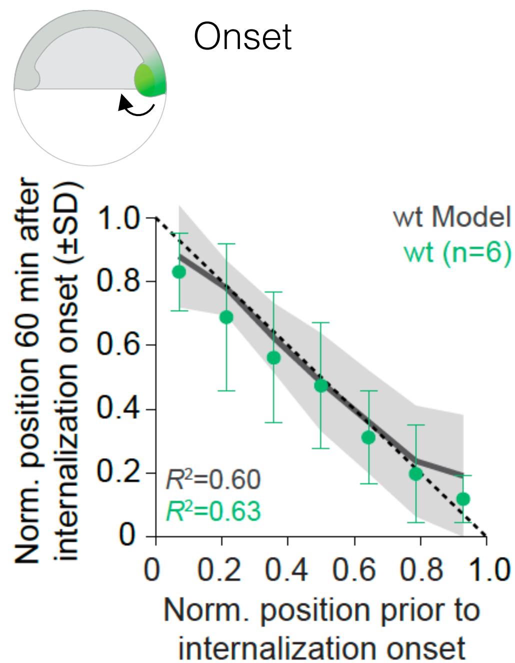


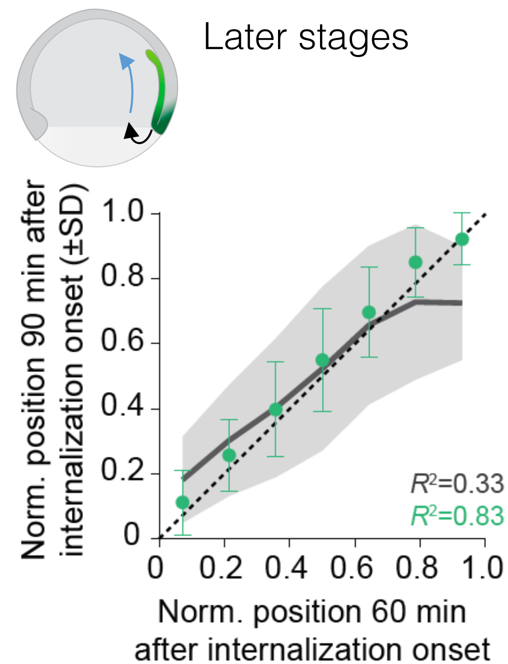
Motility-driven unjamming triggers tissue-scale internalization.

Is it sufficient?

Positional order is preserved even at later stages of mesendoderm internalization in vivo







Collective internalization critically requires tight cell-cell adhesion…

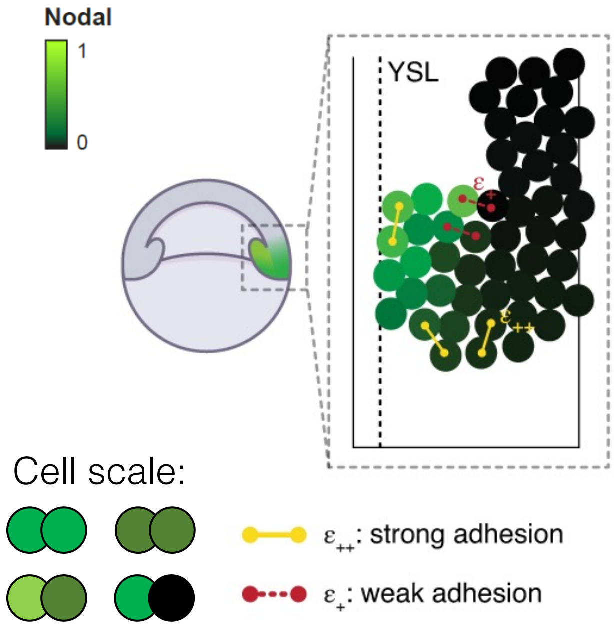
But how to ensure the leaders pull the right followers?

In collaboration with Édouard Hannezo (IST Austria).

*YSL: Yolk syncytial layer (In!)

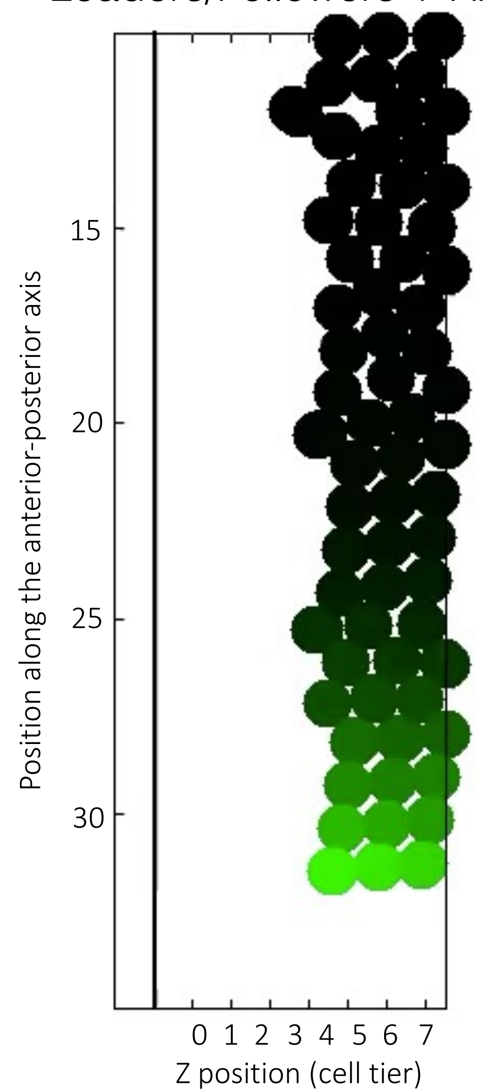
Combining a spatiotemporal pattern of leader/followers with heterotypic adhesion fully recapitulates gastrulation movements

2D particle-based model



Heterotypic adhesion

Leaders/Followers + HA



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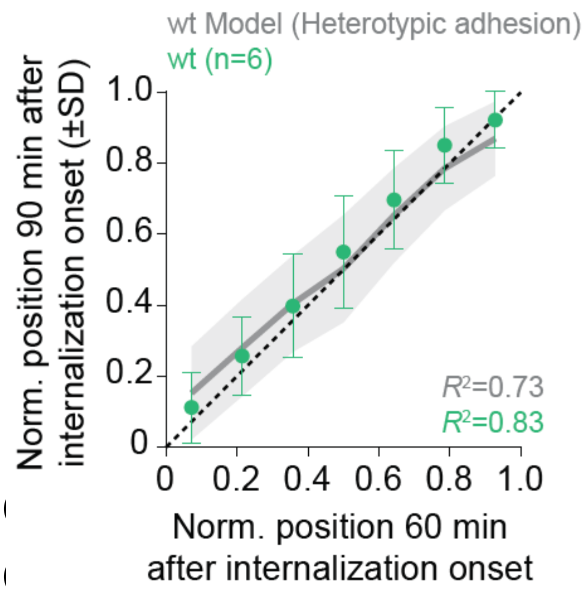
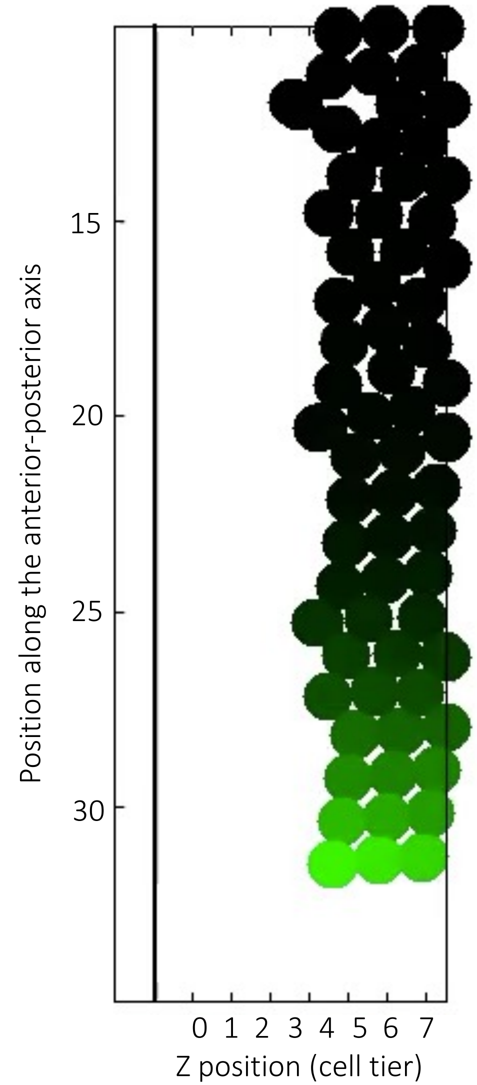


Heterotypic adhesion

Leaders/Followers + HA

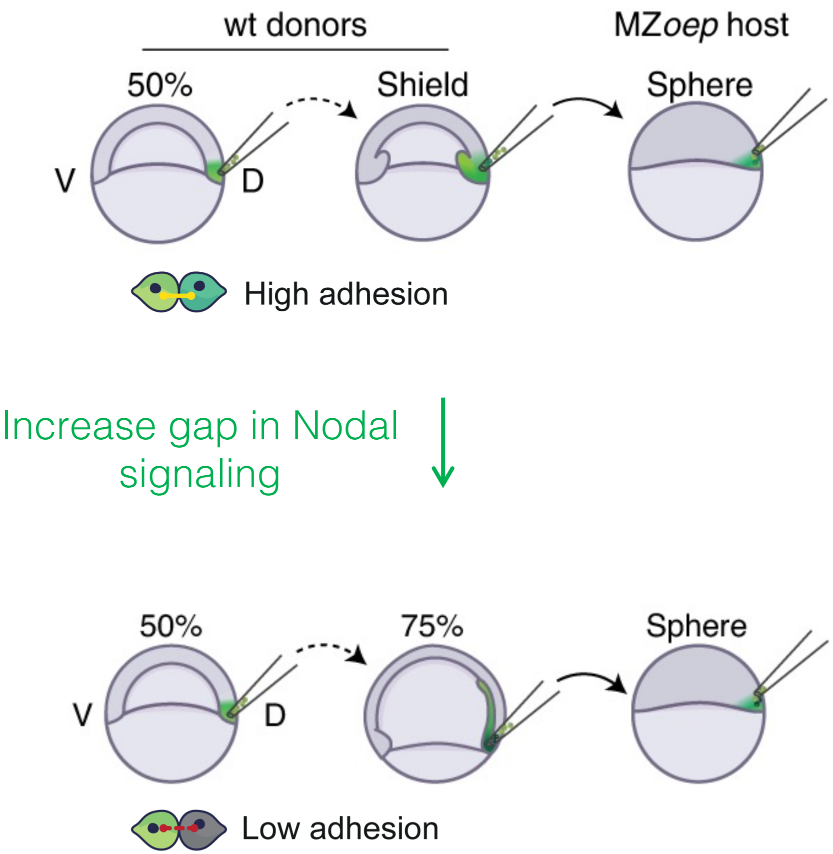


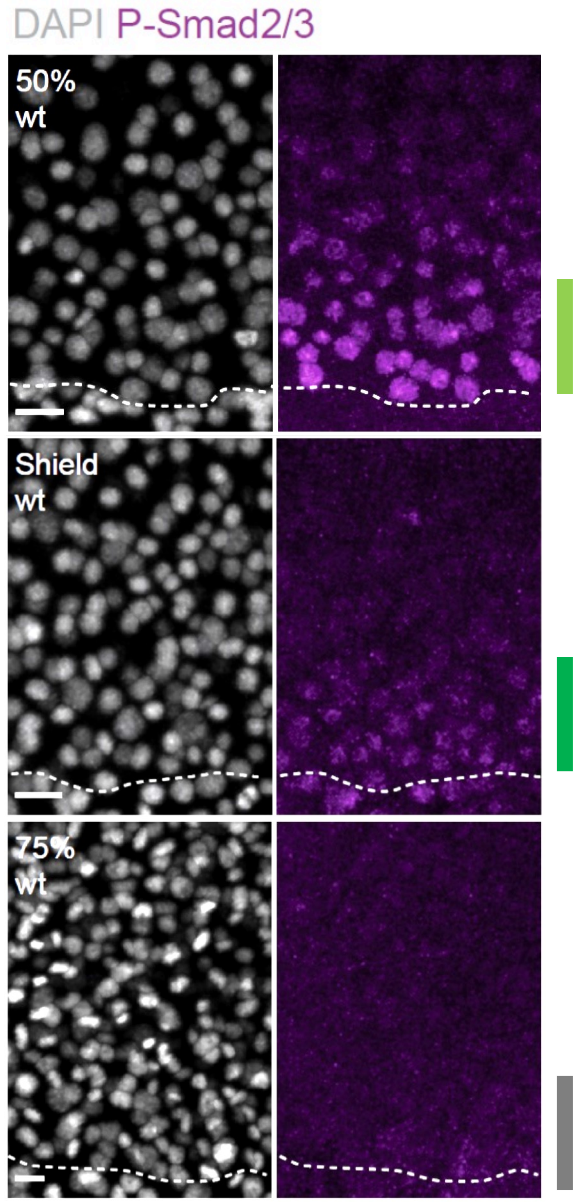
Signatures of heterotypic adhesion in vivo?

In collaboration with Édouard Hannezo (IST Austria).

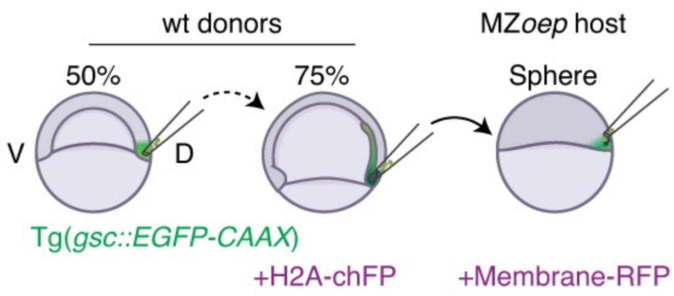
*YSL: Yolk syncytial layer (In!)

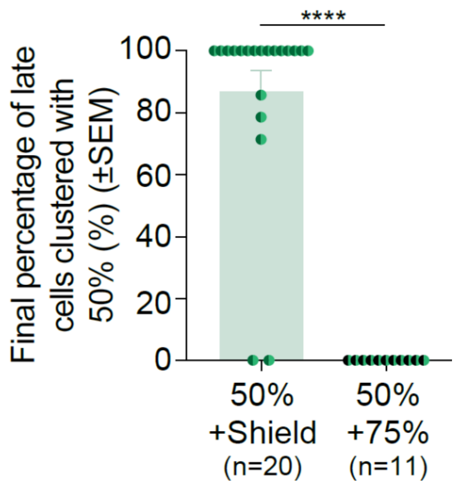
Using transplantation assays to determine cell-cell adhesion strength amongst mesendoderm cells

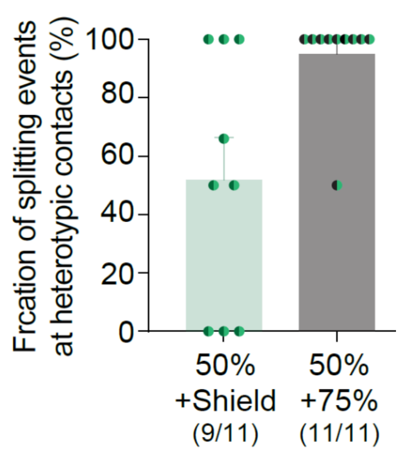


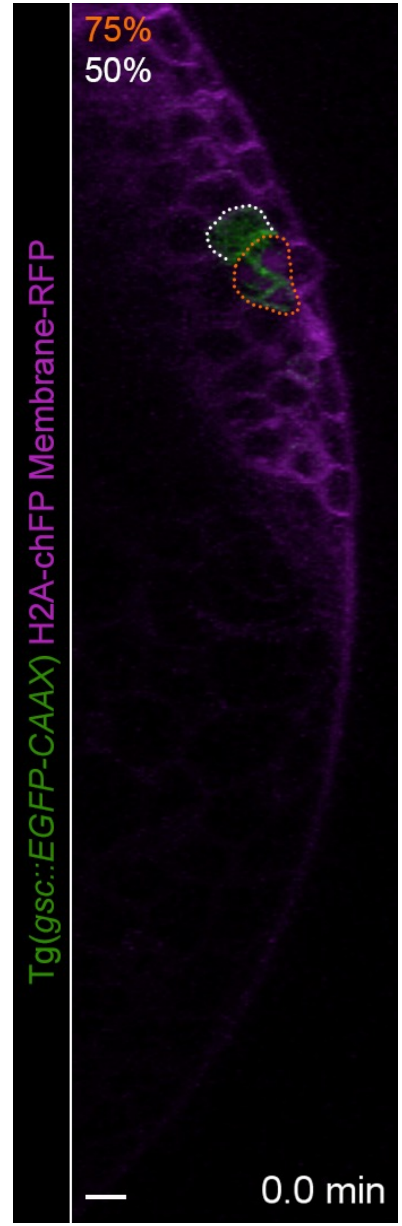


Widening the gap in Nodal signaling is sufficient to impair mesendoderm cohesion







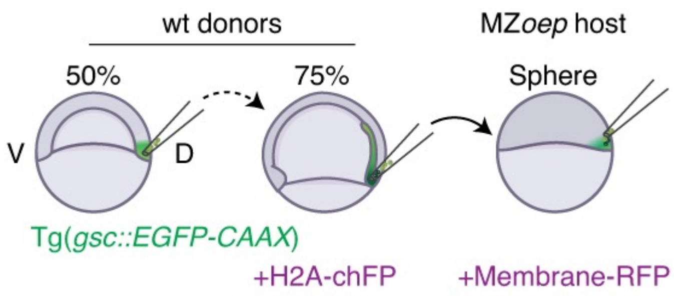


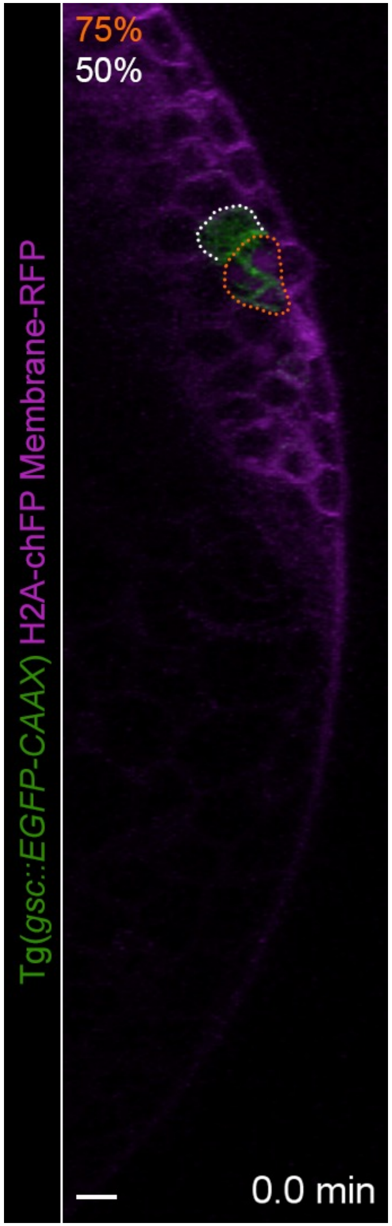
This preferential loss of heterotypic contacts,

suggests that mesendoderm cells can indeed discriminate among its 'followers'.

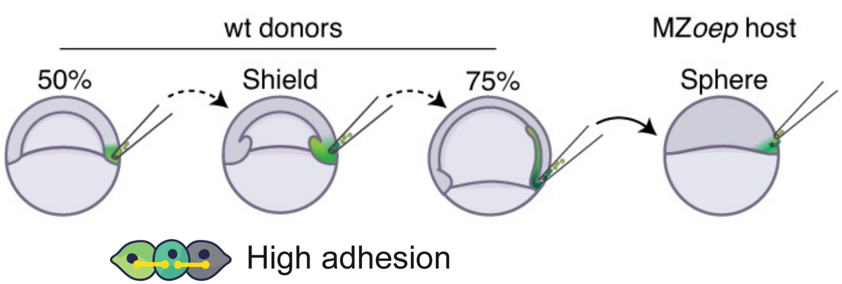
*YSL = Yolk Syncticial Layer (In!)

Widening the gap in Nodal signaling is sufficient to impair mesendoderm cohesion



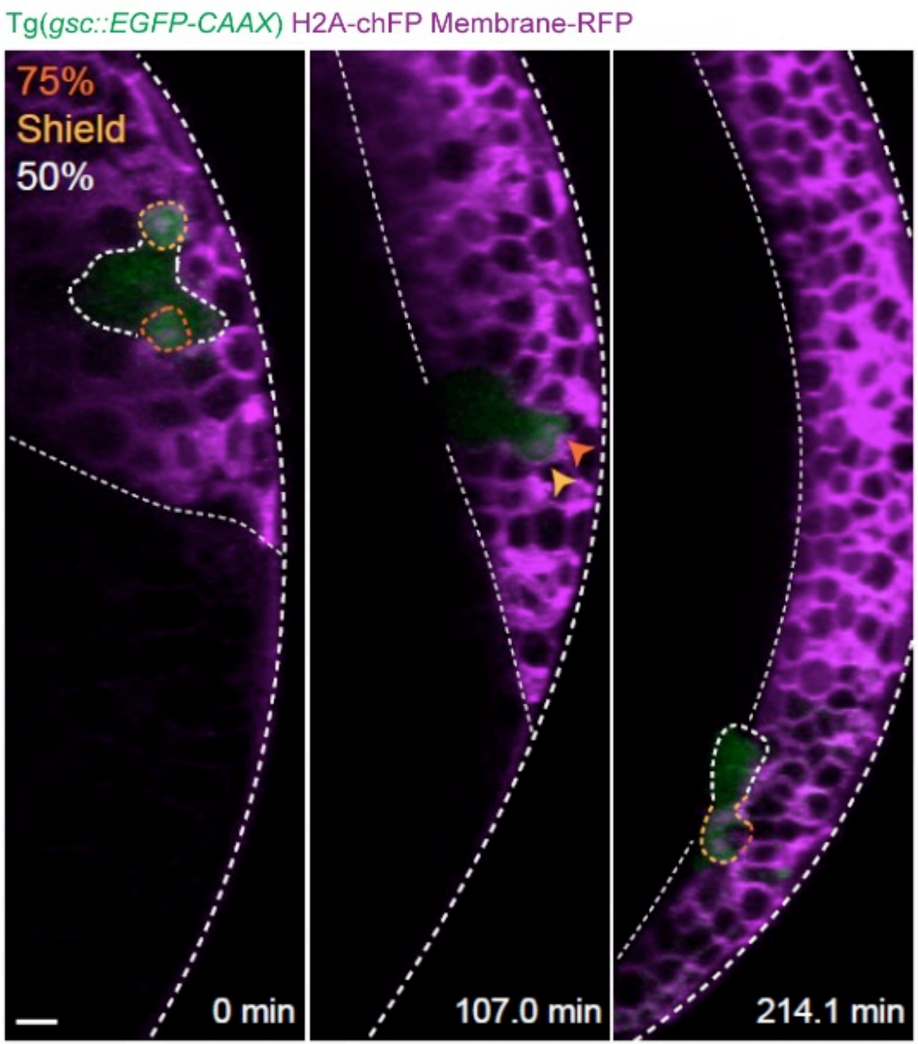


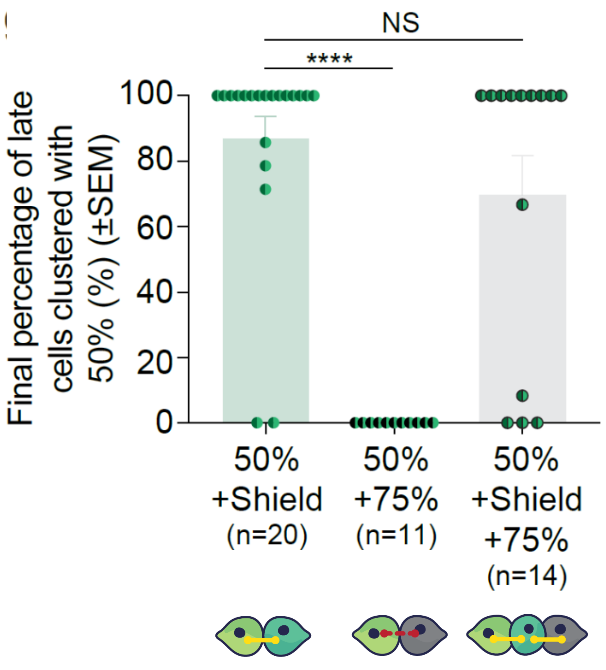
Can we rebuild tissue-level adhesion?



*YSL = Yolk Syncticial Layer (In!)

Re-establishing the Nodal gradient is sufficient to rescue mesendoderm cohesion





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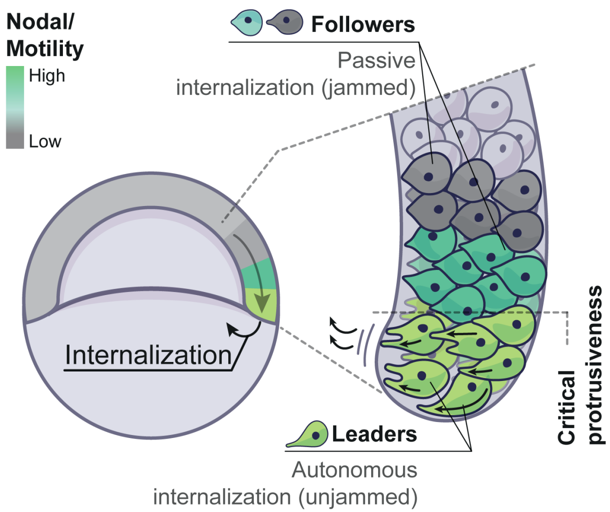
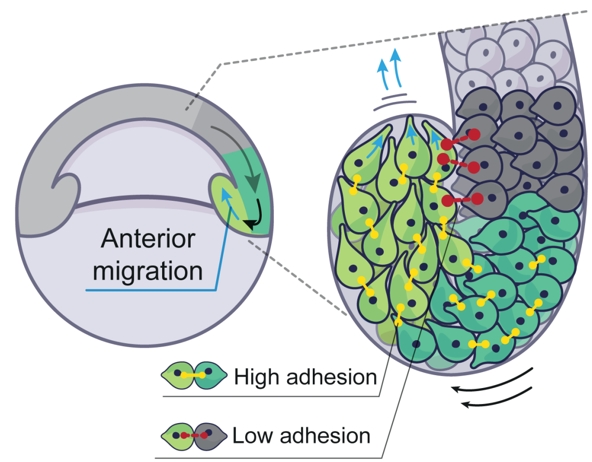
Summary III:

Nodal signaling triggers ordered mesendoderm internalization, via motility-driven unjamming



Motility-driven unjamming triggers tissue-scale internalization.



An adhesion code links the leaders to the right follower cells.

Self-preserving gradient!

Couples tissue patterning + morphogenesis across scales.

<u>Heterotypic adhesion</u> is emerging as a highly efficient mechanism to achieve tissue segregation.

(Canty et al, 2017, Sahu et al. 2021, Barua and Winklbauer. 2022, Guo et al. 2022)

Acknowledgements

Heisenberg Team

Roland Kardos

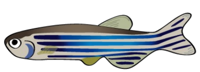
Feyza Nur Arslan

Alexandra Schauer

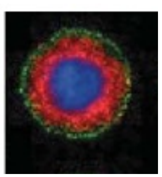
….

Édouard Hannezo

Uday Guabbala



Zebrafish embryo



Gastruloid disc

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(incoming)