

Shaping the amniote embryo: Cellular mechanisms and mechanics of morphogenesis



Octavian Voiculescu

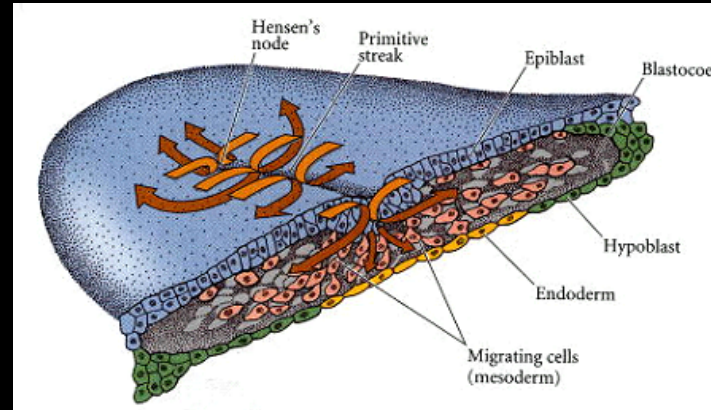
**University College London
Cell & Developmental Biology**

**University of Cambridge
Physiology, Development and Neuroscience**

Gastrulation

What is it?

Generating (two) inner layers from the superficial one



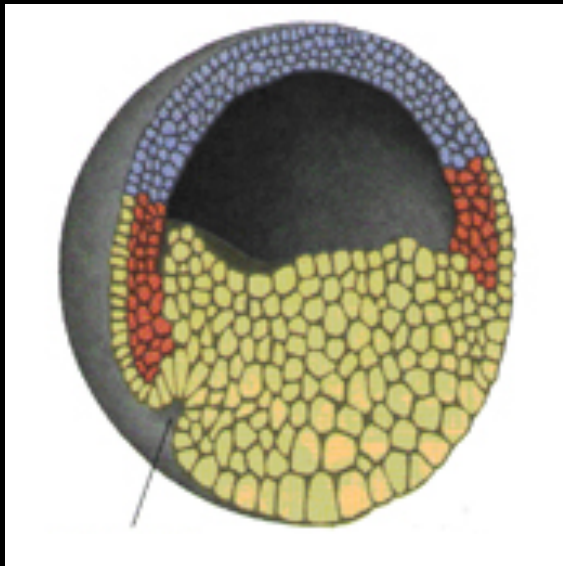
How important is it?

Poses non-trivial questions & is the perfect start point in biology

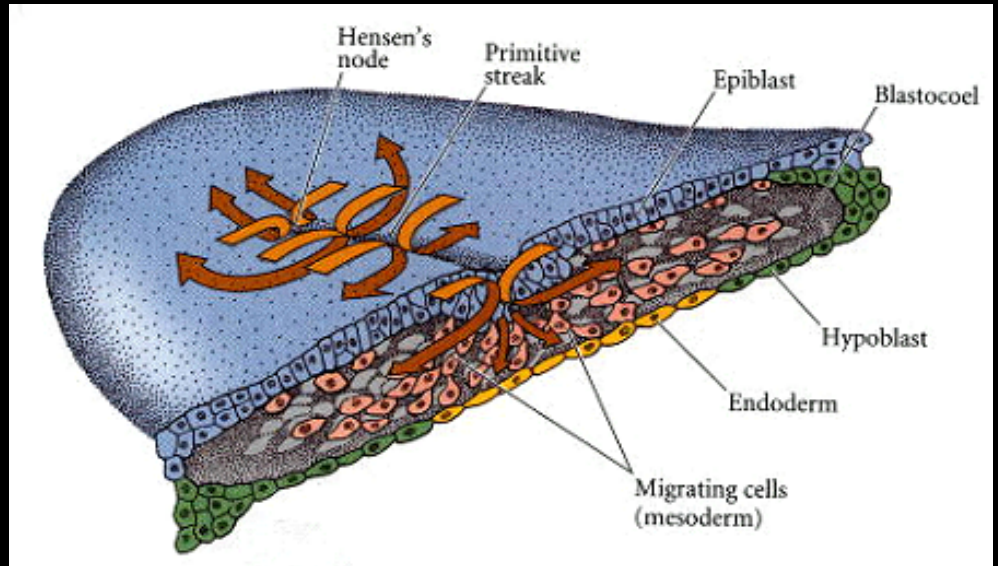
Even the embryos DO care!

How do they do it?

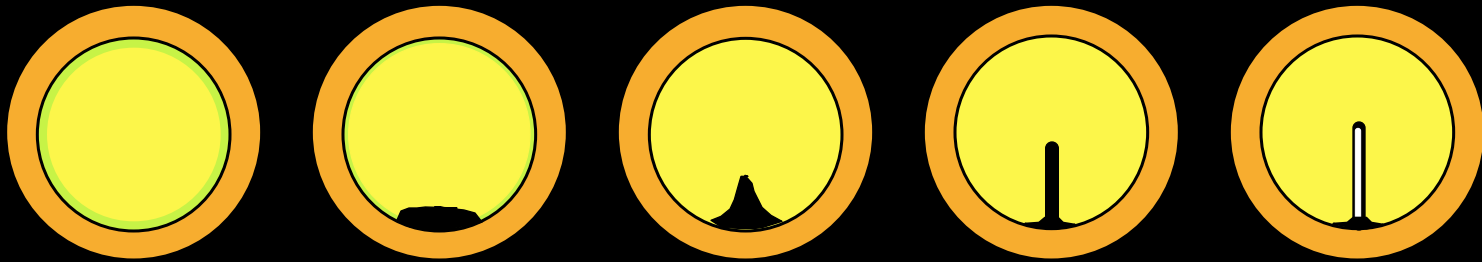
Anamniotes gastrulate through an
“equatorial” blastopore



Amniotes gastrulate through a
“radial” Primitive Streak



Formation of the primitive streak



Polonaise movements

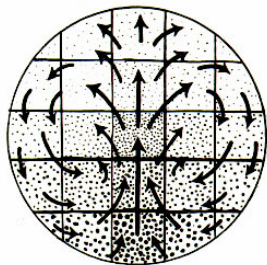


Abb. 91. Vgl. Abb. 1. Schema der Keimfeldbewegungen während der Entstehung des Primitivstreifens: Die Bezirke des formlosen Keimfeldes. Die Pfeile geben die Richtung an, in der sich die Teile des Keimfeldes verschieben werden.

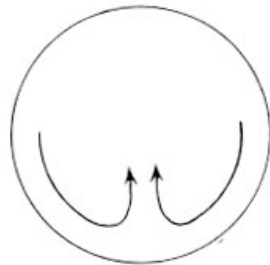
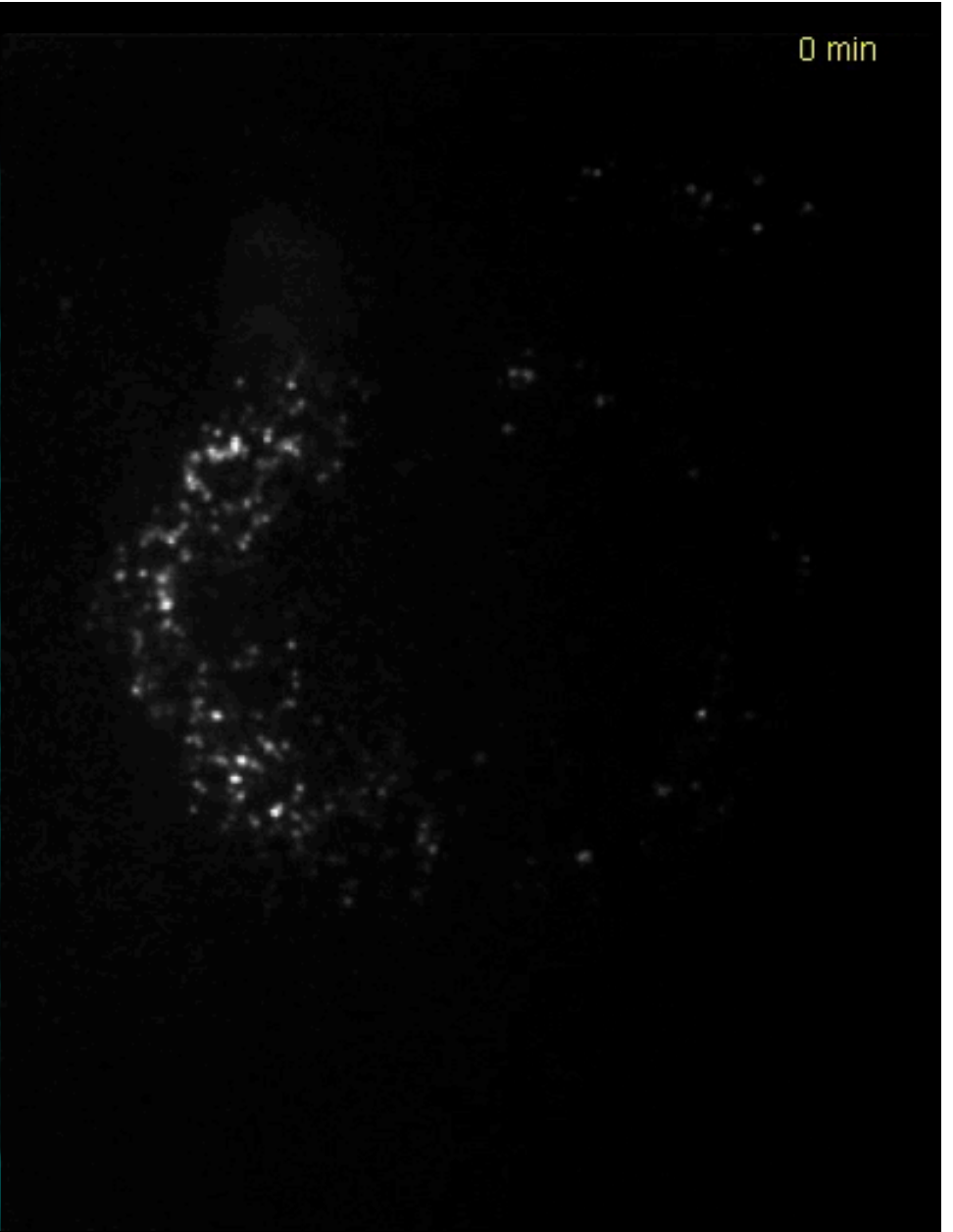
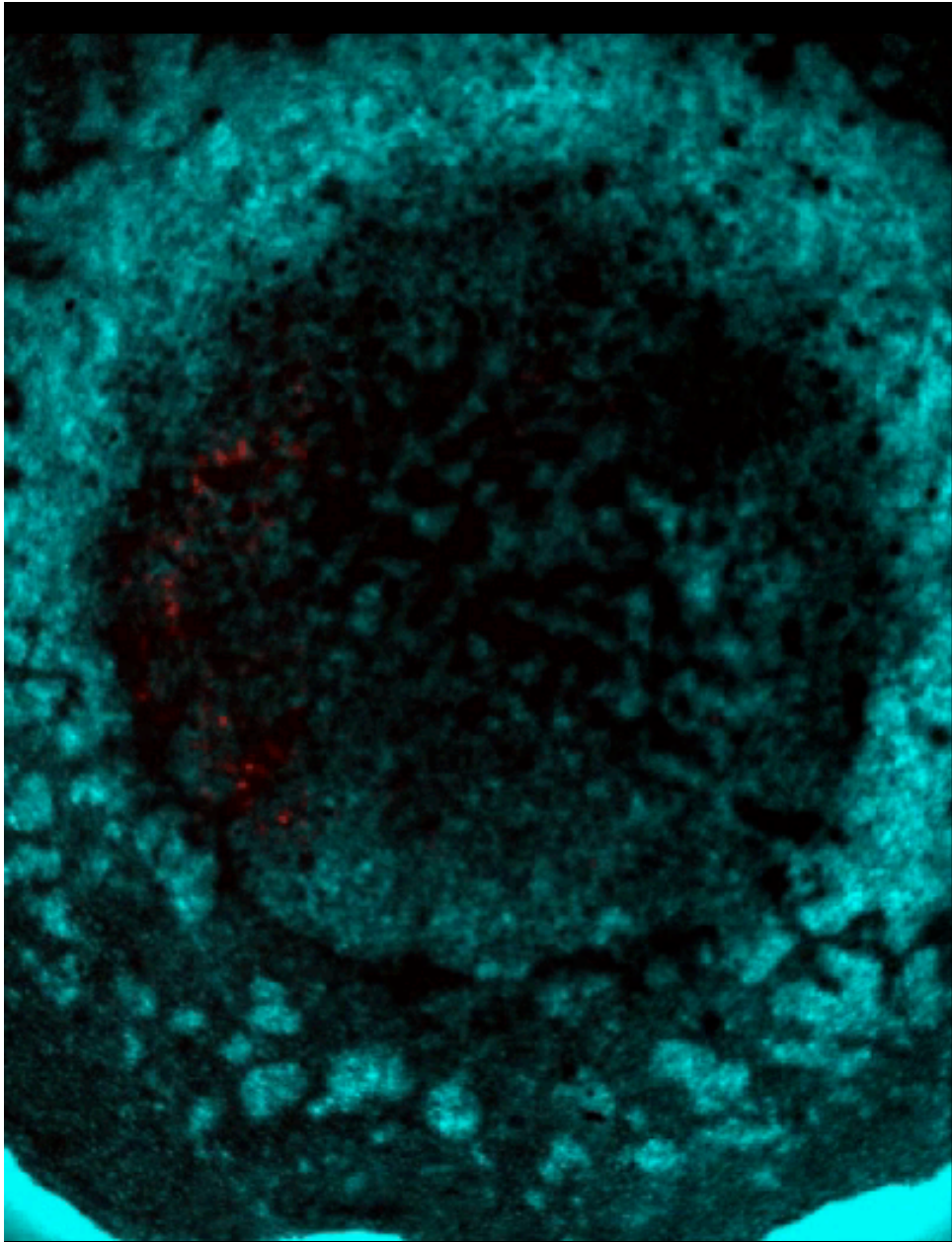


Abb. 3. Schema der polonäseartigen Doppelströmung bei der Bildung des Primitivstreifens.

L. Gräper (1929)

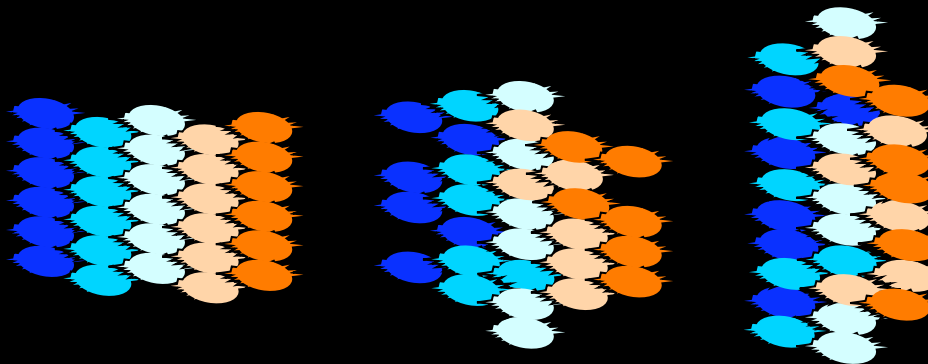
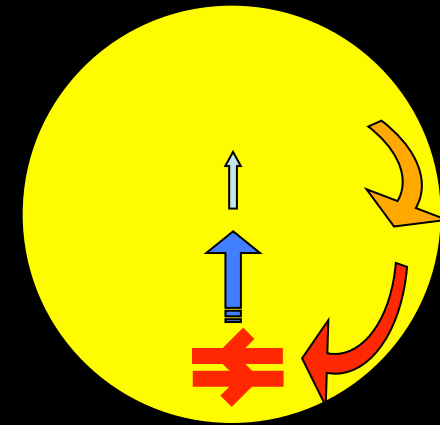
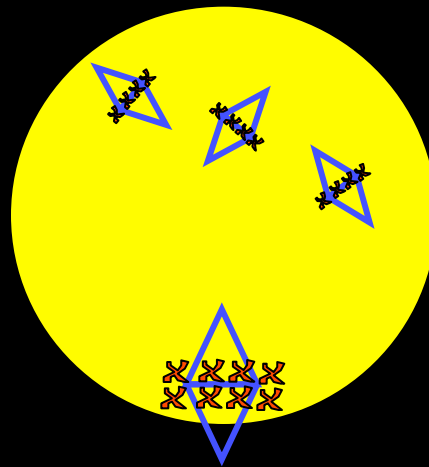


0 min

Voiculescu et al. (2007) *Nature* **449**: 1049

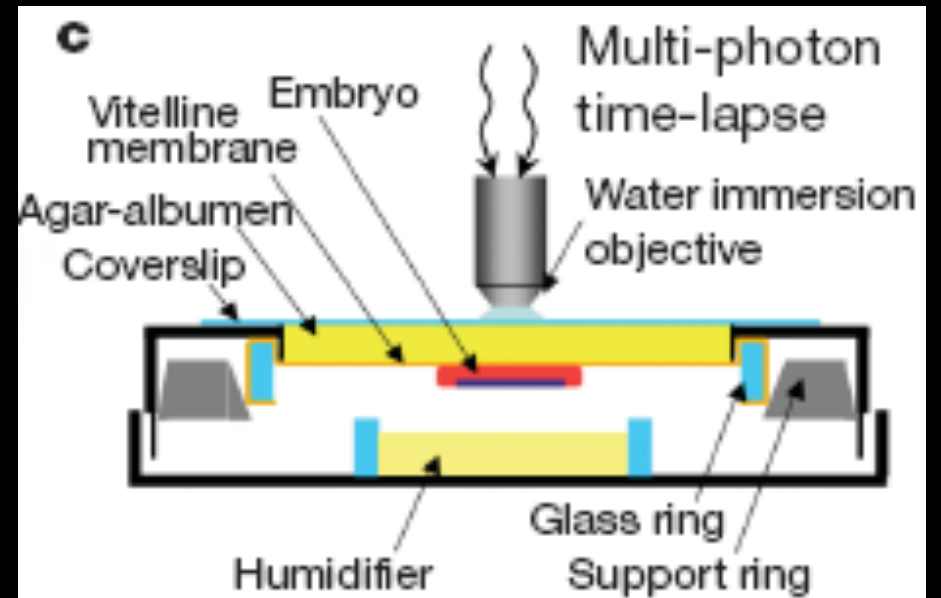
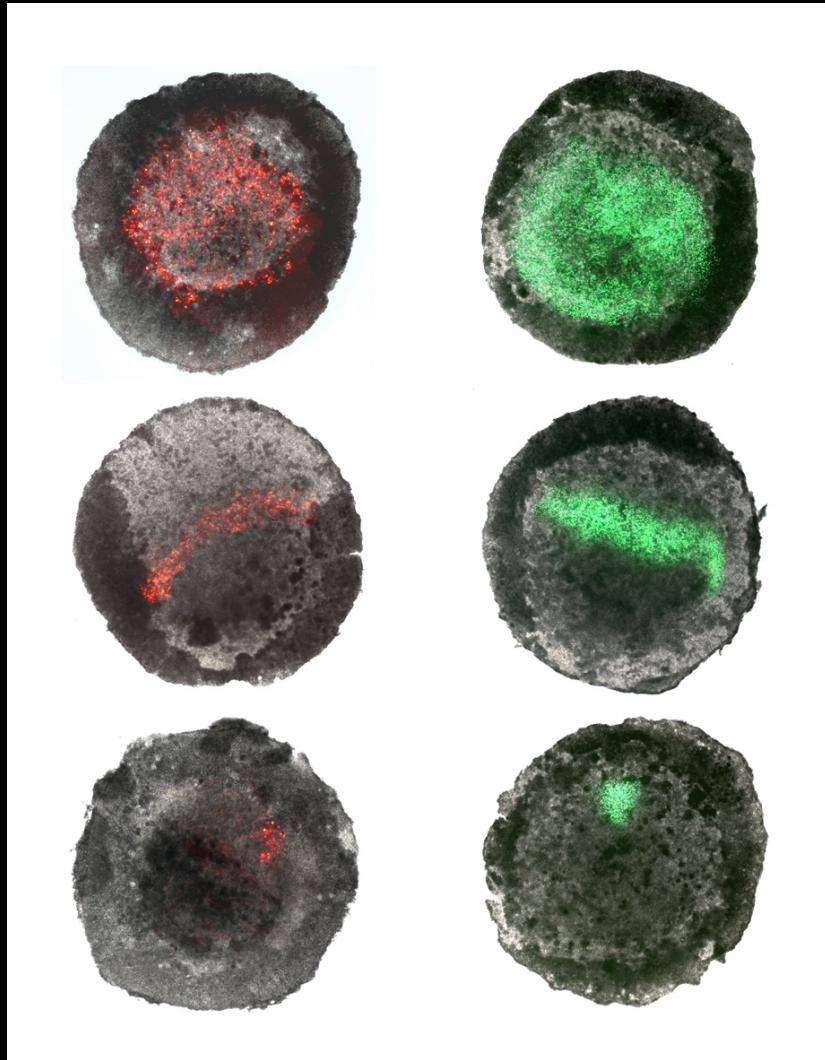
Proposed mechanisms:

1. Epiboly with spatial constraints at the margins (Duval, 1880s)
2. Oriented cell divisions at the posterior midline (Wei&Mikawa, late 1990s)
3. Attraction & Repulsion (nowadays, C. Weijer's chemotaxis)
4. Other? *E.g.*, cell intercalation at the posterior was proposed.





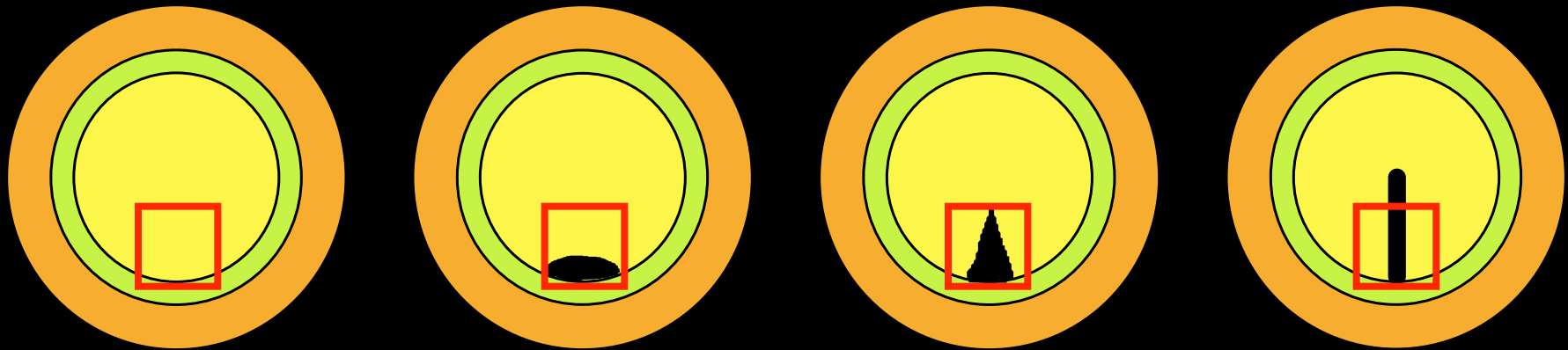
Our real teacher has been and still is the embryo, who is, incidentally, the only teacher who is always right."
Viktor Hamburger



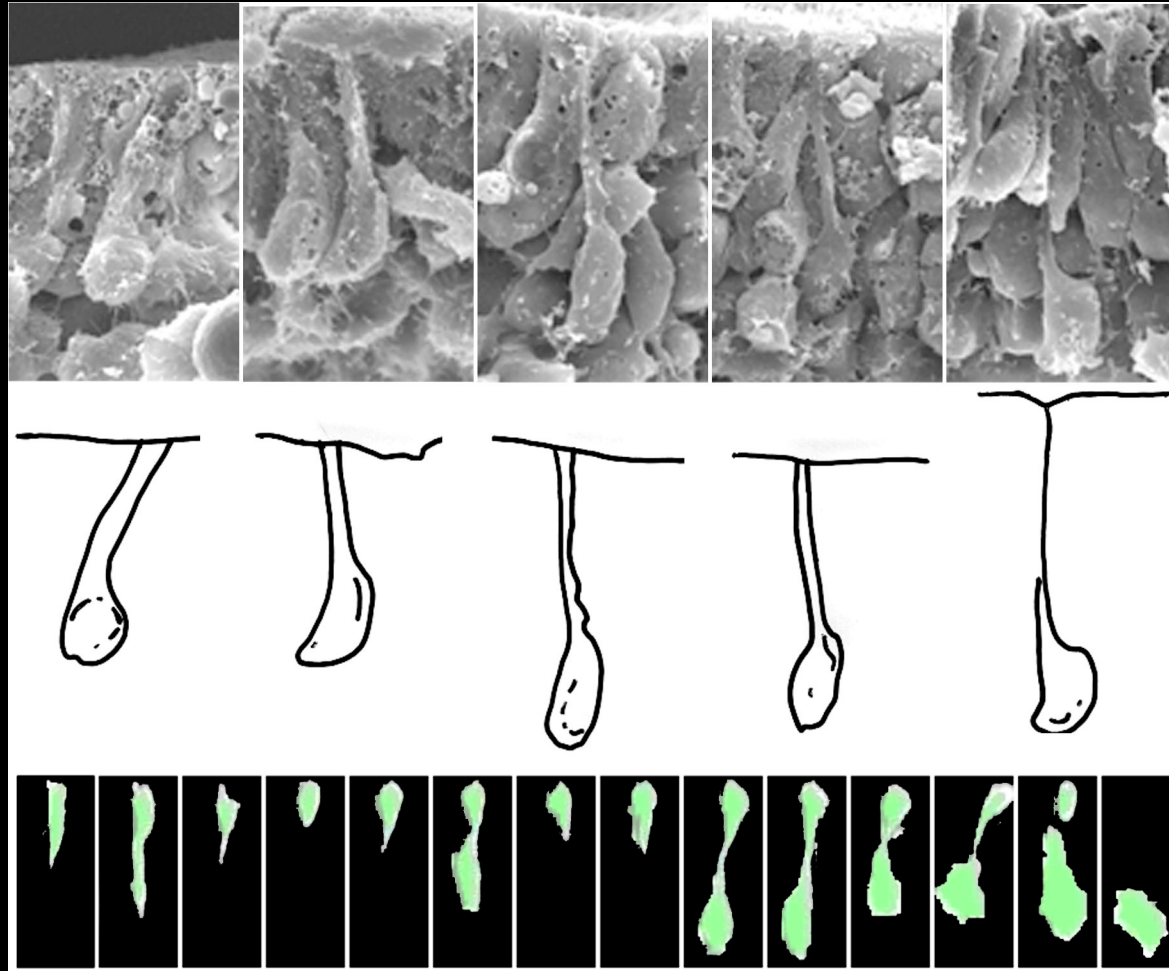
Voiculescu et al. (2007) *Nature* **449**: 1049

Voiculescu et al. 2008, *Nat Protoc* **3**: 419

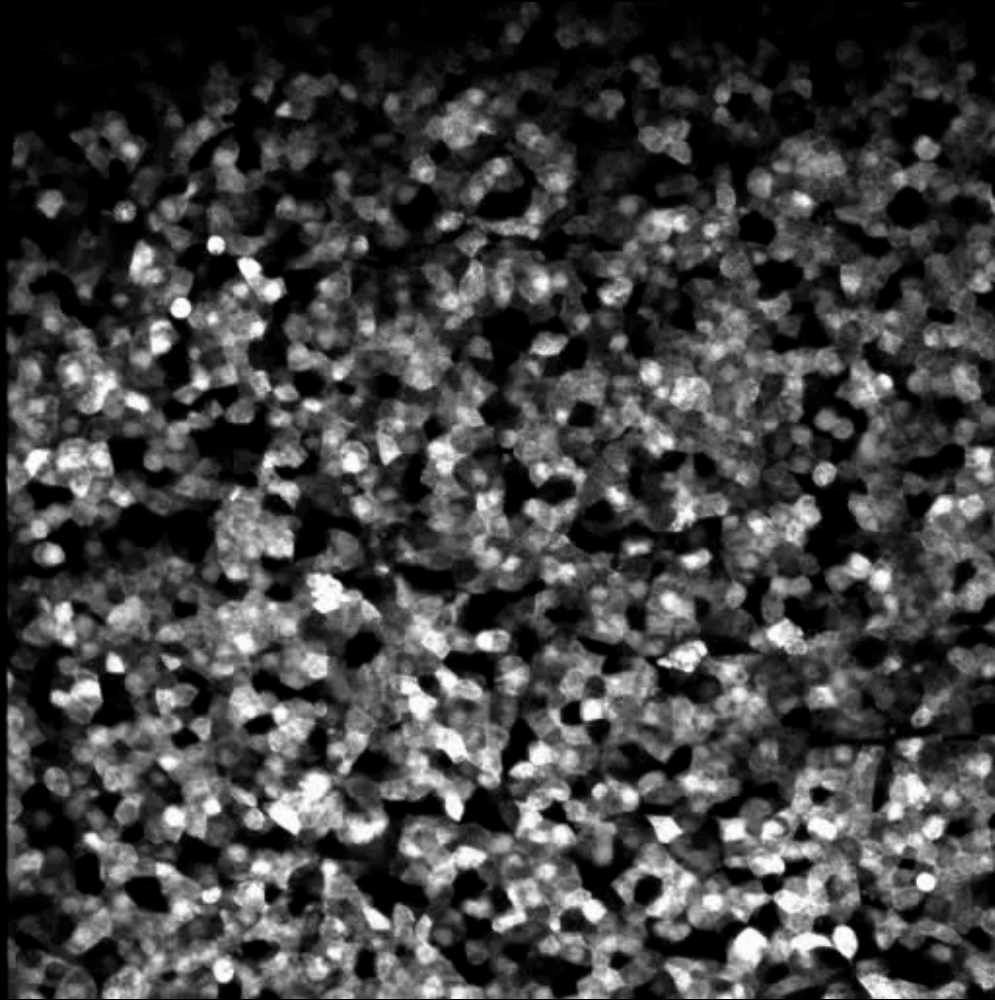
I. The EMT events of gastrulation



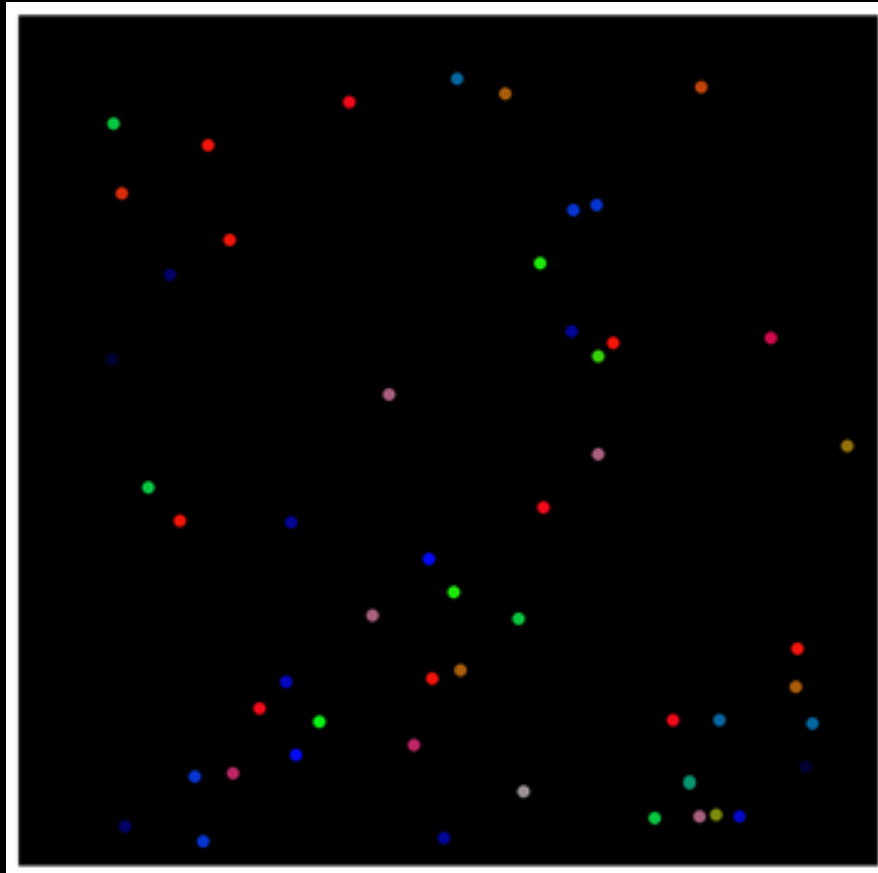
Cells ingress by individual EMT events



Ingression events start before gastrulation...



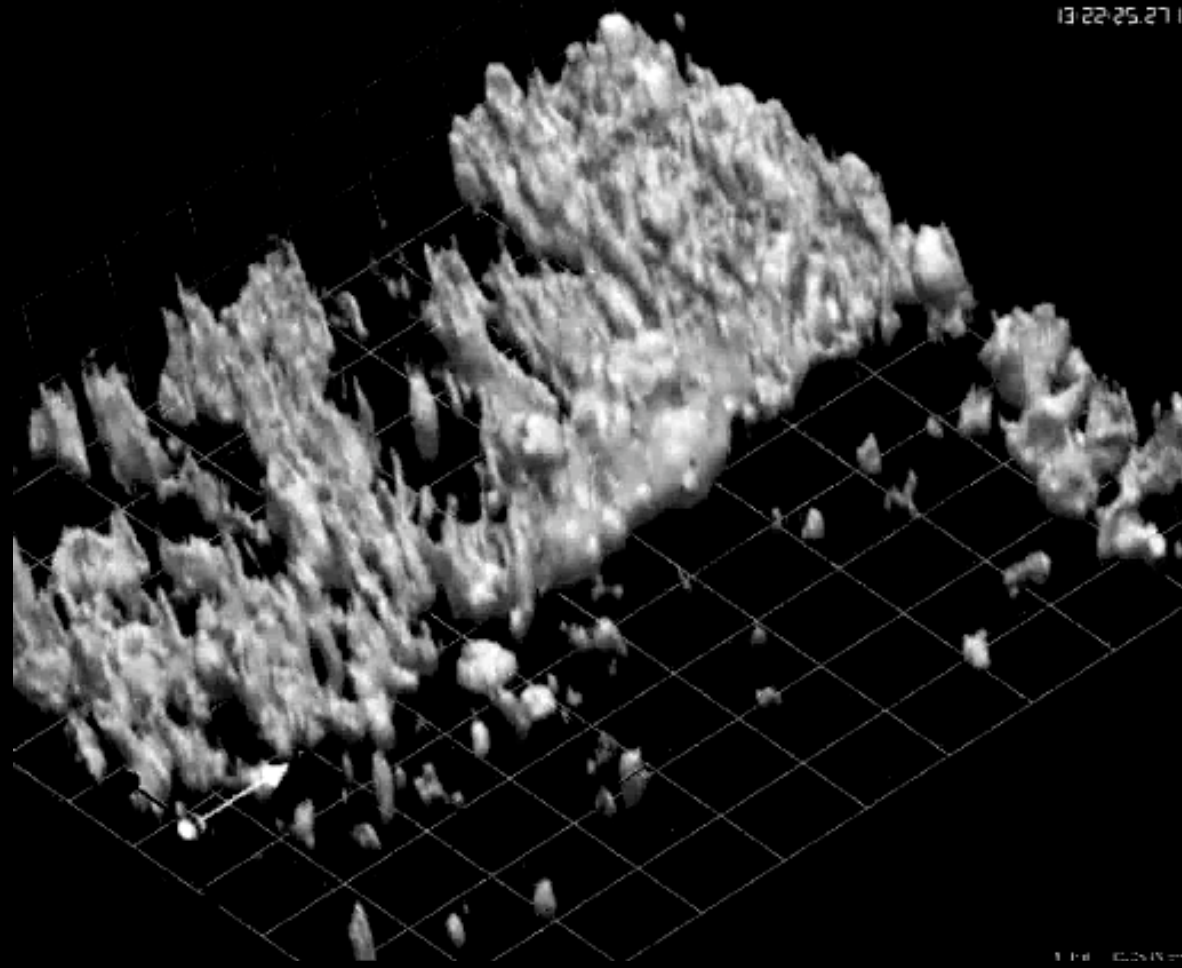
...and accelerate and cluster as the streak forms



0 80 160 240 320
time of ingress

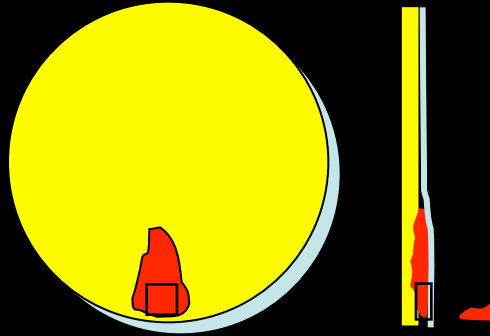


**Afterwards, ingression remains stochastic,
in the streak and outside of it**

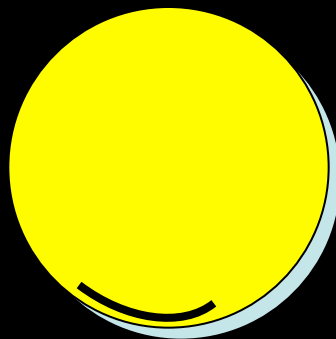


Ventral view of a full primitive streak

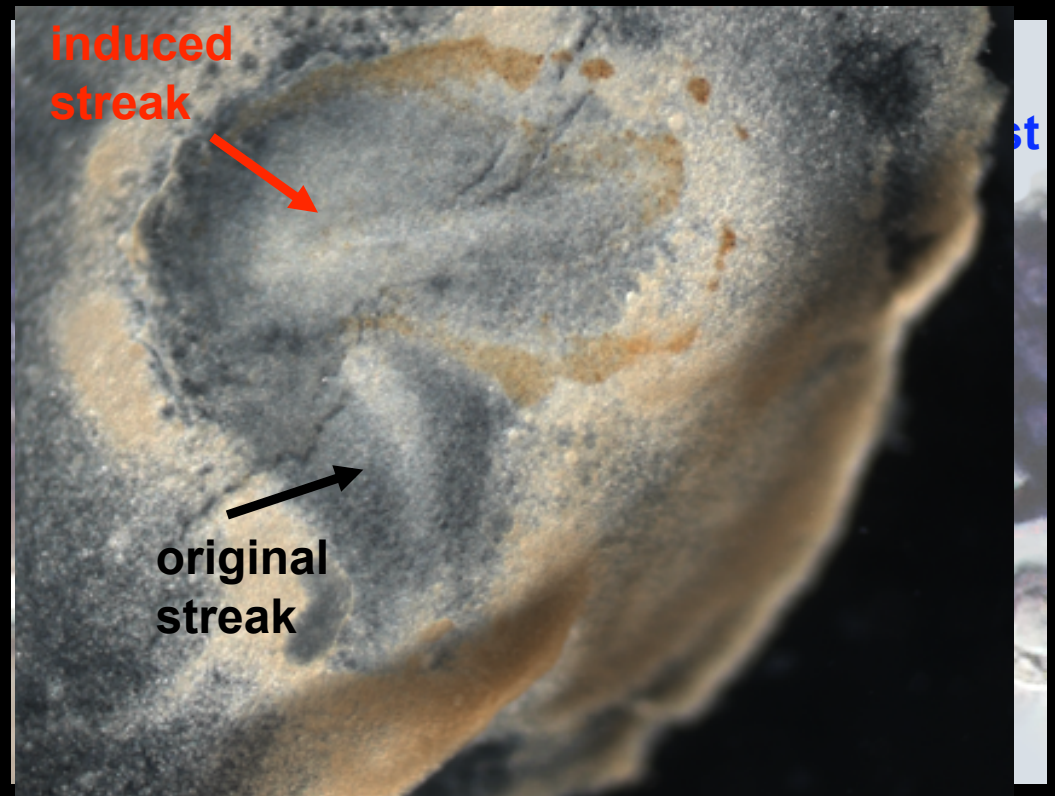
Do ingressing cells favour the ingression of neighbouring cells?



Early streak
Quail embryo



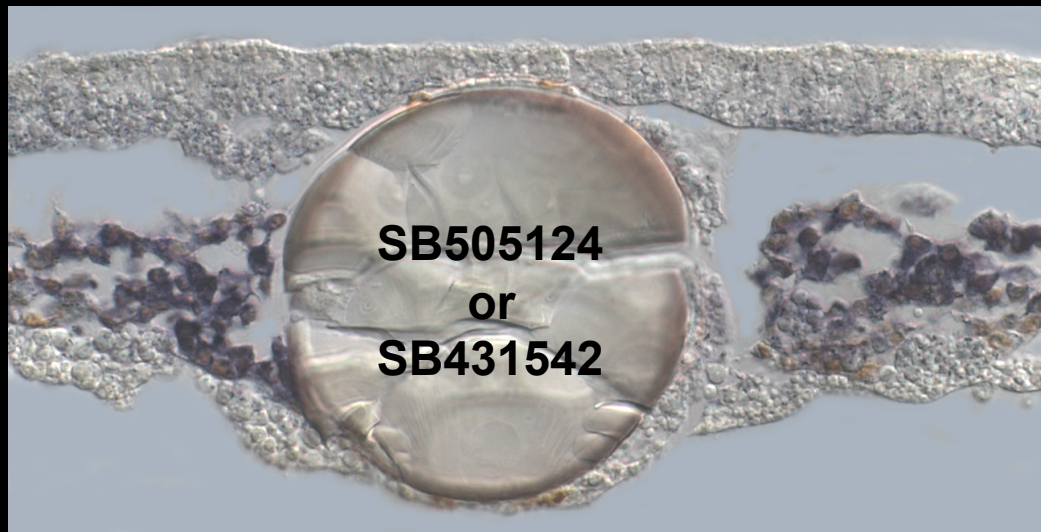
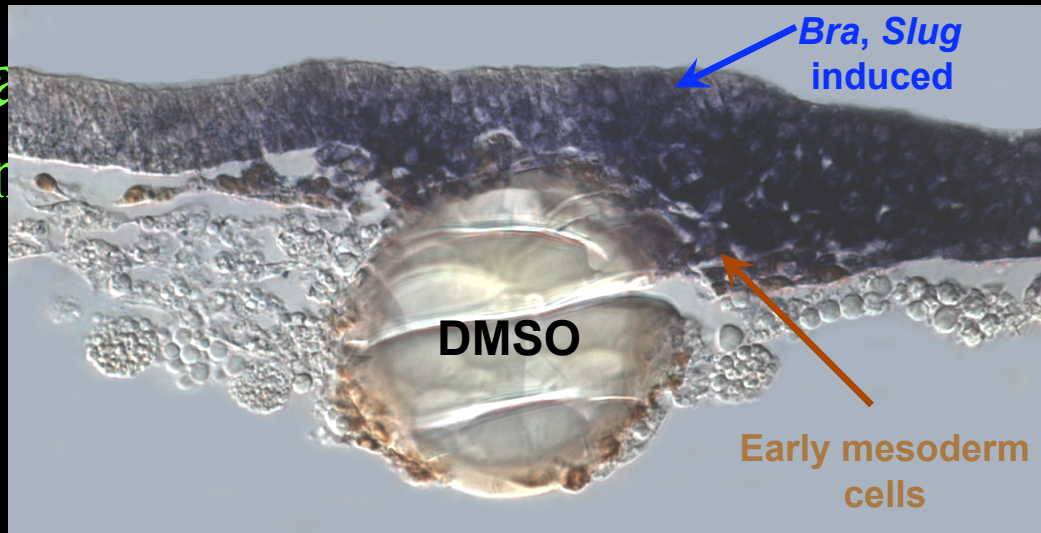
Pre-streak
Chick embryo



Do ingressing cells communicate by cell-cell signalling to induce ingression in adjacent epiblast?

Nodal
(but not)

induction
activities)



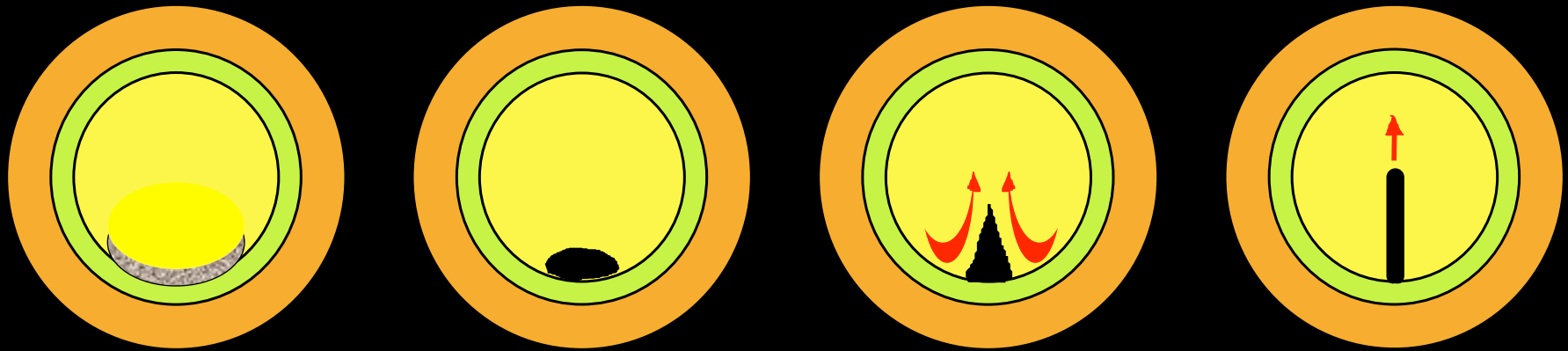
Conclusions I: Formation of the Primitive Streak

Cells ingress stochastically, as individuals

**Gastrulation starts before primitive streak formation –
early, isolated cells delaminate infrequently**

**In the presence of Nodal, ingressing cells cooperate to
induce more ingression in the overlying epiblast**

The primitive streak forms by a feed-forward mechanism



see Bertocchini & Stern (2002) *Dev Cell*

Q: What shapes the Primitive Streak?

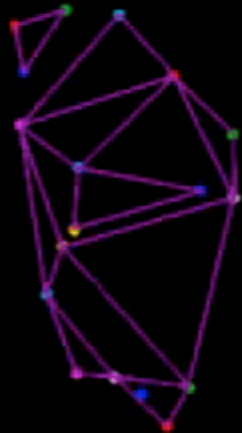
Can cell divisions drive the streak elongation?

0 min

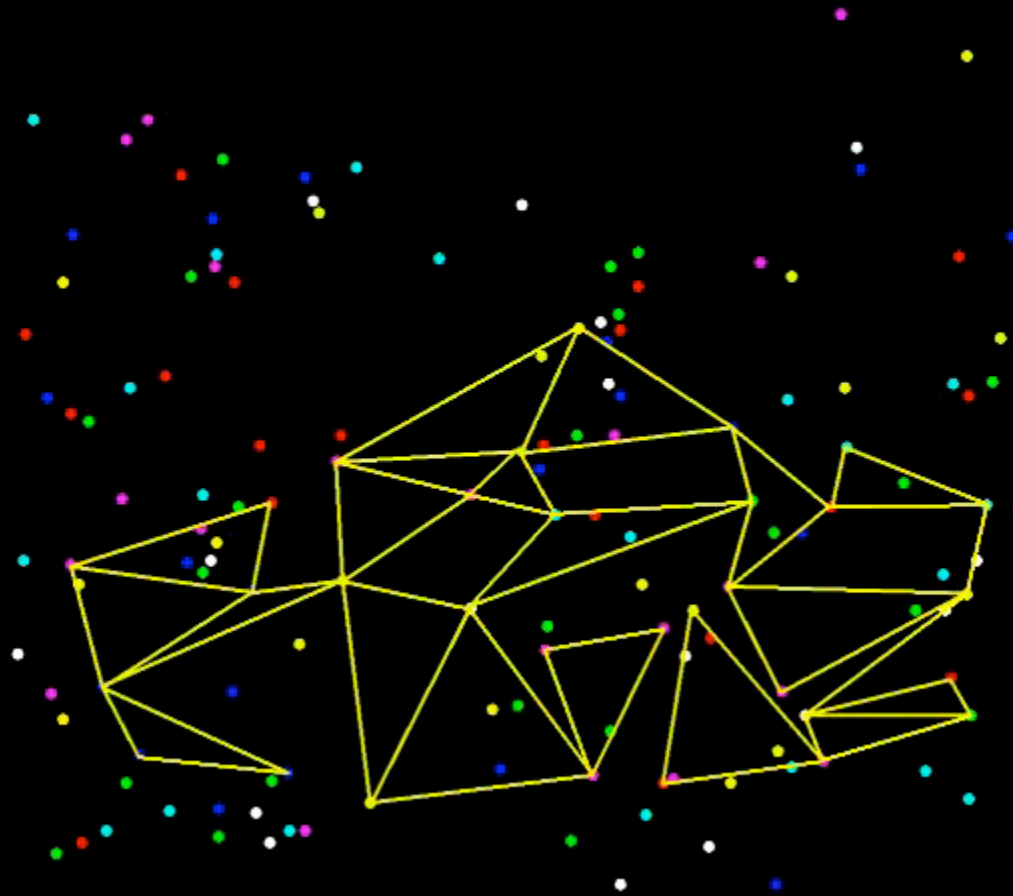


Relative cell movements

0 min

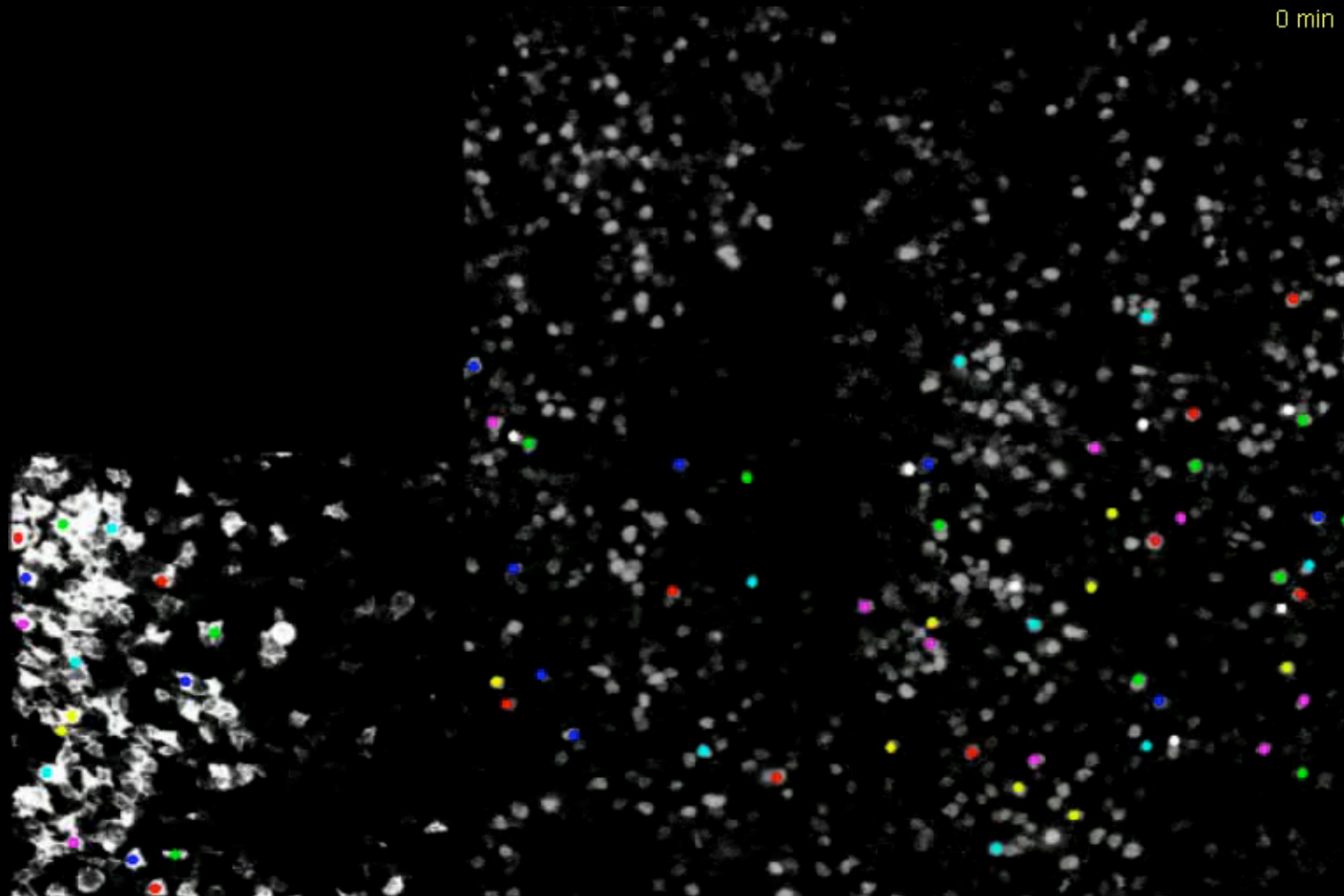


Cells in a lateral domain



Cells contributing to the early streak

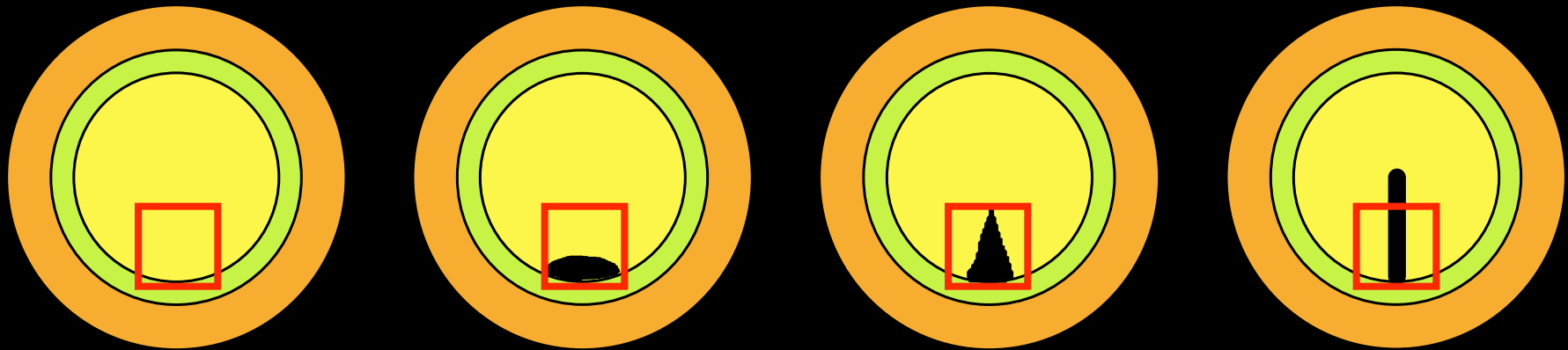
Cell movements (displacements)



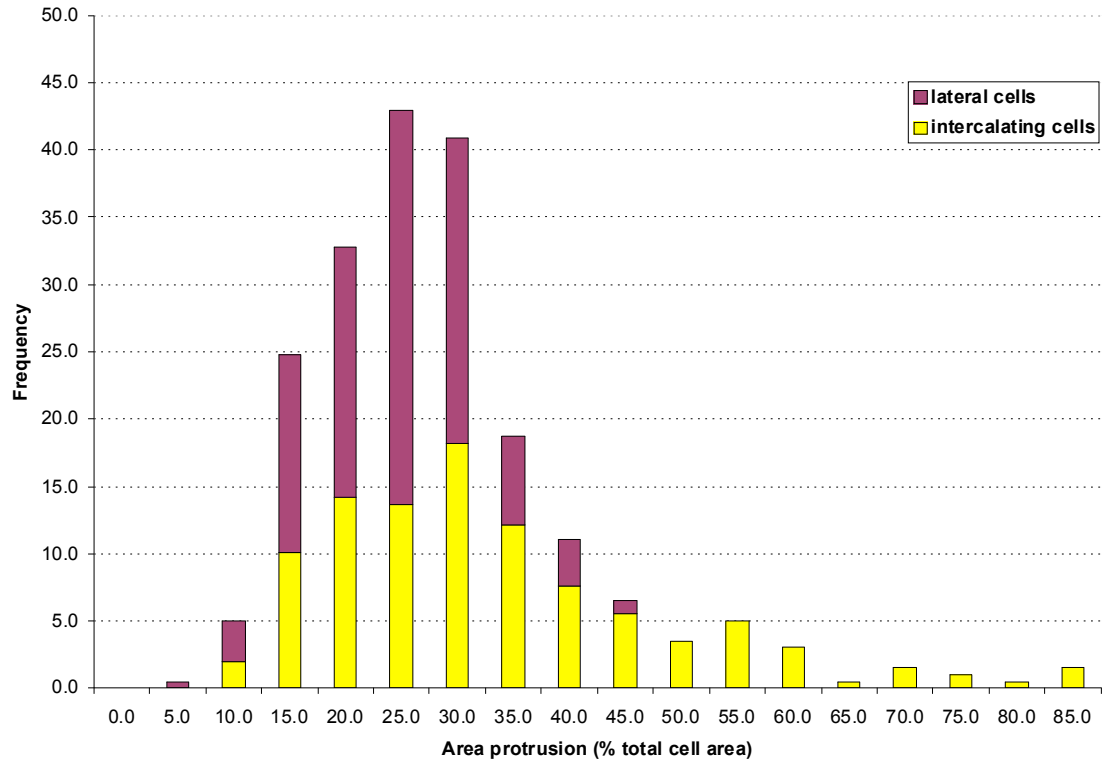
Cells in a lateral domain

Cells contributing to the early streak

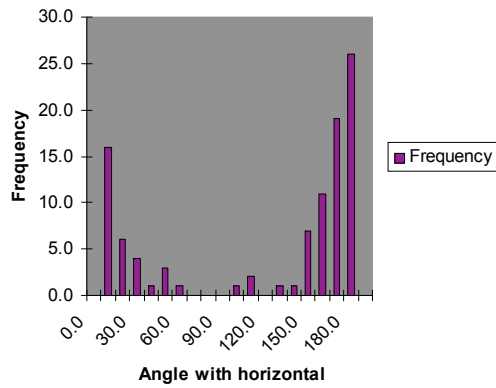
Medio-lateral cell intercalation in the streak-forming region



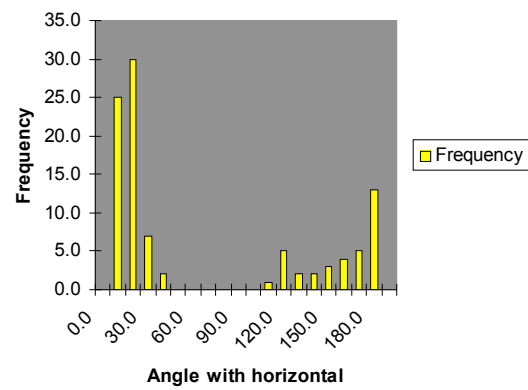
Magnitude of Cell Protrusions



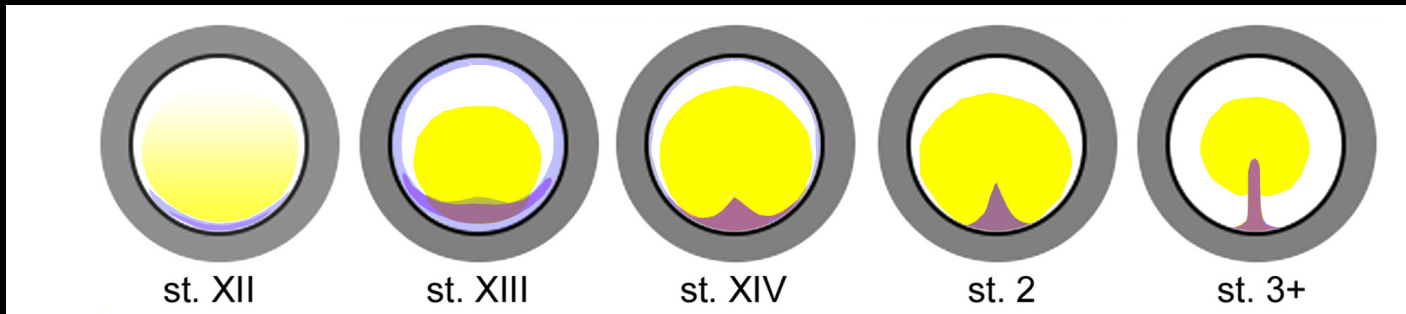
Angles Lateral cells



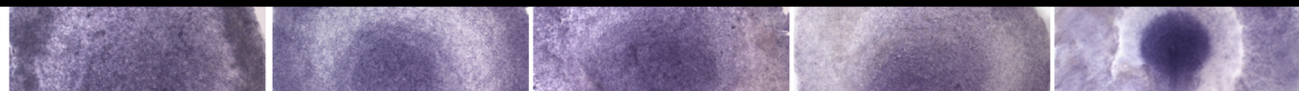
Angles CE cells



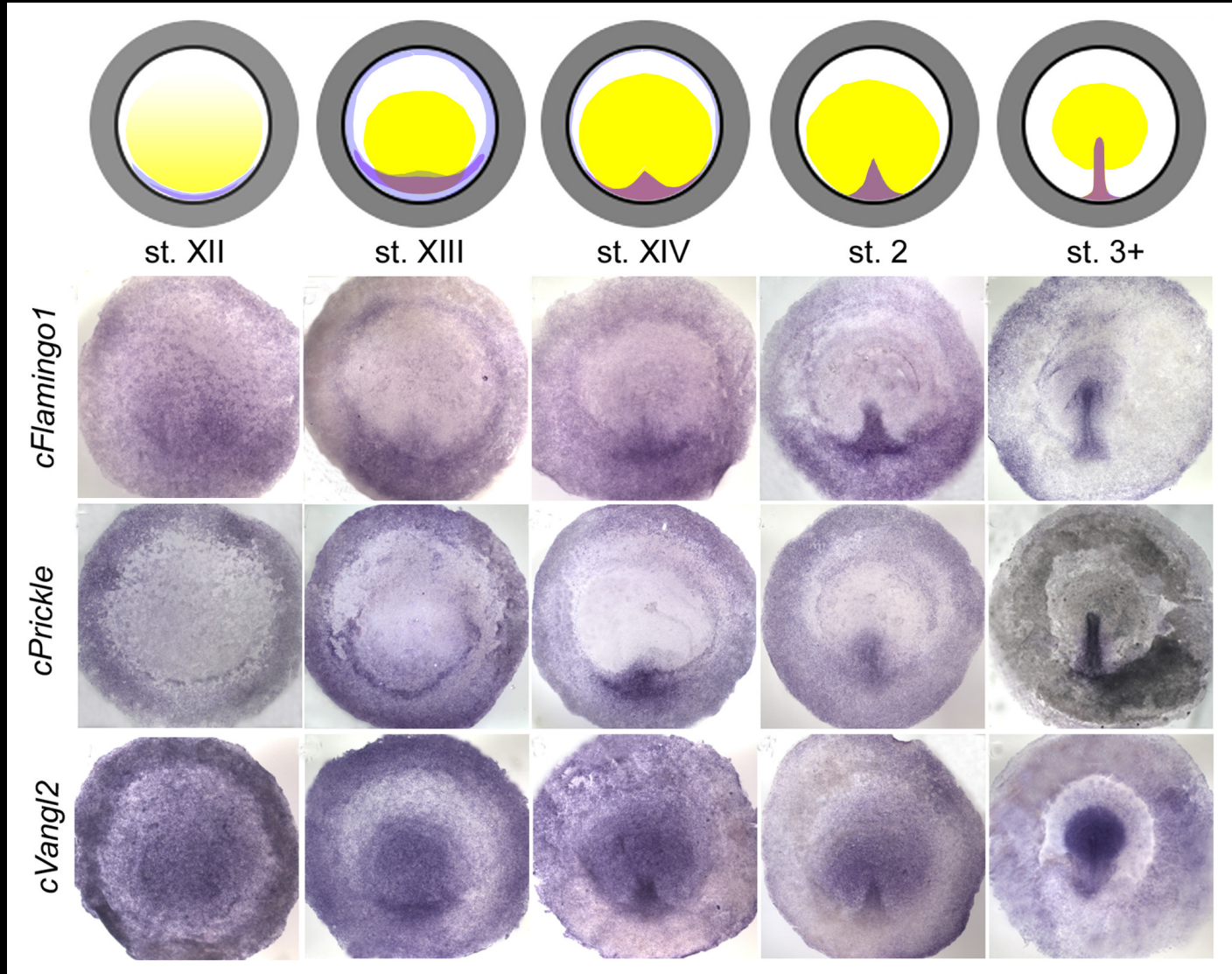
Overlapping expression of Wnt-PCP components



Vangl

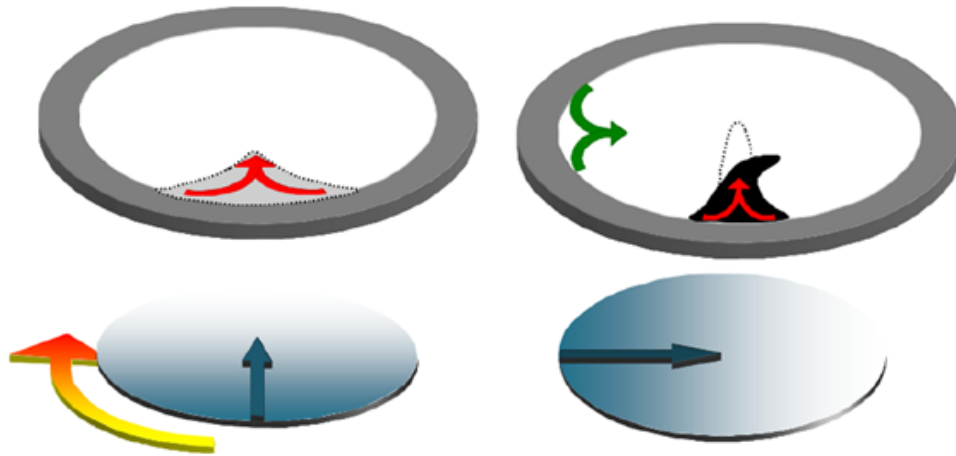


Overlapping expression of Wnt-PCP components



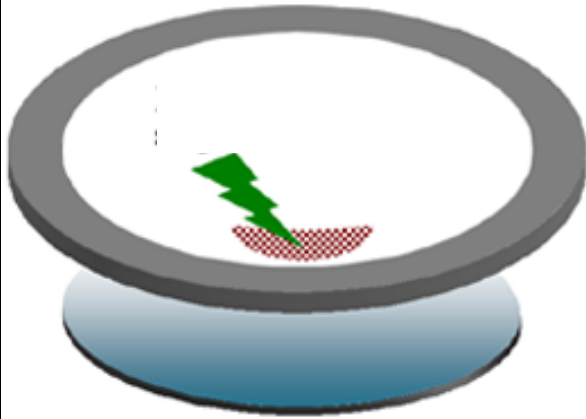
What position is the ventral PCP component? (FGF8)

Hypoblast rotation



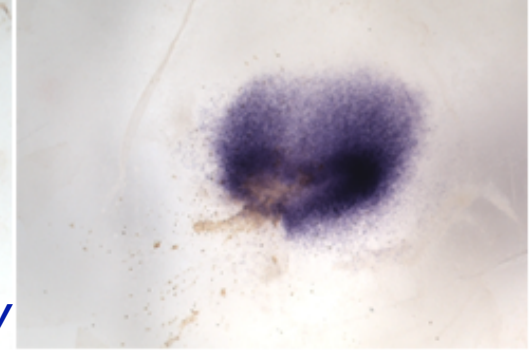
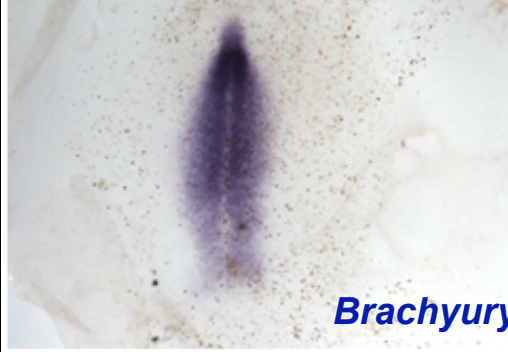
Is Wnt-PCP activity required ?

**Electroporation
stage XII**



Control (active β -catenin)

dishevelled Δ DEP



Conclusions II: The Shaping of the Primitive Streak

**Is controlled by oriented cell intercalation,
dependent on Wnt-PCP activity**

**It is positioned by the hypoblast – a new,
extra-embryonic layer of amniotes (via FGF8)**

**This intercalation is distinct from other intercalation events:
occurs earlier, in a tight epithelium**

... and independently of mesendoderm formation

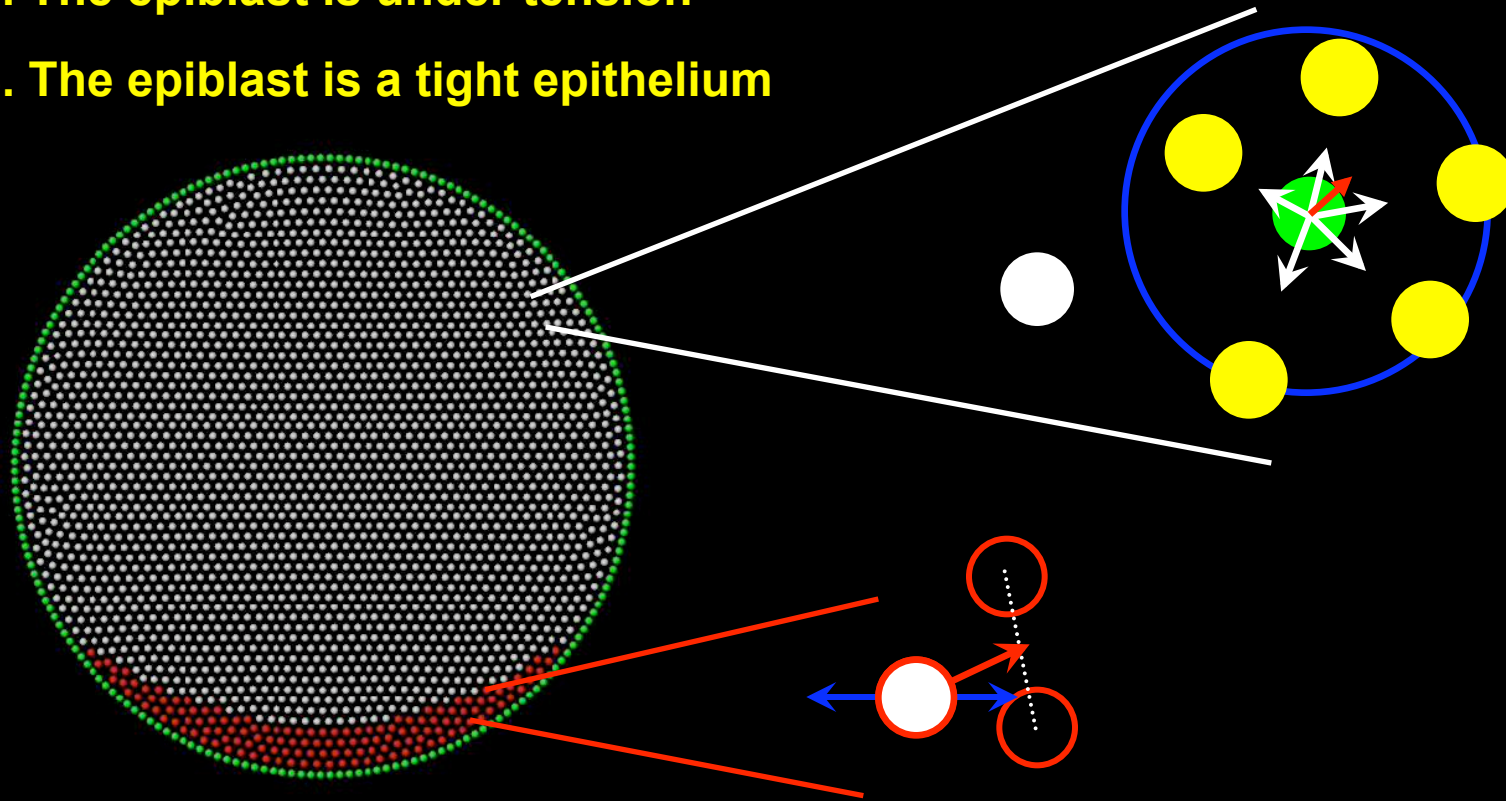
III: Morphogenetic movements

Q: What is the contribution of these components?

Are they sufficient to drive the movements in the whole epiblast?

Computer model of chick gastrulation

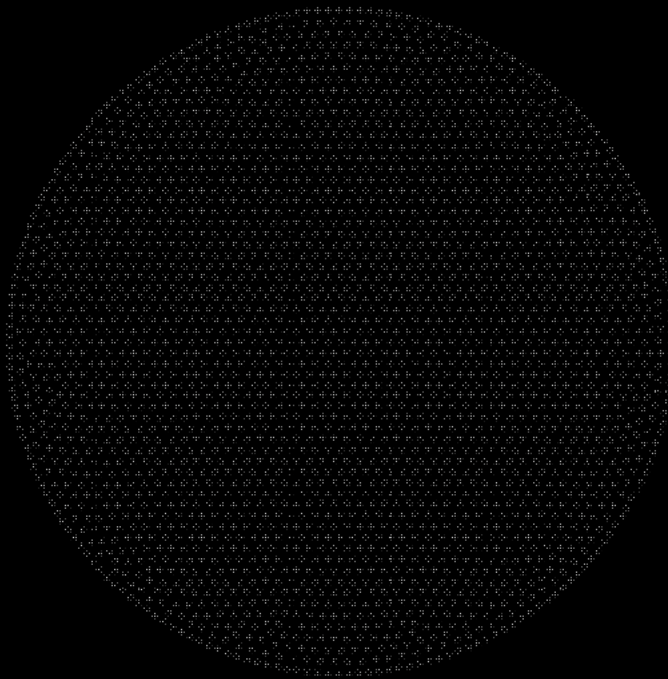
1. The epiblast is composed of cells
2. The epiblast is under tension
3. The epiblast is a tight epithelium



4. Cells have a preferred direction of intercalation in the streak-forming region
5. Ingressing cells cooperate in the posterior crescent

Model predictions

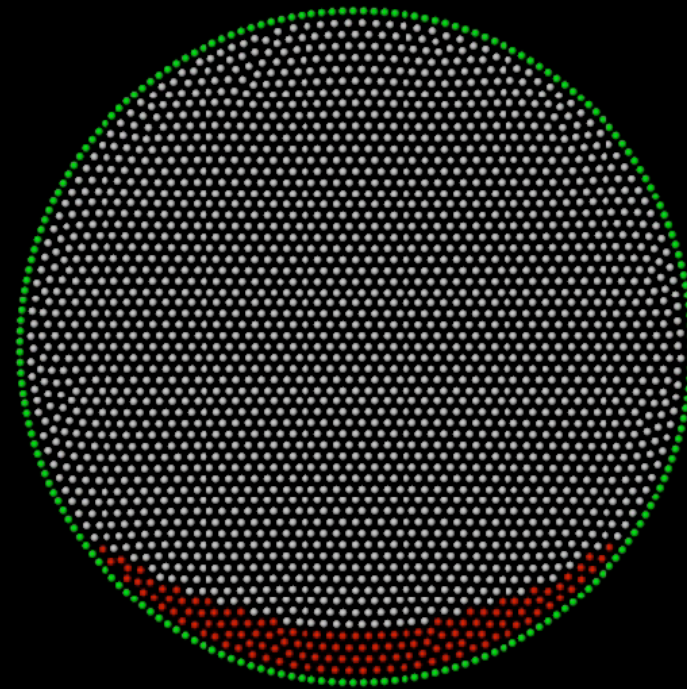
epiblast movements



0h 0m

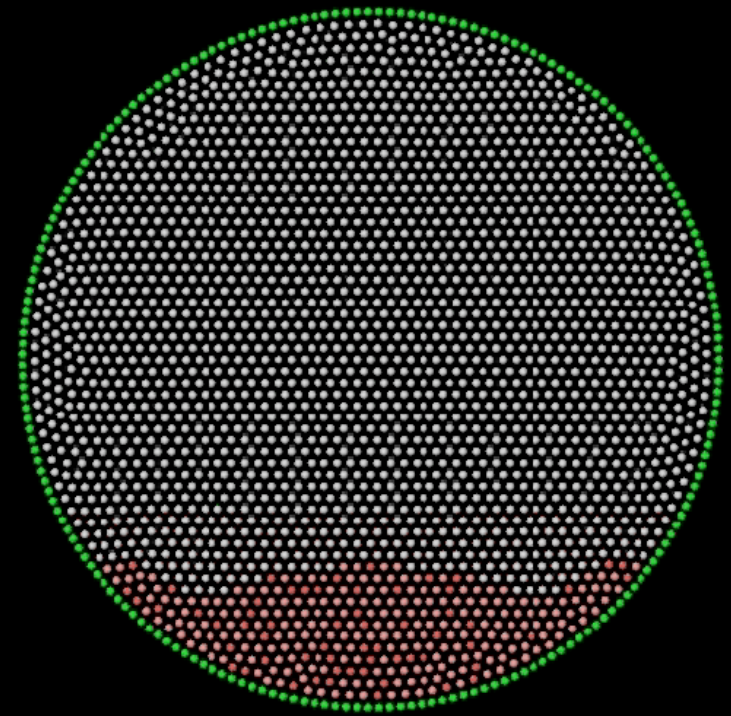
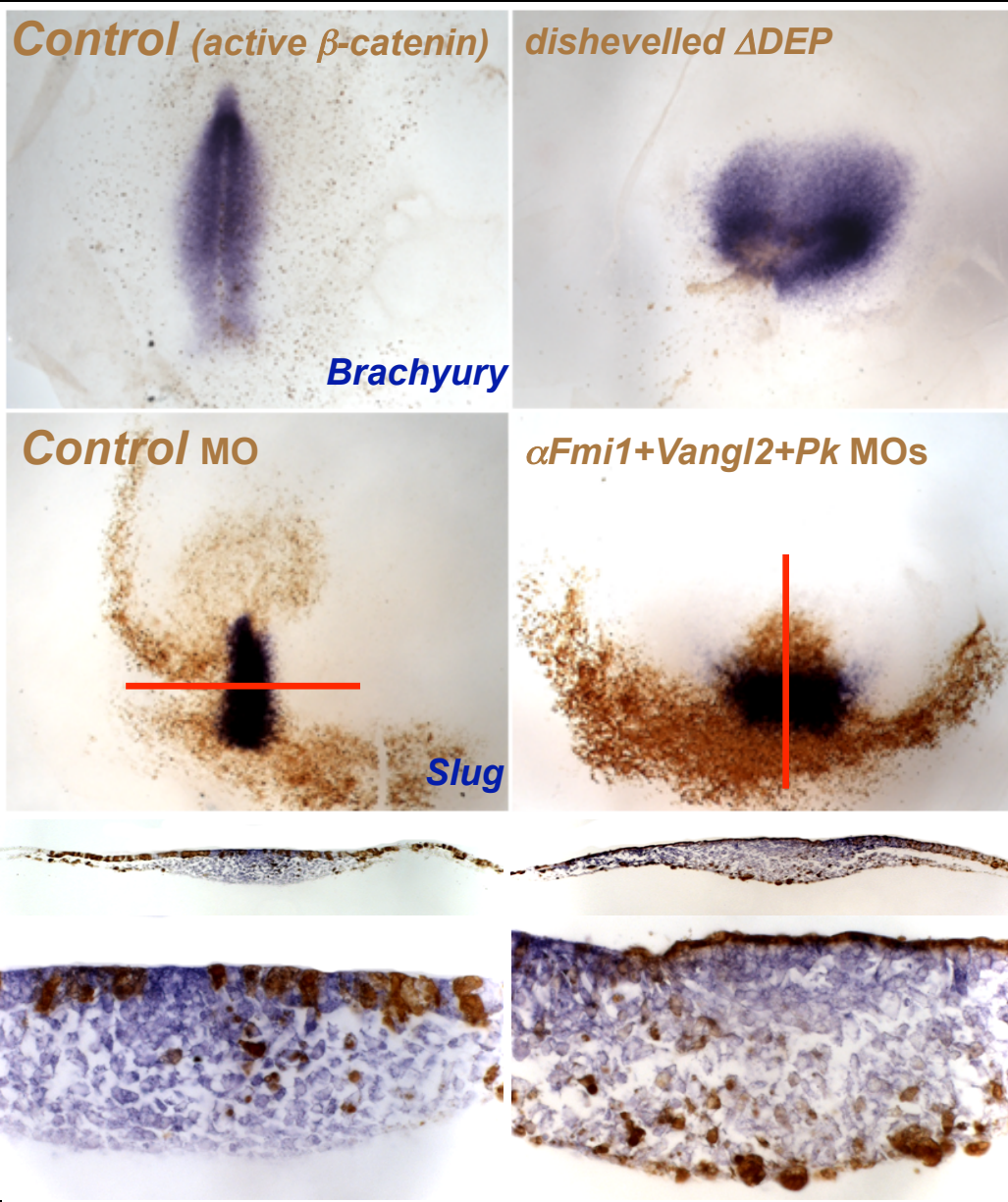
Stage XI 0h 0m

pattern of ingression



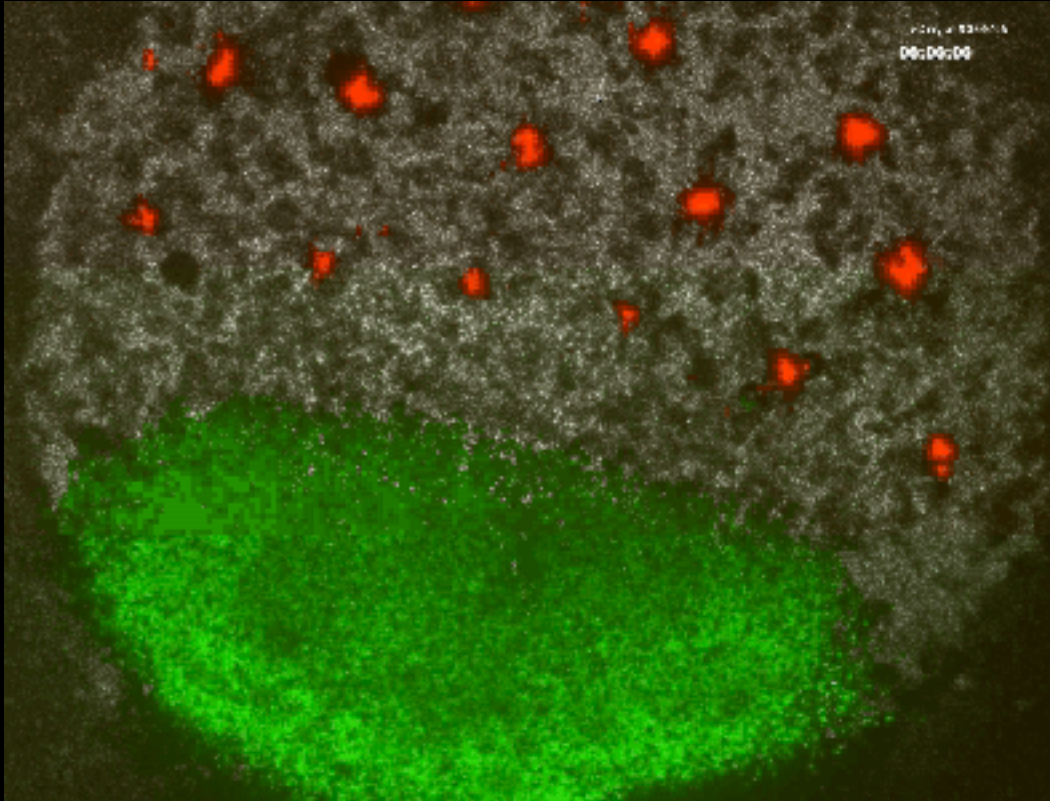
Stage XI

Effect of blocking Wnt-PCP intercalation

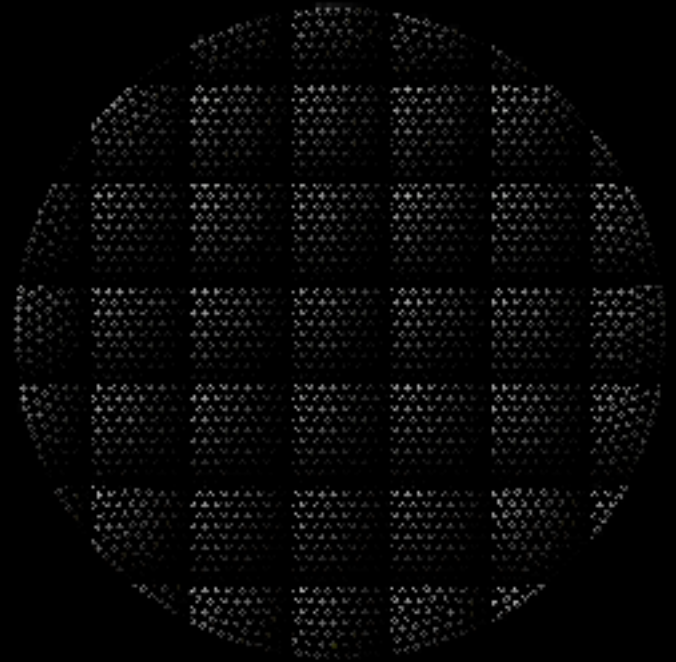


0h 0m

Stage XI



0h 00m



Stage XI

Conclusions III: A model of chick gastrulation

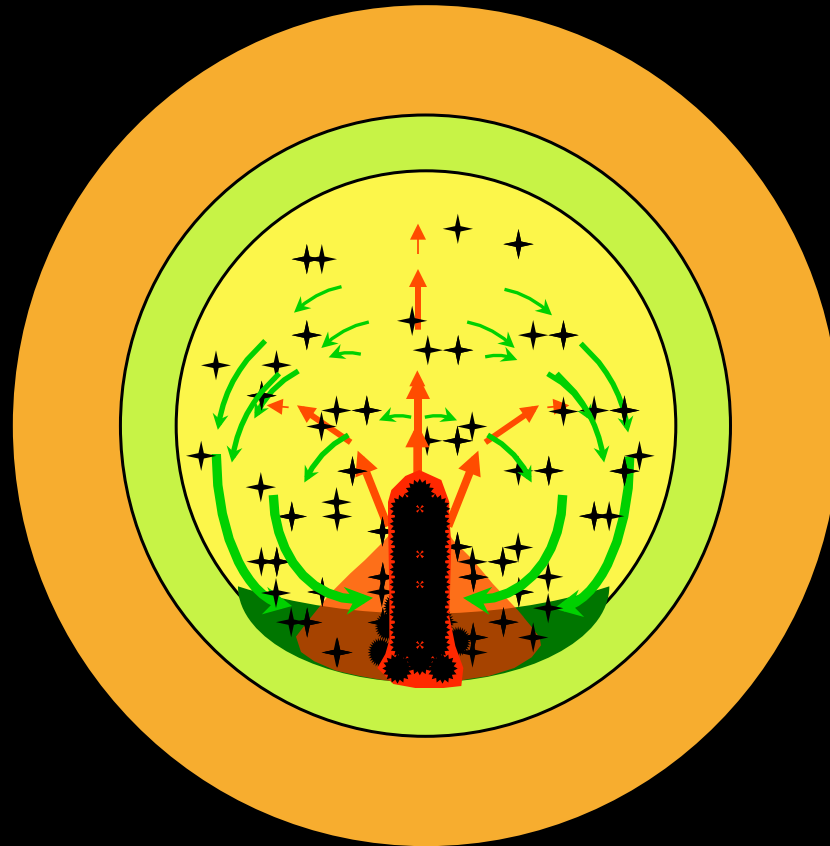
The model can explain the morphogenetic movements in the whole embryo

Model predictions agree with experimental data

**Can be extremely useful to
direct the experimental observations
interpret experimental data**

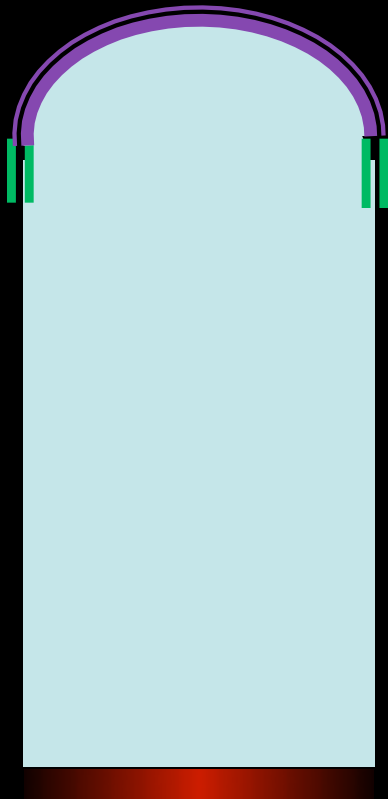
Can be gradually refined

Model of chick (amniote) gastrulation

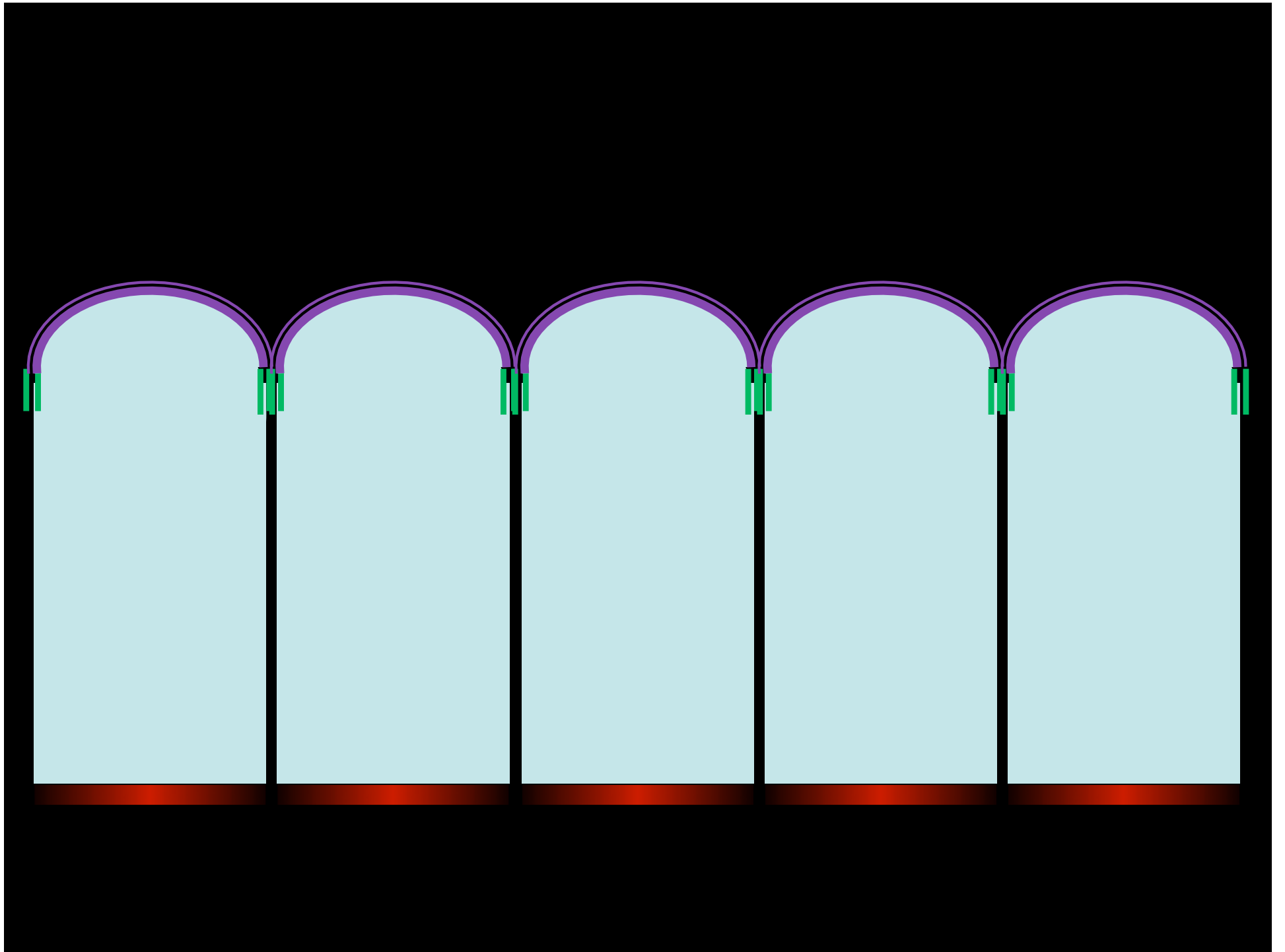


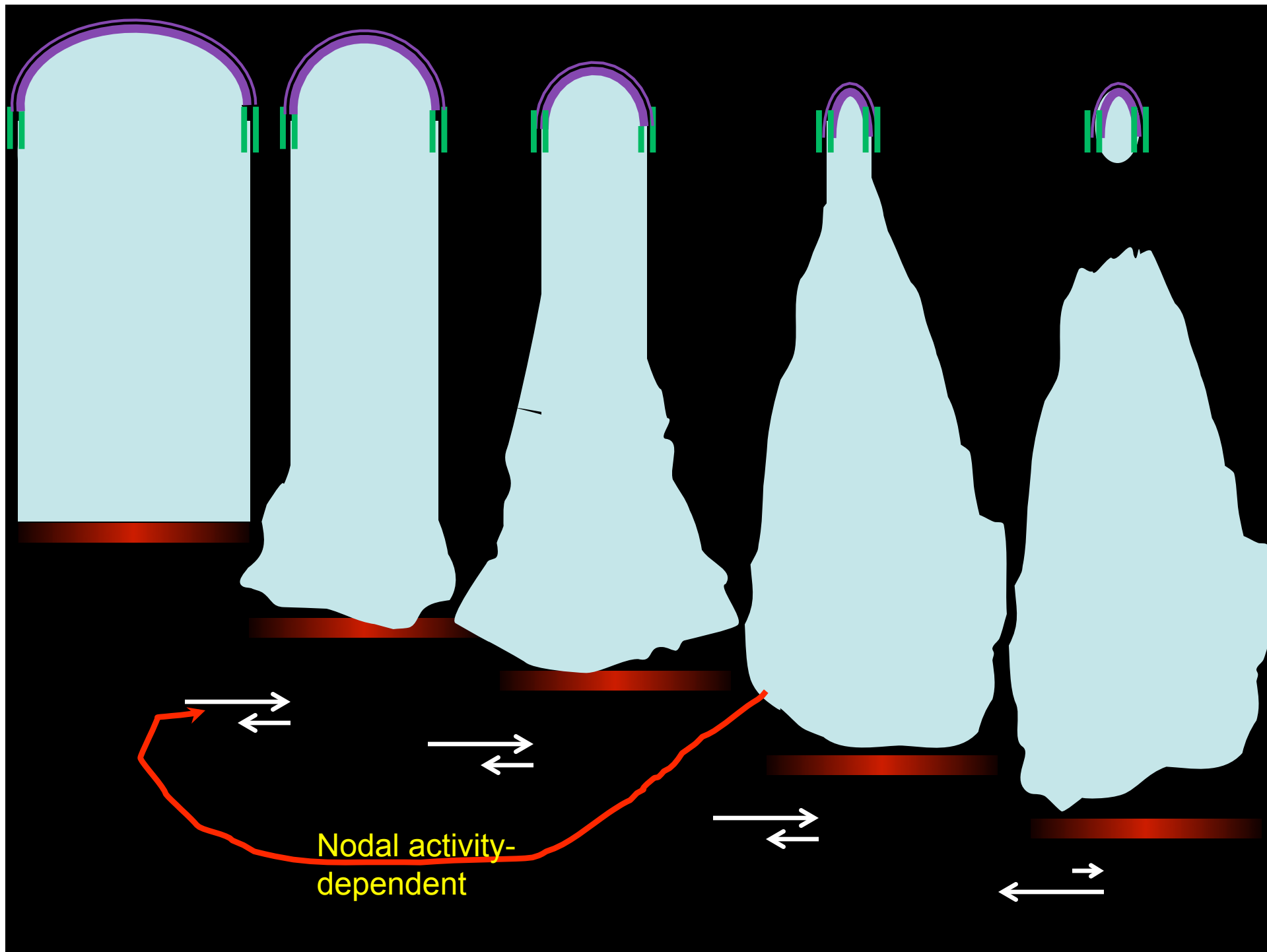
THE model of chick gastrulation

Should we build one, and how?



- A Cell is Polar is Epithelial
- Has Basal, Lateral & Apical Surfaces
- Cortical tension?
- Adheres to Cells via adherens junctions
- Adhesive force?
- Secretes ECM fibrils and Adheres to them at the Basal Surface
- ECM is elastic and fluid
- Force between fibrils?





Questions to address quantitatively:

Is intercalation polarised adhesion, polarised cortical tension, or both?

What is the ratio between oriented and non-oriented tension?

How are these parameters explain the natural variability of shapes but also make the process robust?

Acknowledgements

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