Modelling mammalian early embryonic development and patterning in vitro with stem cells

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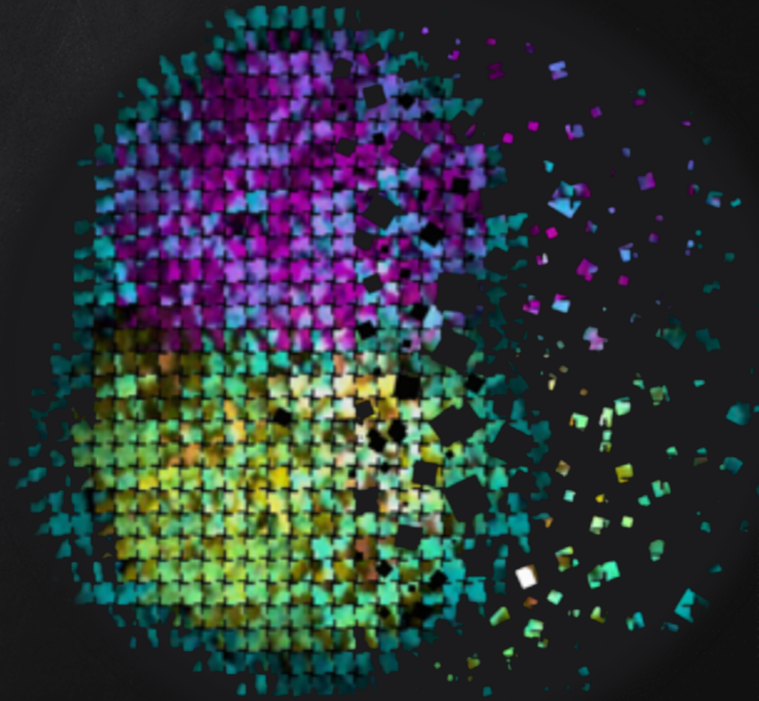
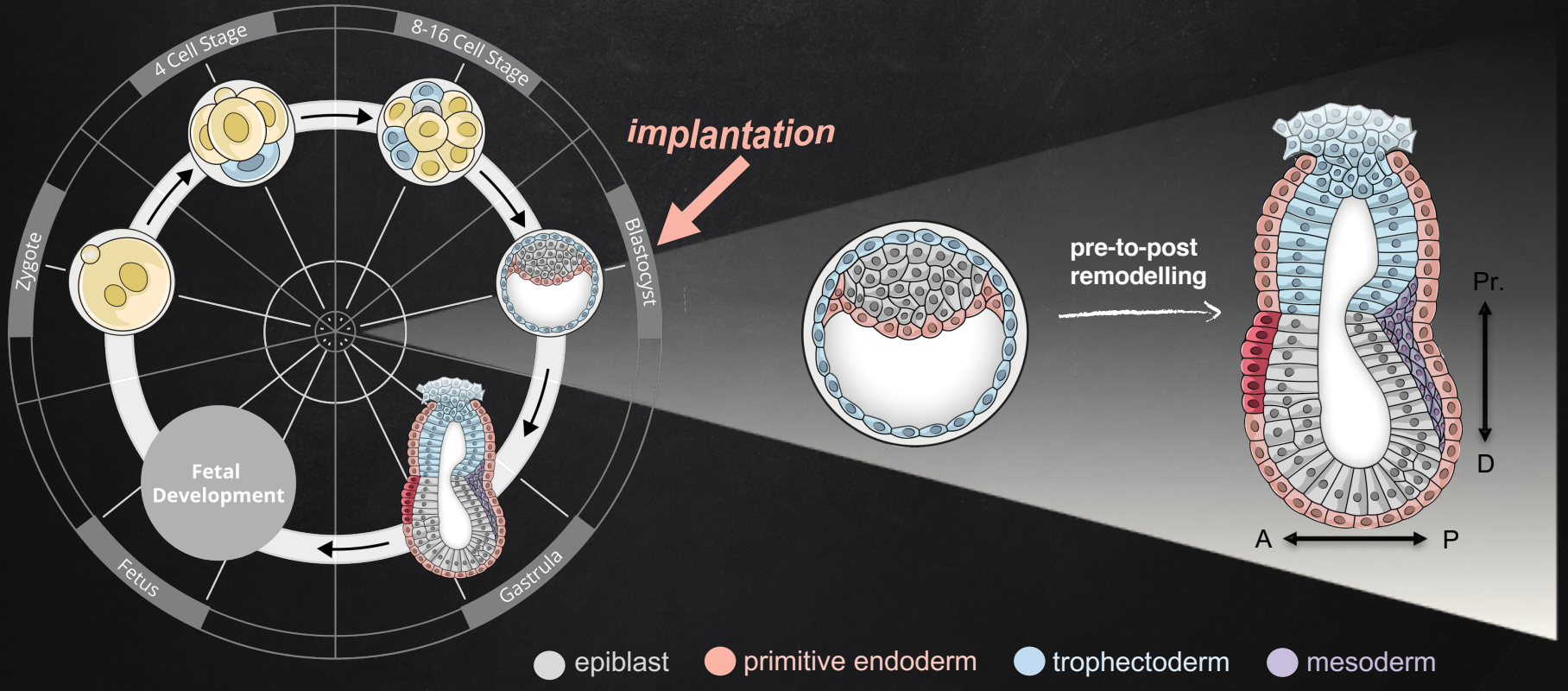
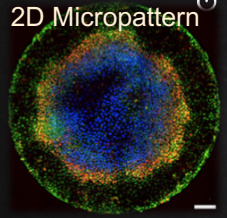
Yale University
School of Medicine

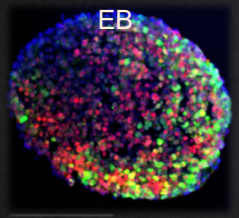


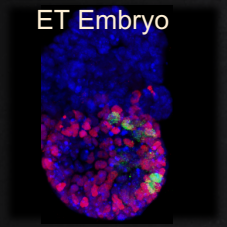
Early embryo development

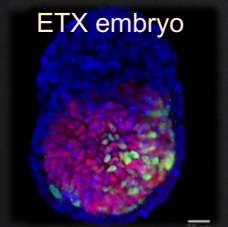


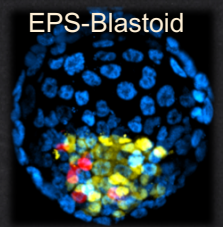
in vitro stem cell-based modelling of early embryogenesis



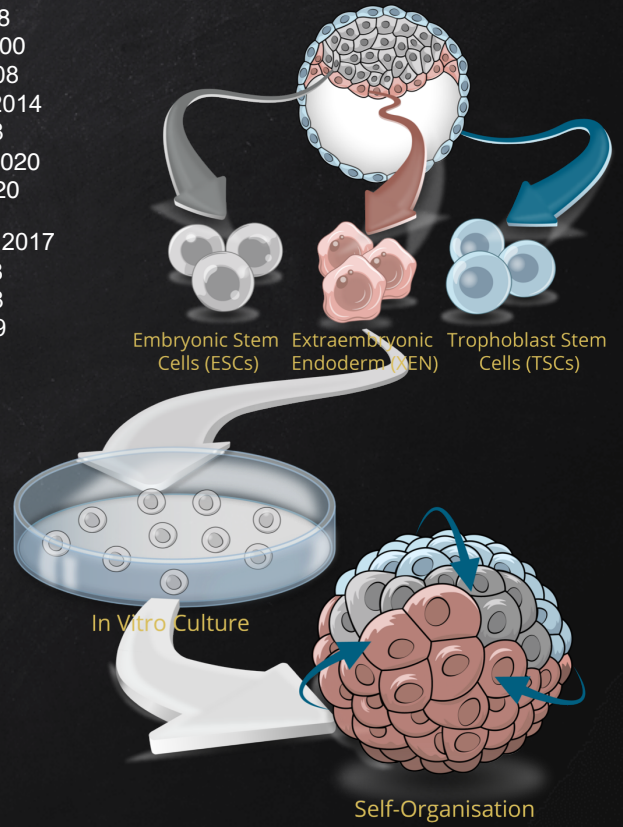








Warmflash et al., 2014
Morgani et al., 2018
Desbaillets et al., 2000
ten Berge et al., 2008
Van den Brink et al., 2014
Beccari et al.,2018
van den Brink et al., 2020
Veenvliet et al.., 2020
Morris et. al. 2020
Harrison, Sozen et al., 2017
Sozen et al., 2018
Rivron et al., 2018
Sozen et al. 2019
Li et al., 2019



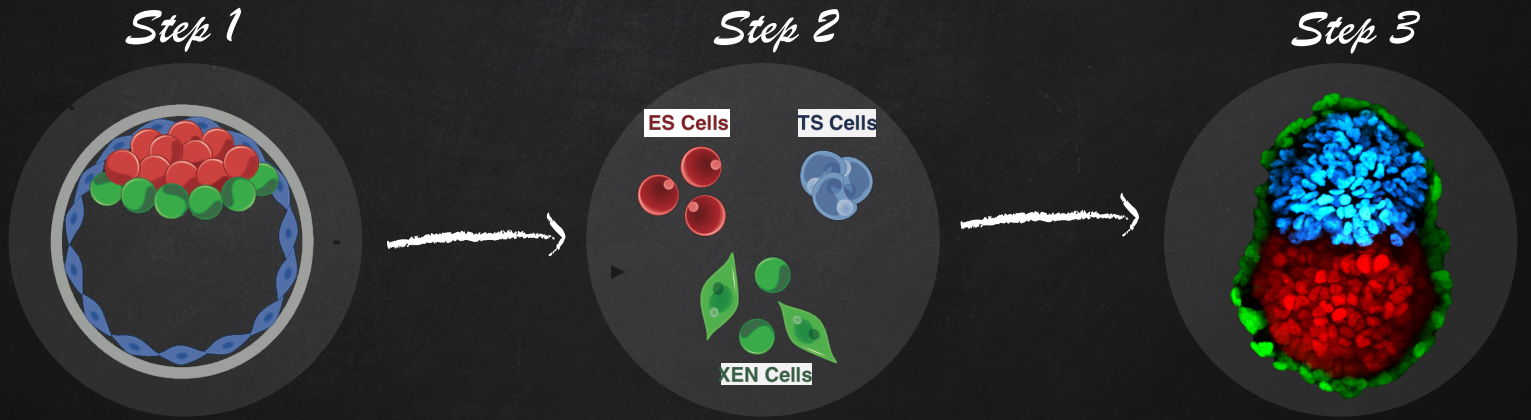
Existing models

- ***ESCs-only:*** Micropatterns, EBs, Gastruloids,
- ***ESCs+ExSCs:*** ET, ETX, Blastoid
- ***EPSCs:*** EPS-Blastoid(s)

Significance

- *In vitro* patterning in space and time
- Hidden dev. events
- Bottom-up approach

Deconstructing the mammalian early patterning while reconstructing it with stem cells



Harrison SE. & Sozen B. et al 2017 | **Science**
Harrison SE. & Sozen B. et al 2018 | **Nature Prot.**
Sozen B.& Amadei G. et al 2018 | **Nature Cell Biology**
Sozen B. & Cox A. et al 2019 | **Dev. Cell**

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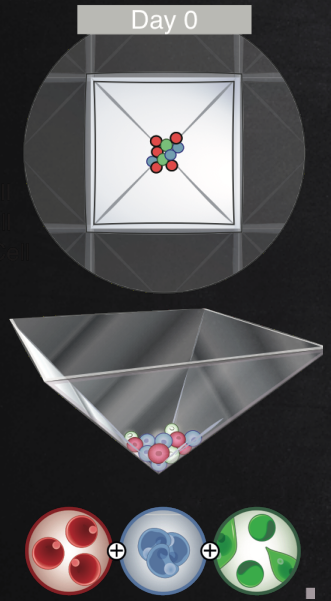
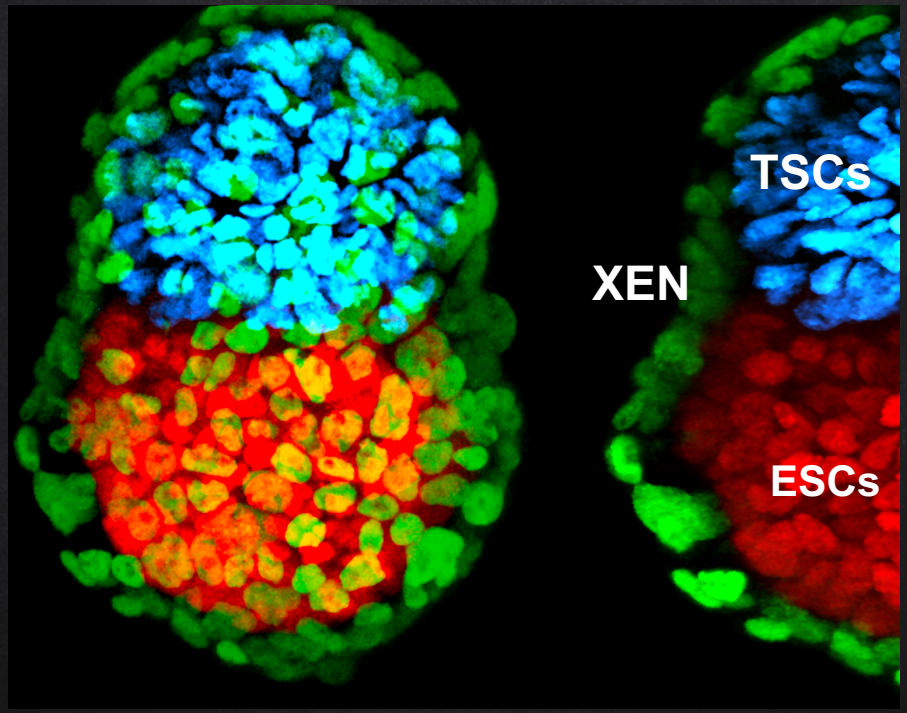
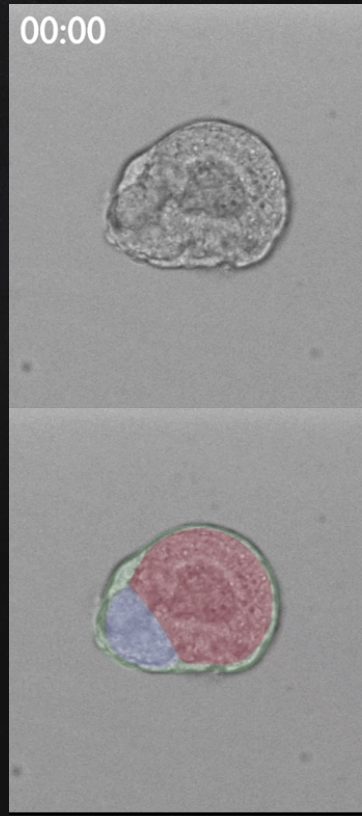
Reconstructing the mammalian embryo: ES + TS + XEN Cells





OCT4 AP2g GATA6

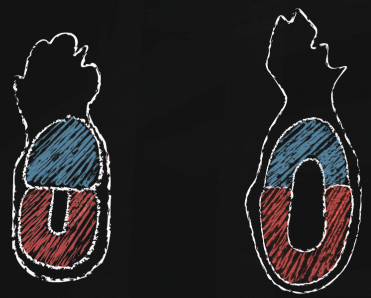
From Day 3 to 5



symmetry breaking

Implantation

Pre-gastrula



- Anterior-posterior patterning
- Proamniotic cavity formation

Gastrulation

Early

- Primitive streak formation
- Germ-layer formation

Mid

- Node formation
- Left-right patterning

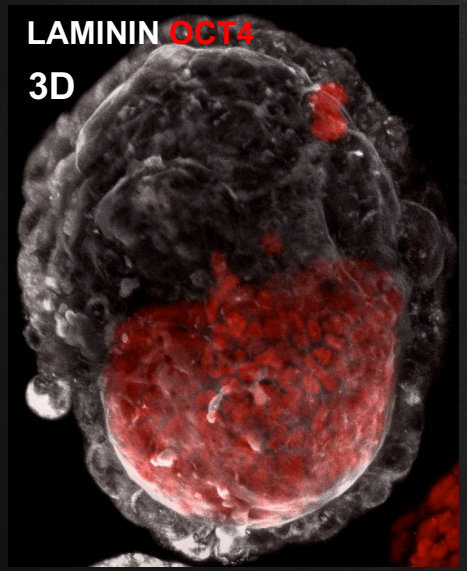
Late

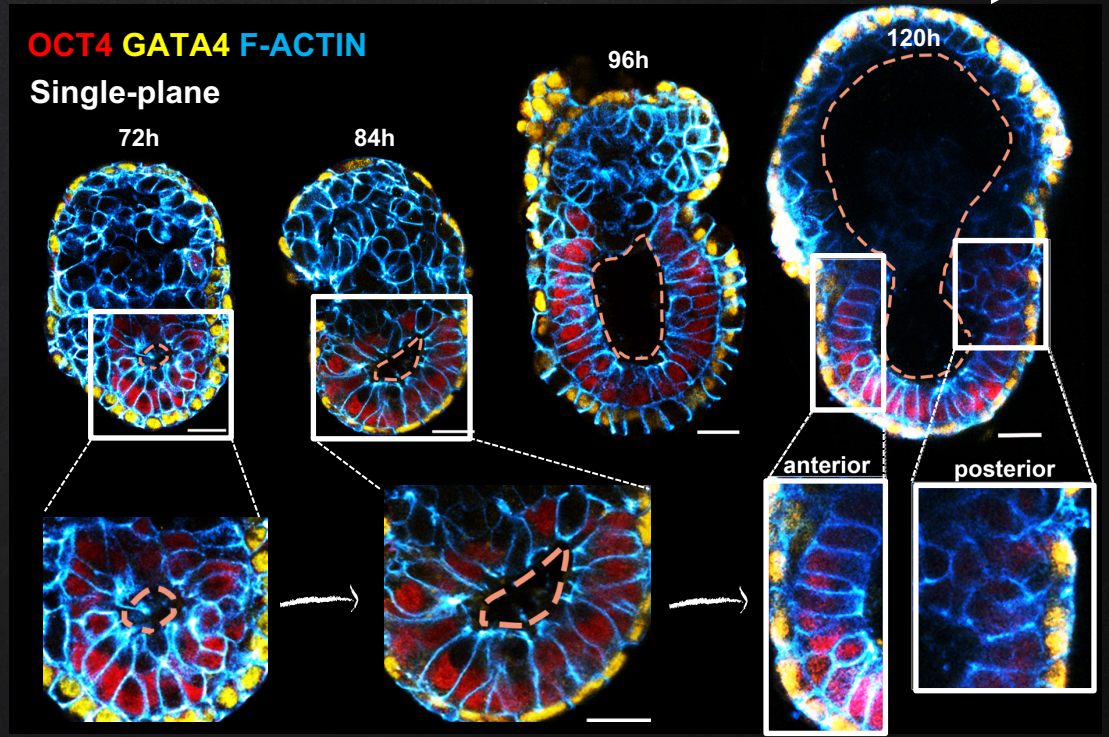
Organogenesis

- Neural plate formation

Pre-gastrulation patterning in stem cell model embryos

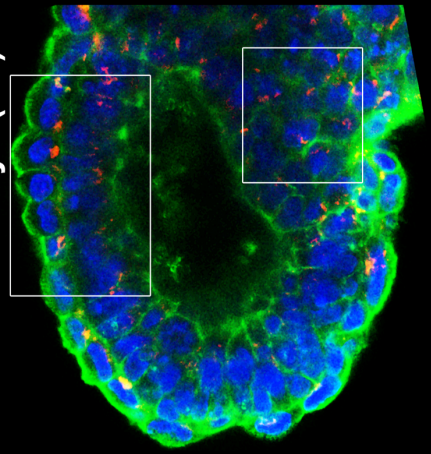
Basement membrane formation → **Lumenogenesis and PAC formation**



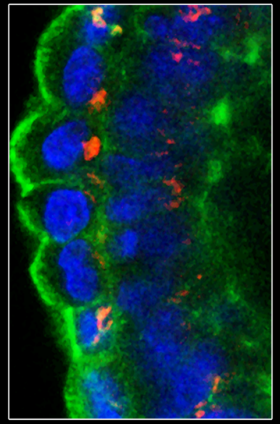


F-ACTIN DAPI GM130

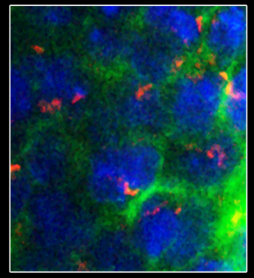
ETX Embryo (D5)



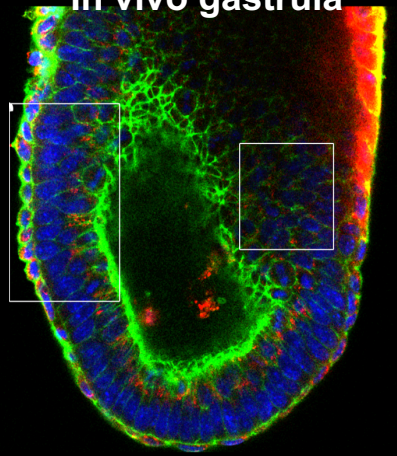
Anterior



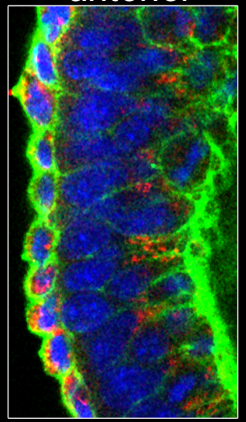
Posterior



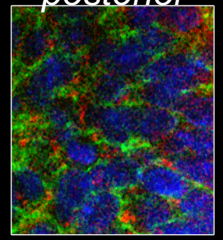
In vivo gastrula



anterior

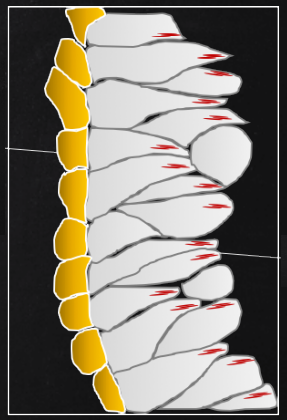


posterior

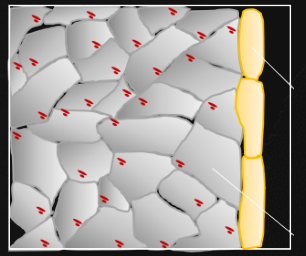


Cellular polarity changes in patterning

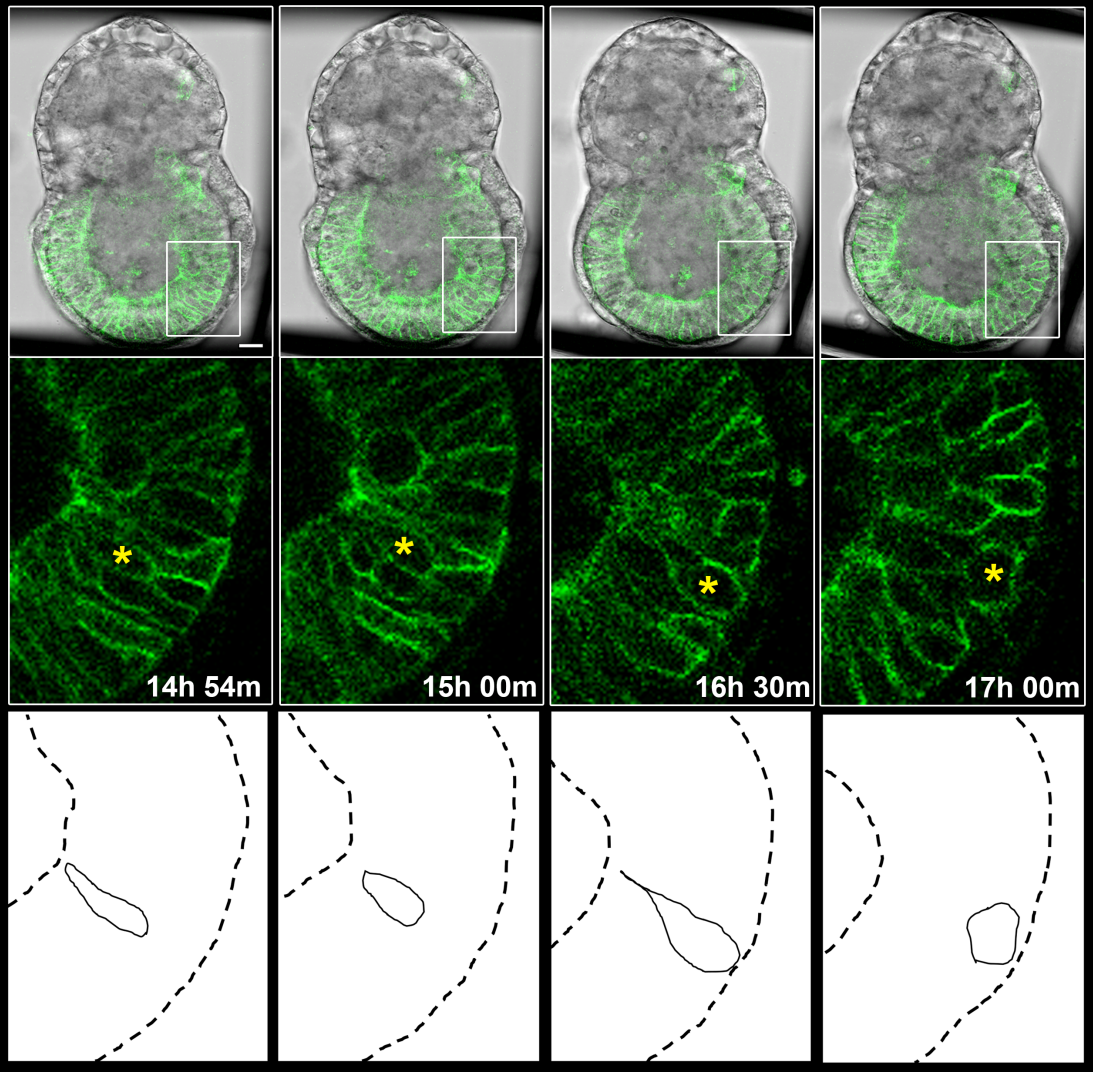
Anterior

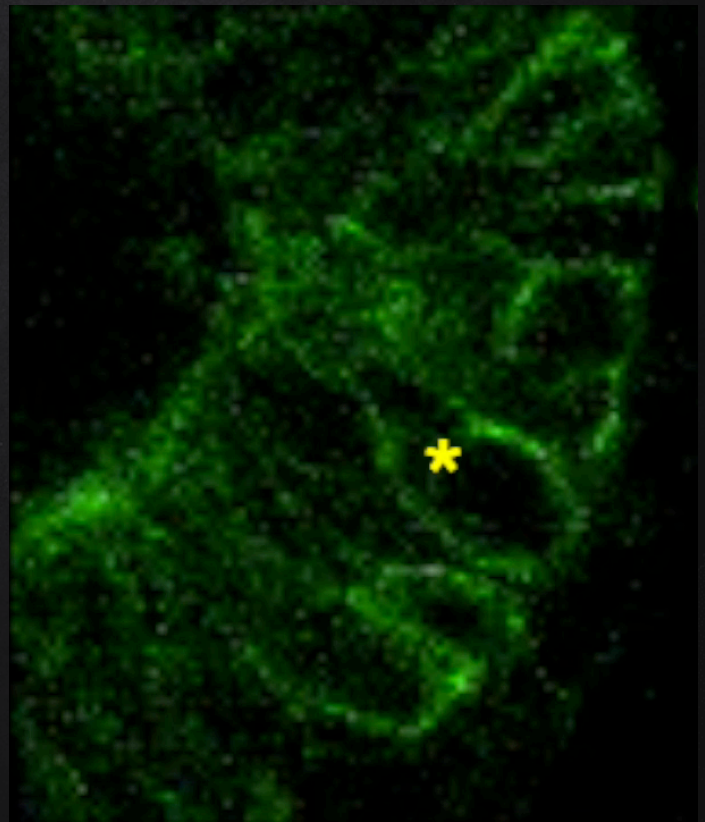


Posterior



Cellular movements during patterning





symmetry breaking

Implantation

Gastrulation

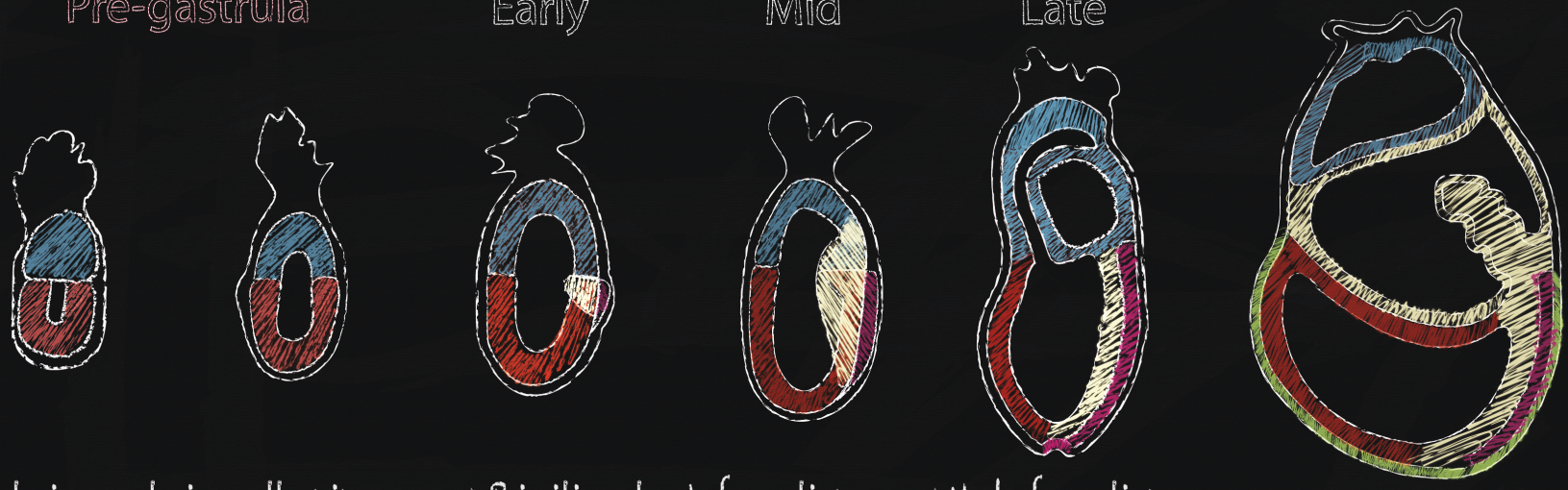
Organogenesis

Pre-gastrula

Early

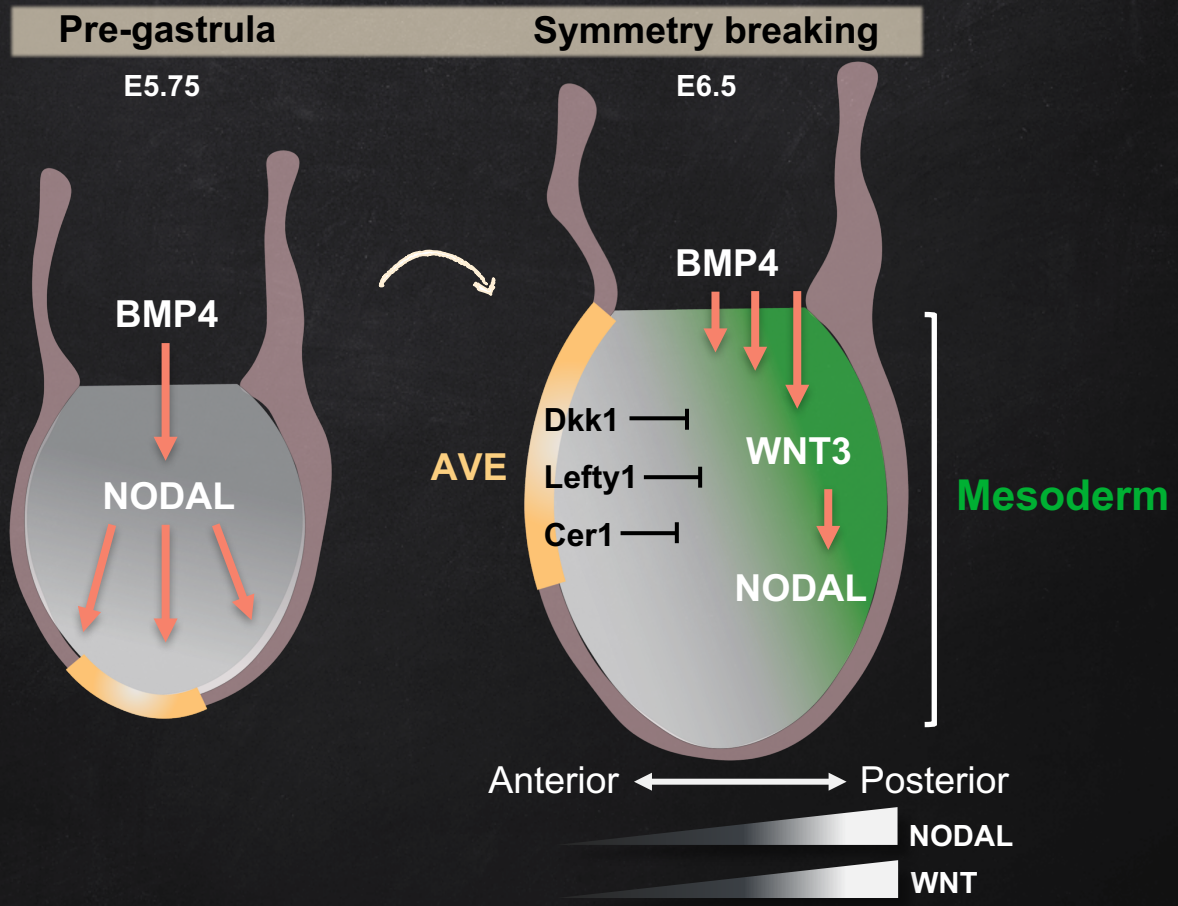
Mid

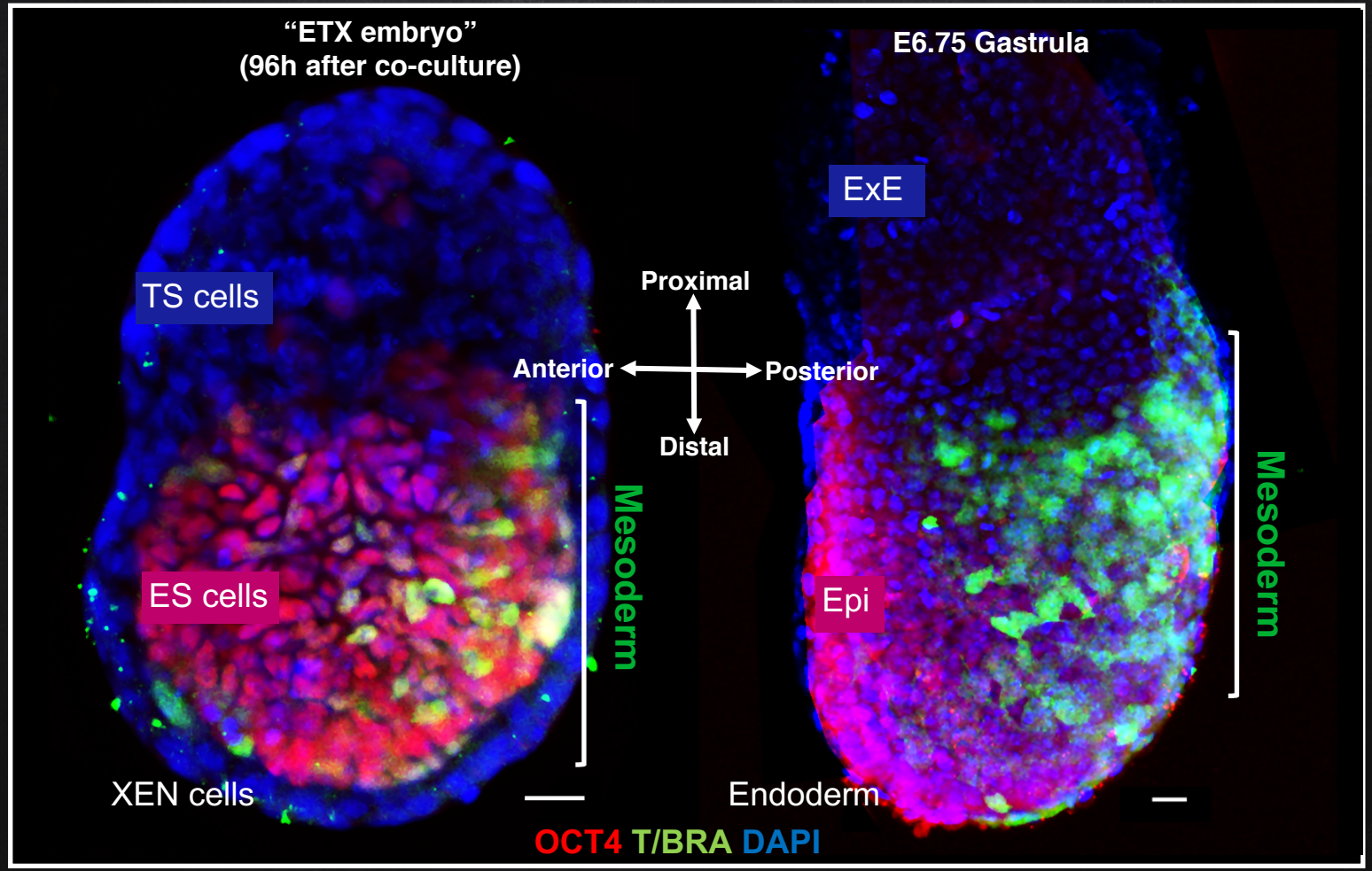
Late

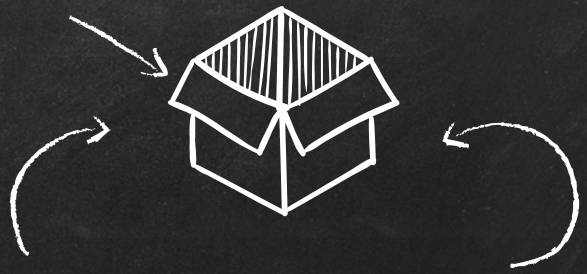


- Anterior-posterior patterning
- Proamniotic cavity formation
- Primitive streak formation
- Germ-layer formation
- Node formation
- Left-right patterning
- Neural plate formation

Mesoderm induction and morphogen gradients




"ETX embryo"
(96h after co-culture)
E6.75 Gastrula
ExE
TS cells
Proximal
Anterior
Posterior
Distal
Mesoderm
ES cells
Epi
Mesoderm
XEN cells
Endoderm
OCT4 T/BRA DAPI



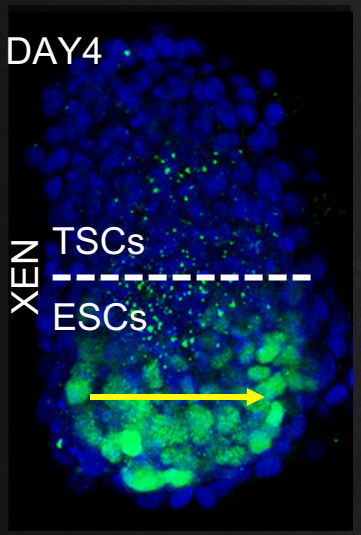
How do stem cell embryos break the symmetry and gain axis specification?

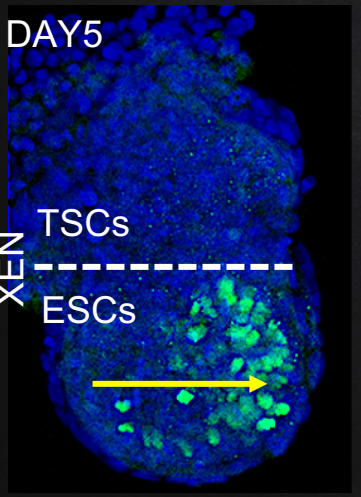
Potential mechanisms:

Morphogen gradients?

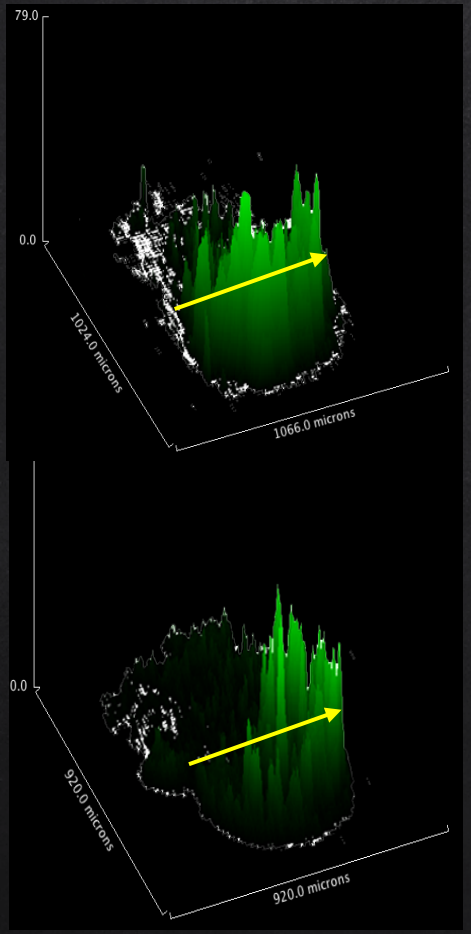
Spontaneous symmetry breaking?

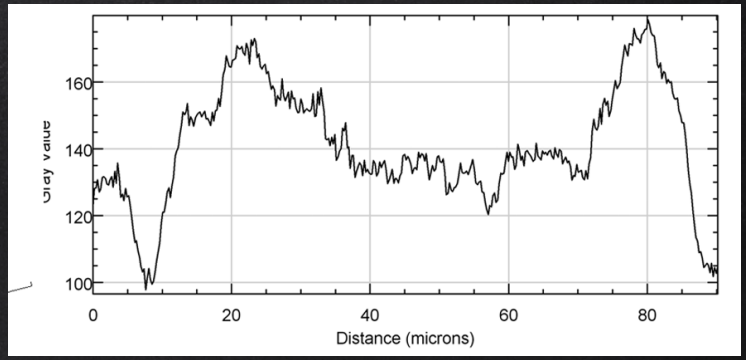
Signalling gradients

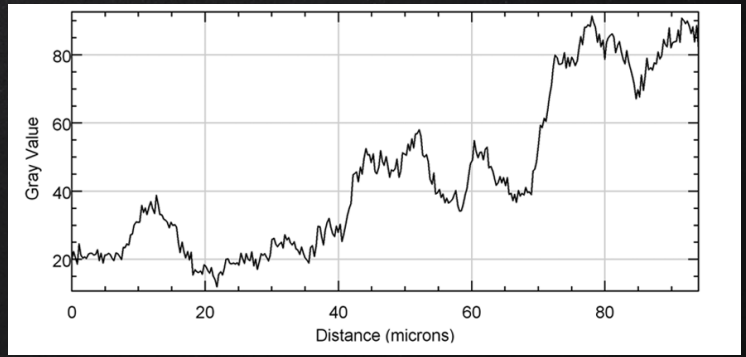


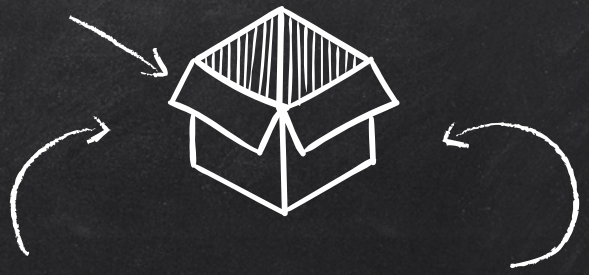


NODAL
Intensity [AU]



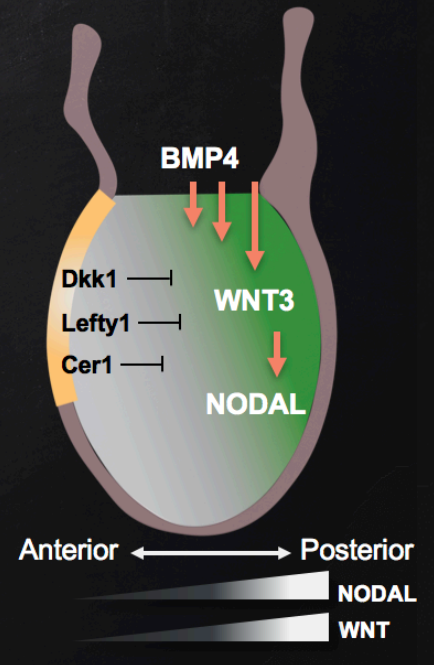




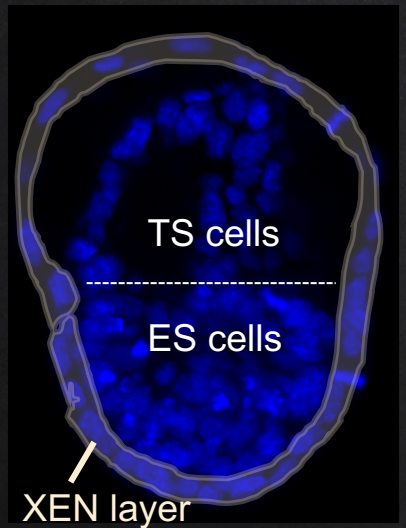


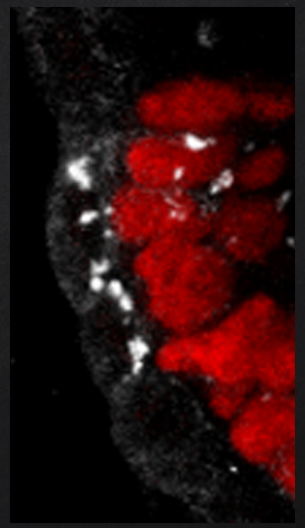
What establishes the asymmetric signalling activity?

- ✗ Extra-embryonic cues?
- ✗ AVE organiser specification?
- ✗ Spontaneous?

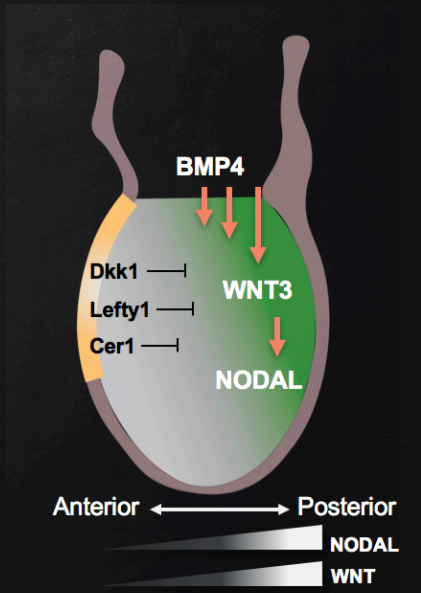


Does AVE function in integrated stem cell model embryo system?





LEFTY1 OCT4 DAPI



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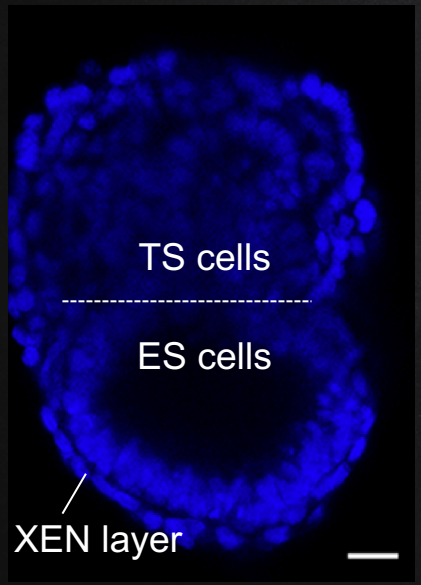
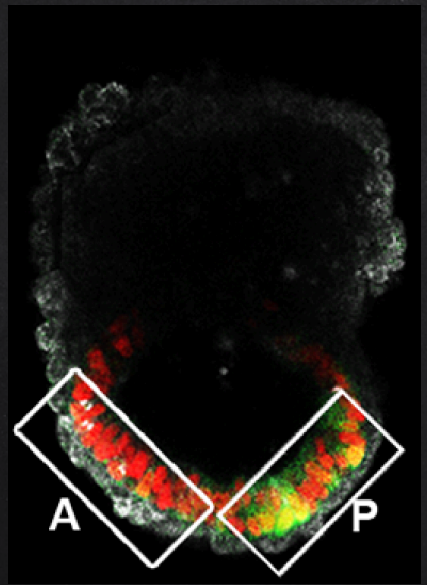
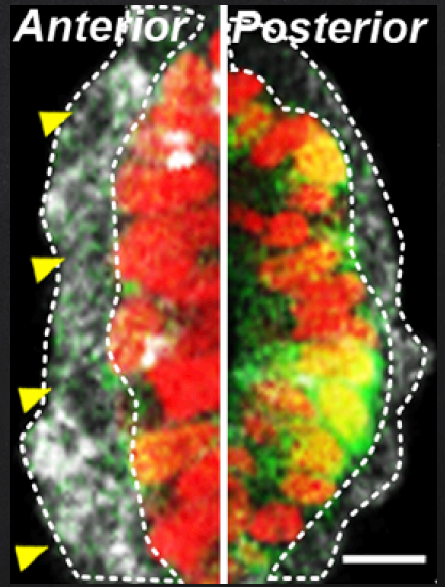
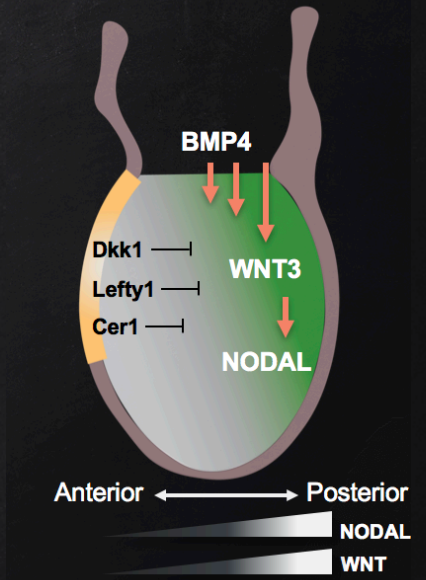
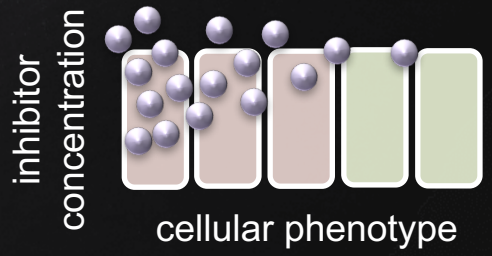






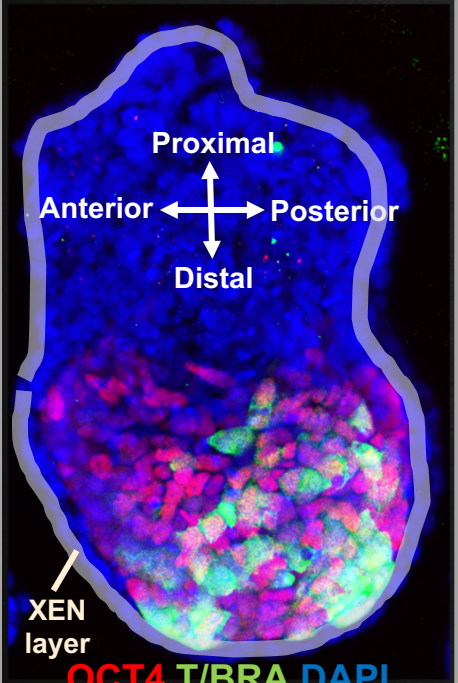
LEFTY1 OCT4 T/BRA DAPI





Symmetry breaking w/o AVE cues…

ETX embryo with no AVE

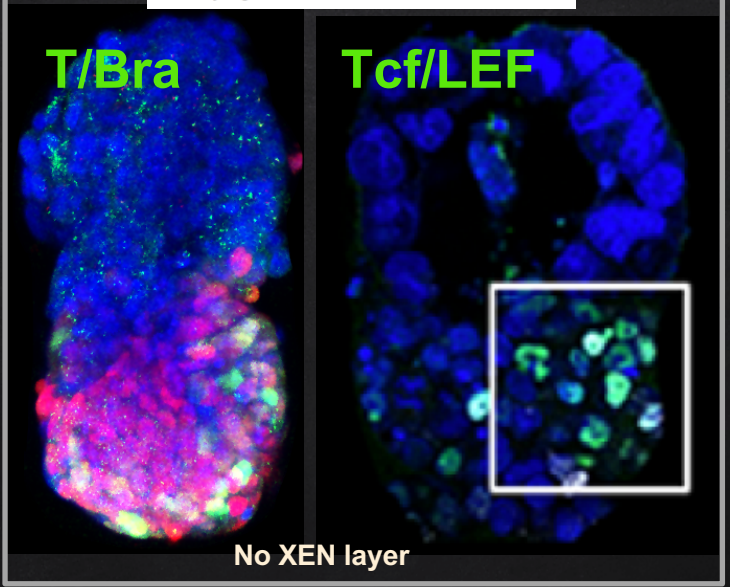


ET embryo with ESCs-TSCs

DEVELOPMENTAL BIOLOGY

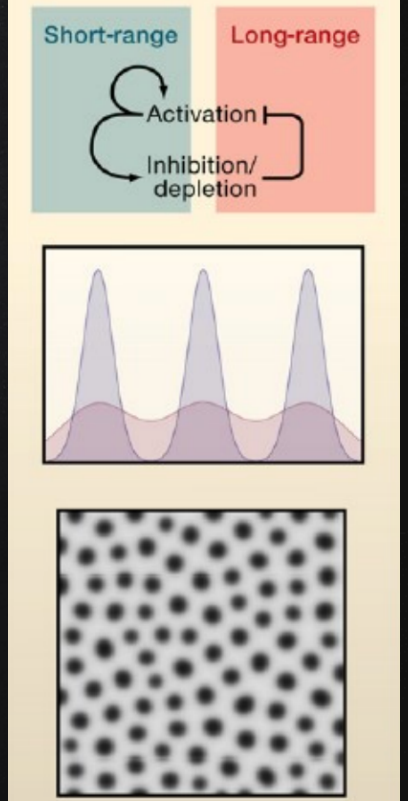
Science AAAS

Assembly of embryonic and extraembryonic stem cells to mimic embryogenesis in vitro

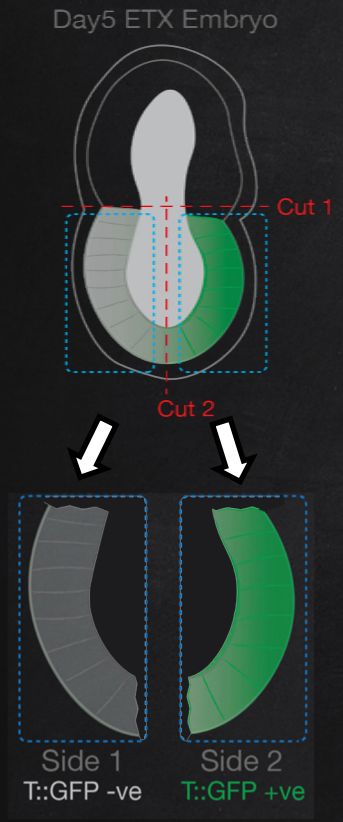


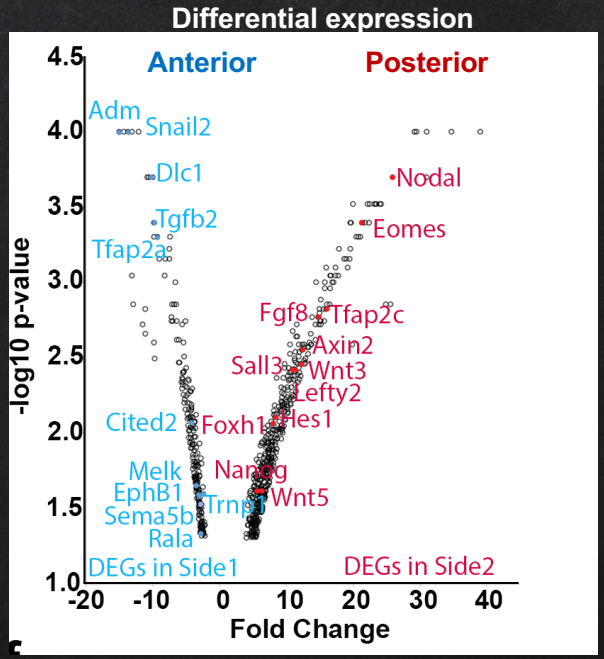
←?→

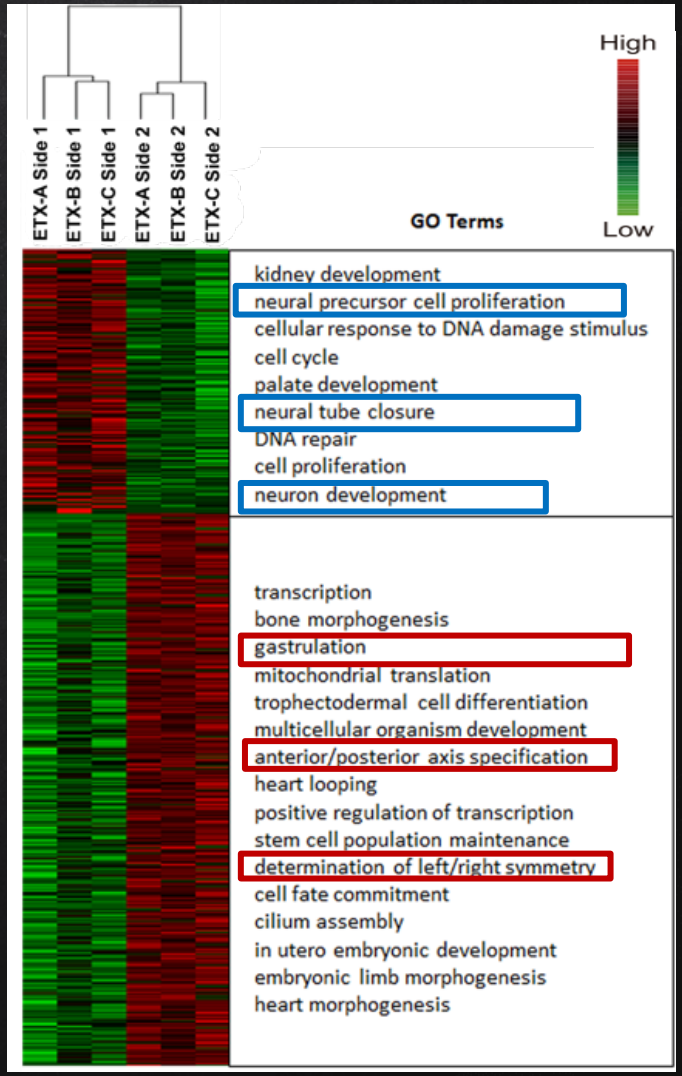
Self-organised



Comparison of gene expression patterns



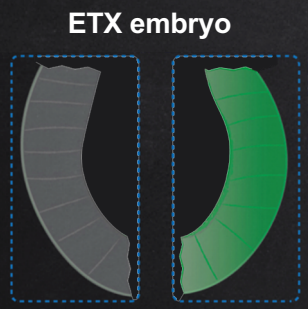




Sozen B.& Amadei G. et al | **Nature Cell Biology**

Comparison of gene expression patterns

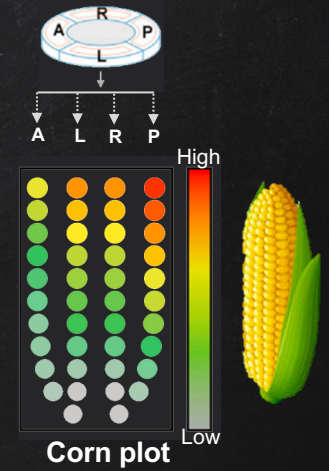
Principle:

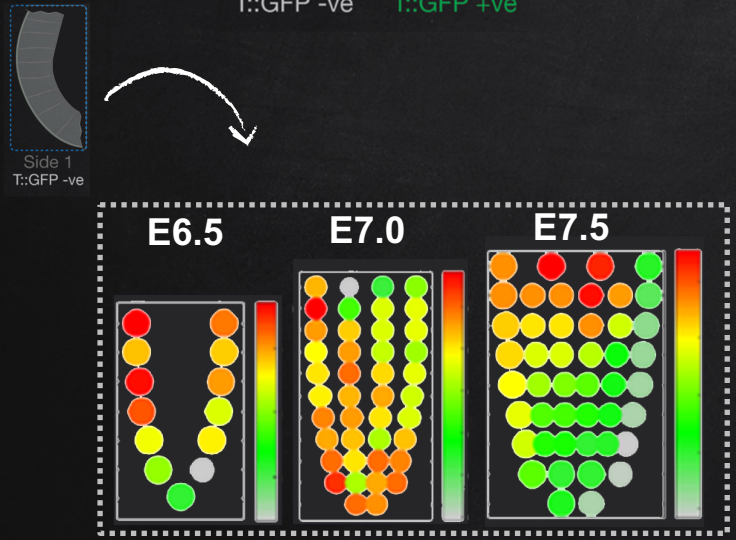


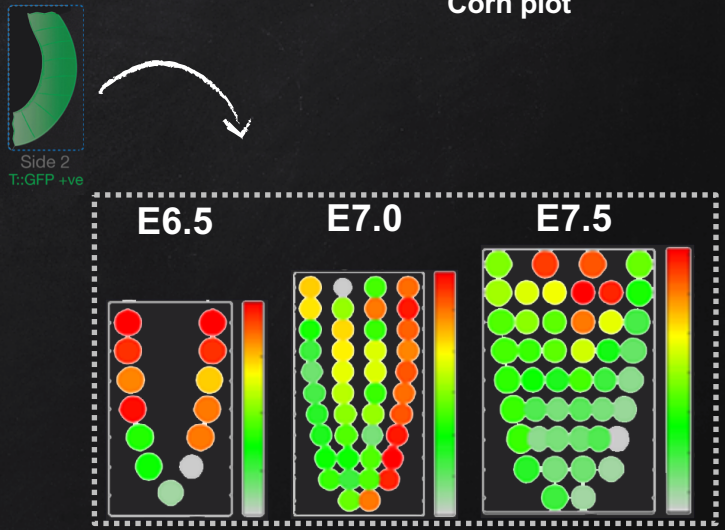
vs

Peng et al. 2014

visualizing the outcome on ‘corn plots’



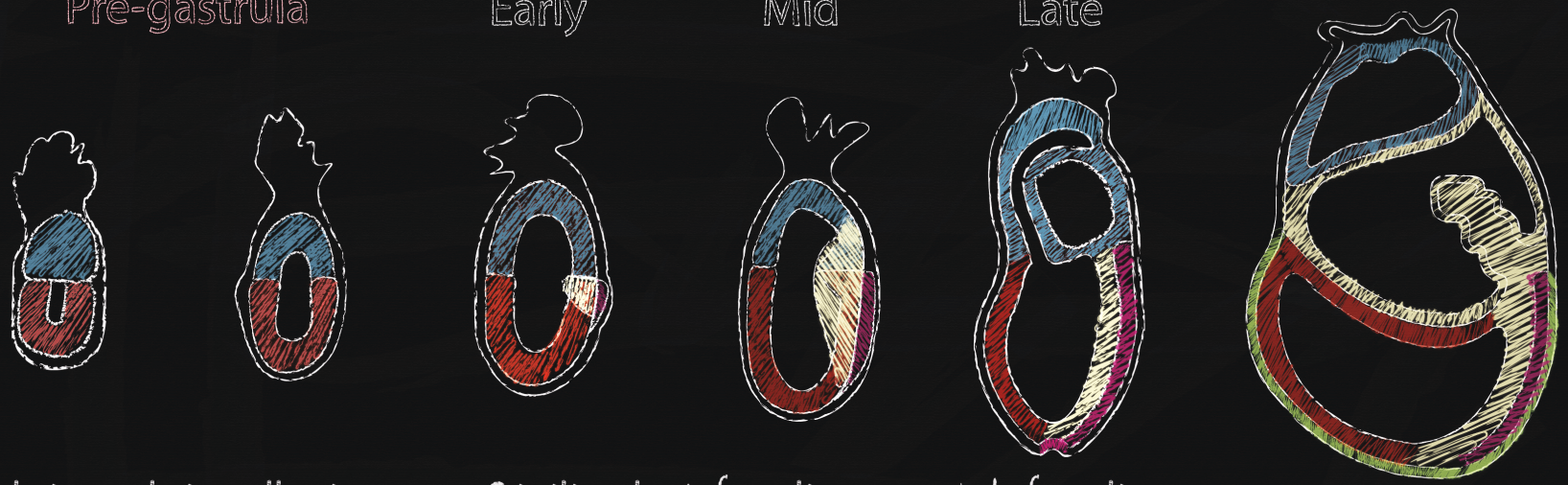




symmetry breaking

Implantation — Gastrulation — Organogenesis

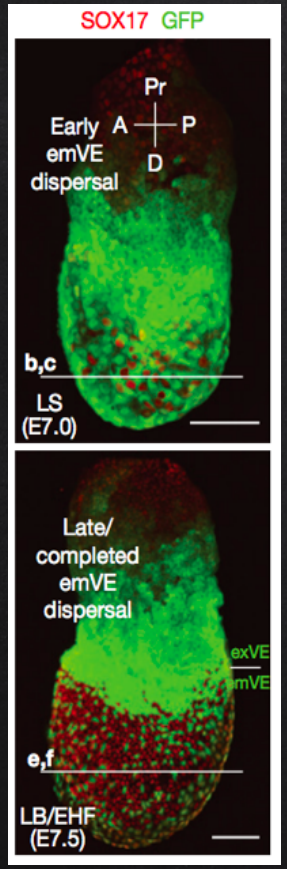
Pre-gastrula | Early | Mid | Late



- Anterior-posterior patterning
- Proamniotic cavity formation
- Primitive streak formation
- Germ-layer formation
- Node formation
- Left-right patterning
- Neural plate formation

3rd Germ Layer: Definitive Endoderm (DE) Specification

natural embryo



Vinotti M. et al, Nat. Cell Bio., 2014

Developmental milestones recapitulated *in vitro* with stem cells:

Limitations:

- Efficiency
- Anteriorisation is not robust
- EMT events limited
- Biomechanical features

Open questions:

- Alternative contributing mechanisms to axis patterning?
- What is the self-organization potential of the extraembryonic lineages?
- Contribution of the biophysical mechanisms?

thanks!

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Diversity is the power!