

Week 13 — Thursday, 18 November

26. Finish limb - start ES/iPSCs and organoid

Remember to turn on zoom



Put in your pods  
Start recording  
Share screen!

- Tuesday's and today's beSocratic + "Blast from the Past" activities due Tuesday - when we return (today's posted at 5PM)
- Over the break: read first page (the rest is optional): [Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment](#)
- For every processes, think of how you would know exactly what is going on - what techniques (in general) could you use
  - What can you learn from single cell RNA seq, chromatin IP and cross-linking, mass spec, reporter assays, etc.

- TEAMS devchat reminder

<https://www.microsoft.com/en-us/microsoft-teams/download-app>

Blast from the past 2: In simple diagrams (→) starting with a signal, a receptor, and a response propose simple positive and negative molecular feedback systems.

Describe factors that might speed up (A) or slow down (B) the feedback response signal.

Then provide an example of a biological system that would benefit from positive or negative (your choice) feedback signalling?

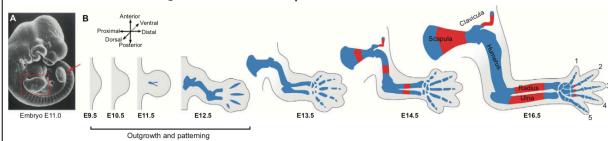


hint: How can the timing of feedback effects be controlled? and to what purpose?

We will go over the rest on  
Tuesday the 30th of November

Questions?

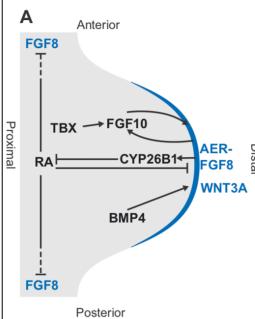
An overview of the stages of limb development.



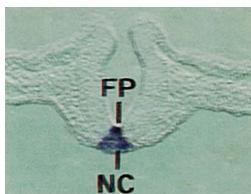
- Morphological changes occurring during forelimb development
- Hindlimb bud development (not shown) is delayed by half a day in comparison with the forelimb bud.
- The proximal scapula (shoulder blade) and clavicle are located in the body.

Next generation limb development and evolution: old questions, new perspectives  
Aimée Zuniga\*

Scheme of a mouse limb bud: mesenchyme (grey), the apical ectodermal ridge (AER, blue) and the zone of polarizing activity (ZPA, red).



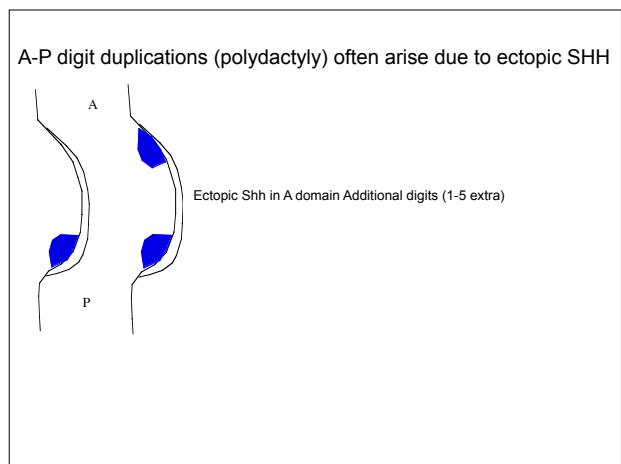
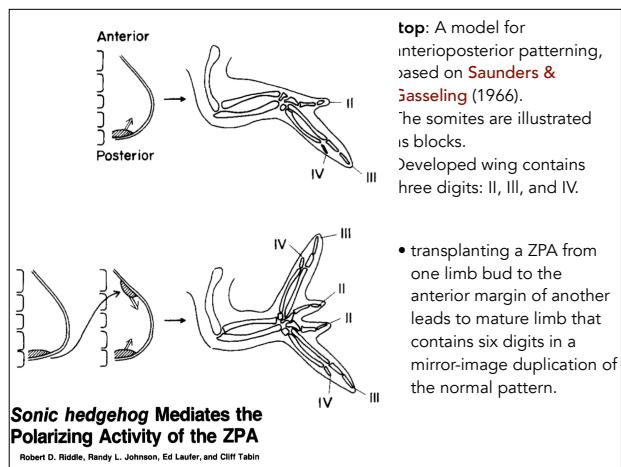
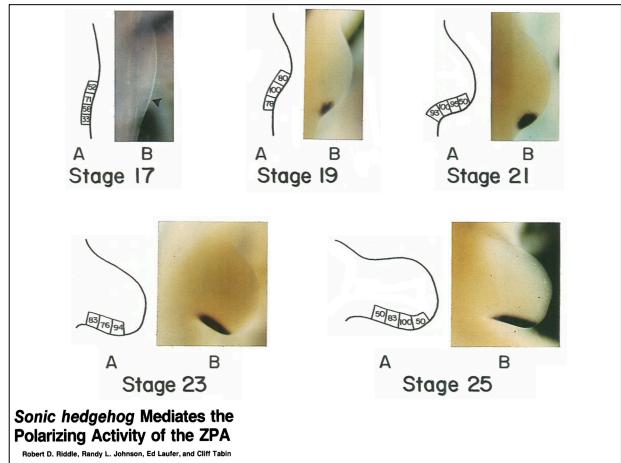
- Position determined by HOX expression
- Antagonism between retinoic acid (RA) & FGF8 patterns the limb field along the primary axis.
- Limb bud emergence is controlled by TBX and Fgf10 regulated genes.
- Fgf10 signaling from the mesenchyme and Fgf8 from progenitors of the AER marker establishes an epithelial-mesenchymal feedback loop that initiates limb bud outgrowth.
- Antagonistic interactions between RA (proximal mesenchyme), AER-derived FGF8.
- CYP26B1 expression is up-regulated by FGF8 signaling and in turn degrades RA in the distal mesenchyme.
- limb elongates

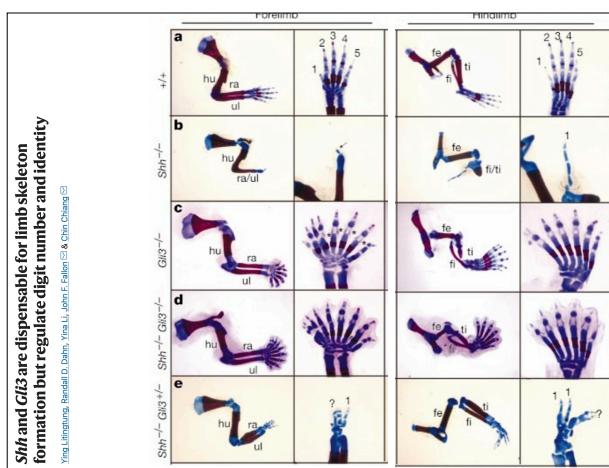
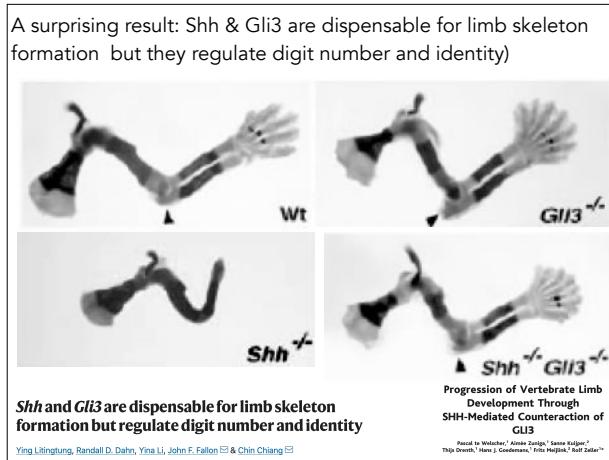
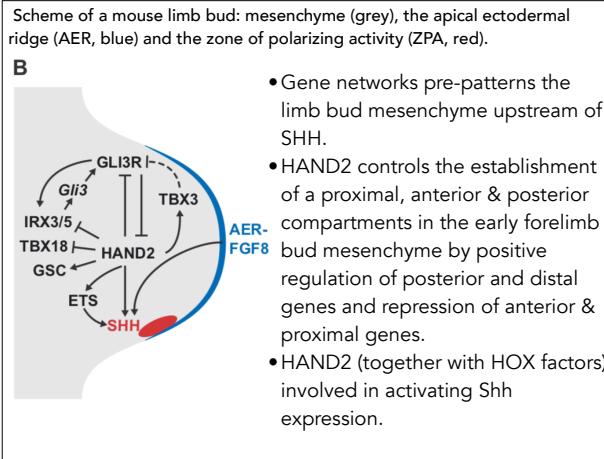


- Used Drosophila Hh to isolate mouse Hh homology (Shh).
- Expression visualized by *in situ* hybridization.

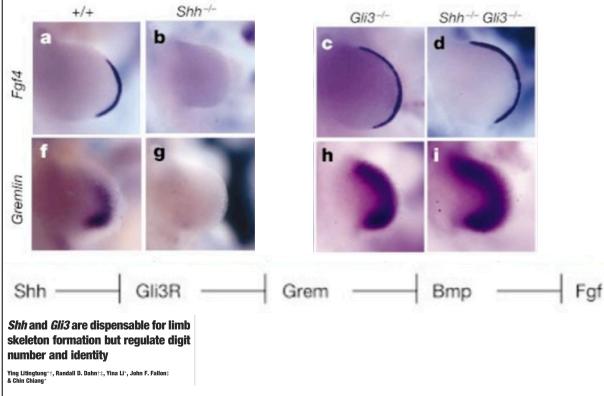
**Sonic hedgehog Mediates the Polarizing Activity of the ZPA**

Robert D. Riddle, Randy L. Johnson, Ed Laufer, and Cliff Tabin

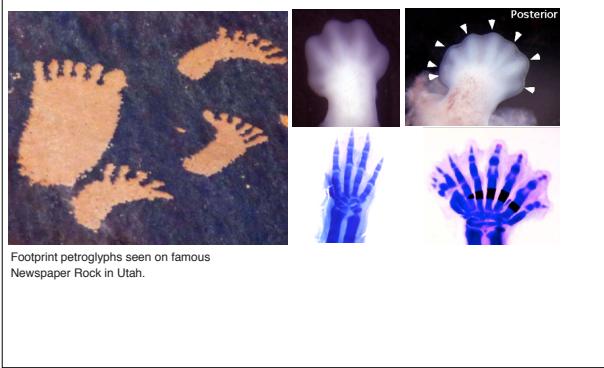




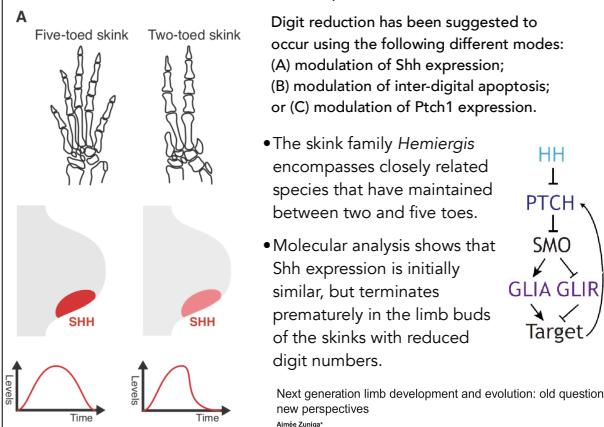
Shh maintains ridge (AER) function by relieving an antagonistic regulatory cascade.



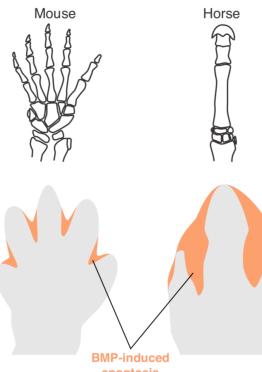
Different modes of digit reduction in tetrapods.



Different modes of digit reduction in tetrapods.



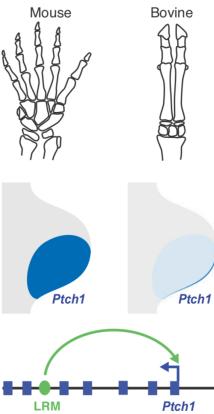
### Different modes of digit reduction in tetrapods.



- Scheme of a *Mus musculus* (mouse) and *Equus ferus* (horse) forelimb.
- The area of BMP-induced apoptosis is significantly increased in the interdigital mesenchyme of horse limb buds in to the mouse.

Next generation limb development and evolution: old questions, new perspectives  
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### Different modes of digit reduction in tetrapods.



- Scheme of a *Mus musculus* (mouse) & *Bos taurus* (bovine) forelimb skeleton.
- Ptch1 expression in the mesenchyme of bovine limb buds is much reduced in comparison to its mouse counterpart.
- A limb bud-specific cis-regulatory module (LRM) controls the transcriptional upregulation of Ptch1 in the distal mesenchyme of mouse limb buds.
- The bovine LRM is non-functional and fails to upregulate expression in limb bud mesenchyme.
- The failure to upregulate mesenchymal Ptch1 expression leads to early loss of AP asymmetry in the artiodactyl (cloven hoof) limb buds (bovine and pig).

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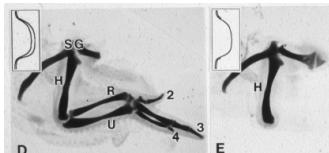
### Outgrowth of the limb (dependent on apical epidermal ridge)

pioneered by John Saunders

THE PROXIMO-DISTAL SEQUENCE OF ORIGIN OF THE PARTS OF THE CHICK WING AND THE ROLE OF THE ECTODERM

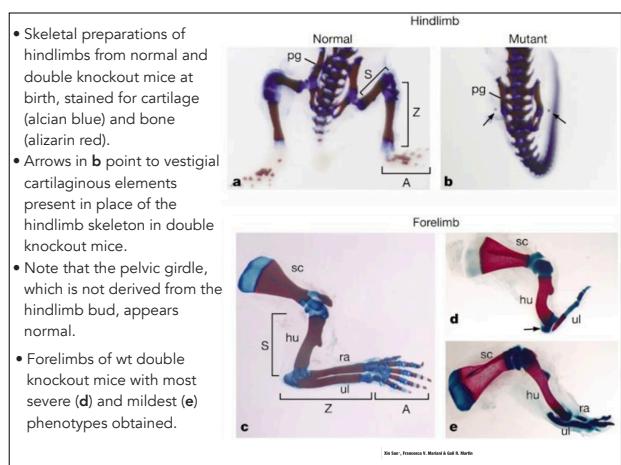
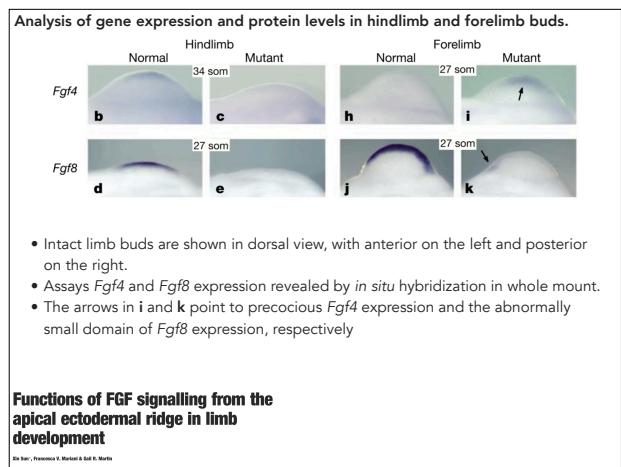
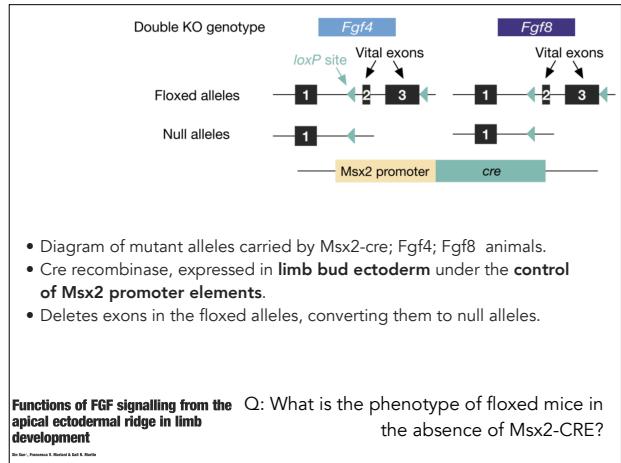
JOHN W. SAUNDERS, JR.  
Department of Biology, The Johns Hopkins University, Baltimore, Maryland

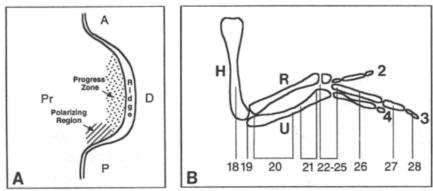
1 THE JOURNAL OF EXPERIMENTAL ZOOLOGY  
Vol. 108, No. 3, August, 1948



STAGE OF OPERATION	WING PARTS FORMED
4	
5	
6	
7	

FGFs expressed in the AER

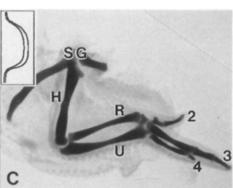




- Schematic of an early stage limb bud showing the apical ectodermal ridge and two functionally distinct regions of the limb bud mesenchyme, the progress zone and the polarizing region.
- Schematic drawing of a normal limb at approximately 10 days of development; each numbered line indicates the approximate proximodistal level at which limb truncation would occur following removal of the ridge at the stages designated.

**FGF-4 Replaces the Apical Ectodermal Ridge and Directs Outgrowth and Patterning of the Limb**

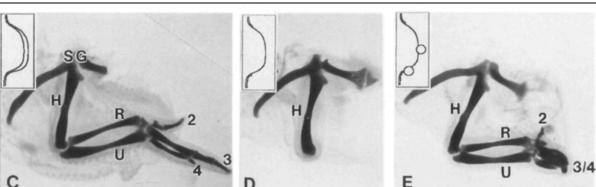
Lee Hineseder,<sup>1,2</sup> Cheryl Tickle,<sup>1</sup> Astrid Vogel,<sup>1</sup> and Gert R. Martin<sup>1</sup>  
<sup>1</sup>Imaging Facility, Institute for the Primary Signal Response  
<sup>2</sup>Sanford-Burnham Medical Research Institute, La Jolla, CA 92037, USA



- CONTROL: The photographs illustrate the extent of limb development 6-7 days after experimental manipulation, and each is representative of the denoted treatment.
- The limbs have been stained to reveal the skeletal pattern

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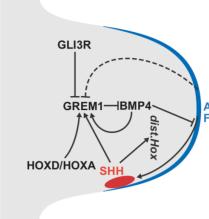
- D) Ridge removal at stage 20.
- (E) Ridge removal at stage 20, followed by application of one FGF bead to posterior and one to apical mesenchyme.

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Scheme of a mouse limb bud: mesenchyme (grey), the apical ectodermal ridge (AER, blue) and the zone of polarizing activity (ZPA, red).

C



- Distal limb bud outgrowth is driven by SHH/GREM1/AER-FGF feedback signaling.
- HOXD/HOXA transcriptional regulators positively regulate Grem1 expression.
- SHH propagates the expression of Grem1 and distal Hox genes.
- As BMPs repress AER-FGF gene expression, Grem1 is essential to propagate these feedback signaling interactions.
- Ultimately, the SHH/GREM1/AER-FGF loop is terminated by down-regulation of Grem1 expression by GLI3R (anterior mesenchyme) and increasing inhibition by AER-FGFs

## ES & iPS cells

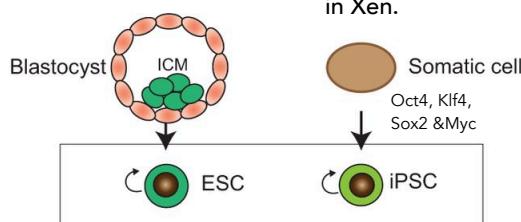
**Embryonic stem cells (ES cells):** derived from mammalian embryo inner cell mass  
begin as totipotent - can be differentiated *in vitro*

**induce pluripotent stem cells (iPS cells):** derived from differentiated cells, driven to become pluripotent, can differentiate into many cell types.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676.

ES and iPSC

process similar to  
nuclear transplantation  
in Xen.



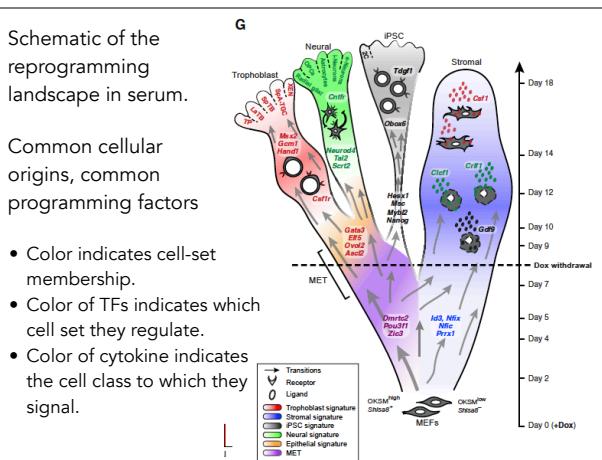
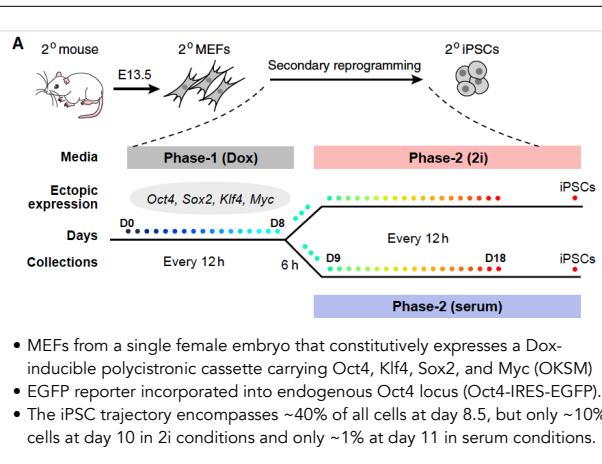
Q: how might type of somatic cells influence efficiency of reprogramming?

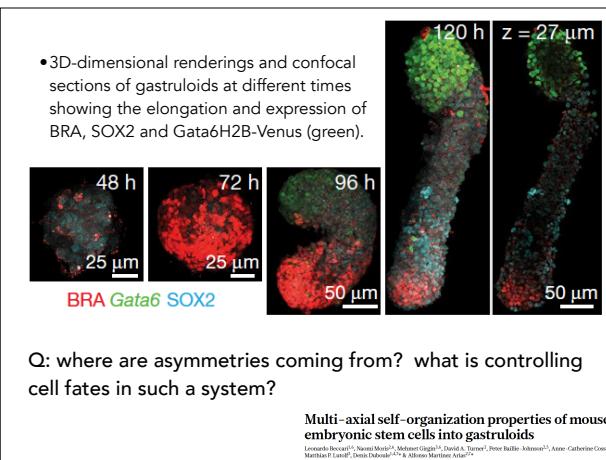
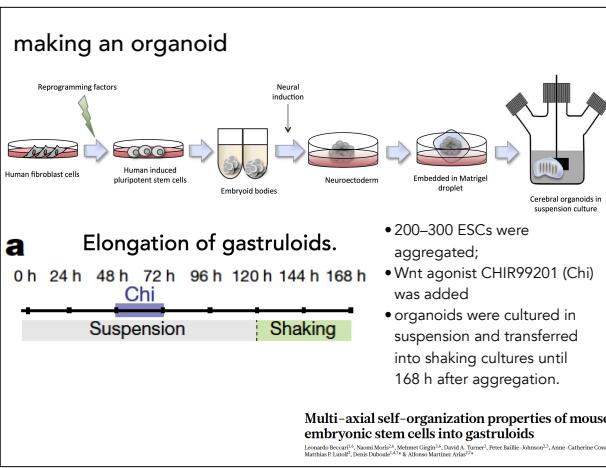
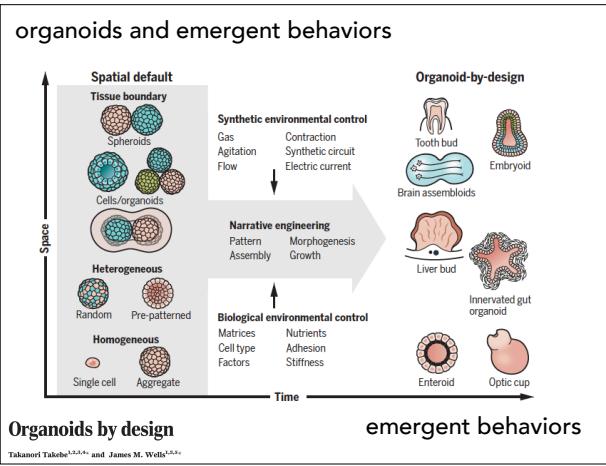
How were reprogramming factors identified?  
(hints and trial and error)

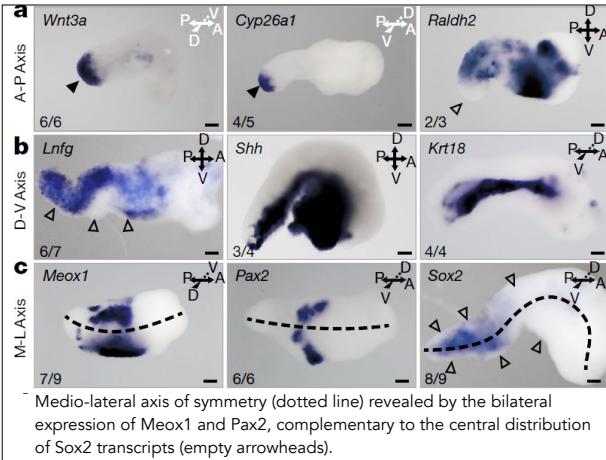
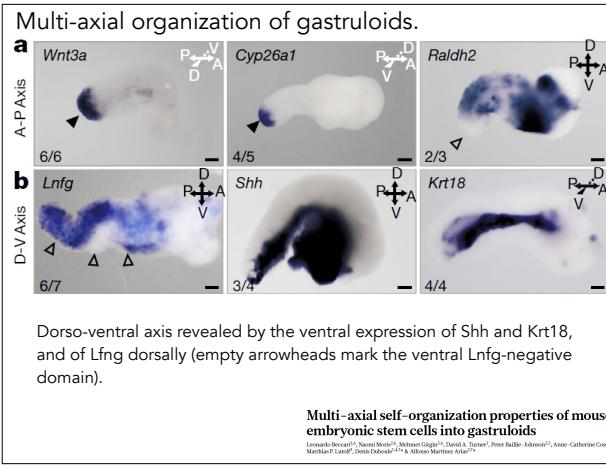
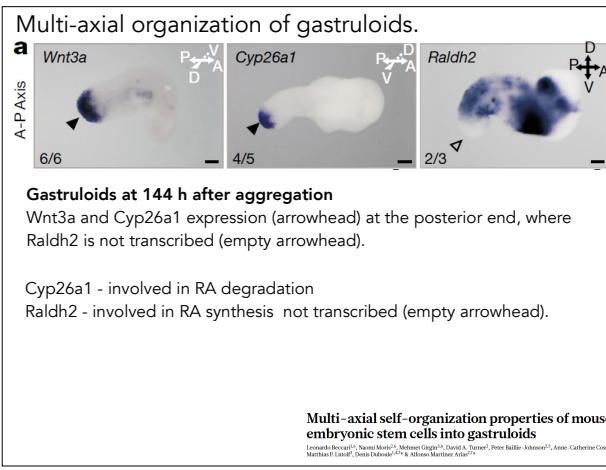
## Genome editing reveals a role for OCT4 in human embryogenesis

Norah M. E. Fogarty<sup>1</sup>, Afsnan McCarthy<sup>1</sup>, Kirsten E. Snijders<sup>2</sup>, Benjamin E. Powell<sup>1</sup>, Nada Kubikova<sup>4</sup>, Paul Blakeley<sup>1</sup>, Rebecca Lea<sup>1</sup>, Kay Elder<sup>3</sup>, Sissy E. Wamaitha<sup>1</sup>, Daesik Kim<sup>6</sup>, Valdene Macaulay<sup>1</sup>, Jens Kleinjung<sup>7</sup>, Jin-Soo Kim<sup>6,8</sup>, Dagan Wells<sup>4</sup>, Ludovic Vallier<sup>2,9,10</sup>, Alessandro Bertero<sup>3,4</sup>, James M. A. Turner<sup>3</sup> & Kathy K. Niakan<sup>1</sup>

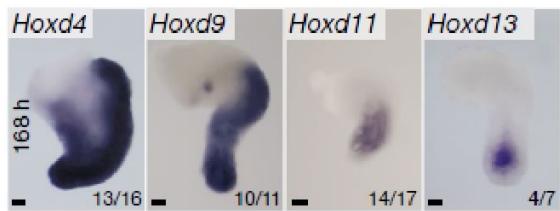
- OCT4-targeted human embryos initiate blastocyst formation, the inner cell mass (ICM) forms poorly, and embryos subsequently collapse – associated with the down-regulation of genes associated with all three preimplantation lineages, including NANOG (epiblast), GATA2 (trophectoderm) and GATA4 (primitive endoderm).







In situ hybridization of 168 h AA gastruloids using probes for various Hoxd genes.

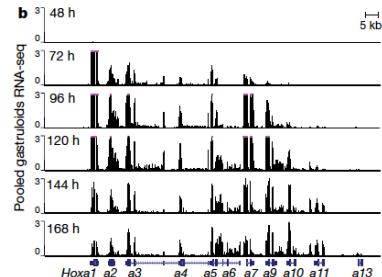


Expression becomes spatially restricted along the antero-posterior axis in parallel with the respective position of the genes in the cluster. For each gene, the proportion of gastruloids displaying the reported expression pattern is shown in the bottom right corner of the image, expressed as a fraction of the total number of gastruloids analysed. Scale bar, 100  $\mu$ m.

Multi-axis self-organization properties of mouse embryonic stem cells into gastruloids

Lennart Boonen<sup>1</sup>, Nanni Meini<sup>2</sup>, Barbara Giugni<sup>1</sup>, David Lévy<sup>1</sup>, Frédéric Baril<sup>1</sup>, Joaquim<sup>1</sup>, Anne-Catherine Goss<sup>1</sup>,

Martina P. Lenz<sup>1</sup>, Denis Duboule<sup>1,2,\*</sup> & Alfonso Martinez Arias<sup>1,2</sup>



Transcript profiles over the HoxA cluster, using time-sequenced pooled gastruloids.

A progressive wave of transcription through Hoxa genes is observed between the 72 h and 168 h after aggregation time points.

The arrangement of the Hoxa cluster is shown schematically below the x axis.

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Hoping you have a safe and  
rejuvenating Thanksgiving break