Single-Cell Revolution: Embryogenesis at High-Resolution

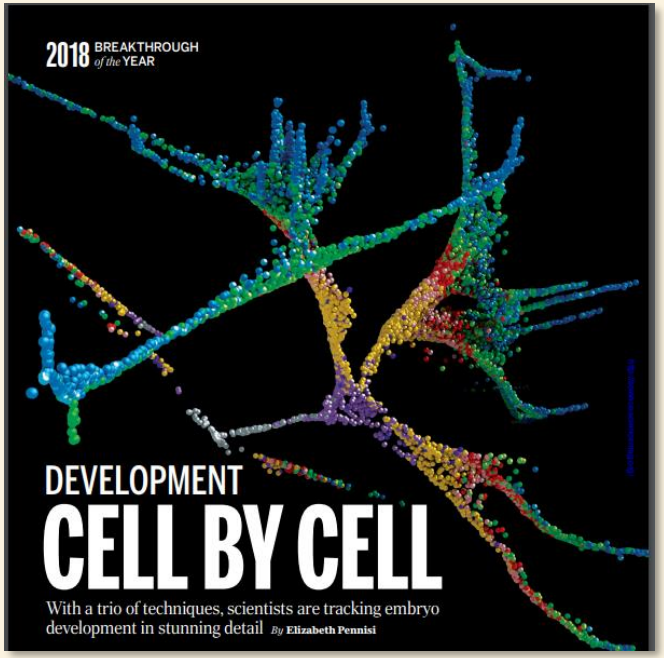
Symposium organized by the BDRP Science Committee

July 2, 2020

12:15 – 2:15 pm

(virtual)

Profiling development at the single-cell level



Three technical pillars:

- scRNAseq analysis *(cell-state landscape)*

- light sheet microscopy *(cell-imaging in situ)*

- CRISPR/*Cas9* *(functional analysis)*

https://science.sciencemag.org/content/sci/362/6421/1344.full.pdf

Symposium Overview

What does scRNAseq bring to 21st Century embryology and birth defects research?

Teratogenesis at the Single-Cell Level: Opportunities and Challenges
Thomas Knudsen, USEPA/ORD-CCTE

Development and Disease at Single Cell Resolution
Malte Spielmann, Max Planck Institute for Molecular Genetics

What scRNAseq Is Now Telling Us about Embryology
Sean Megason, Harvard Medical School

How Single-Cell Profiling Data Can Be Applied to Improve Children's Health
Elaine Faustman, University of Washington

Society for Birth Defects Research & Prevention
EST. 1960 AS THE TERATOLOGY SOCIETY

TERATOGENESIS AT THE SINGLE-CELL LEVEL: OPPORTUNITIES AND CHALLENGES

Thomas B. Knudsen, PhD
Developmental Systems Biologist
US EPA, Center for Computational Toxicology and Exposure
Research Triangle Park, NC 27711
knudsen.thomas@epa.gov
ORCHID 0000-0002-5036-596x

Disclosure Statement

DISCLAIMER:

The views expressed in this presentation are my own and do not reflect Agency policy.

DISCLOSURE:

Editor-in-Chief of '*Current Research in Toxicology*' (Elsevier).

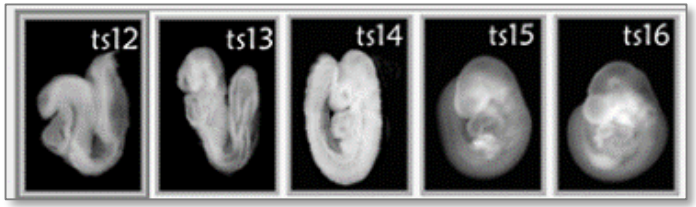
CONFLICTS of INTEREST:

None.

Single-cell RNA-seq (scRNAseq)

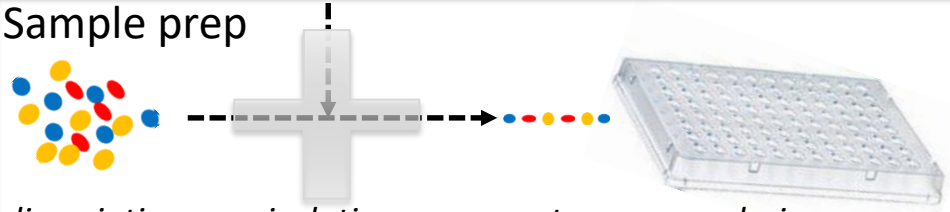
- The fundamental unit of form and function in embryology is ‘the cell’, each with its own unique history (lineage) and environment (microphysiology).
- NexGen sequencing (RNA-seq) averages the transcriptome across a composite system but cannot decode individual lineages or state dynamics.
- With methods to separate and tag individual cells, scRNAseq enables annotation of tens of thousands of cells in parallel from a composite sample.
- Computational methods are then used to unravel cellular complexity and reconstruct gene expression dynamics for quantitative lineage tracing.

scRNAseq Workflow



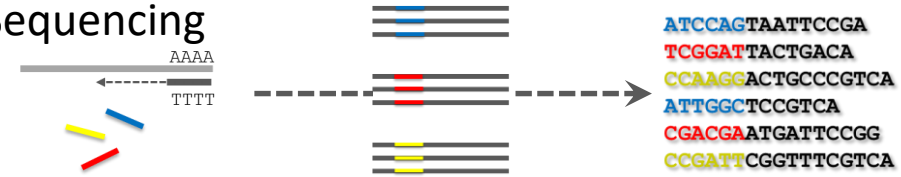
https://www.emouseatlas.org/emap/ema/home.php

Sample prep



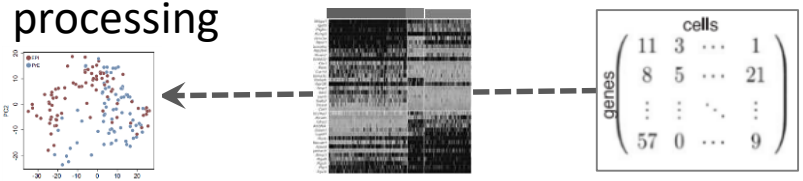
dissociation *isolation* *capture* *lysis*

Sequencing



cDNA barcoding *barcoded library* *NexGen sequencing*

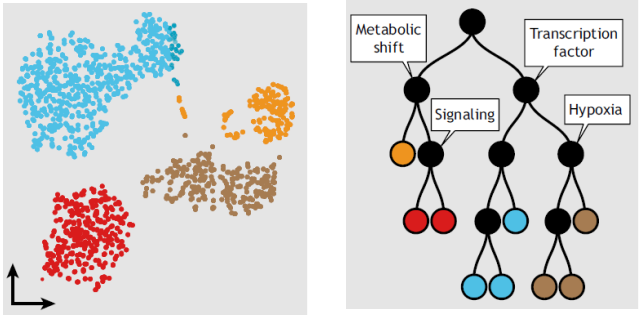
Data processing



HVGs *DEGs* *read count matrix*

Deconvolution

t-SNE: t-distributed stochastic neighbor embedding



t-SNE plot *lineage/state*

McKenna and Gagnon (2019) Development 146

Challenges

- Cell separation tools: how well does the scRNA-ome survive preparation protocols required to generate isolated cells from a composite system?
- Bioinformatics: how can individual cell provenance be retained and annotations decoded while deep-sequencing thousands of cells in parallel?
- Analysis: how to distinguish highly-variable genes (HVGs) from technical variation (eg, PCR artifacts and over-sequencing)?
- Computational: how to ~~validate~~ qualify transitional cell types, map them to developmental trajectories, and spatially reconstruct a composite system?

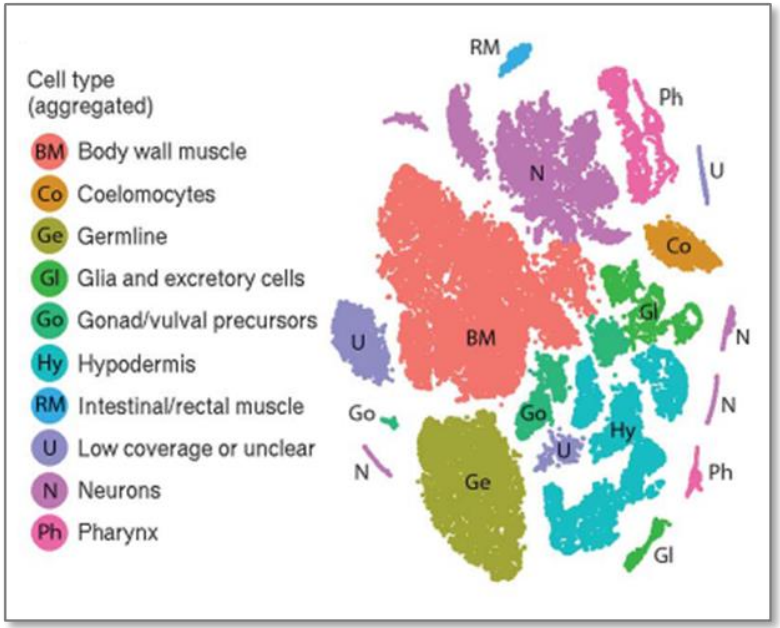
Opportunites

- Comprehensive ‘cell atlases’ for modeling gene expression dynamics as cells acquire specified fates during morphogenesis and differentiation; examples:
  - *simple model organisms (planaria, C elegans, Drosophila)*
  - *experimental embryology (ascidian, zebrafish, frog, chick)*
  - *mammalian embryos (mouse, human)*
  - *differentiating embryonic stem cell lines (mouse, human)*
  - *and more ...*

- Despite many studies to date that have used genomic approaches to characterize dysmorphogenesis, very few examples using scRNAseq.

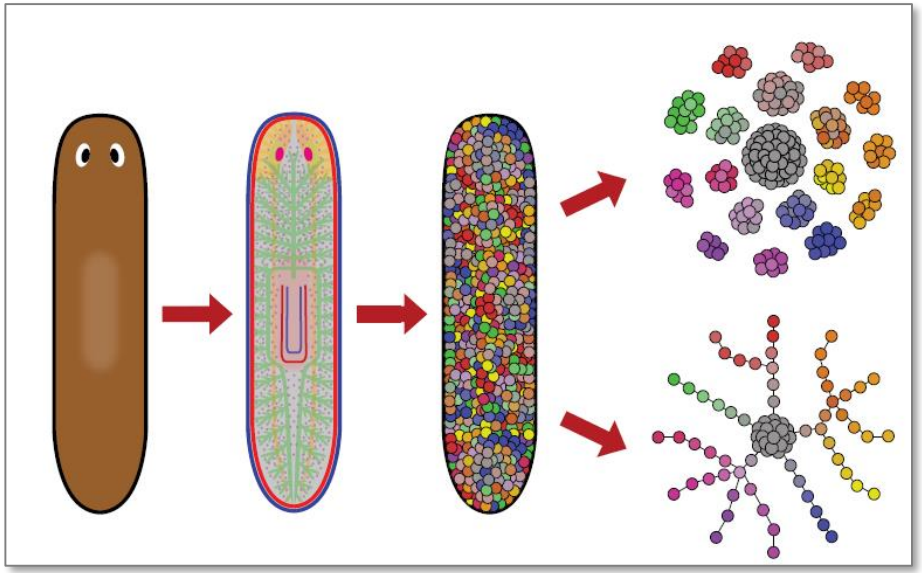
Roundworm & Flatworm

t-SNE plot decomposing *C elegans* into 29 cell clusters at L2 larval stage.



Cao et al. (2017) Science 357

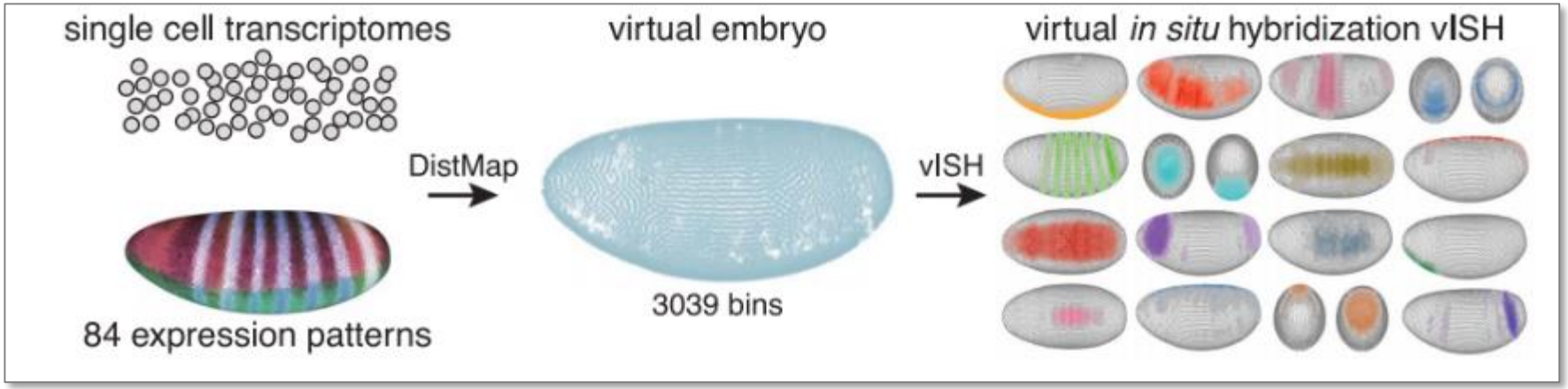
Planaria (regenerative) decomposed to multiple cell states → reconstitution of lineages by divergence of transcriptomes.



Plass et al. (2018) Science 360

Drosophila

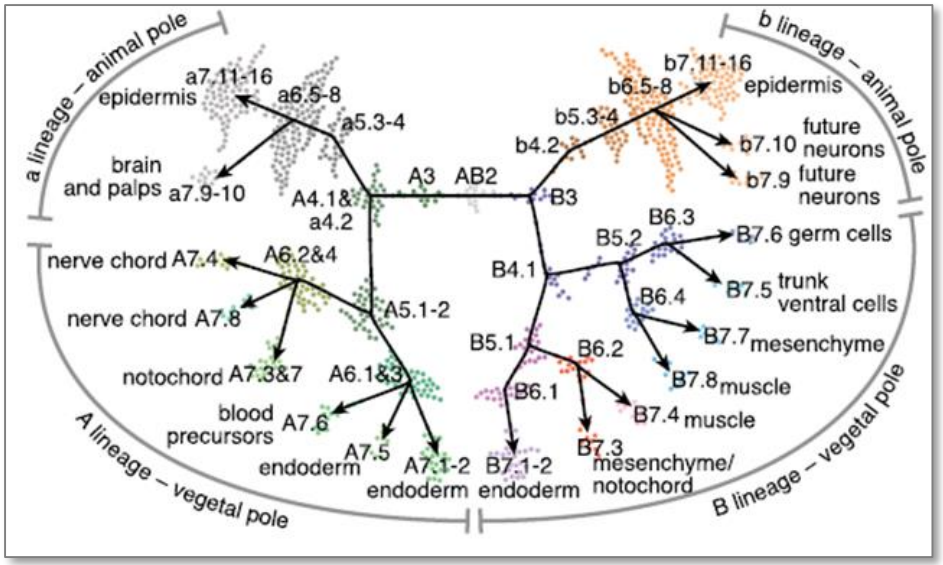
- scRNAseq profiles decomposed into 84 cell clusters that could be annotated by well-correlated marker genes (coverage = 87% of cells, >8k genes per cell);
- spatial reconstruction mapped expression patterns in a ‘virtual embryo’ that predicts developmental trajectories linked to key transcription factors and signal gradients.



Karaiskos et al. (2017) Science 358

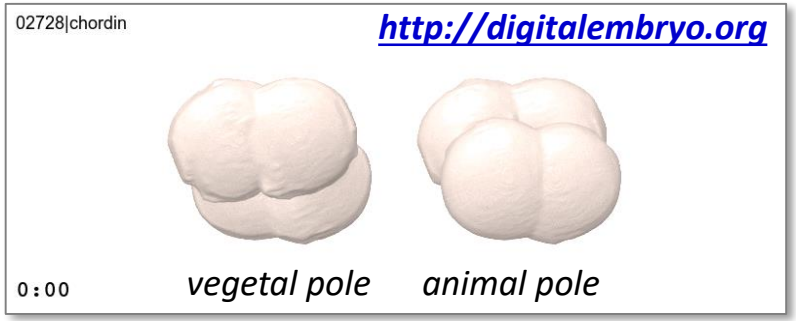
Ascidian

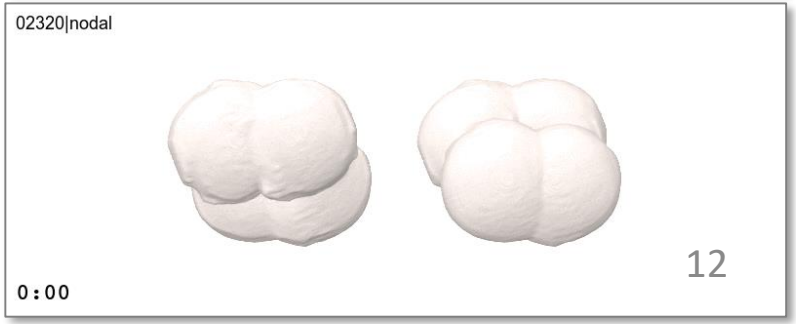
Lineage tree from 1,042 cells (>8K genes per cell) → gene expression history (scRNAseq) and physical position (DLSM) of every cell from the 4- to 64-cell stage.



Sladitschek et al. (2020) Cell 181

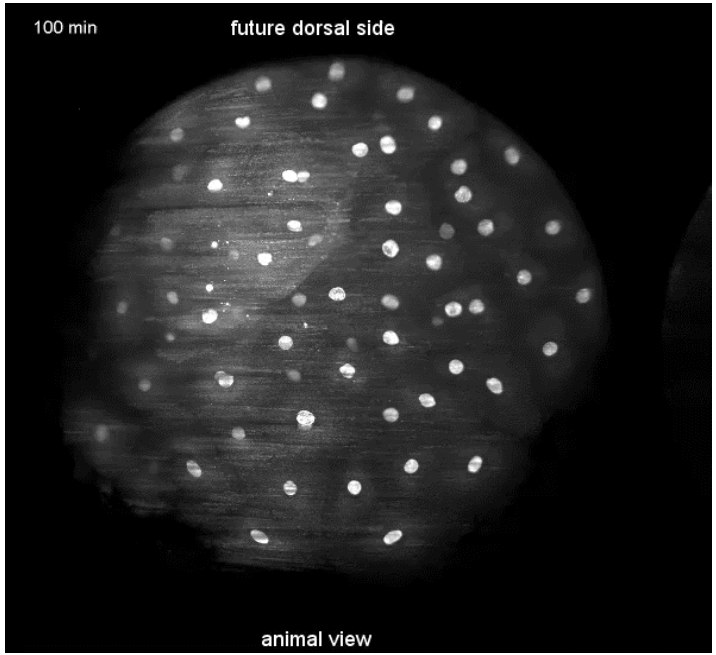
vISH: reconstruction of expression dynamics up to gastrulation; two genes (chordin, nodal) selected from the online digital library.



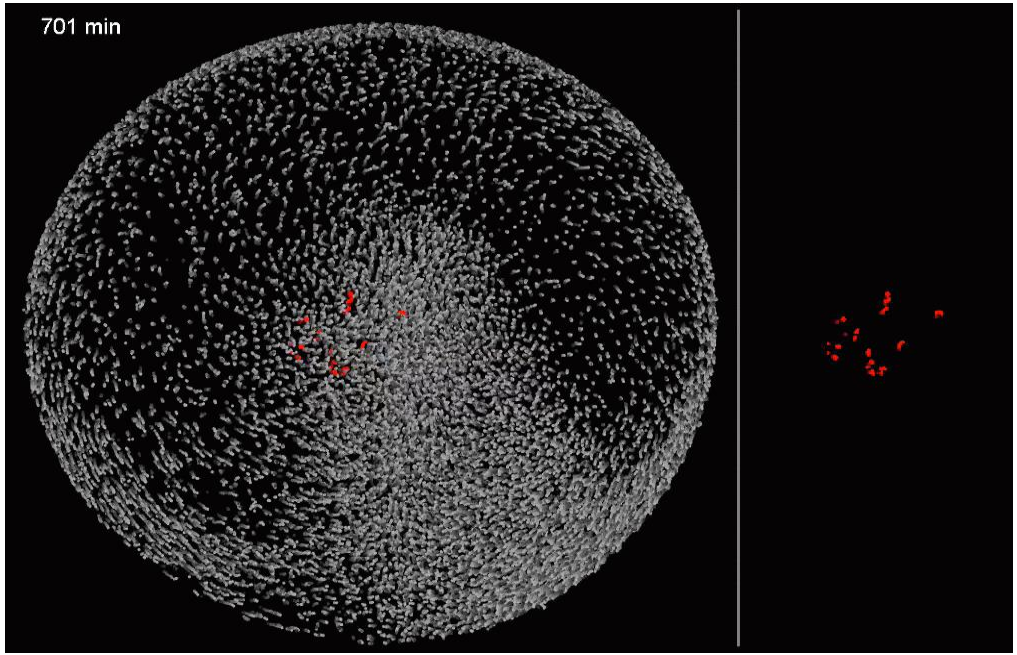


Zebrafish - *at single-cell resolution*

Digital LSM reconstruction at 90 sec intervals through 18 hpf.



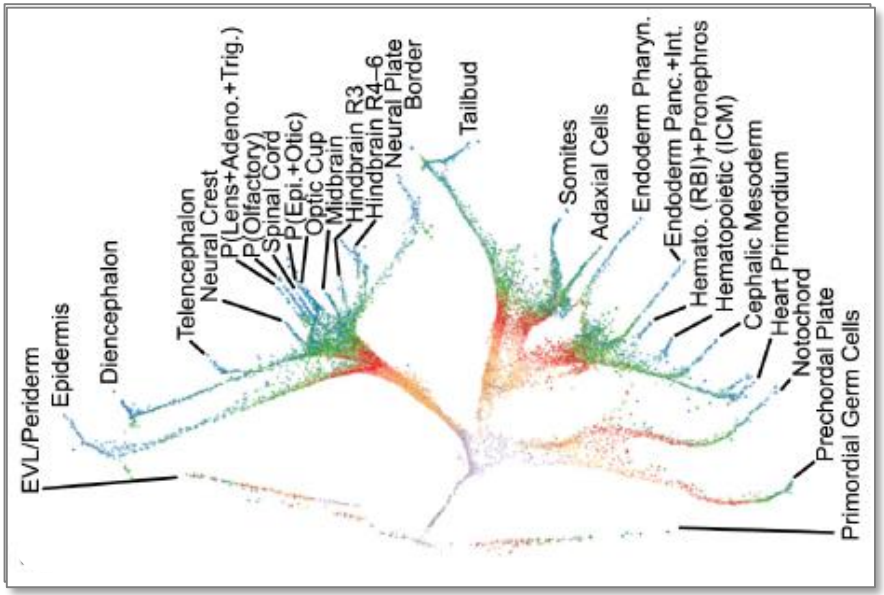
Reverse engineering the physical position of cells forming the optic vesicle (red).



Keller et al. (2008) Science 322

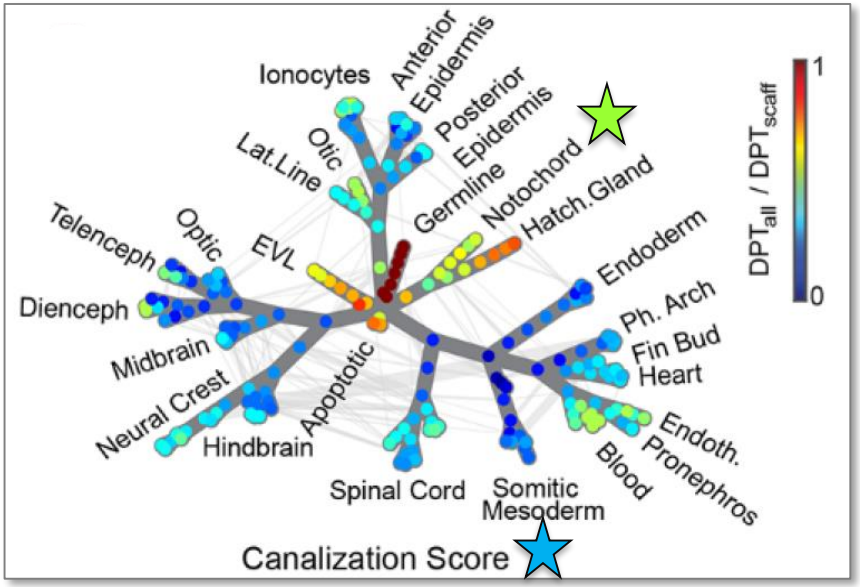
Zebrafish

Lineage tree: 25 cell types built on 38,731 cells captured 3.3 hpf (pluripotent blastula) to 12 hpf (6-somite pharyngula).



Farrell et al. (2018) Science 360

Canalization tree built on 92,000 cells 4 hpf through organogenesis (24 hpf); note buffering notochord > somitic lineages.

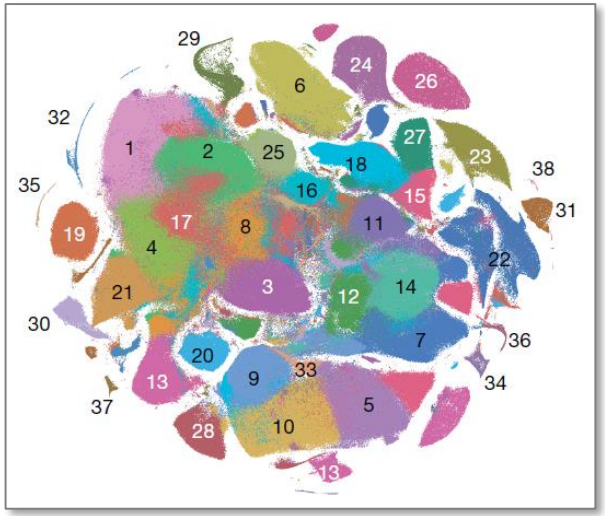


Wagner et al. (2018) Science 360

From Science cover 2018

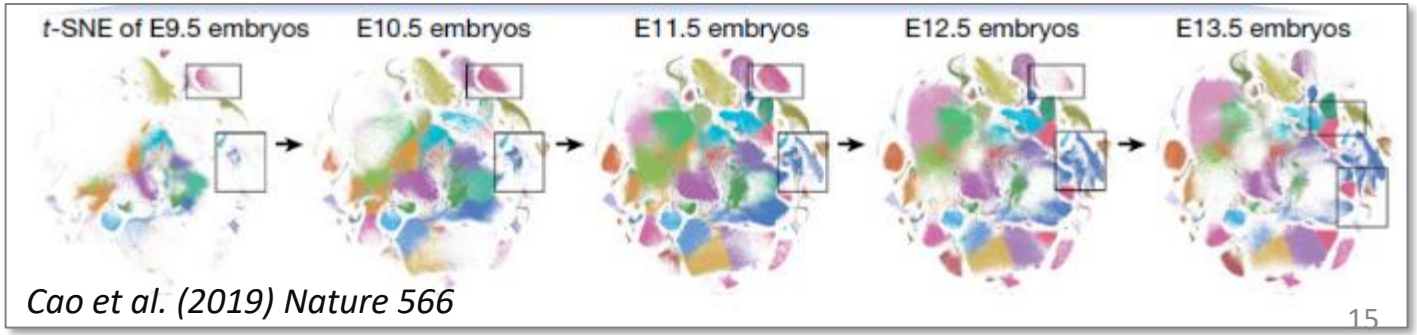
Mouse - *organogenesis*

1–Connective tissue progenitors
2–Chondrocytes and osteoblasts
3–Intermediate mesoderm
4–Jaw and tooth progenitors
5–Excitatory neurons
6–Epithelial cells
7–Radial glia
8–Early mesenchyme
9–Neural progenitor cells
10–Postmitotic premature neurons
11–Oligodendrocyte progenitors
12–Isthmic organizer cells
13–Myocytes
14–Dorsal neural tube cells
15–Inhibitory neurons
16–Stromal cells
17–Osteoblasts
18–Inhibitory neuron progenitors
19–Premature oligodendrocytes
20–Endothelial cells
21–Chondroctye progenitors
22–Definitive erythrocyte lineage
23–Schwann cell precursors
24–Sensory neurons
25–Limb mesenchyme
26–Primitive erythroid lineage
27–Inhibitory interneurons
28–Granule neurons
29–Hepatocytes
30–Notochord and floor plate cells
31–White blood cells
32–Ependymal cells
33–Cholinergic neurons
34–Cardiac muscle lineage
35–Megakaryocytes
36–Melanocytes
37–Lens
38–Neutrophils



t-SNE annotated 38 cell clusters during organogenesis, the period when most organ systems form:

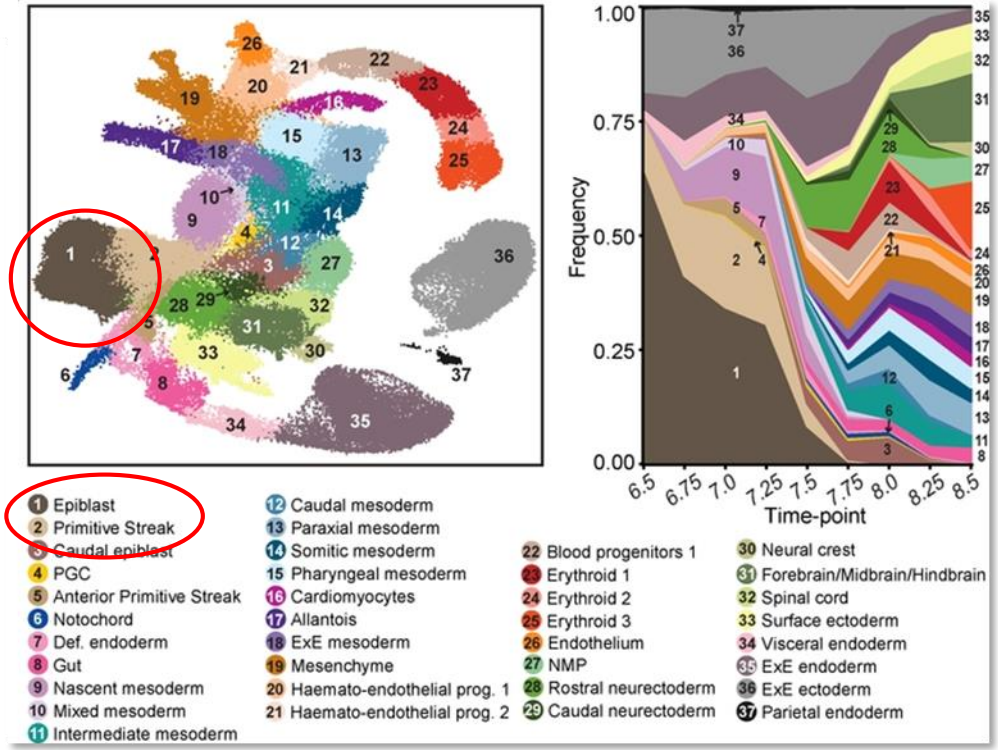
- GD 9.5 (151K cells)
- GD 10.5 (370K cells)
- GD 11.5 (603K cells)
- GD 12.5 (468K cells)
- GD 13.5 (435K cells)



Cao et al. (2019) Nature 566

Mouse - *gastrulation*

- The vast majority of cell lineages derive from the epiblast during gastrulation;
- t-SNE lineage staged from 116,312 cells epiblast (GD 6.5) to headfold (GD 8.5);
- body's fundamental genomic blueprint is also decoded during this period;
- while autopoiesis rules the epiblast each cell's destiny determined by its position.



Pijuan-Sala et al. (2019) Nature 566

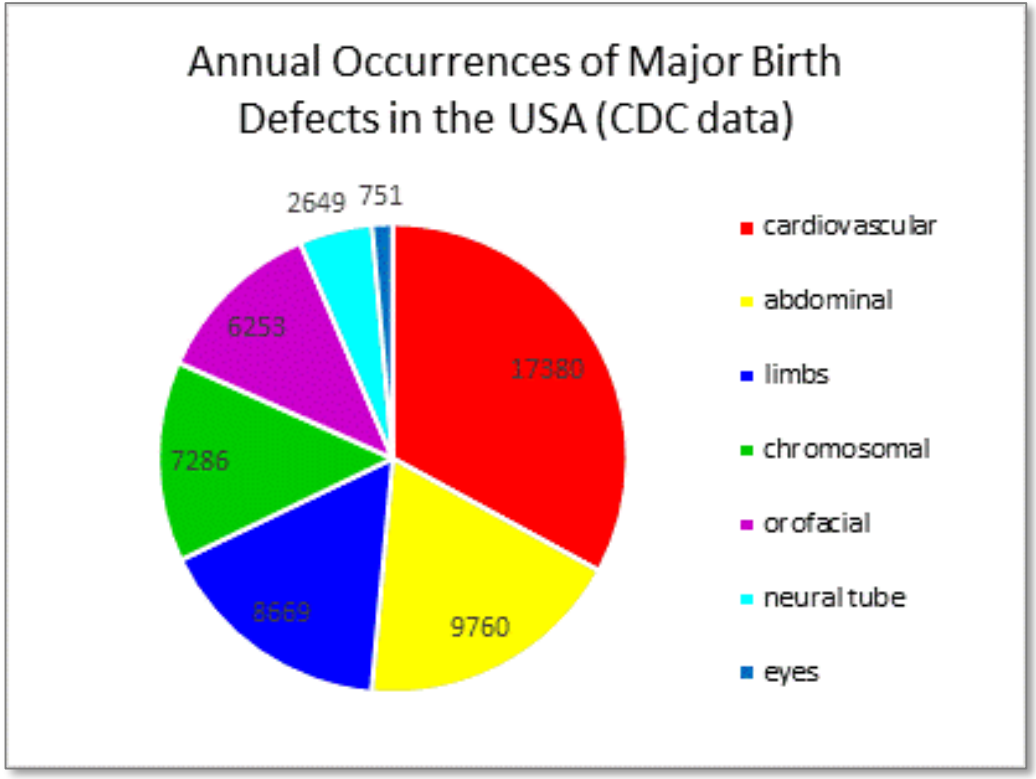
Mouse - *at single-cell resolution*

In toto live cell imaging atlas reveals individual cellular dynamics by position, fate, movement, and division (GD 6.5 – GD 8.5).

rostral

caudal

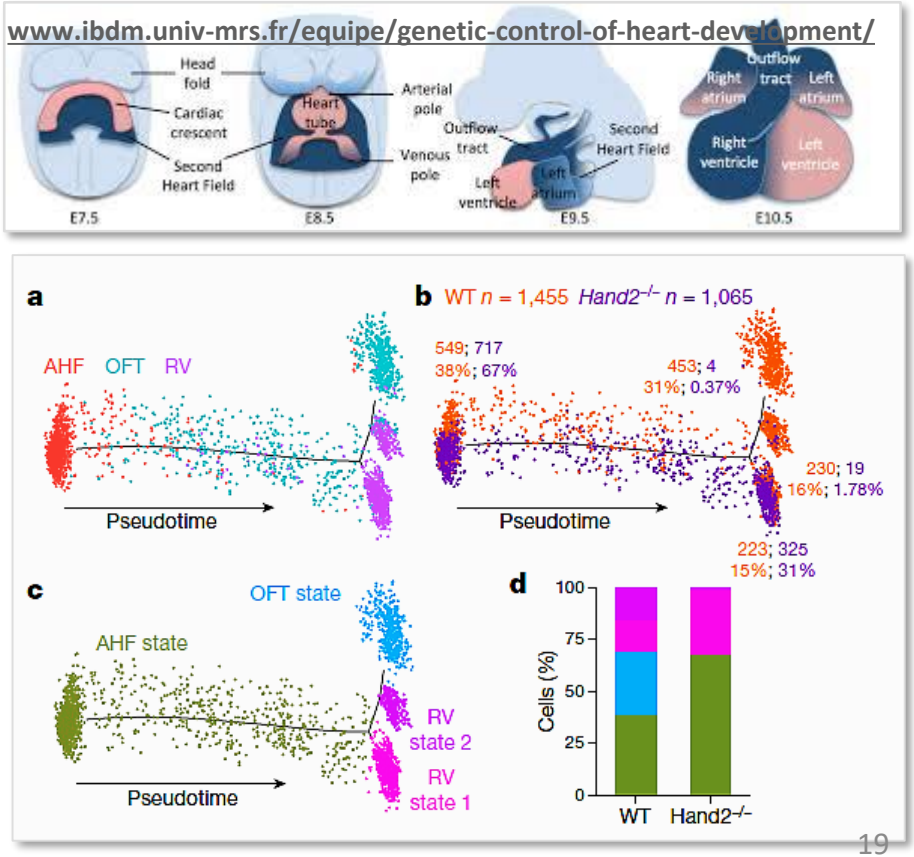
Birth Defects - *1 in 33 babies in the USA*



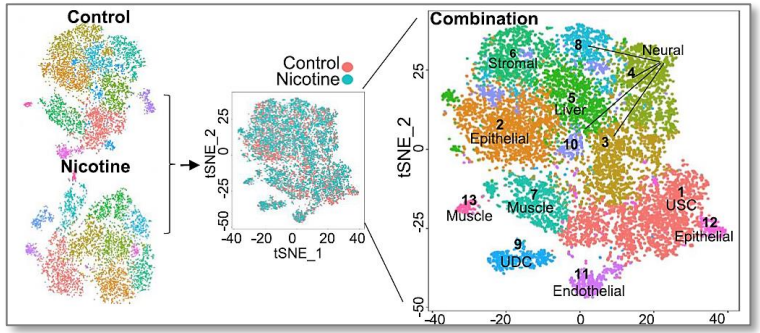
Congenital Heart Defects

- scRNAseq on 36,000 cells from the cardiogenic region (GD 7.75 to 9.25);
- analysis pointed to *Hand2* specification of myocardial cells in the OFT;
- however, *Hand2-null* mice display RV hypoplasia in addition to OFT defects;
- scRNAseq of *Hand2(-/-)* rudiments pointed to retinoic acid (ATRA) signaling;
- altered ATRA distribution 'posteriorized' RV to an atrial-like morphology.

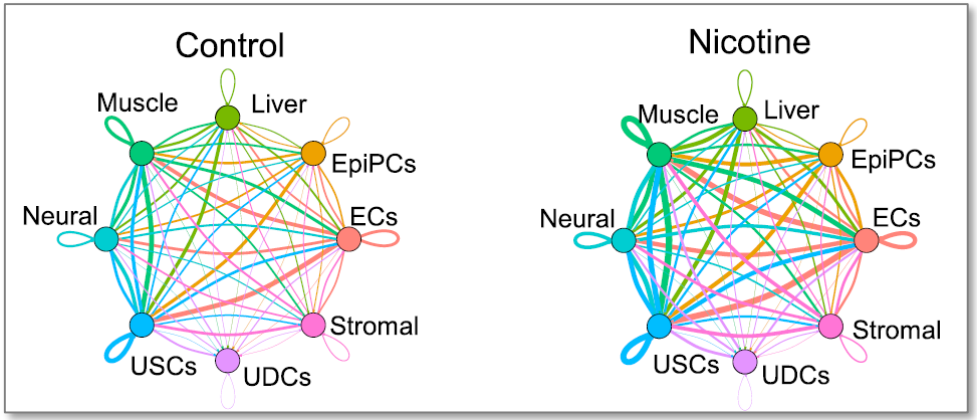
de Soysa et al. (2019) Nature 572



Nicotine and hESC differentiation



- t-SNE plots from hESC-derived embryoid bodies on day 21 of culture;
- control condition (n= 6,847 individual cells) and 10 µM nicotine (n= 5,646 cells) exposure.



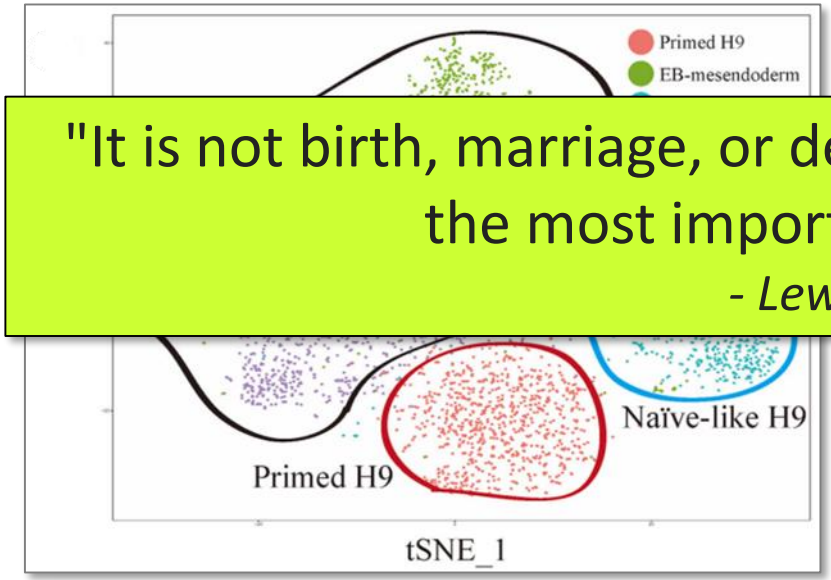
- Nicotine increased specific cell-to-cell communication pathways;
- network connects ligands from to receptors for target cell populations.

Guo et al. (2019) Stem Cell Reports 12

Pluripotency – *embryonic stem cells and the epiblast*

hESCs (0- 8 days in culture)

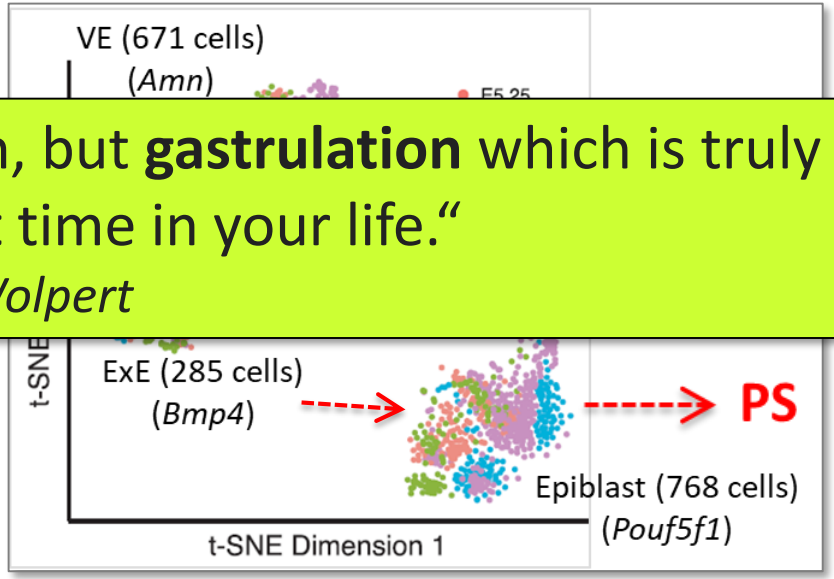
tSNE map of 4822 barcoded progenitors at naïve (n=1491), primed (n=695), and embryoid body (EB, n=2636) stages.



Han et al. (2018) Genome Biol 19

Epiblast (GD 5.5 – GD 6.5)

t-SNE map of 1,724 cells from 28 mouse embryos as they acquire the propensity for 'Primitive Streak' formation.

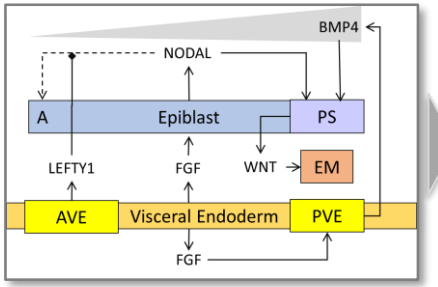


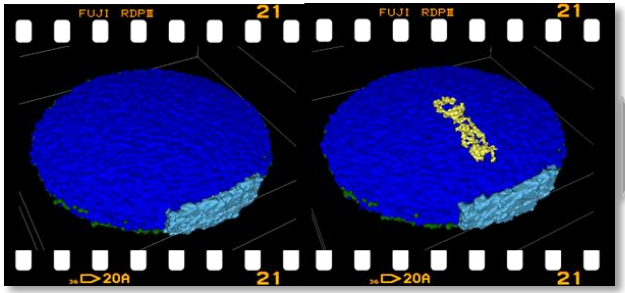
Cheng et al. (2019) Cell Reports 26

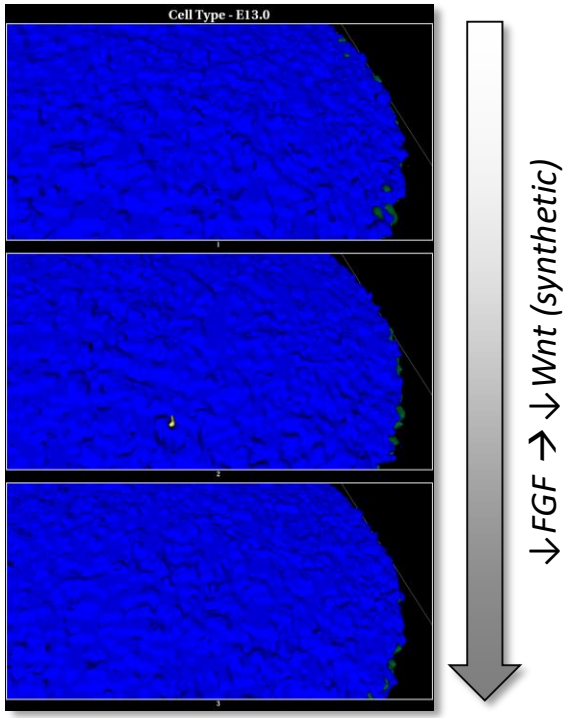
"It is not birth, marriage, or death, but **gastrulation** which is truly the most important time in your life."

- Lewis Wolpert

Quasi-gastrulation – *epiblast in silico*







- When and where an epiblast cell migrates through the PS ultimately determines it's regional destiny;
- morphological programming logic of the epiblast can be tapped to virtually reconstruct gastrulation *in silico*;
- simulation manifold for developmental computation with data from chemical effects on pluripotent hESCs.

Knudsen et al. (2020) SOT ePoster #2043

Single-cell profiling for DevTox

- Developmental toxicology has yet to experience scRNAseq profiling as a next-generation blueprint for chemical testing.
- ‘Cell atlases’ (e.g., HCA, MCA) for modeling gene expression dynamics as cells acquire specified fates during morphogenesis and differentiation.
- Precision toxicology due to unprecedented resolution to examine biological systems and their perturbation by chemicals.
- Computational approaches needed for data integration/clustering, HVGs for state landscape, models for cell-swarming and positional information, …).

References for Images

Slide 07 McKenna and Gagnon (2019) Recording development with single cell dynamic lineage tracing. Development 146, dev169730. doi:10.1242/dev.169730 (10 pages).
Slide 10 Cao et al. (2017) Comprehensive single-cell transcriptional profiling of a multicellular organism. Science 357: 661–667.
Slide 10 Plass et al. (2018) Cell type atlas and lineage tree of a whole complex animal by single-cell transcriptomics. Science 360, eaaq1723 (10 pages).
Slide 11 Karaiskos et al. (2017) The Drosophila embryo at single-cell transcriptome resolution. Science 358: 194-199.
Slide 12 Sladitschek et al. (2020) MorphoSeq: full single-cell transcriptome dynamics up to gastrulation in a Chordate. Cell 181: 922–935.
Slide 13 Keller et al. (2008) Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. Science 322: 1065-1069.
Slide 14 Farrell et al. (2018) Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. Science 360: doi:10.1126/science.aar3131.
Slide 14 Wagner et al. (2018) Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo. Science 360: 981-987.
Slide 15 Cao et al. (2019) The single-cell transcriptional landscape of mammalian organogenesis. Nature 566: doi.org/10.1038/s41586-019-0969-x.
Slide 16 Pijuan-Sala et al. (2019) A single-cell molecular map of mouse gastrulation and early organogenesis. Nature 566: doi.org/10.1038/s41586-019-0933-9.
Slide 17 McDole et al. (2018) *In toto* imaging and reconstruction of postimplantation mouse development at the single cell level. Cell 175: 859–876.
Slide 19 de Soysa et al. (2019) Single-cell analysis of cardiogenesis reveals basis for organ-level developmental defects. Nature 572: 120-124.
Slide 20 Guo et al. (2019) Single-cell RNA sequencing of human embryonic stem cell differentiation delineates adverse effects of nicotine on embryonic development. Stem Cell Reports 12: 772–786.
Slide 21 Han et al. (2018) Mapping human pluripotent stem cell differentiation pathways using high throughput single-cell RNA-sequencing. Genome Biol. 19: 47. doi.org/10.1186/s13059-018-1426-0.
Slide 21 Cheng et al. (2019) Single-cell RNA-seq reveals cellular heterogeneity of pluripotency transition and X chromosome dynamics during early mouse development. Cell Reports 26:2593–2607.
Slide 22 Knudsen et al. (2020) Developmental computation with embryonic stem cells (SOT 2020 ePoster #2043).