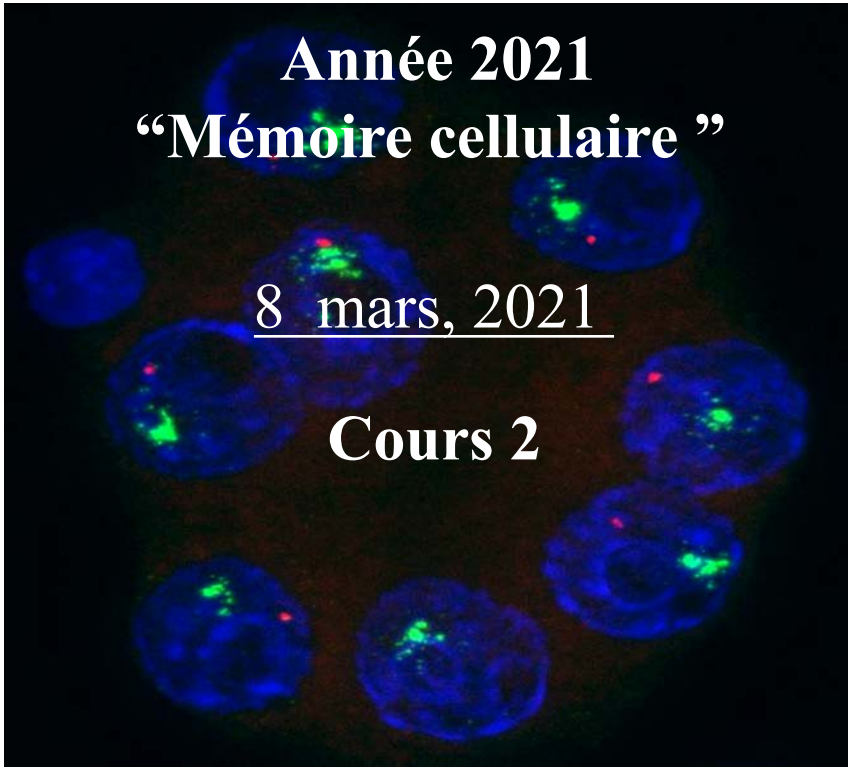
CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE



Stabilité et plasticité au cours du développement

Stability and plasticity during embryonic development

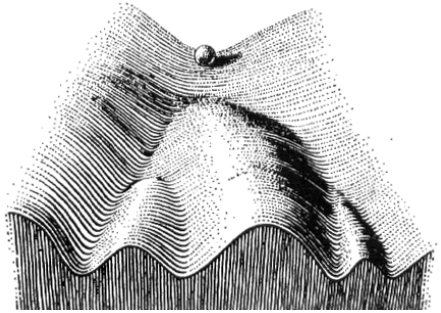
COLLÈGE DE FRANCE 1530

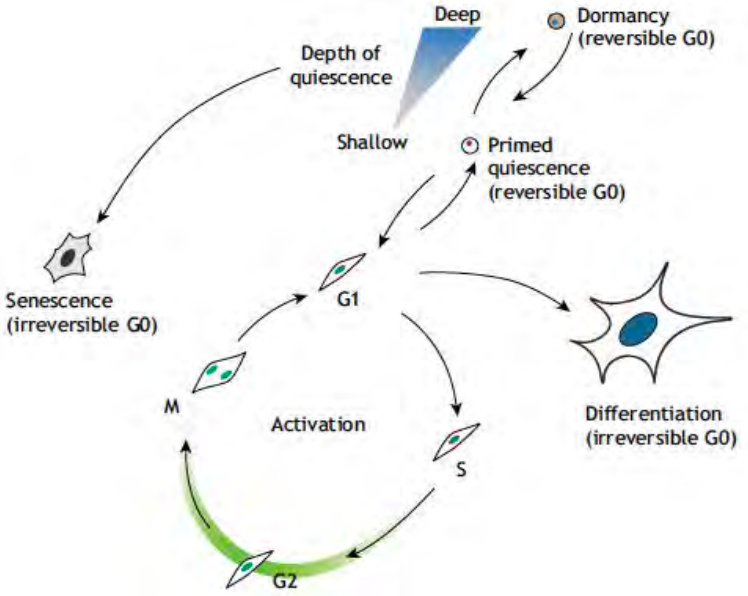
Last Week

Cellular Memory: remembrance of cell identity, cell states, cell functions

The maintenance of an activity state of a gene once it is triggered by stress, a change in gene expression, a developmental signal, an environmental cue, or the production of a small RNA.

During development
Over cell divisions
In non-dividing cells



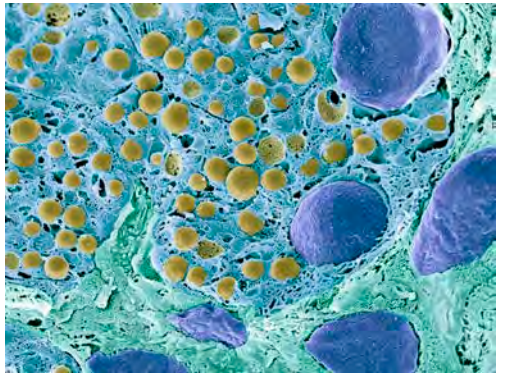


COLLÈGE DE FRANCE 1530

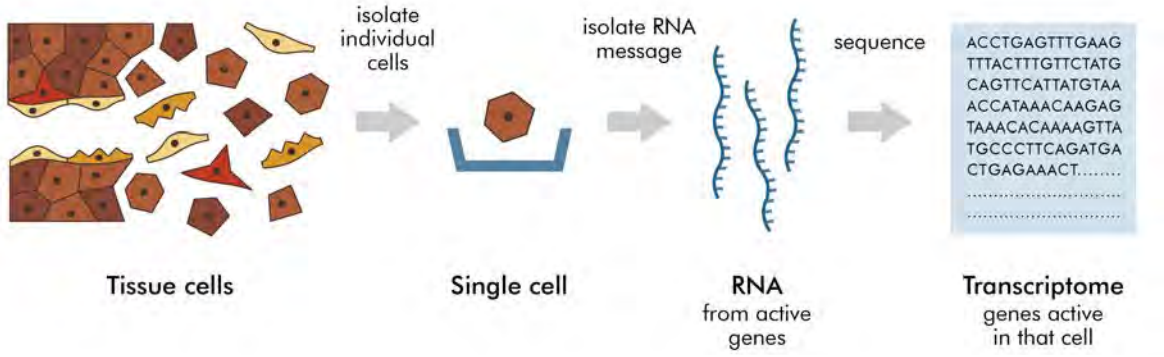
Last Week

Cellular Memory: remembrance of cell identity, cell states, cell functions

Cell identity:



In the retina alone, there are around 100 functionally-distinct retinal cell types



Courtesy of John Marioni

HUMAN CELL ATLAS

www.humancellatlas.org

COLLÈGE DE FRANCE 1530

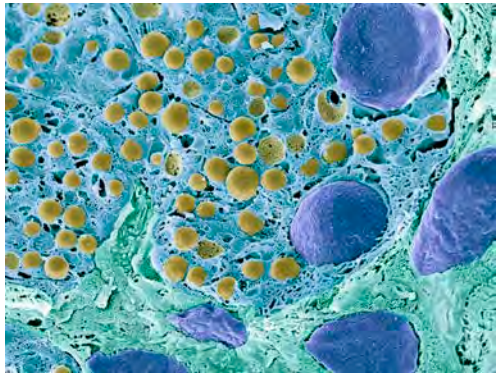
Last Week

Cellular Memory: remembrance of cell identity, cell states, cell functions

Cell identity:

The Human Cell Atlas is a new, large international initiative involving collaboration between many laboratories worldwide that will aim to produce a deep catalogue of the cell types within a human, thereby answering many questions:

1. How many cell types are there?
2. In each cell type, which combination of genes are switched on? What's the blueprint for that cell?
3. Where are these cell types in 3D space?
4. How do these cell types change during development or other processes?



In the retina alone, there are around 100 functionally-distinct retinal cell types

HUMAN CELL ATLAS

www.humancellatlas.org

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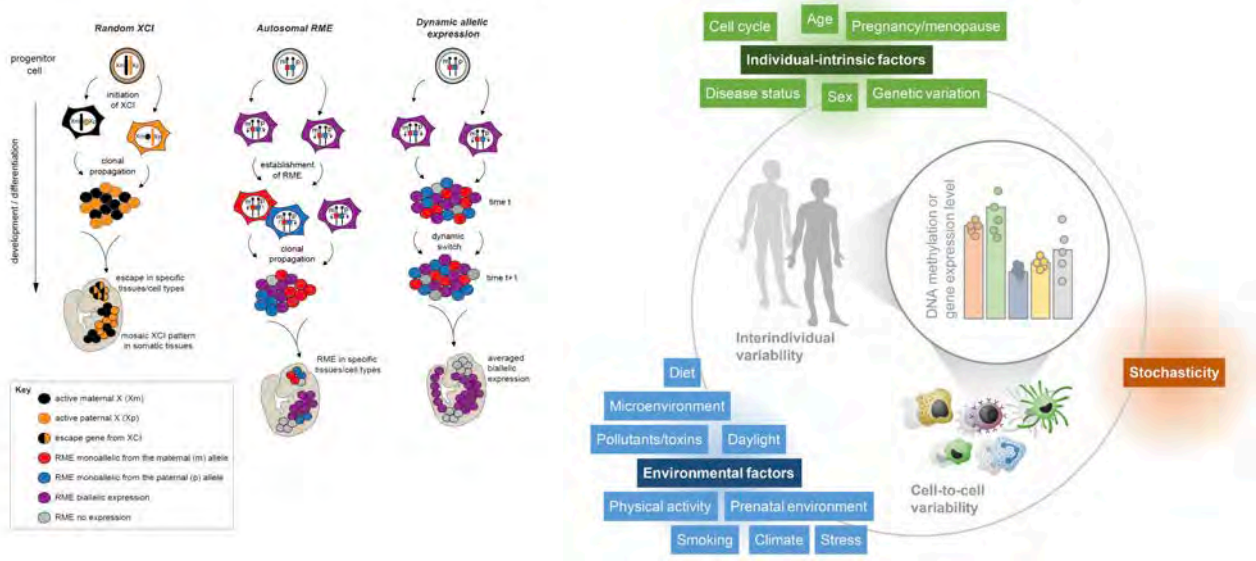
Courtesy of John Marioni

Last Week

Cellular Memory: remembrance of cell identity, cell states, cell functions

Cell identity: hundreds or thousands of different cell types must retain the memory of their state

Cell to cell variability: deterministic, stochastic, envrionmentally triggered



Ecker, S., Pancaldi, V., Valencia, A., Beck, S. & Paul, D. S. Epigenetic and transcriptional variability shape phenotypic plasticity. Bioessays 40, 1700148 (2018).

Last Week

Cellular Memory: remembrance of cell identity, cell states, cell functions

Cell identity: hundreds or thousands of different cell types must retain the memory of their state

Cell to cell variability: deterministic, stochastic, envrionmentally triggered

Mechanisms of cellular memory:

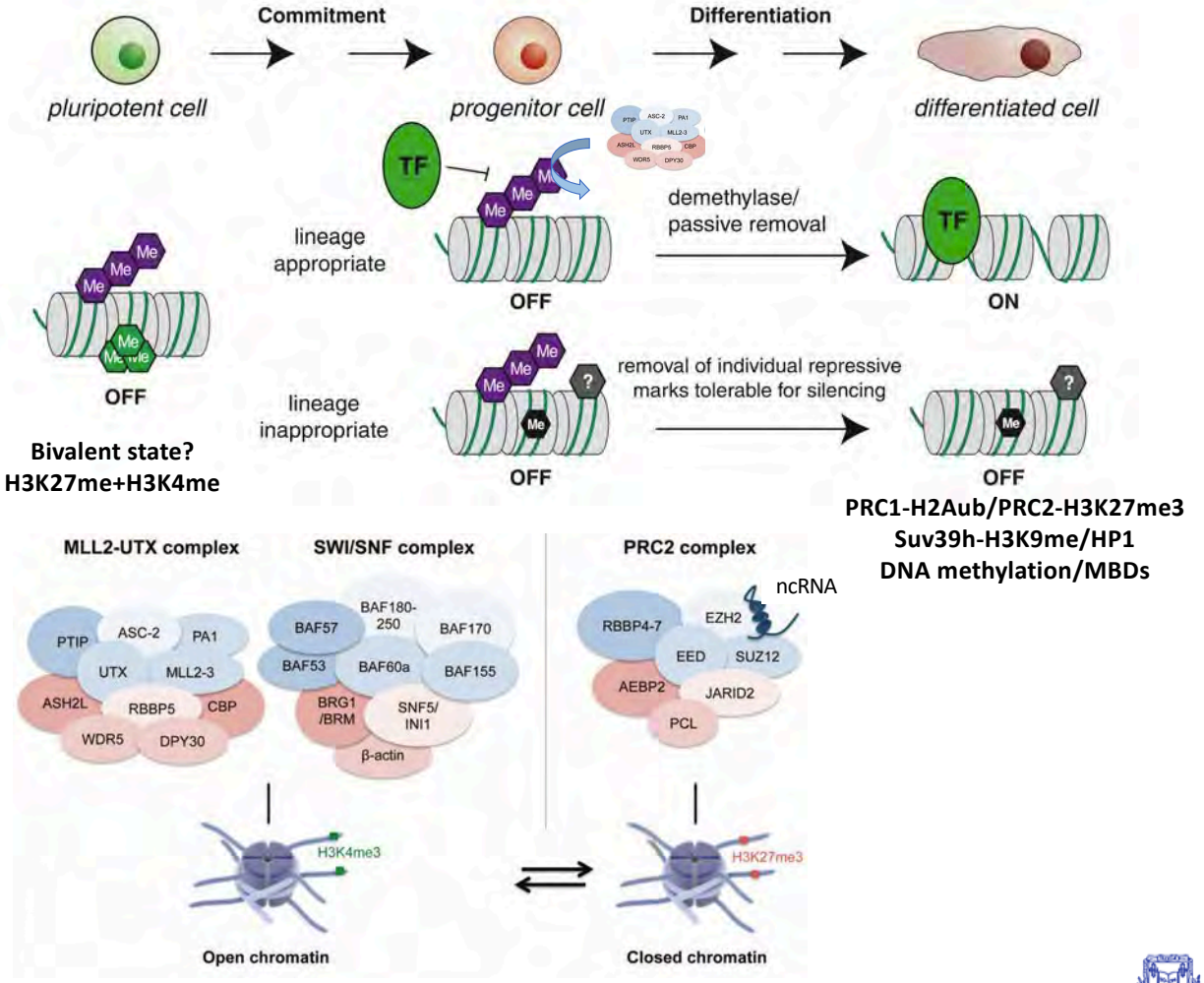
- Transcriptional circuits and feedback loops
- Self-templating epigenetic mechanisms including DNA methylation, chromatin, prions

Memory of repressive (heterochromatic) or active (euchromatin) states in dividing cells:

- chromatin domains enable self-templating repression
- transcription and TFs required for active memory

Cellular memory in Non-dividing Cells

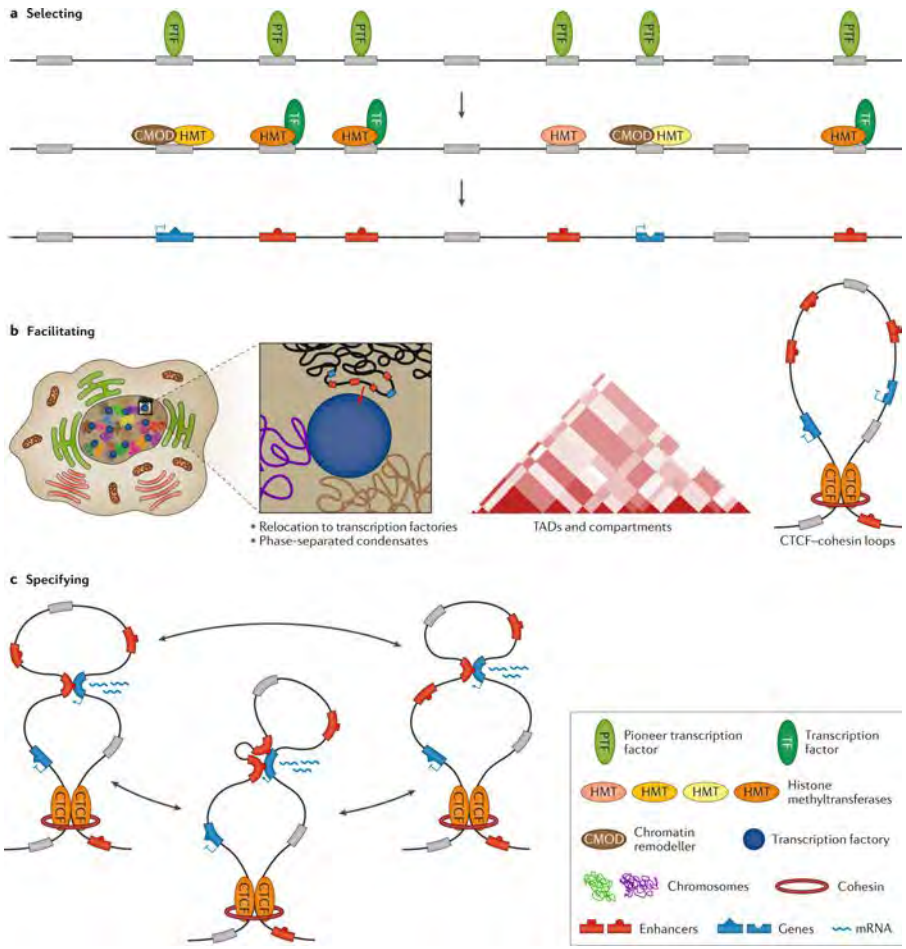
Chromatin: enabling developmental transitions and memorising activity states?



E. Heard, 8 mars, 2021

COLLÈGE DE FRANCE 1530

Chromatin and chromosome organization to enable long-range enhancer–promoter contacts for gene expression control



a | The combined action of pioneer transcription factors, chromatin remodelling enzymes, transcription factors and chromatin-modifying enzymes, such as histone methyltransferases, bookmark cell-type-specific regulatory elements through epigenetic modifications and the creation of binding sites for chromatin proteins (‘selecting’).

b | Relocation of genomic loci to transcription factories or phase-separated condensates, topologically associating domains (TADs), compartments and loops do not confer regulatory contact specificity directly but create microenvironments that reduce the search space and thereby increase the chance of encounters between regulatory elements in the 3D nuclear space (‘facilitating’).

c | Enhancer–promoter contact specificity within chromosomal domains (such as TADs and loops) is achieved by interactions between *trans*-acting factors bound to regulatory elements (‘specifying’), allowing for dynamic transitions between genome conformations.
CTCF, CCCTC binding factor.

Schoenfelder and Fraser, Nature Reviews Genetics 2019

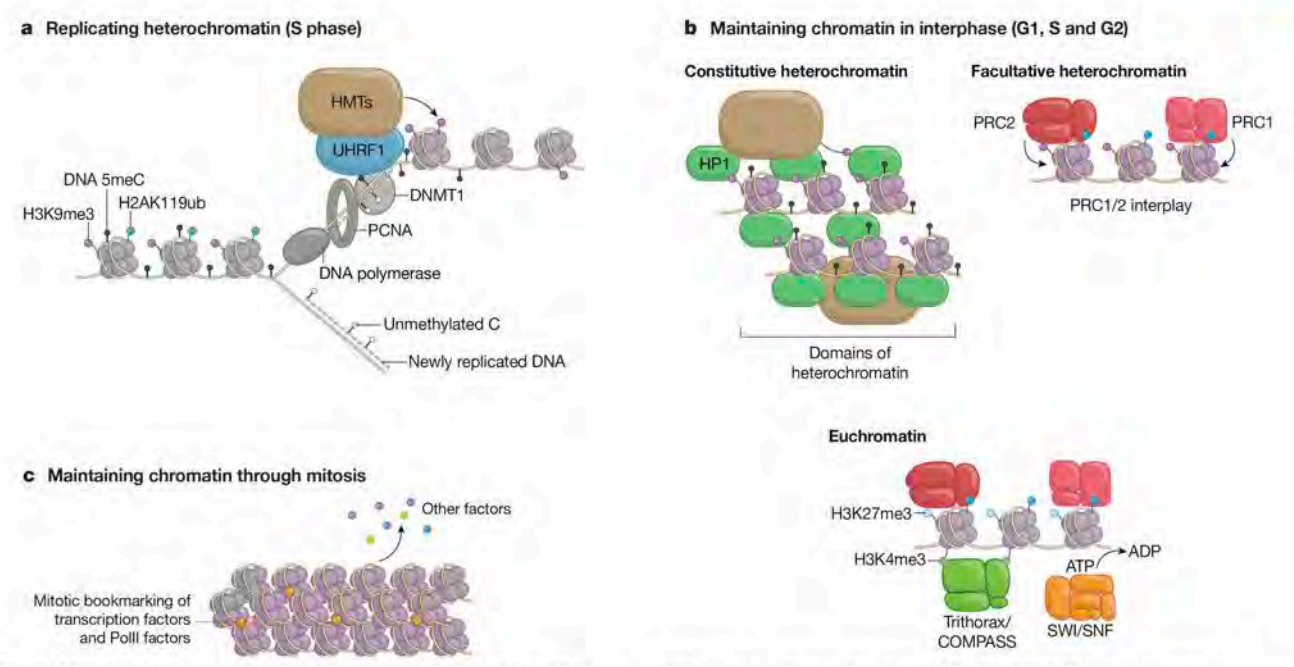
Propagating chromatin states in dividing cells

Gene expression memory during DNA Replication:

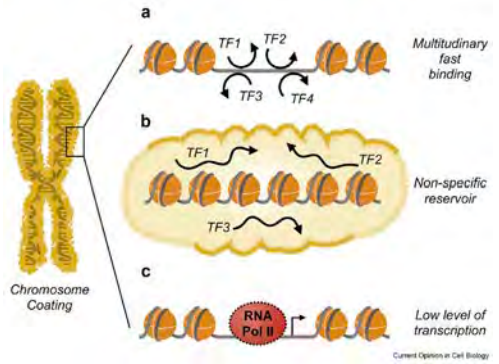
- Repressive chromatin domains are propagated
- Active domains require transcription and TFs

Gene expression memory during Mitosis:

- Repressive chromatin marks
- Active bookmarking factors



From Cavalli and Heard, Nature 2019



Gonzales et al. Curr Op Cell Biol 2021

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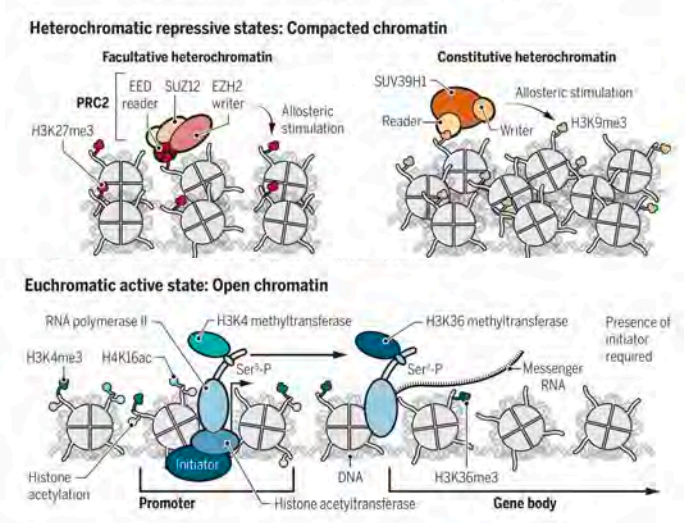
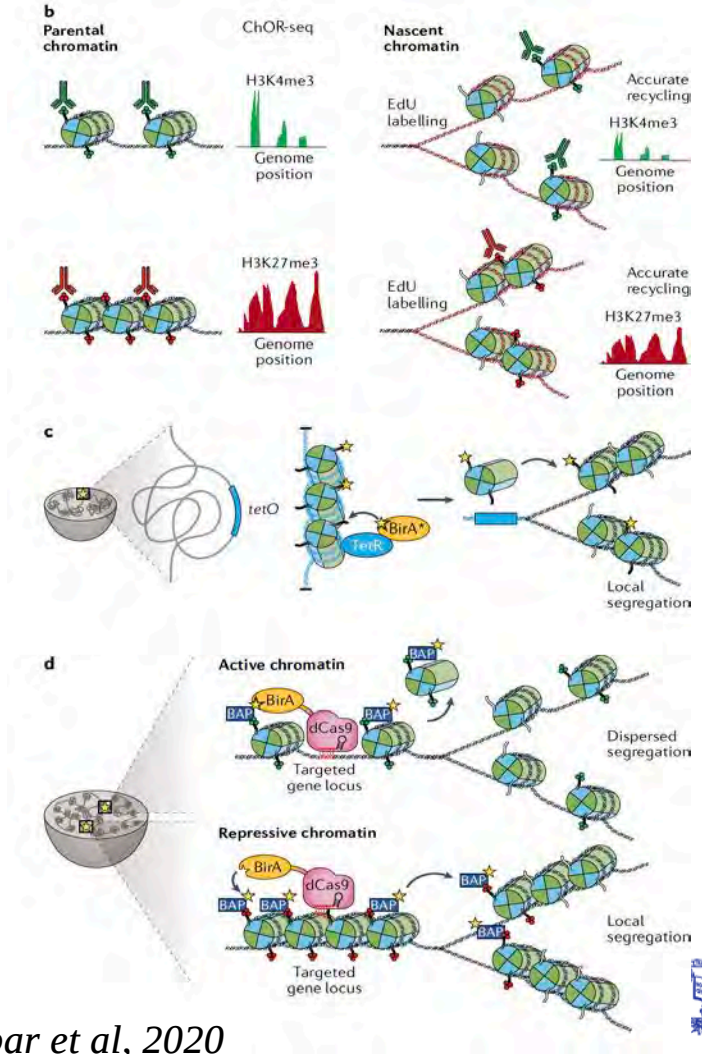
Propagating chromatin and gene expression states in dividing cells

Only repressive chromatin marks appears to be self-templating (ie truly epigenetic?) through the cell cycle Readers/Writers



and Erasers!

Passive versus acative loss of epigenetic marks (DNA and histone modifications)



Escobar et al. 2020

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Propagating chromatin and gene expression states in dividing cells

Gene expression memory during DNA Replication:

- Repressive chromatin domains are propagated
- Active domains require transcription and TFs

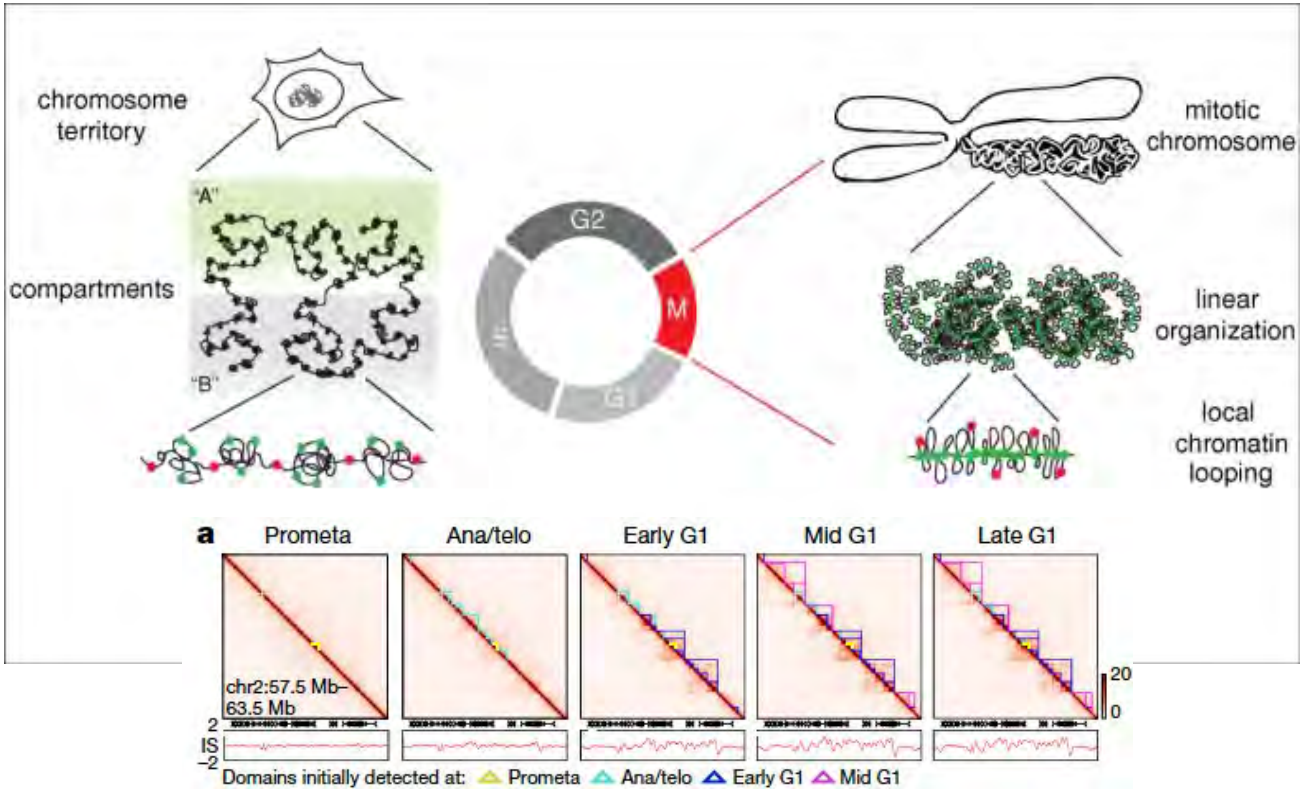
Gene expression memory during Mitosis:

- Repressive chromatin marks
- Active bookmarking factors

Chromosome Folding :

TADs are diminished during S phase and lost during Mitosis but CTCF remains

TAD progressively build up during G1
Compartments rapidly re-establish in G1



Nagano et al, 2018; Gibcus et al, 2018; Zhang et al, 2019

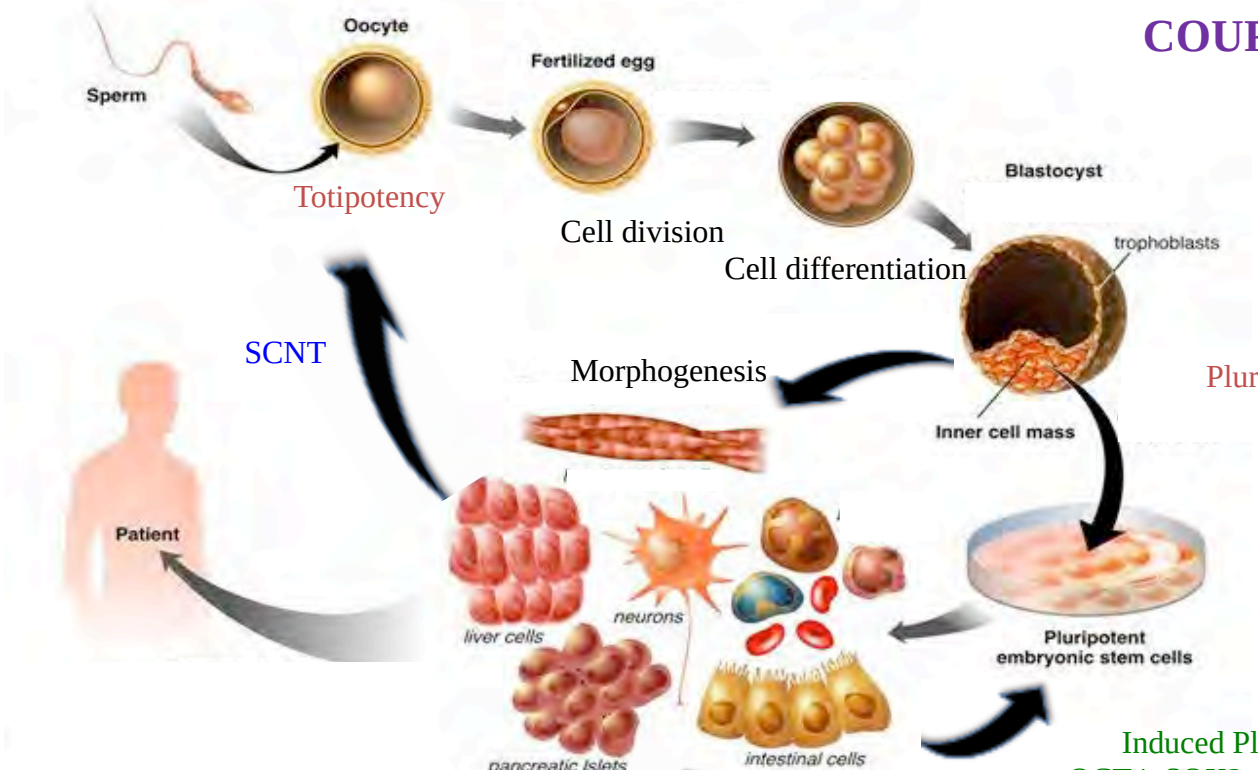
COLLÈGE DE FRANCE 1530

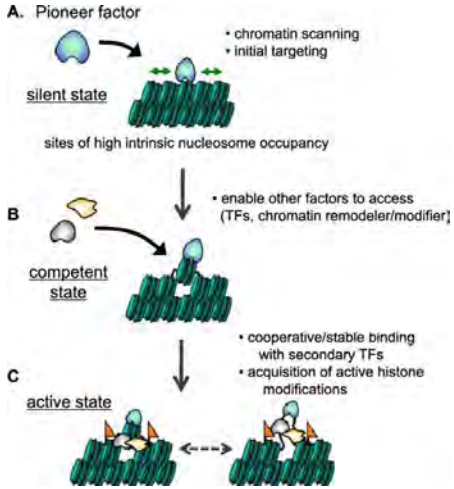
COURS II

1. Cellular memory during embryogenesis: stability and plasticity
2. Tracing cell identity and cell fate during embryogenesis
3. Establishing cellular memory during development
4. Epigenetic dynamics during early mouse development
5. Strategies that enable cellular memory: the epigenetic machineries
6. Lessons from X-chromosome inactivation

Stability and Plasticity
Embryonic Stem Cells (ESCs) and Induced Pluripotent Stem (iPS) Cells

COURS 2014



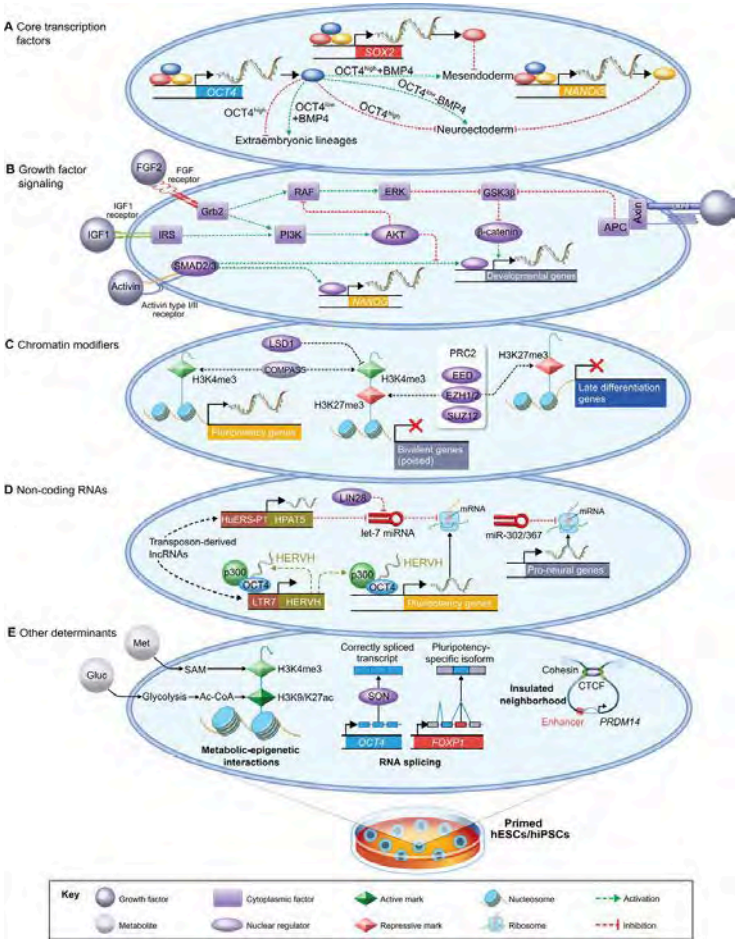


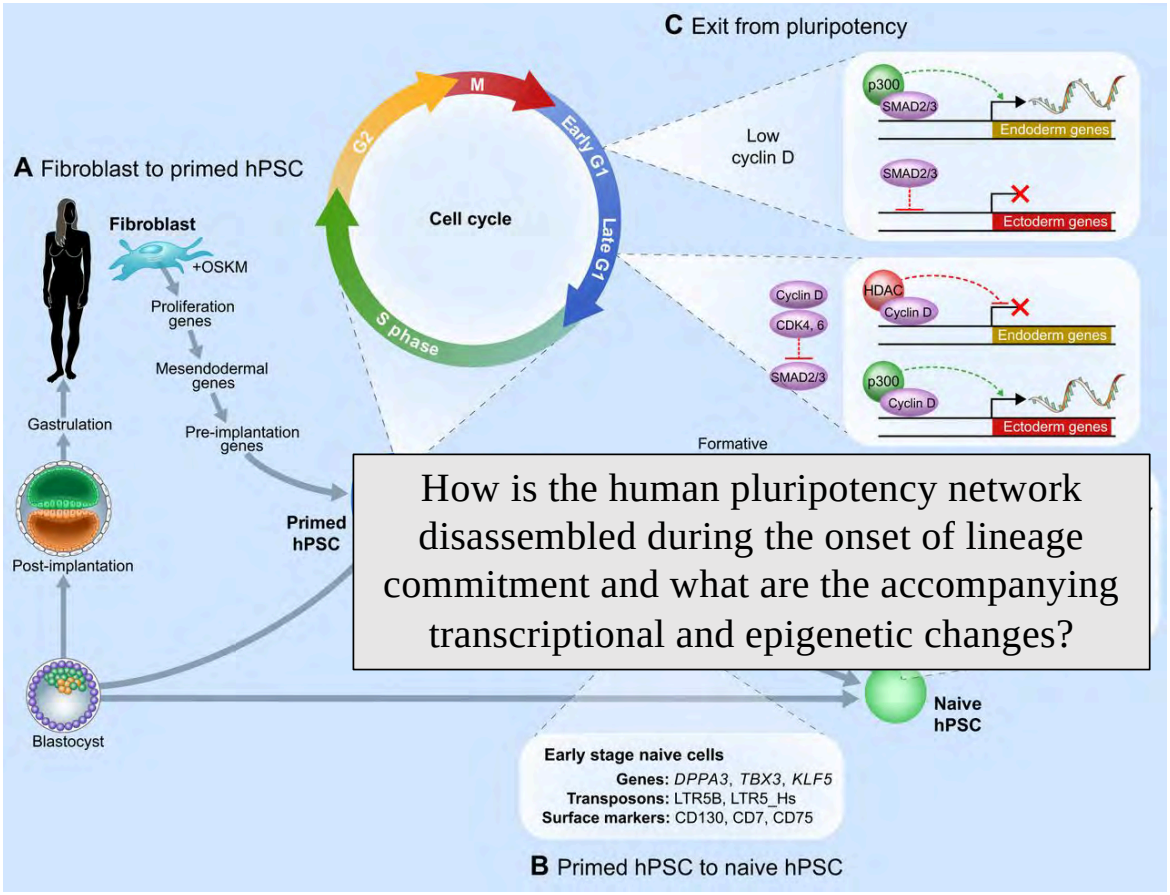
Decades of research were dedicated to studies of cell fate changes during development and led to the view that, in vivo, differentiated cells are irreversibly committed to their fate.

However, reprogramming of somatic cells by transfer into enucleated oocytes pioneered by John Gurdon and colleagues in the 1950s (Gurdon et al., 1958), fusion with other cell partners (Blau et al., 1983), and ectopic transcription factor expression (Davis et al., 1987; Takahashi and Yamanaka, 2006) revealed a remarkable plasticity of the differentiated state.

E. Heard,

Molecular Dynamics during the transition in and out of Human Pluripotency





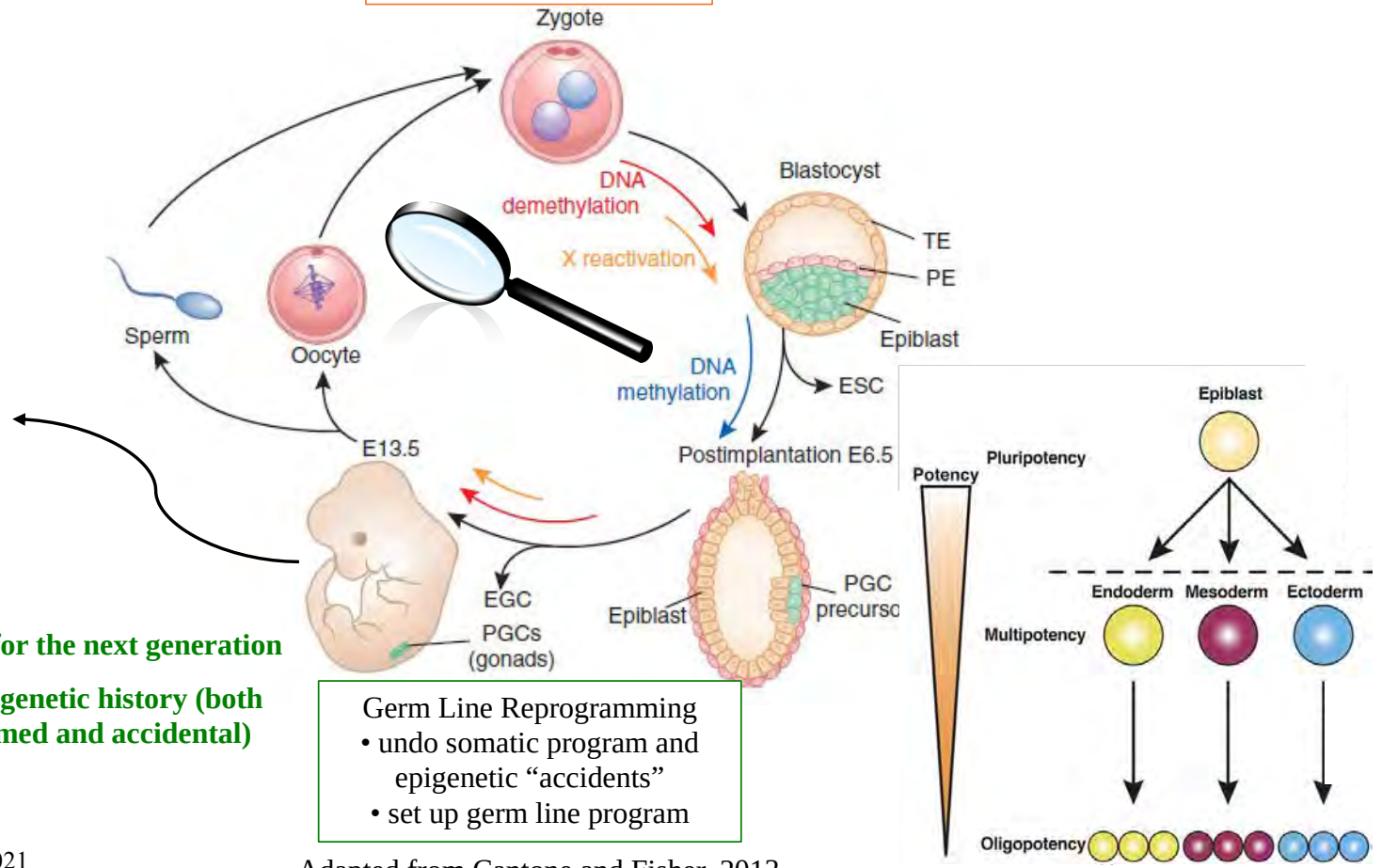
Theunissen and Jaenisch, Development 2017

Mammalian Development: Cellular memory is progressively established and globally erased multiple times

- Prepare for development (epigenesis)
- Preserve some epigenetic marks (parental imprints), erase others

Zygotic Reprogramming
• undo gamete programs
• set up totipotency

COURS 2015



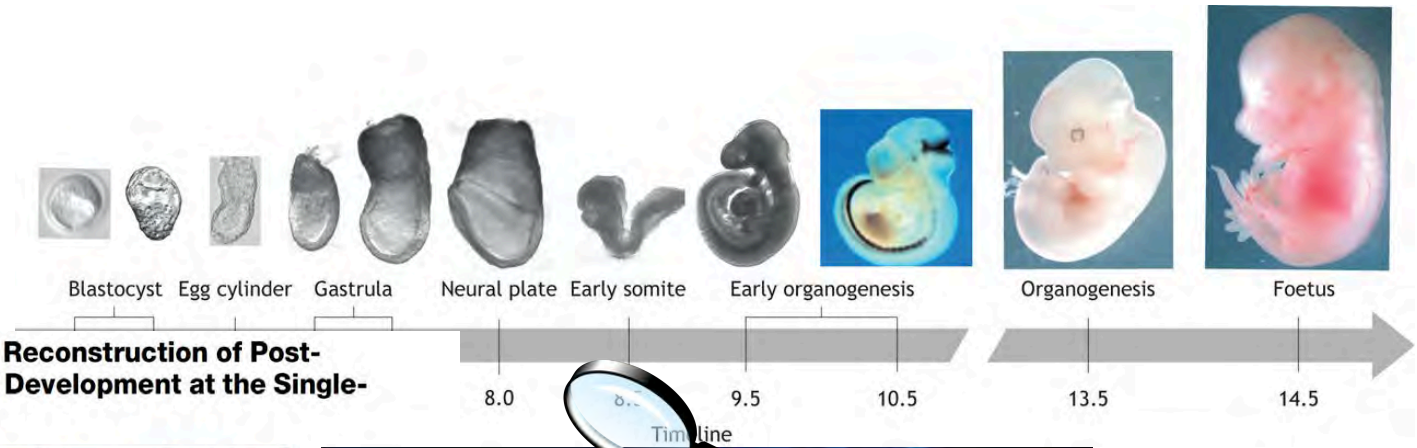
- **Prepare for the next generation**
- **Erase epigenetic history (both programmed and accidental)**

Germ Line Reprogramming
• undo somatic program and epigenetic "accidents"
• set up germ line program

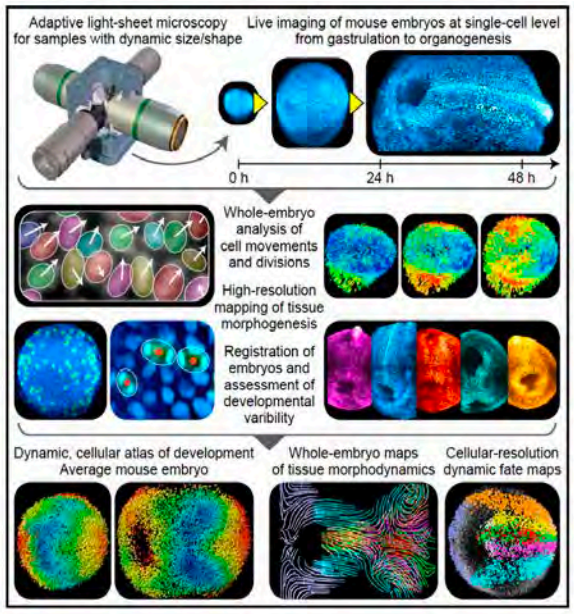
E. Heard, 8 mars, 2021

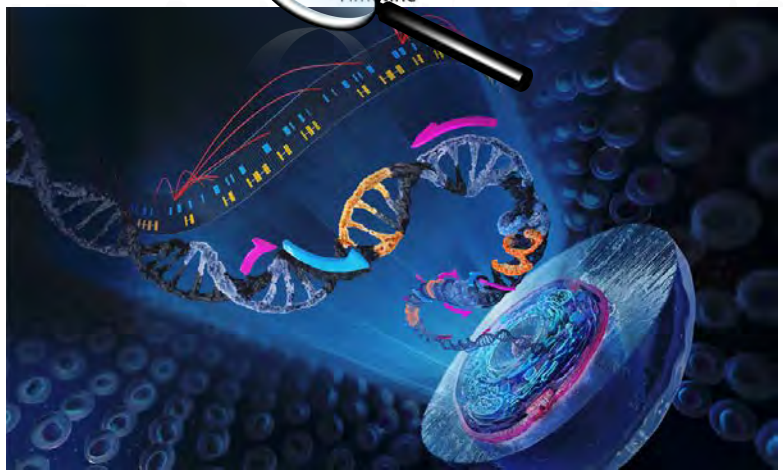
Adapted from Cantone and Fisher, 2013

(Re-)Exploring Mammalian Development at the Single Cell Level



In Toto Imaging and Reconstruction of Post-Implantation Mouse Development at the Single-Cell Level





An illustration of single-cell RNA sequencing (scRNA-seq), a powerful analysis method that gives researchers detailed insights into levels of gene expression in individual cells. Credit: Tobias Wüstefeld/BlueClay Studios

Patrick P. L. Tam, and Joshua W. K. Ho Development 2020;147:dev179788

Single cell RNA-sequencing (scRNA-seq) technology has matured to the point that it is possible to generate large single cell atlases of developing mouse embryos. These atlases allow the dissection of developmental cell lineages and molecular changes during embryogenesis. When coupled with single cell technologies for profiling the chromatin landscape, epigenome, proteome and metabolome, and spatial tissue organisation, these scRNA-seq approaches can now collect a large volume of multi-omic data about mouse embryogenesis. In addition, advances in computational techniques have enabled the inference of developmental lineages of differentiating cells, even without explicitly introduced genetic markers.

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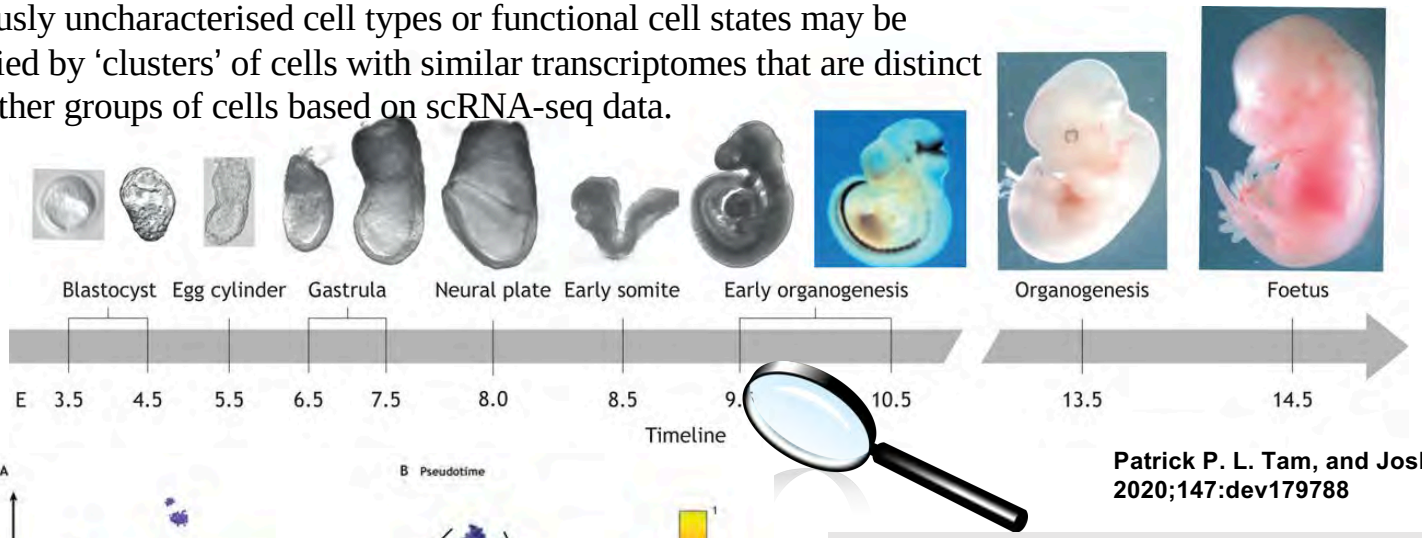
(Re-)Exploring Mammalian Development at the Single Cell Level

Previously uncharacterised cell types or functional cell states may be identified by 'clusters' of cells with similar transcriptomes that are distinct from other groups of cells based on scRNA-seq data.



Patrick P. L. Tam, and Joshua W. K. Ho Development 2020;147:dev179788

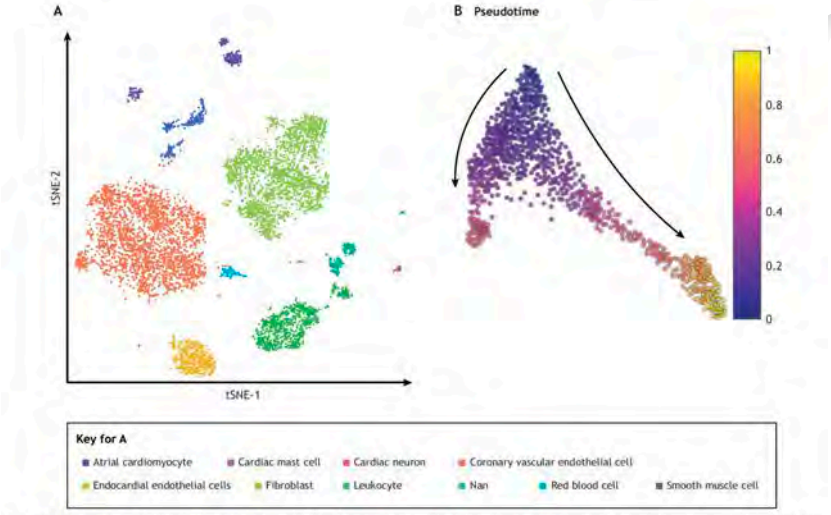


Fig. 2. Visualization of the single cell transcriptome data. (A) A representative tSNE plot illustrating the identification of cell clusters in a mouse single cell atlas (droplet based transcriptomic profiles of heart and aorta in Tabula Muris Senis). (B) A representative plot showing inferred pseudo-time (shown in a colour scale) trajectory of mouse embryonic E3.5-E4.5 cells from inner cell mass, epiblast and primitive endoderm (Nowotschin et al., 2019). This trajectory suggests the branching of one initial cell type into two differentiating cell types.

(t-SNE) t-Distributed Stochastic Neighbour Embedding is a non-linear dimensionality reduction algorithm used for exploring high-dimensional data. It maps multi-dimensional data to two or more dimensions suitable for human observation.

t-SNE is also a method to reduce the dimension. One of the major differences between PCA and t-SNE is it preserves only local similarities whereas PA preserves large pairwise distance maximize variance.

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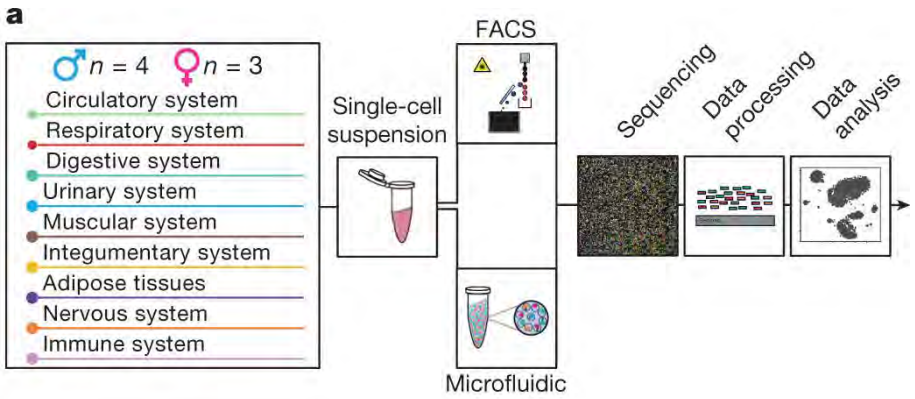
Tabula Muris, or ‘Mouse Atlas’ of Adult Mice

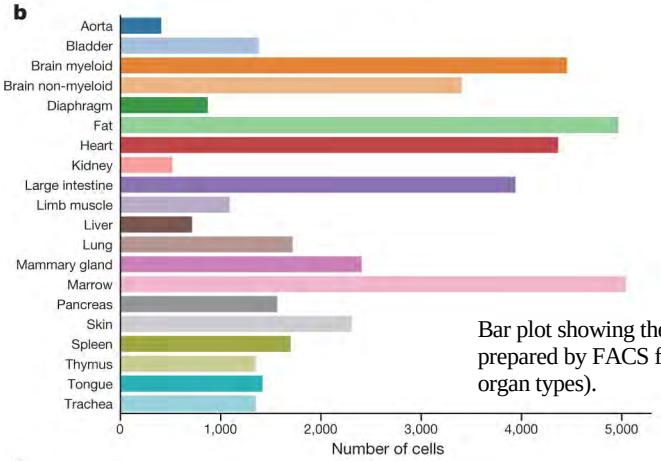
Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris

The Tabula Muris Consortium. Nature , 2018

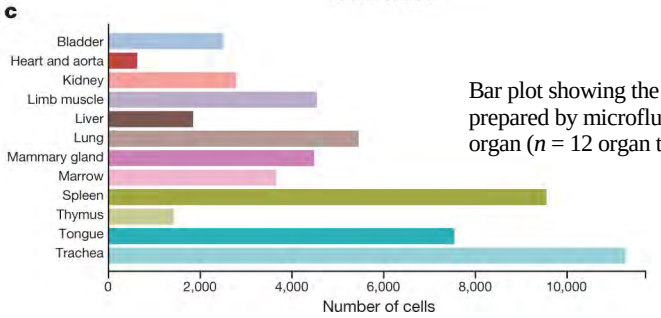
Here we present a compendium of single-cell transcriptomic data from the model organism *Mus musculus* that comprises more than 100,000 cells from 20 organs and tissues. These data represent a new resource for cell biology, reveal gene expression in poorly characterized cell populations and enable the direct and controlled comparison of gene expression in cell types that are shared between tissues, such as T lymphocytes and endothelial cells from different anatomical locations.



20 organs from four male and three female mice were analysed. After dissociation, cells were sorted by FACS and, for some organs, captured in microfluidic oil droplets. Cells were lysed, transcriptomes amplified and sequenced, reads mapped, and data analysed.



Bar plot showing the number of sequenced cells prepared by FACS from each organ (n = 20 organ types).



Bar plot showing the number of sequenced cells prepared by microfluidic droplets from each organ (n = 12 organ types).

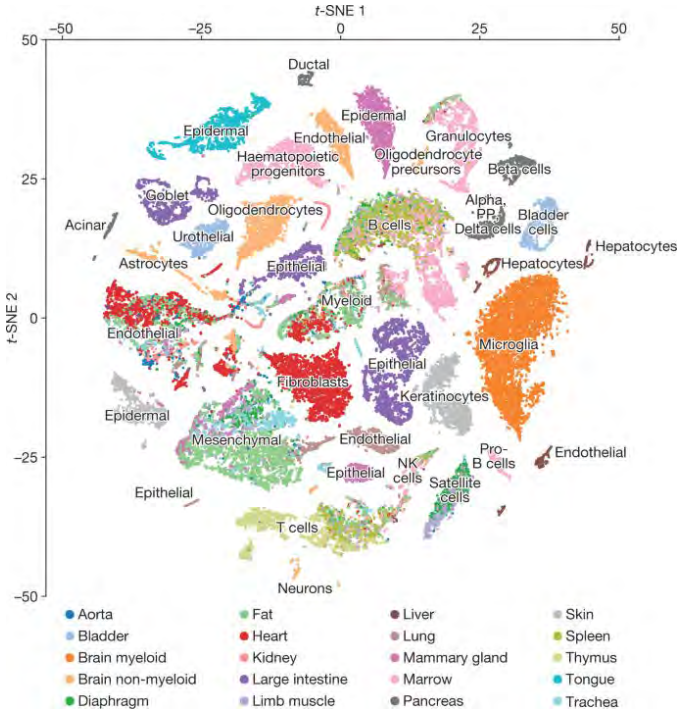
E. Heard, 8 mars, 2021

Tabula Muris, or ‘Mouse Atlas’ of Adult Mice

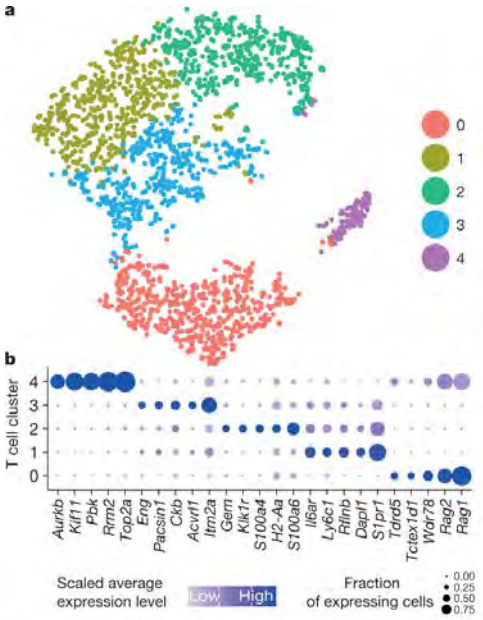
Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris

The Tabula Muris Consortium. Nature , 2018

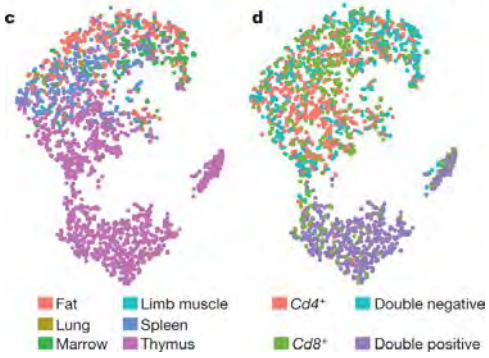
Here we present a compendium of single-cell transcriptomic data from the model organism *Mus musculus* that comprises more than 100,000 cells from 20 organs and tissues. These data represent a new resource for cell biology, reveal gene expression in poorly characterized cell populations and enable the direct and controlled comparison of gene expression in cell types that are shared between tissues, such as T lymphocytes and endothelial cells from different anatomical locations.



t-SNE plot of all cells collected by FACS, coloured by organ, overlaid with the predominant cell type composing each cluster; n = 44,949 individual cells.



To demonstrate an example of investigating common cell types across organs, we collectively analysed all FACS cells annotated as T cells, which revealed five clusters (Fig. 4). Cluster 0 comprises thymic cells undergoing VDJ recombination characterized by the expression of *Rag1*, *Rag2* and *Dntt*, and includes uncommitted double-positive T cells (*Cd4*$^+$ and *Cd8a*$^+$). Cluster 4 contains predominantly proliferating thymic T cells, which may represent pre-T cells expanding after VDJ recombination. Clusters 1–3 contain mostly single-positive T cells (*Cd4*$^+$ or *Cd8a*$^+$). Cluster 3 contains *Cd5*^{hi} thymic T cells that are possibly undergoing positive selection, whereas Cluster 2 contains mostly non-thymic T cells expressing the high-affinity IL2 receptor (encoded by the genes *Il2ra* and *Il2rb*), which suggests that they are activated. Notably, they also express MHC class II genes (*H2-Aa* and *H2-Ab1*). Although this is known in human T cells, MHC class II was previously thought to be restricted to professional antigen-presenting cells in mice[22]. Finally, Cluster 1 also represents mature T cells, but primarily splenic.



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E. Heard, 8 mars, 2021

Single Cell Landscape of Mammalian Organogenesis

ARTICLE

https://doi.org/10.1038/s41586-019-0969-x

The single-cell transcriptional landscape of mammalian organogenesis

Junyue Cao[1,2,10], Malte Spielmann[1,10], Xiaojie Qiu[1,2], Xingfan Huang[1,3], Daniel M. Ibrahim[4,5], Andrew J. Hill[1], Fan Zhang[6], Stefan Mundlos[4,5], Lena Christiansen[6], Frank J. Steemers[6], Cole Trapnell[1,7,8]* & Jay Shendure[1,7,8,9]*

Mammalian organogenesis is a remarkable process. Within a short timeframe, the cells of the three germ layers transform into an embryo that includes most of the major internal and external organs. Here we investigate the transcriptional dynamics of mouse organogenesis at single-cell resolution. Using single-cell combinatorial indexing, we profiled the transcriptomes of around 2 million cells derived from 61 embryos staged between 9.5 and 13.5 days of gestation, in a single experiment. The resulting 'mouse organogenesis cell atlas' (MOCA) provides a global view of developmental processes during this critical window. We use Monocle 3 to identify hundreds of cell types and 56 trajectories, many of which are detected only because of the depth of cellular coverage, and collectively define thousands of corresponding marker genes. We explore the dynamics of gene expression within cell types and trajectories over time, including focused analyses of the apical ectodermal ridge, limb mesenchyme and skeletal muscle.

Single-cell RNA-seq of two million cells

Single-cell combinatorial indexing is a methodological framework involving split-pool barcoding of cells or nuclei.

Single-cell combinatorial-indexing RNA-sequencing analysis (sci-RNA-seq) and a conceptually identical method termed SPLiT-seq.

To increase throughput, the authors developed sci-RNA-seq3 whereby:

(i) nuclei are extracted directly from fresh tissues without enzymatic treatment, then fixed and stored

(ii) for the third level of indexing, switched from Tn5 tagmentation to hairpin ligation

(iii) individual enzymatic reactions were optimized

(iv) fluorescence-activated cell sorting was replaced by dilution.

Sonication and filtration steps were added to minimize aggregation.

Even without automation, sci-RNA-seq3 library preparation can be completed through the intensive effort of a single researcher in one week at a cost of less than $0.01 per cell.

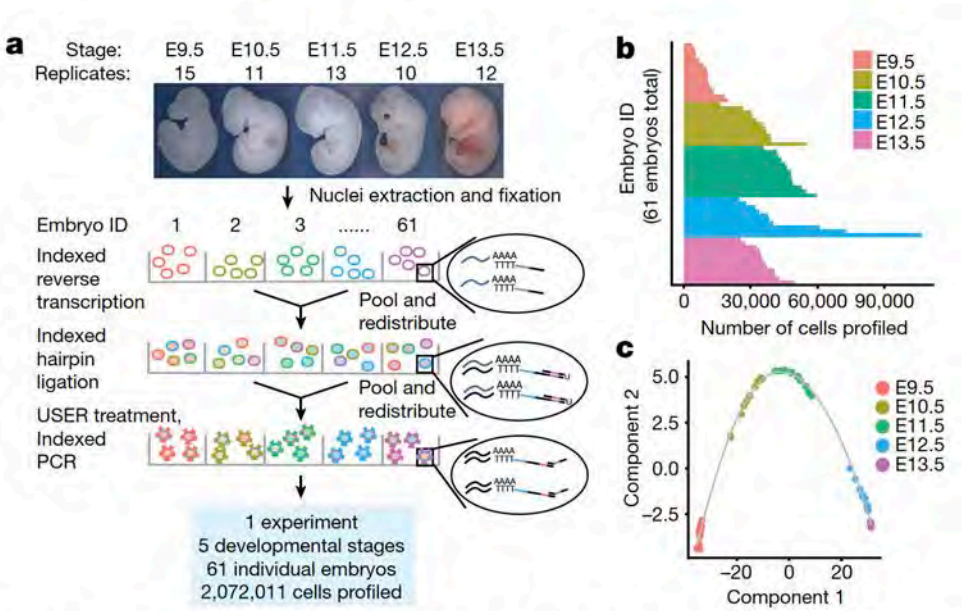

Fig. 1 | sci-RNA-seq3 enables profiling of 2,072,011 cells from 61 mouse embryos across 5 developmental stages in a single experiment. **a**, sci-RNA-seq3 workflow and experimental scheme. USER, uracil-specific excision reagent. **b**, Bar plot showing number of cells profiled from each of 61 mouse embryos. **c**, Pseudotime trajectory of pseudobulk RNA-seq profiles of mouse embryos.

Cao et al., Nature 2019,

COLLÈGE DE FRANCE 1530

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- Identification of cell types and subtypes
- Mouse organogenesis cell atlas (MOCA)
- Characterization of the apical ectodermal ridge (highly specialized epithelium involved in digit development)
- Reconstructing developmental trajectories
- Reconstructing skeletal myogenesis

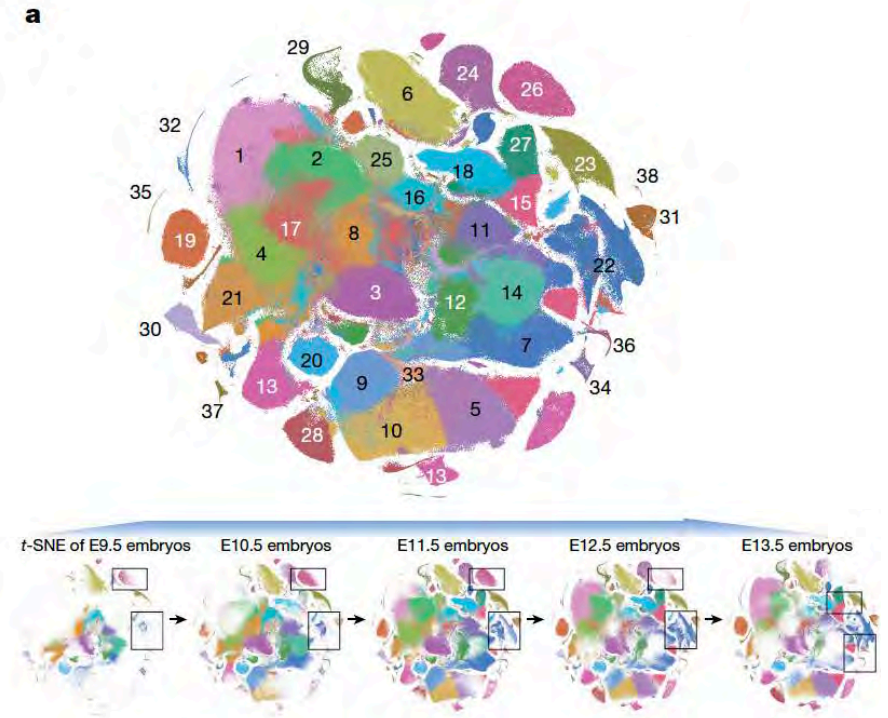


Fig. 2 | Identifying the major cell types of mouse organogenesis. **a**, *t*-SNE visualization of 2,026,641 mouse embryo cells (after removing a putative doublet cluster), coloured by cluster identity (ID) from Louvain clustering (in **b**), and annotated on the basis of marker genes. The same *t*-SNE is plotted below, showing only cells from each stage (cell numbers from left to right: $n = 151{,}000$ for E9.5; 370,279 for E10.5; 602,784

Cao et al., Nature 2019,

COLLÈGE DE FRANCE 1530

Single Cell Landscape of Mammalian Organogenesis

ARTICLE

https://doi.org/10.1038/s41586-019-0969-x

The single-cell transcriptional landscape of mammalian organogenesis

Junyue Cao[1,2,10], Malte Spielmann[1,10], Xiaojie Qiu[1,2], Xingfan Huang[1,3], Daniel M. Ibrahim[4,5], Andrew J. Hill[1], Fan Zhang[6], Stefan Mundlos[4,5], Lena Christiansen[6], Frank J. Steemers[6], Cole Trapnell[1,7,8]* & Jay Shendure[1,7,8,9]*

- Identification of cell types and subtypes
- Mouse organogenesis cell atlas (MOCA)
- Characterization of the apical ectodermal ridge (highly specialized epithelium involved in digit development)
- Reconstructing developmental trajectories
- Reconstructing skeletal myogenesis

Whole-organism single-cell transcriptional atlases will empower reverse genetics—enabling the discovery of subtle defects in the molecular programs or the relative proportions of specific cell types in mutant contexts

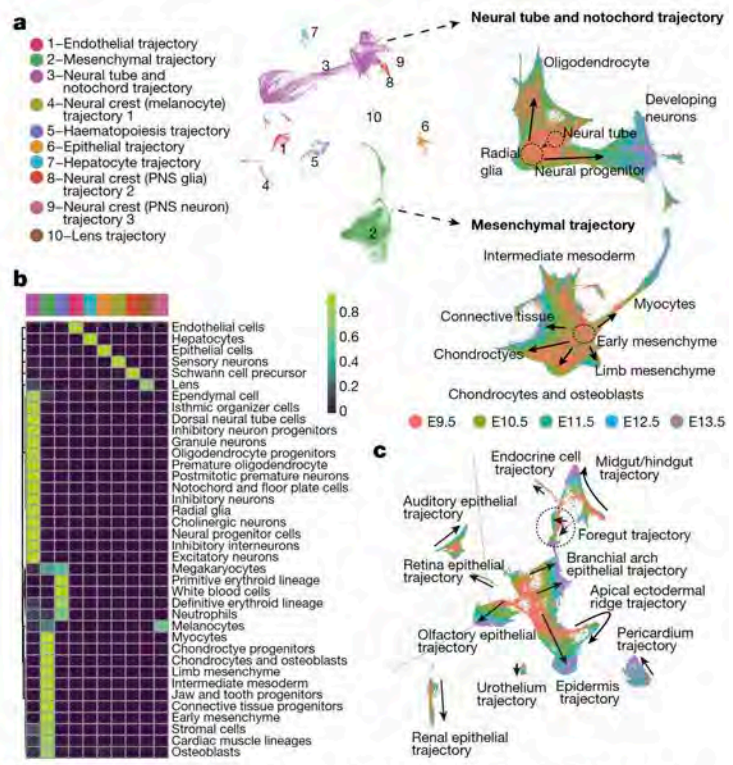


Fig. 4 | Characterization of ten major developmental trajectories present during mouse organogenesis. a, UMAP 3D visualization of our overall dataset. Left, views from one direction; bottom: zoomed view of neural tube–notochord (top) and mesenchymal (bottom) trajectories, coloured by development stage. **b**, Heat map showing the proportion of cells from each of the 38 major cell types (rows) assigned to each of the 10 major trajectories (columns, colour key in **a**, left). **c**, UMAP 3D visualization of epithelial subtrajectories coloured by development stage (colour key in **a**, right).

Cao et al., Nature 2019,

Towards a multi-omic atlas of mouse embryonic development

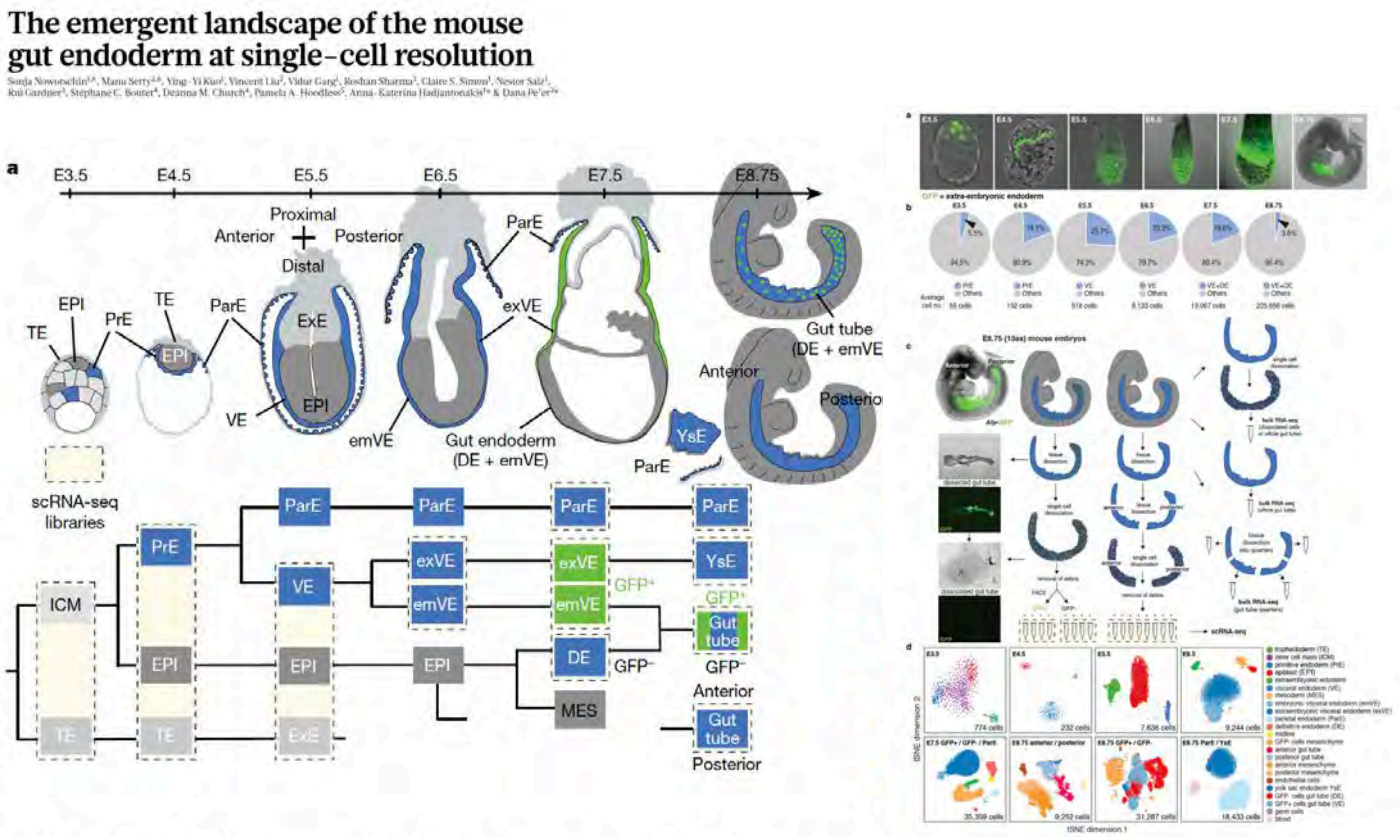


Fig. 1 | Single-cell map of the mouse endoderm, from blastocyst to midgestation. a, Schematic highlighting embryonic stages sampled, lineage relationships and single-cell libraries collected across sequential stages. **b**, *t*-SNE plot of all samples; each dot represents a single cell that is colour-coded by cell type. DE, definitive endoderm; TE, trophectoderm; ParE, parietal endoderm; VE, visceral endoderm; YsE, yolk sac endoderm.

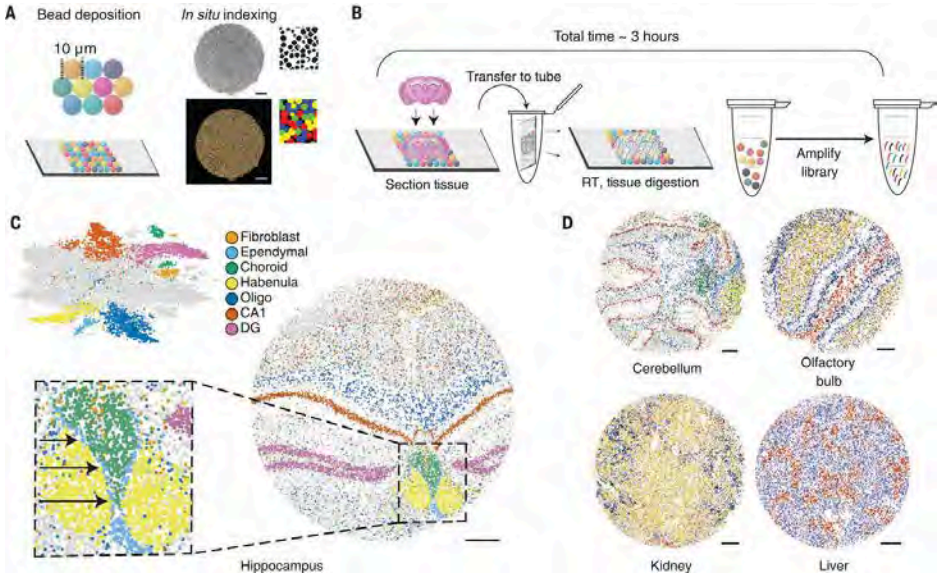
- Delineate the ontogeny of the mammalian endoderm by generating 112,217 single-cell transcriptomes, which represent all endoderm populations within the mouse embryo until midgestation.
- Use graph-based approaches to model differentiating cells, which provides a spatio-temporal characterization of developmental trajectories and defines the transcriptional architecture that accompanies the emergence of the first (primitive or extra-embryonic) endodermal population and its sister pluripotent (embryonic) epiblast lineage.
- Uncover a relationship between descendants of these two lineages, in which epiblast cells differentiate into endoderm at two distinct time points—before and during gastrulation.
- Trajectories of endoderm cells were mapped as they acquired embryonic versus extra-embryonic fates and as they spatially converged within the nascent gut endoderm, which revealed these cells to be globally similar but retain aspects of their lineage history.
- Observed regionalized identity of cells along the anterior–posterior axis of the emergent gut tube, which reflects their embryonic or extra-embryonic origin, and the coordinated patterning of these cells into organ-specific territories.

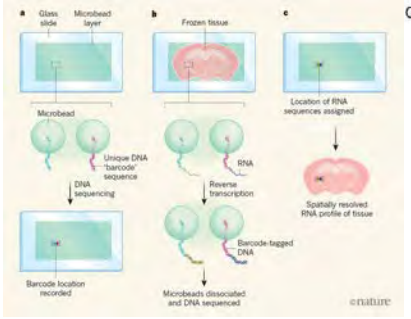
E. Heard, 8 mars, 2021

Nowotschin et al, The emergent landscape of the mouse gut endoderm at single-cell resolution. Nature 2019

Ultimately: Spatial-omics atlases

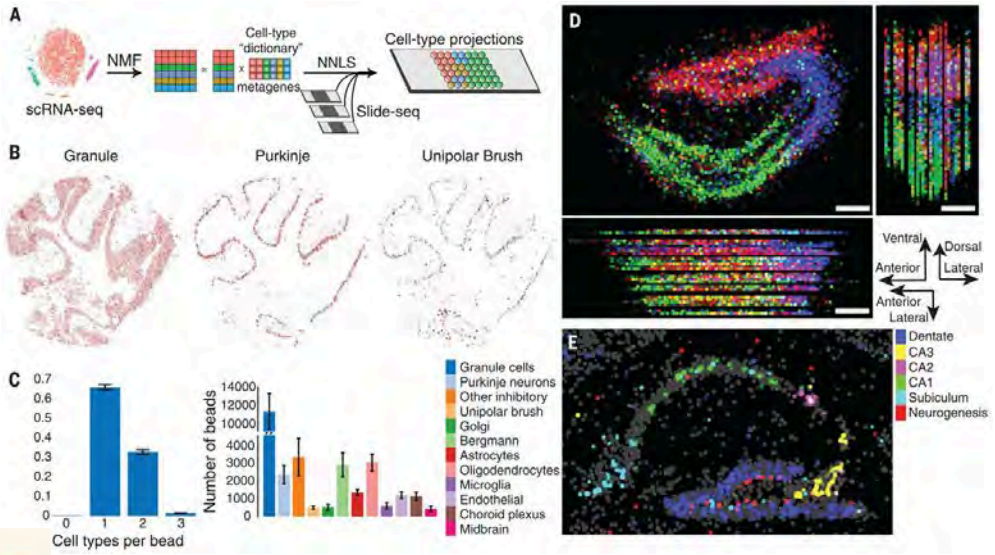
High-resolution RNA capture from tissue by Slide-seq.





Rodriques et al. Science 2019;363:1463-1467

Localization of cell types in the cerebellum and hippocampus using Slide-seq.



COLLÈGE DE FRANCE 1530

New Technologies to follow Cellular Memory

Single-cell epigenomics: Recording the past and predicting the future

Gavin Kelsey,[1,2]*† Oliver Stegle,[3,4]*† Wolf Reik[1,2,5]†

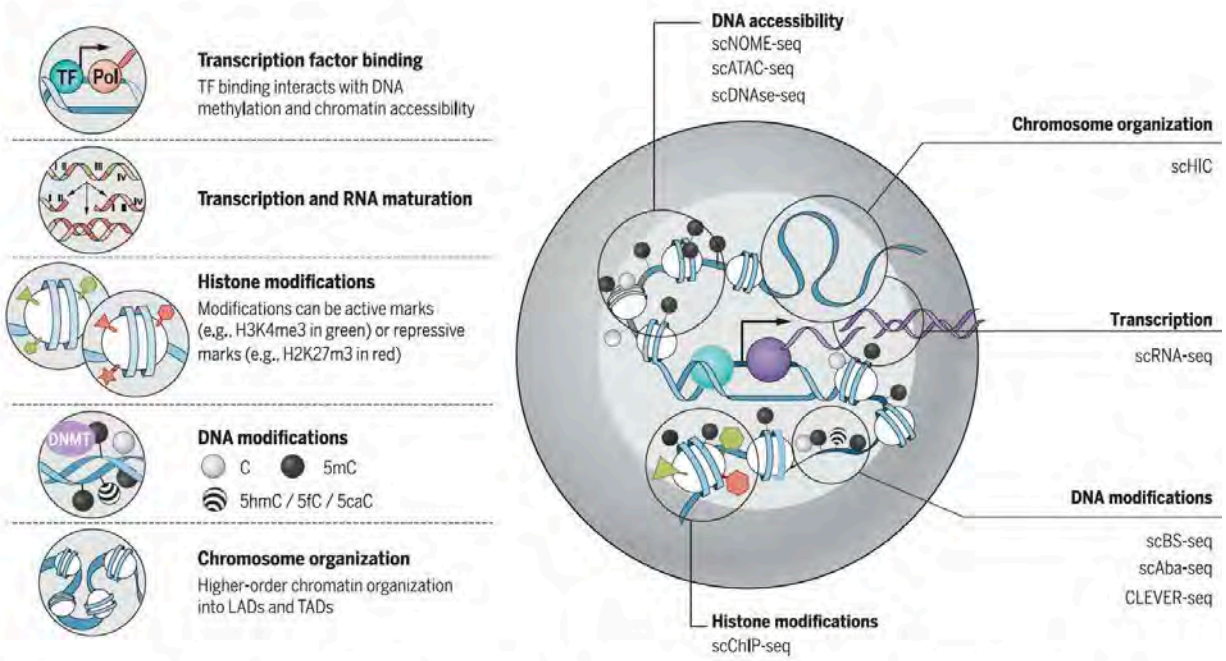
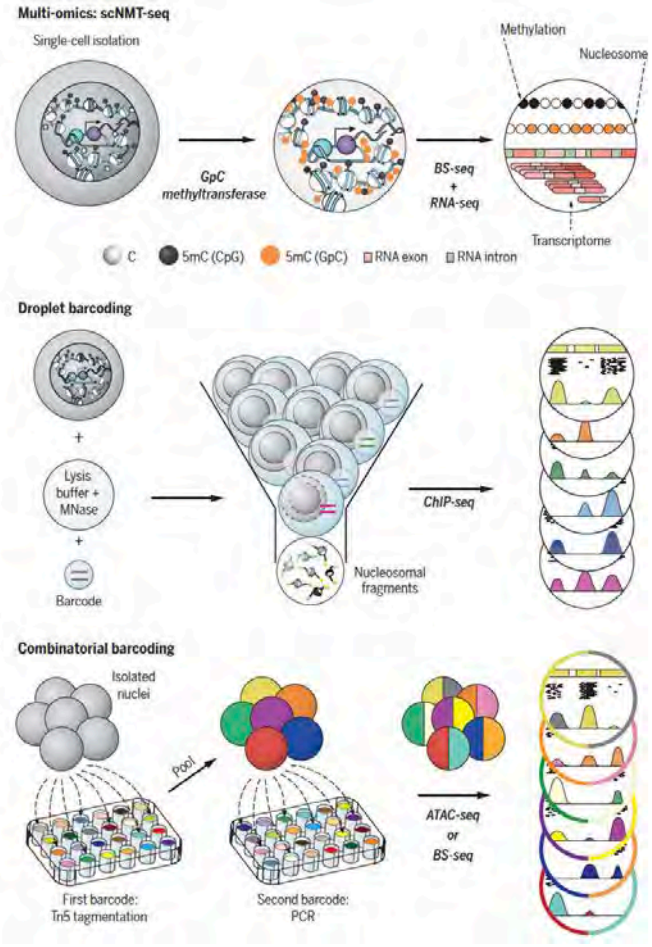


Fig. 1. Single-cell methods and heterogeneity of different molecular layers. (**Left**) Overview of different molecular layers that can be assayed using single-cell protocols. (**Right**) A cell with different layers of multi-omics measurements, as defined on the left. Concordance or heterogeneit respectively may exist between the different layers, and this can be recorded by single-cell sequencing and computationally evaluated.

- With RNA sequencing analysis, one can detect the genes that are turned on in each group of cells. Then integrate all the transcriptomes together to generate a 3D map of gene expression in the embryo.
- Single-cell multi-omics technologies can explore different layers of genomic output—and hence cell identity and function—can be recorded simultaneously.
- Integrating various components of the epigenome into multi-omics measurements allows the study of cellular heterogeneity at different time scales and the discovery of new layers of molecular connectivity between the genome and its functional output.
- Measurements that are increasingly available range from those that identify transcription factor occupancy and initiation of transcription to long-lasting and heritable epigenetic marks such as DNA methylation.
- Together with techniques in which cell lineage is recorded, this multilayered information will provide insights into a cell's past history and its future potential.
- This will allow new levels of understanding of cell fate decisions, identity, and function in normal development, physiology, and disease.

E. Heard, 1 mars, 2021

New Technologies to follow Cell Identity and Cell Fate



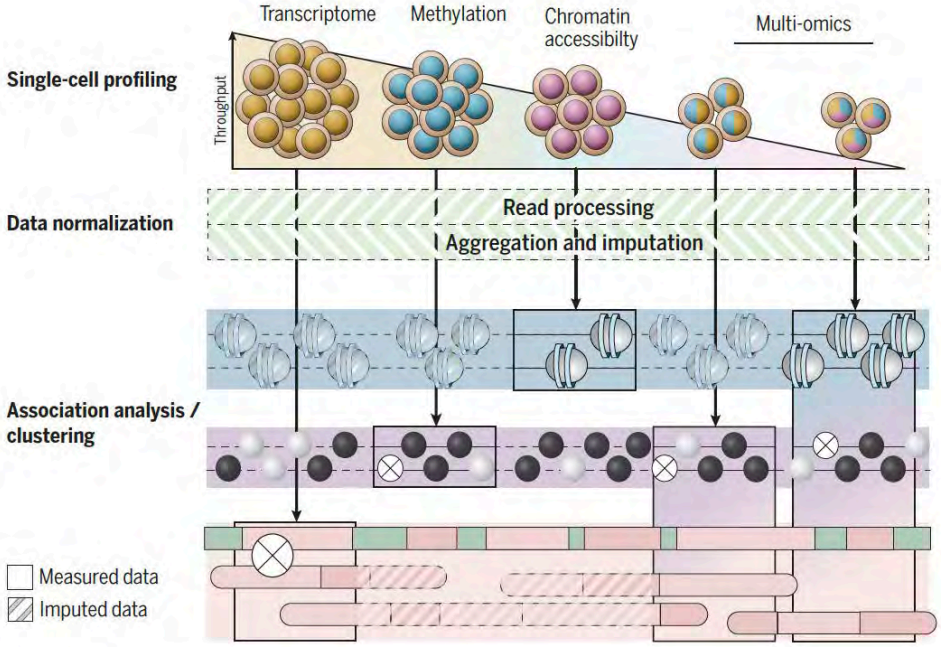
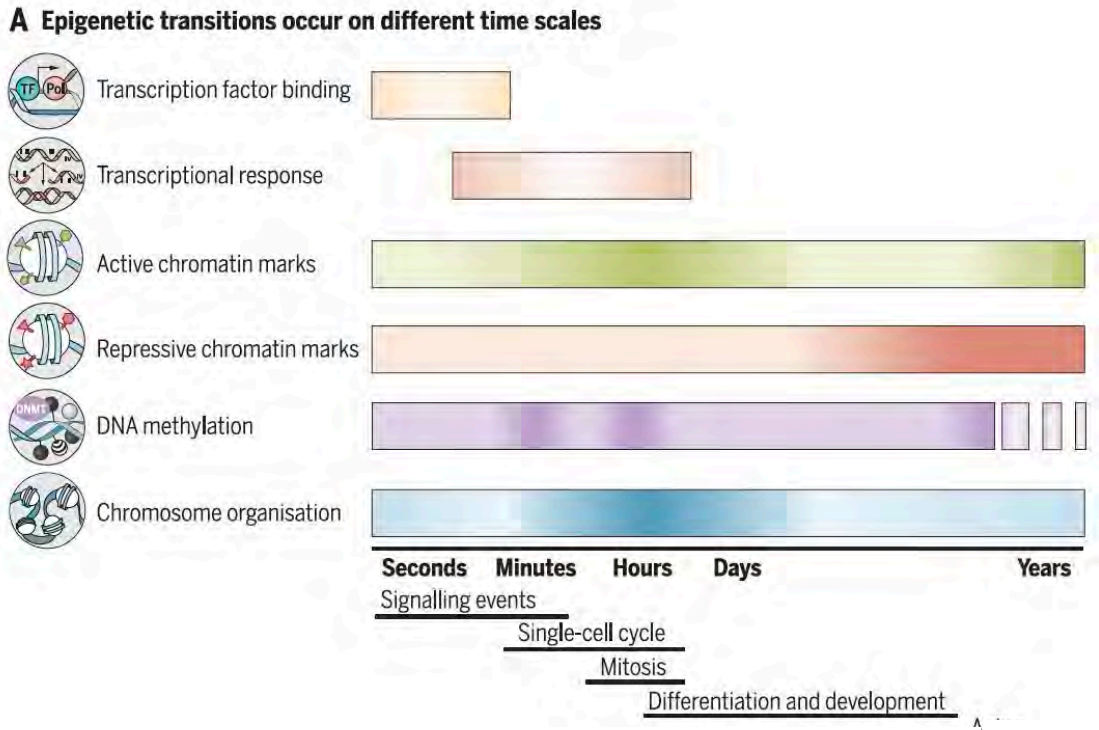
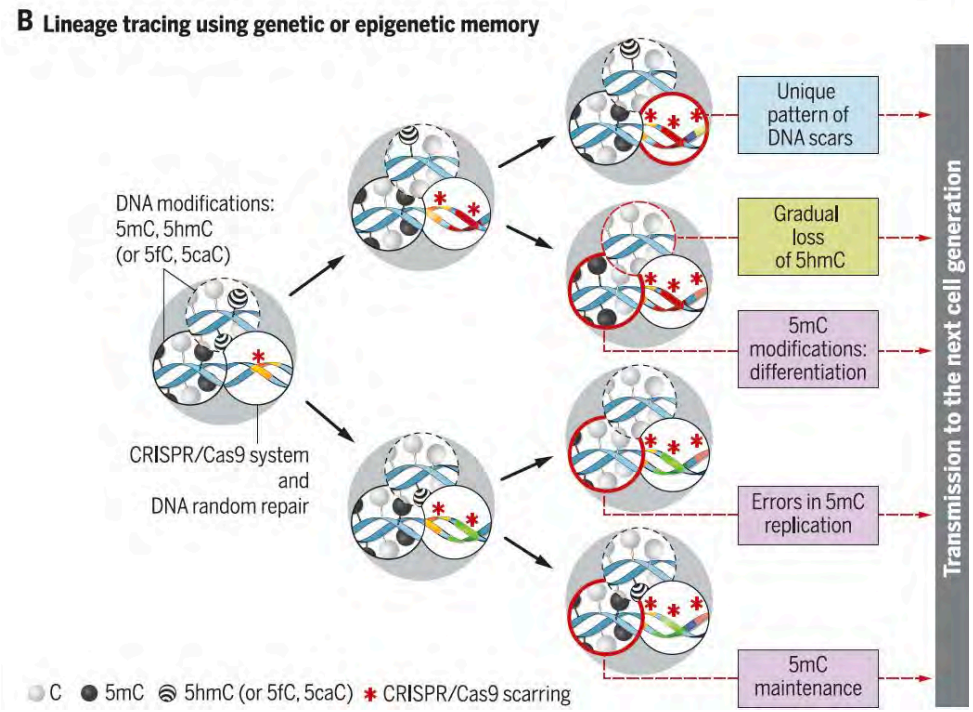


Fig. 3. Multi-omics and computational methods. Shown are typical trade-offs between single-cell RNA-seq, single-cell epigenome protocols, and multi-omics methods that provide readouts from multiple molecular layers in parallel. Consequently, it is commonly required to integrate data from different sequencing protocols. Raw sequence reads from these methods are deduplicated and aggregated into locus-specific readouts, with an optional imputation step to complete missing information. Associations between molecular layers can be used for completing missing data and allow for discovering regulatory associations.

New Technologies to follow Cellular Memory during Development





- Different layers of information can in principle be recorded by single-cell multi-omics, from transcription factor binding and transcriptional responses to long-term epigenetic memory such as is possible with DNA methylation.
- Timing and transitions in information—range from seconds to years.
- With aging, fidelity of epigenetic information such as DNA methylation may degrade, leading to increased cell-to-cell heterogeneity.

E. Heard, 8 mars, 2021

- Lineage tracing using genetic or epigenetic memory.
- Cell lineage can be traced by CRISPR scarring approaches in which each cell and its descendants within a lineage are linked by unique mutations or barcodes.
- DNA modifications may also be used to track lineage based on their inheritance and on errors in their maintenance at DNA replication.
- Nonheritable modifications (5hmC, 5fC, and 5caC) have a short-term lineaging potential, whereas heritable modifications (5mC) have long-term non-invasive lineaging potential

Tracing cellular development in mouse development

Article

Multi-omics profiling of mouse gastrulation at single-cell resolution

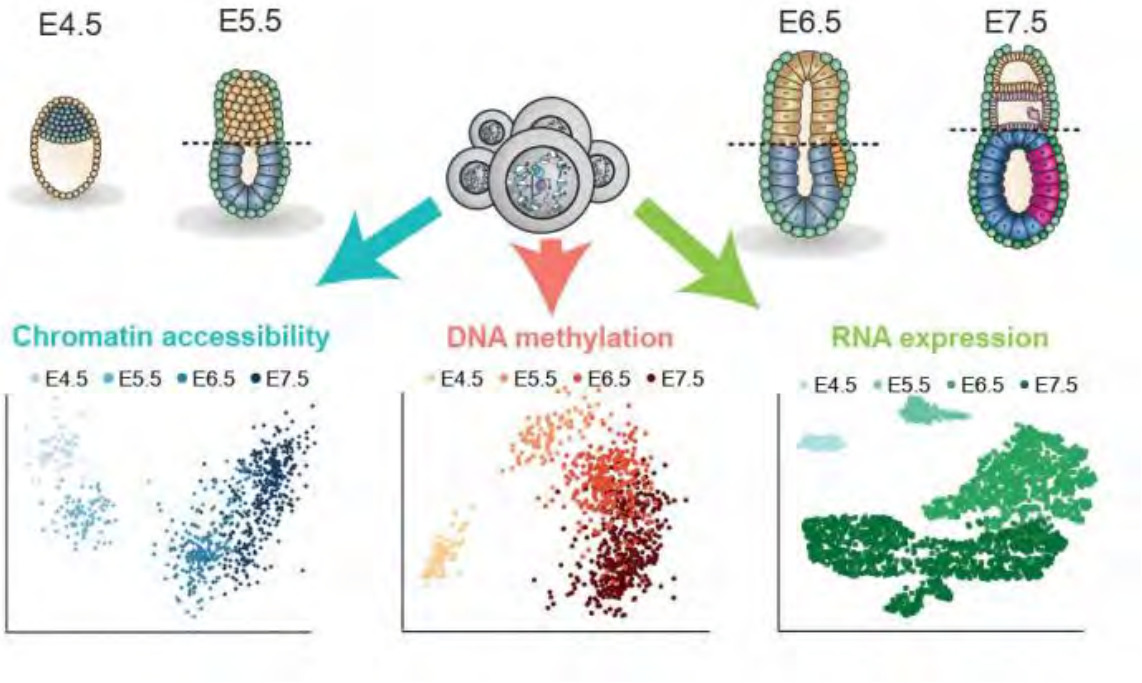
Single-cell analysis of the earliest cell fate decisions in development

Multi-omics analysis of the process establishing the mammalian body plan

• Uniting three different parameters in single cells taken from mouse embryos reveals more about how foundational cell identities are established in early development

•The first single-cell multi-omics analysis of gastrulation allows gene expression, DNA methylation and chromatin accessibility to be connected and to thus understand the potential role of the epigenome in cell fate decisions in early development.

•The findings propose how embryonic cells may be deviated away from a default cell state and awoken to new developmental possibilities during gastrulation, and plots the timeline of the epigenetic events controlling cell identity.



Argelaguet, Clark, Mohammed, Stapel et al Nature 2019

Tracing cellular development in mouse development

Article

Multi-omics profiling of mouse gastrulation at single-cell resolution

Single-cell analysis of the earliest cell fate decisions in development

Cell type as a function of stage

• Uniting three different parameters in single cells taken from mouse embryos reveals more about how foundational cell identities are established in early development

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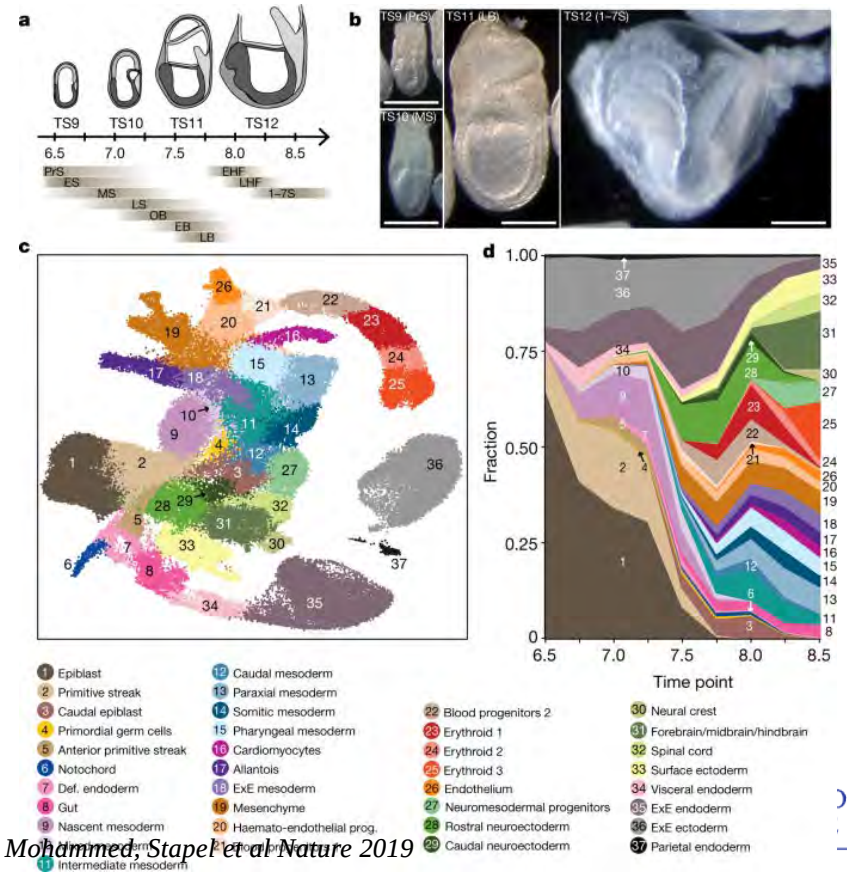

Argelaguet, Clark, Mohammed, Stapel et al Nature 2019

Tracing cellular development in mouse development

Article

Multi-omics profiling of mouse gastrulation at single-cell resolution

Enhancer priming or remodeling define the primary germ layers

• Uniting three different parameters in single cells taken from mouse embryos reveals more about how foundational cell identities are established in early development

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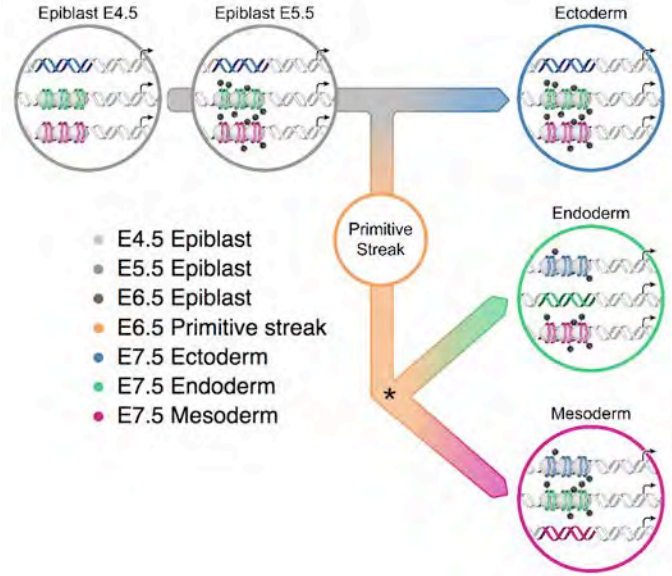
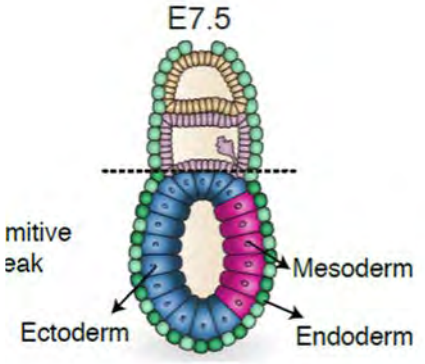
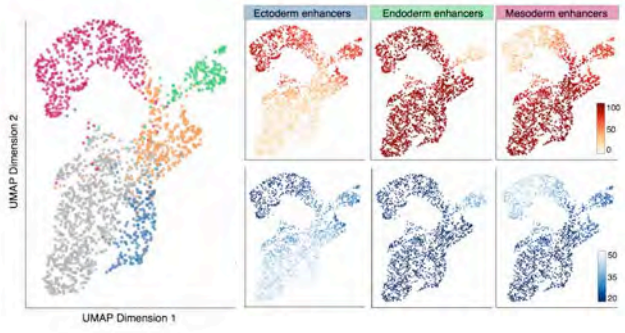






Argelaguet, Clark, Mohammed, Stapel et al Nature 2019

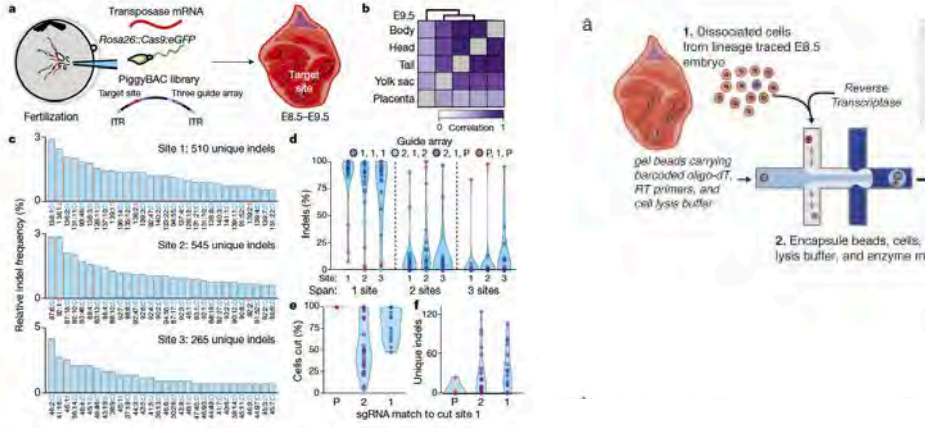
COLLÈGE DE FRANCE 1530

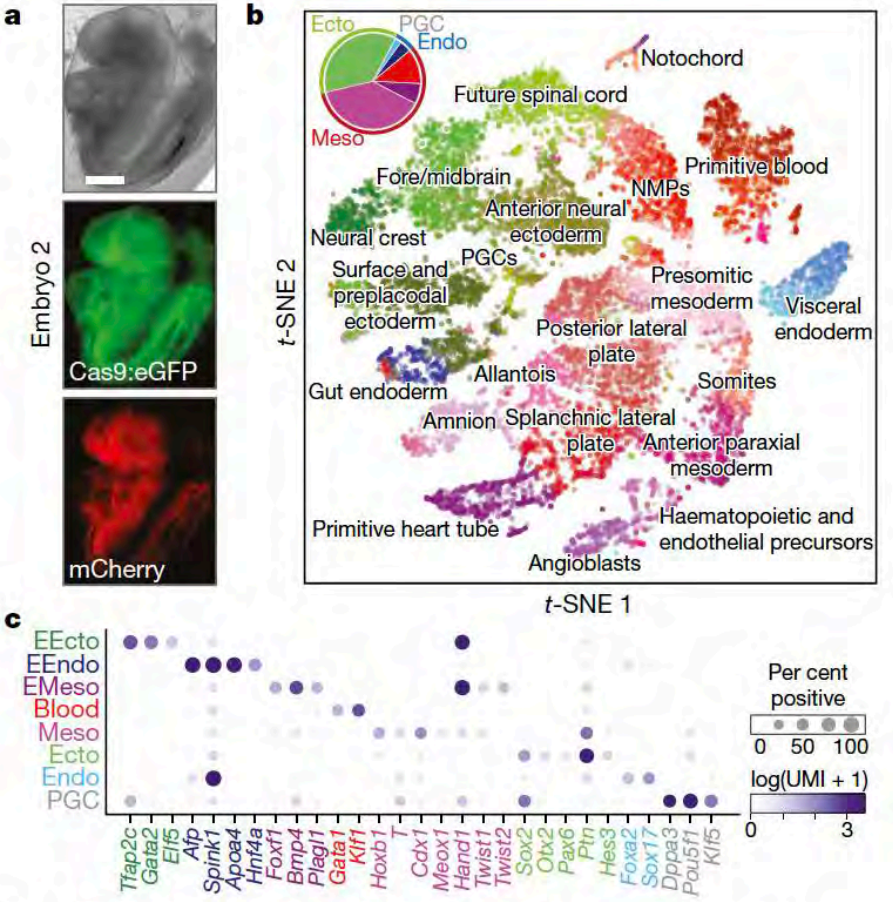
Tracing Cellular Memory during Development

Molecular recording of mammalian embryogenesis

Michelle M. Chan[1,2,14], Zachary D. Smith[3,4,5,14], Stefanie Grosswendt[6], Helene Kretzmer[6], Thomas M. Norman[1,2], Britt Adamson[1,2,13], Marco Jost[1,2,7], Jeffrey J. Quinn[1,2], Dian Yang[1,2], Matthew G. Jones[1,2,8], Alex Khodaverdian[9,10], Nir Yosef[9,10,11,12], Alexander Meissner[3,4,6*] & Jonathan S. Weissman[1,2*]

Ontogeny describes the emergence of complex multicellular organisms from single totipotent cells. This field is particularly challenging in mammals, owing to the indeterminate relationship between self-renewal and differentiation, variation in progenitor field sizes, and internal gestation in these animals. Here we present a flexible, high-information, multi-channel molecular recorder with a single-cell readout and apply it as an evolving lineage tracer to assemble mouse cell-fate maps from fertilization through gastrulation. By combining lineage information with single-cell RNA sequencing profiles, we recapitulate canonical developmental relationships between different tissue types and reveal the nearly complete transcriptional convergence of endodermal cells of extra-embryonic and embryonic origins. Finally, we apply our cell-fate maps to estimate the number of embryonic progenitor cells and their degree of asymmetric partitioning during specification. Our approach enables massively parallel, high-resolution recording of lineage and other information in mammalian systems, which will facilitate the construction of a quantitative framework for understanding developmental processes.





A molecular recorder as a continuously evolving lineage tracer to observe the fate map that underlies mouse embryogenesis

COLLÈGE DE FRANCE 1530

Single Cell Tracing Discoveries: Plasticity
Reactivation of the Pluripotency network prior to Neural Crest Formation

RESEARCH ARTICLE

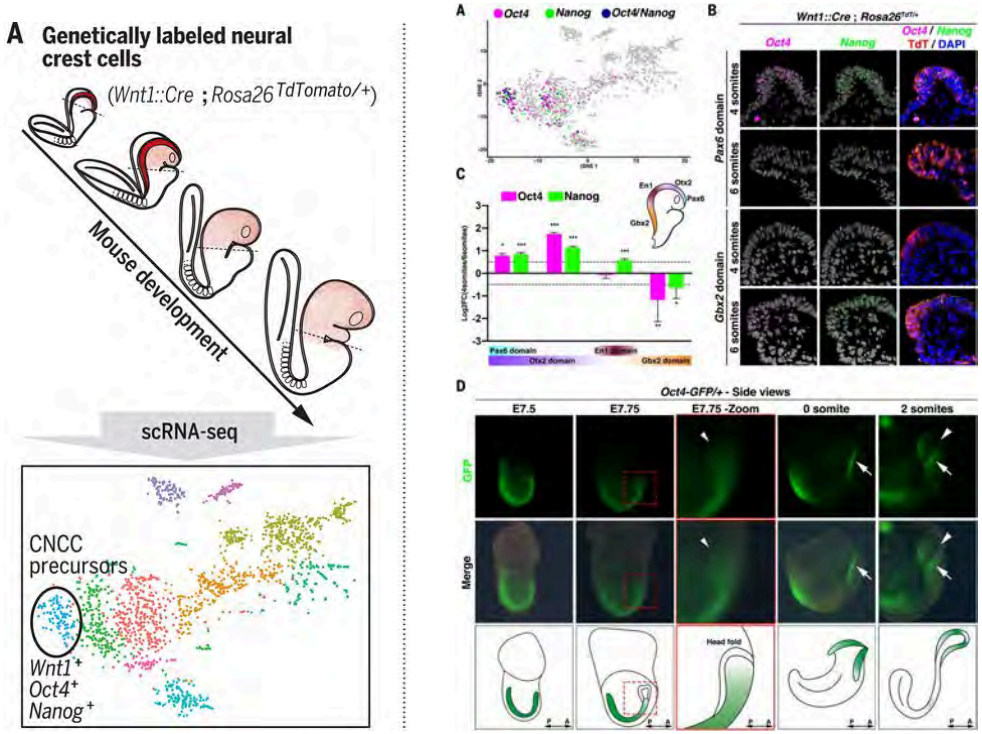
DEVELOPMENTAL BIOLOGY

Reactivation of the pluripotency program precedes formation of the cranial neural crest

Antoine Zalc[1]*, Rahul Sinha[2]*, Gunsagar S. Gulati[2], Daniel J. Wesche[2], Patrycja Daszczuk[1], Tomek Swigut[1], Irving L. Weissman[2], Joanna Wysocka[1,2,3,4]†

RESULTS

We found that premigratory CNCCs are heterogeneous and carry positional information reflective of their origin in the neuroepithelium, but this early positional information is subsequently erased, with delaminating CNCCs showing a relatively uniform transcriptional signature that later rediversifies as CNCCs undergo first commitment events. We identify an early precursor population that expresses canonical pluripotency transcription factors and gives rise to CNCCs and craniofacial structures. Rather than being maintained from the epiblast, pluripotency factor Oct4 is transiently reactivated in the prospective CNCCs after head-fold formation, and its expression shifts from the most anterior to the more posterior part of the cranial domain as development progresses. Oct4 is not required for the induction of CNCCs in the neuroepithelium, but instead is important for the specification and survival of facial mesenchyme, thus directly linking this pluripotency factor with the expansion of CNCC cellular potential. Open chromatin landscapes of Oct4$^+$ CNCC precursors are consistent with their neuroepithelial origin while also broadly resembling those of pluripotent epiblast stem cells. In addition, we saw priming of distal regulatory regions at a subset of loci associated with future neural crest migration and mesenchyme formation.



(A) Single-cell RNA (scRNA) sequencing of genetically labeled murine CNCCs over 14 hours of development revealed rapid transcriptional changes and identified a precursor population expressing pluripotency factors.

E. Heard, 8 mars, 2021

COLLÈGE DE FRANCE 1530

Reactivation of the Pluripotency network prior to Neural Crest Formation

RESEARCH ARTICLE

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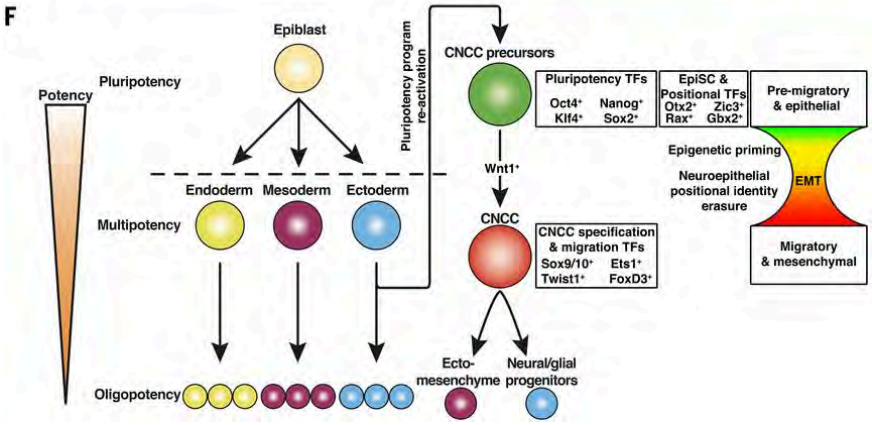
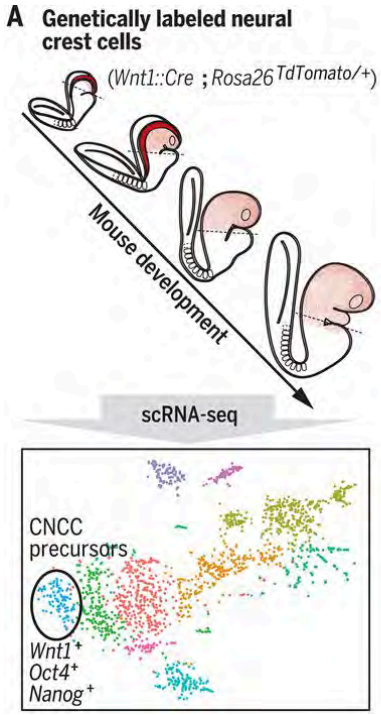
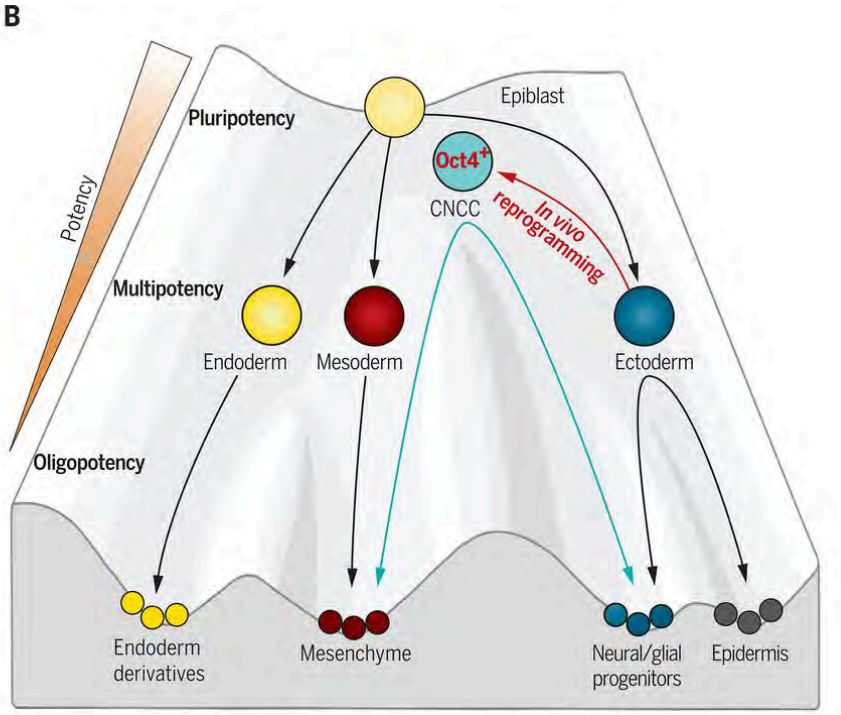
Reactivation of the pluripotency program precedes formation of the cranial neural crest

Antoine Zalc[1]*, Rahul Sinha[2]*, Gunsagar S. Gulati[2], Daniel J. Wesche[2], Patrycja Daszczuk[1], Tomek Swigut[1], Irving L. Weissman[2], Joanna Wysocka[1,2,3,4]†

During development, cells progress from a pluripotent state to a more restricted fate within a particular germ layer. However, cranial neural crest cells (CNCCs), a transient cell population that generates most of the craniofacial skeleton, have much broader differentiation potential than their ectodermal lineage of origin. Here, we identify a neuroepithelial precursor population characterized by expression of canonical pluripotency transcription factors that gives rise to CNCCs and is essential for craniofacial development. Pluripotency factor *Oct4* is transiently reactivated in CNCCs and is required for the subsequent formation of ectomesenchyme. Furthermore, open chromatin landscapes of Oct4$^+$ CNCC precursors resemble those of epiblast stem cells, with additional features suggestive of priming for mesenchymal programs. We propose that CNCCs expand their developmental potential through a transient reacquisition of molecular signatures of pluripotency.







(A) Single-cell RNA (scRNA) sequencing of genetically labeled murine CNCCs over 14 hours of development revealed rapid transcriptional changes and identified a precursor population expressing pluripotency factors.

(B) Uphill on Waddington's epigenetic landscape, reactivation of Oct4 endows CNCC precursors with the ability to form derivatives typical of mesoderm, such as mesenchyme.

E. Heard, 8 mars, 2021

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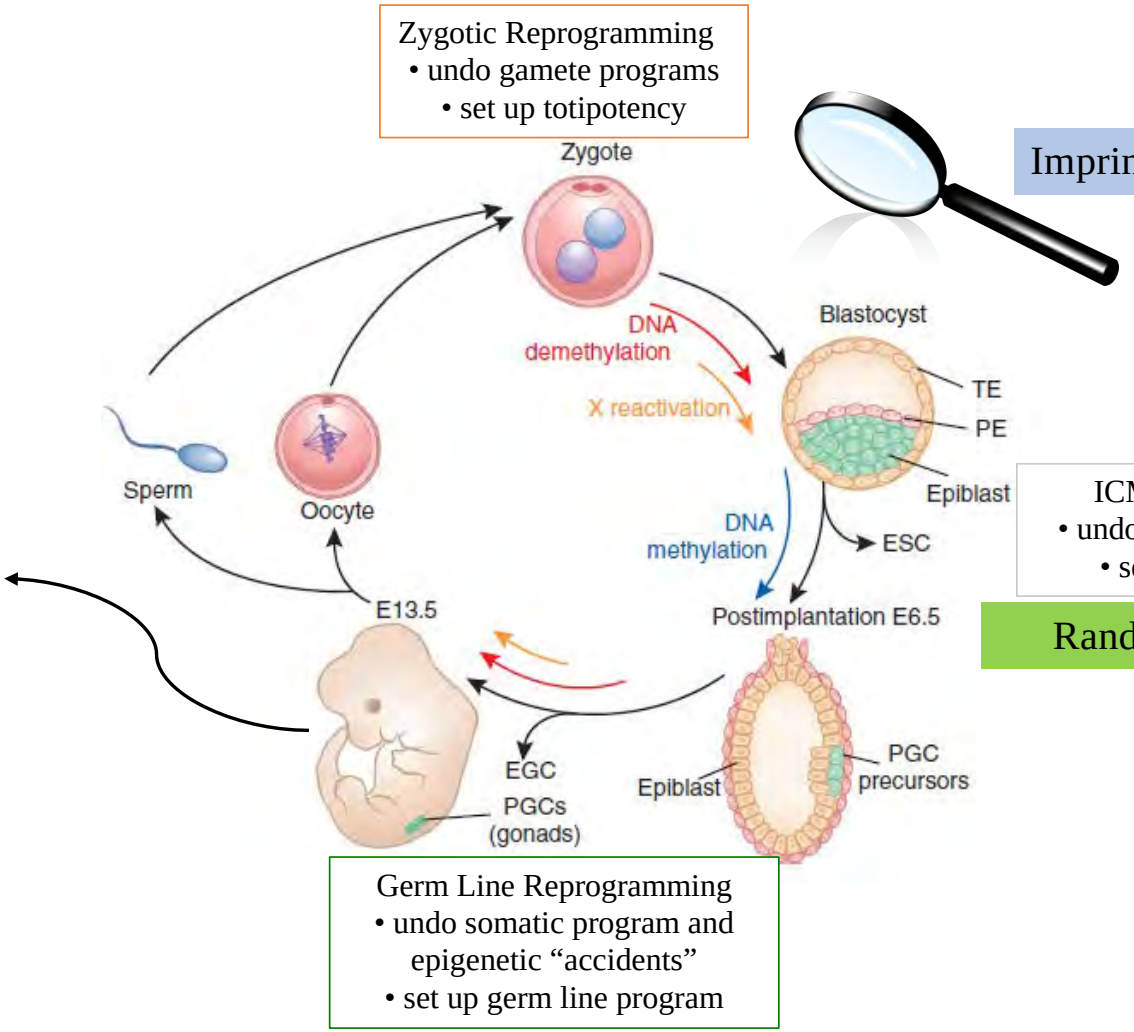
Stability and Plasticity during Mammalian Pre-Implantation Development



Imprinted X inactivation

ICM Reprogramming
• undo/prevent TE program
• set up Pluripotency

Random X inactivation

E. Heard, 8 mars, 2021

Adapted from Cantone and Fisher, 2013

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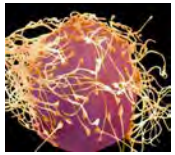
Epigenetic changes mouse preimplantation embryo development

Egg and Sperm: two highly differentiated cell types



Maternal chromatin: maternal proteins, mRNAs
- to enable reprogramming upon fertilization and early development

Highly packaged paternal genome: DNA + protamines
(1-15% histones; small amount of RNA; other factors?)

A very rapid reprogramming process with the two parental genomes exhibit similar chromatin accessibility within 12 hours of fertilization and this reprogramming process is DNA replication-independent. However, the protein factors that mediate the reprogramming process are largely unknown.

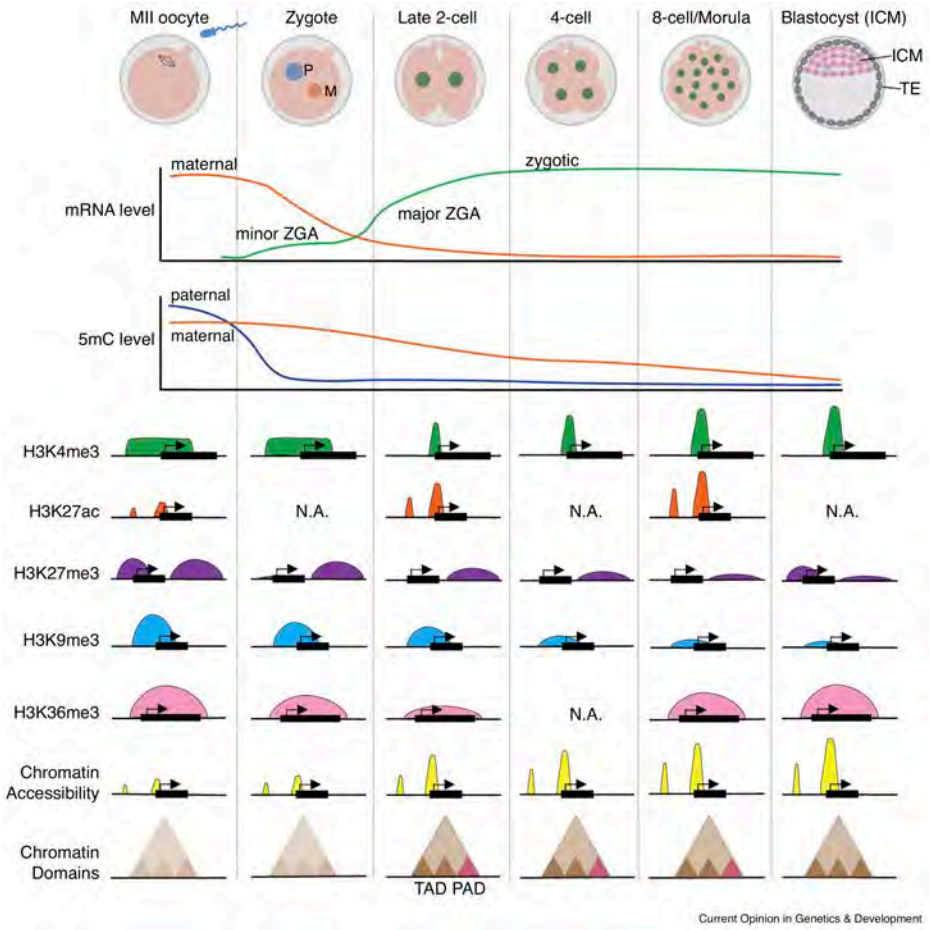
The broad H3K4me3 domains are replaced by the promoter-associated canonical H3K4me3 peaks at the late two-cell stage. Active erasure of the broad H3K4me3 domains appears to be essential for ZGA as knockdown of the H3K4me3 demethylases Kdm5a/b results in preimplantation developmental arrest with defects in activation of a subset of ZGA genes

Maternal H3K27me3- mediated non-canonical imprinting in preimplantation embryos is largely lost in the embryonic lineage after implantation, but maintained in the extraembryonic lineage at certain loci that include the maternal Xist and certain genes important for placenta development

Repressive marks such as H3K27me3, H3K9me3 also exhibit dynamic changes during preimplantation development. While promoter H3K27me3 and H3K9me3 are quickly erased or decreased after fertilization, the broad H3K27me3 domains in distal regions can be maintained until the blastocyst stage. Removal of H3K9me3 is essential for preimplantation development as failure to do so in somatic cell nuclear transfer (SCNT) embryos leads to developmental arrest due to ZGA defects. Whether removal of H3K27me3 is required for preimplantation development still await to be shown.

Depletion of maternal H3K36me3 in oocytes results in the disruption of the maternal epigenome, thus causing defects in ZGA and embryonic development . After fertilization, maternal H3K36me3 is attenuated from the late two-cell stage and disappears at the eight-cell stage, while zygotic H3K36me3 is established gradually during preimplantation development.

Higher order chromatin structure, indicated by compartments and topologically associating domains (TADs), is disordered in MII oocytes and zygotes (light brown triangles with dashed lines) and established from late two-cell stage (brown triangles with solid lines).
Polycomb-associating domains (PADs, red triangles with solid lines) are present transiently during two-cell to eight-cell stages.



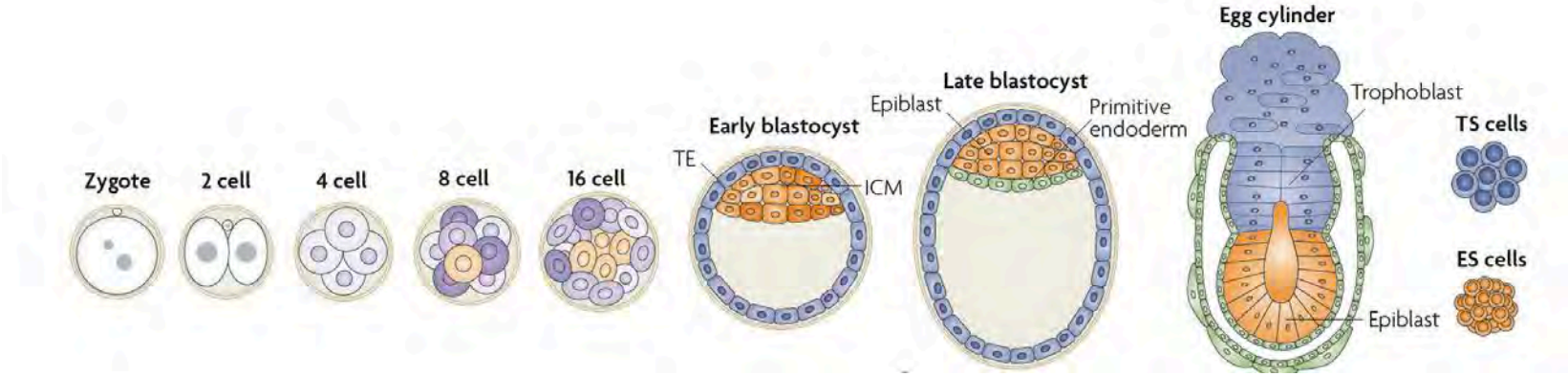
Tranisent , parent-of-origin differences in gene expression and chromatin topology
Next WEEK

E

COLLÈGE DE FRANCE 1530

Establishing and maintaining early lineage decisions during Mouse development

Totipotency in the mouse embryo up to ~4-cell stage



• Progressive restriction of cellular plasticity from 4-cell stage

• Positional cues start to play a role at ~8-16 cell morula stage:

-**inner cells** tend to form inner cell mass (epiblast = soma + germ line; primitive endoderm)

-**outer cells** tend to form trophectoderm TE (extra-embryonic tissues)

• Key transcription factors are essential to determine cell fate and establish cell lineages of the early embryo

• Chromatin factors (eg histone modifiers CARM1, SETDB1, PRC2, G9a) provide permissive (or non-permissive) environment for cell fate, and/or predispose a cell towards a particular lineage.

• Chromatin marks and DNA methylation also progressively lock in active and inactive states

Establishing cell differentiation during development

Instructions for Assembling the Early Mammalian Embryo

Melanie D. White,[1] Jennifer Zenker,[1] Stephanie Bissiere,[1] and Nicolas Plachta[1,2,*]
[1]Institute of Molecular and Cell Biology, A*STAR, 61 Biopolis Drive, Singapore 138673
[2]Department of Biochemistry, NUS, Singapore
*Correspondence: plachtan@imcb.a-star.edu.sg
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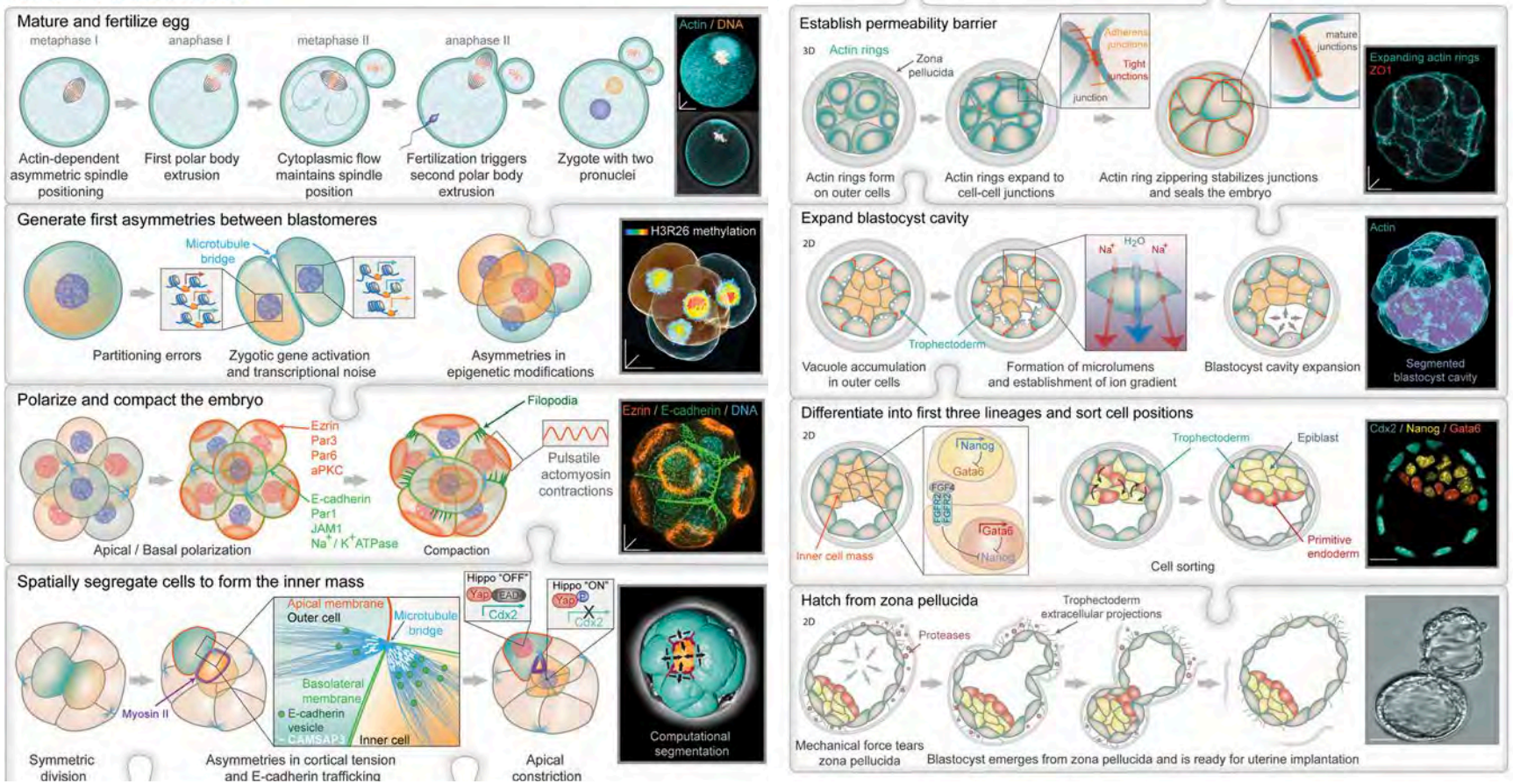
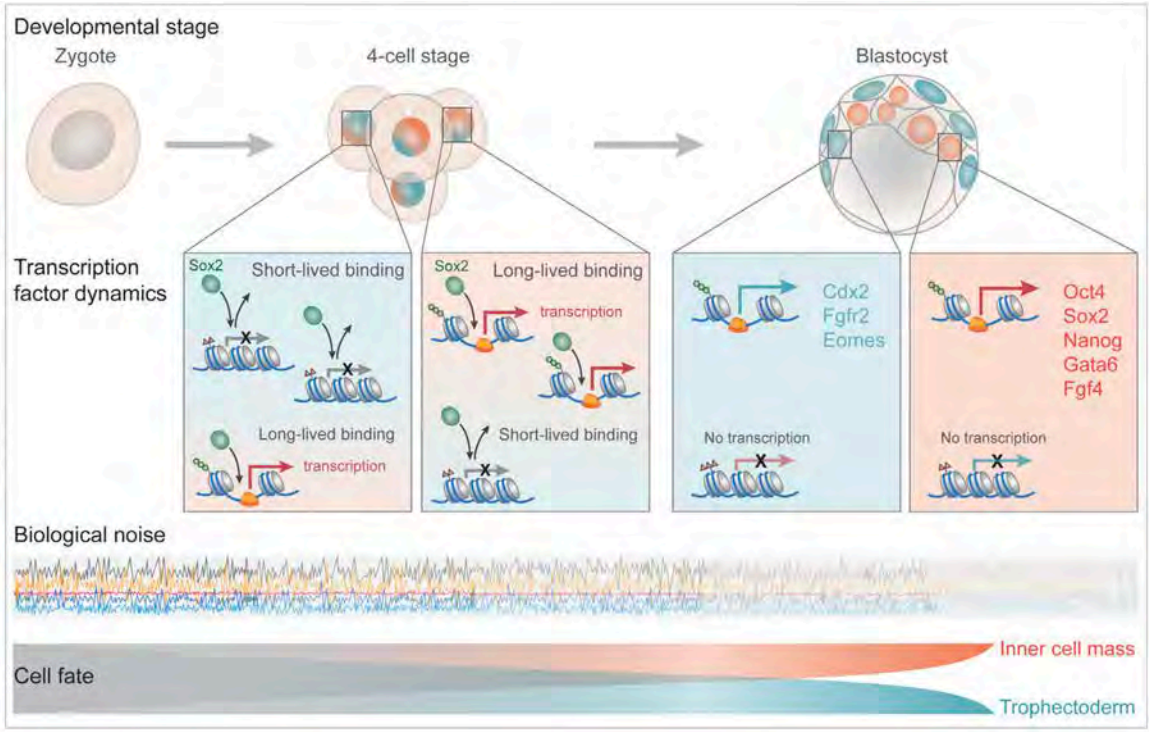


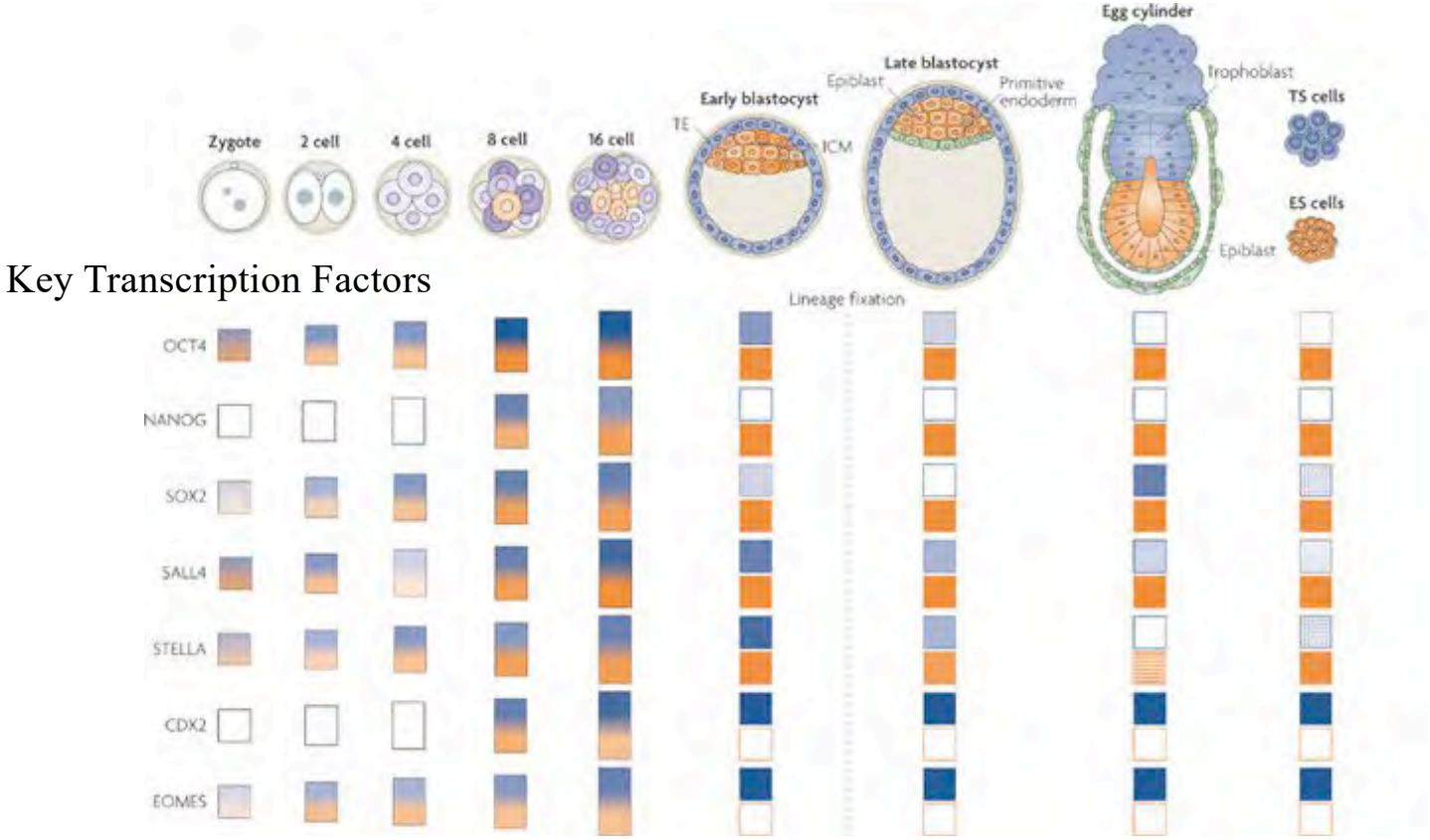
Progressive Refinement of Cell Fate



Reprogramming in Inner Cell Mass to allow Pluripotency

Figure 4. Progressive Refinement of Cell Fate
Biases in cell fate may arise at the four-cell stage due to a combination of biological noise and early heterogeneities between blastomeres in epigenetic modifications (red triangles, green hexagons) and transcription factor binding to DNA. These early differences may be progressively amplified and refined until the first two lineages diverge at the blastocyst stage.

Establishing and maintaining early lineage decisions during Mouse development



• First, permissive chromatin states for lineage determination are established by histone modifications in a tight interplay with transcriptional regulators, including OCT4, NANOG, SOX2, SALL4 as well as TEAD4, CDX2 and EOMES.

• Once lineages have been specified, DNA methylation of key loci, eg Elf5 and Stella, is then required to restrict their differentiation potential and to establish lineage-committed cell populations, the fate allocation of which is stably inherited by all descendants

Developmental phenotypes due to mutation of chromatin modifiers : can have one or more roles in development

	Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
	Histone Modifications					
H3K9me pathways	Glp/Ehmt1	HMTase	Severe growth retardation and lethality at E9.5; reduction of H3K9me1 and H3K9me2 in embryos	ND	yes	Tachibana et al. (2005)
	G9a/Ehmt2	HMTase	Loss of H3K9 methylation in euchromatin; developmental and growth arrest at E8.5	yes	yes	Tachibana et al. (2002)
	Eset/ SETDB1	HMTase	Peri-implantation lethality (between E3.5 and E5.5); defects in ICM outgrowth	yes	no	Dodge et al. (2004)
	Suv39h1 Suv39h2	HMTase	Double knockout shows loss of H3K9 methylation in heterochromatin; polyploidy in MEF cells; chromosome pairing defects during spermatogenesis; male sterility and death of some double-mutant embryos at E14.5	ND	yes	Peters et al. (2001)
H3K27me pathways	Ezh2/ Enx-1	HMTase PRC2 complex	Growth defect of the primitive ectoderm; peri-implantation lethality	yes	no	O'Carroll et al. (2001)
	Eed	PRC2/3 complex	Defective gastrulation; failure to maintain inactive X in trophoblast cells	yes	yes	Shumacher et al. (1996)
	Suz12	PRC2/3 complex	Early postimplantation lethality; gastrulation defects	yes	ND	Pasini et al. (2004)
	YY1	PRC2/3 interaction	Defects in epiblast cell growth/survival; peri-implantation lethality	yes	no	Donohoe et al. (1999)
	Ring1b/ Rnf2	Ubiquitin ligase PRC1 complex	Gastrulation defects; lethality by E9.5	yes	ES viable	Voncken et al. (2003)
	DNA Methylation					
DNA methylation pathways	Dnmt1	DNA MTase	Genome-wide demethylation; developmental arrest at E8.5	yes	yes	Li et al. (1992)
	Dnmt3a	DNA MTase	Malfunction of gut; spermatogenesis defects; postnatal lethality (~4 weeks of age)	yes	yes	Okano et al. (1999)
	Dnmt3b	DNA MTase	Demethylation of minor satellite DNA; mild neural tube defects; embryonic lethality at E14.5–E18.5	yes	yes	Okano et al. (1999)

Full repression of repeats and certain genes

Bivalent states: ready for signal to activate or silence

Primed: setting up chromatin state for later gene expression

Fully active: gene expression

COURS II

1. Cellular memory during embryogenesis: stability and plasticity
2. Tracing cell identity and cell fate during embryogenesis
3. Establishing cellular memory during development
4. Epigenetic dynamics during early mouse development
5. Strategies that enable cellular memory: the epigenetic machineries
6. Lessons from X-chromosome inactivation: stability and plasticity in development

NEXT WEEK:

- more about establishing memory from embryo to soma
- transient allelic effects
- XCI in somatic cells