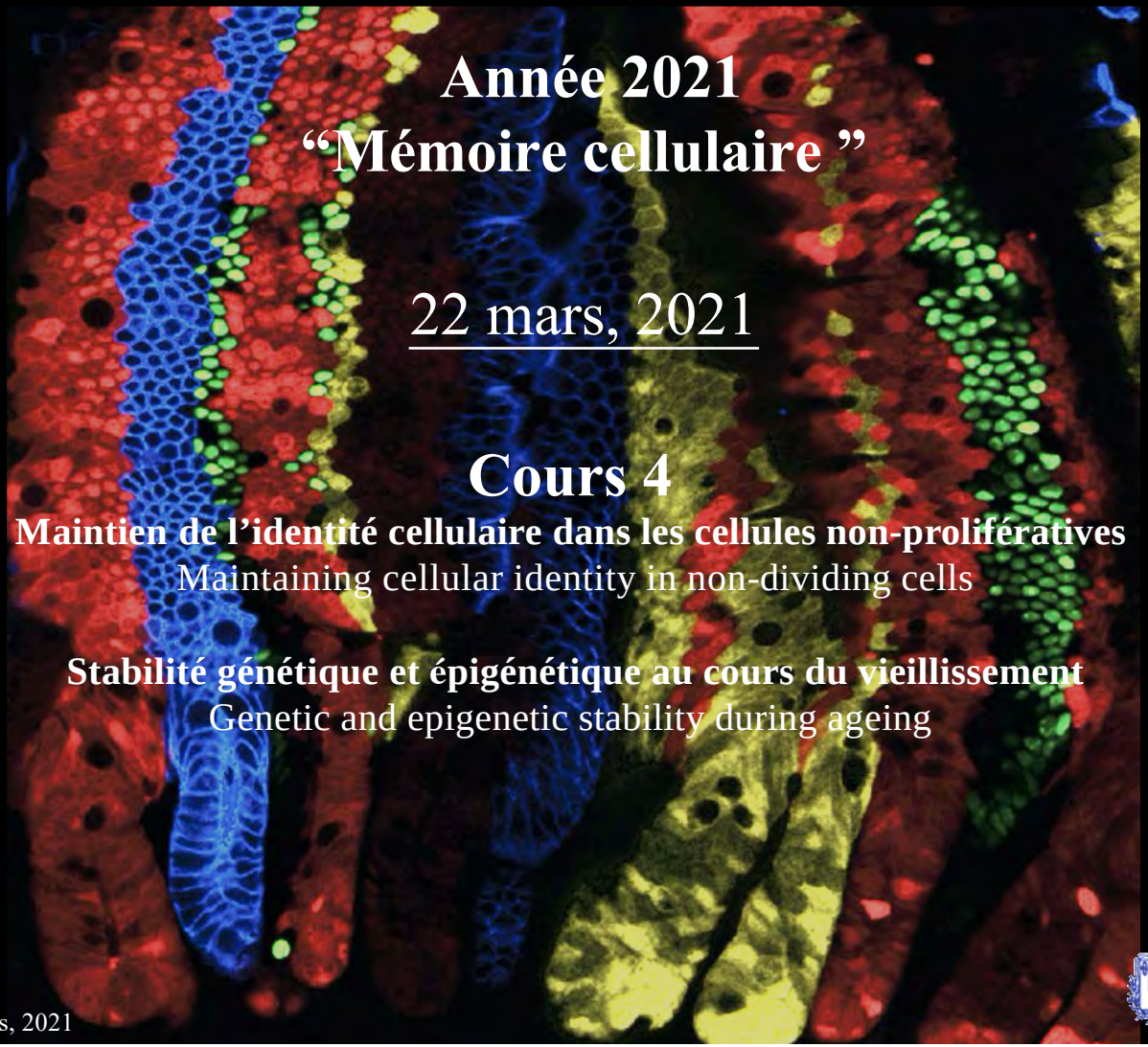
CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE



Année 2021
“Mémoire cellulaire ”

22 mars, 2021

Cours 4

Maintien de l’identité cellulaire dans les cellules non-prolifératives
Maintaining cellular identity in non-dividing cells

Stabilité génétique et épigénétique au cours du vieillissement
Genetic and epigenetic stability during ageing

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CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

COURS 1 (lundi 1er mars 10h-12h)
Introduction

COURS 2 (lundi 8 mars 10h-12h)
Stabilité et plasticité au cours du développement
Stability and plasticity during embryonic development

COURS 3 (lundi 15 mars 10h-12h)
Maintien de l'identité cellulaire dans les cellules non-prolifératives
Maintaining cellular identity in non-dividing cells

COURS 4 (lundi 22 mars 10h-12h)
Stabilité génétique et épigénétique au cours du vieillissement
Genetic and epigenetic stability during ageing

COURS 5 (lundi 29 juin 10h-12h)
Perte d'idéntité cellulaire au cours de la reprogrammation et dans des pathologies
Losing cellular identity during reprogramming and in disease

E. Heard, 22 mars, 2021

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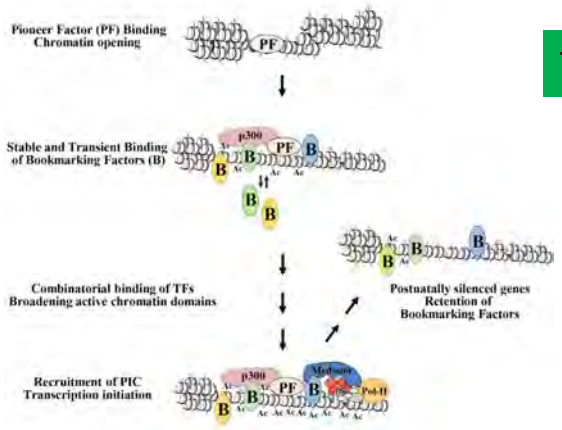
SUMMARY from LAST WEEK
Cellular Memory: stability and plasticity during development

Orchestrating epigenesis

- Focus only on transcription factor (TF) networks, signalling, and chromatin
- Most epigenetic factors (chromatin associated) play multiple different roles throughout development
- Chromatin factors establish the landscape for appropriate developmental gene expression patterns
- Chromatin changes helps maintain appropriate patterns of gene expression
- Establishment of early lineage decisions through transcriptional noise and chromatin remodeling
- Early parental asymmetries in gene expression due to chromatin and 3D organization – transient imprints and X inactivation
- Reactivation of the inactive X by active processes: transcription factors and histone demethylase
- Repressing and activating or priming genes as lineages are established
- Establishing the memory of somatic cell lineages through pioneer and bookmarking transcription factors as well as chromatin

Setting up cellular memory states for later action



Transcription Factor Networks

Transcription Factors

Chromatin

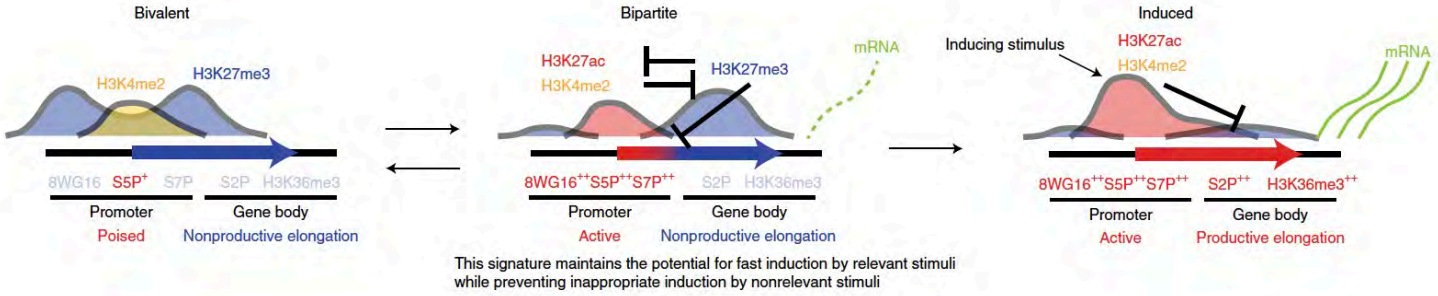
Chromosome architecture

Chromatin

DNA methylation

Bookmarking factors

Pioneer factors



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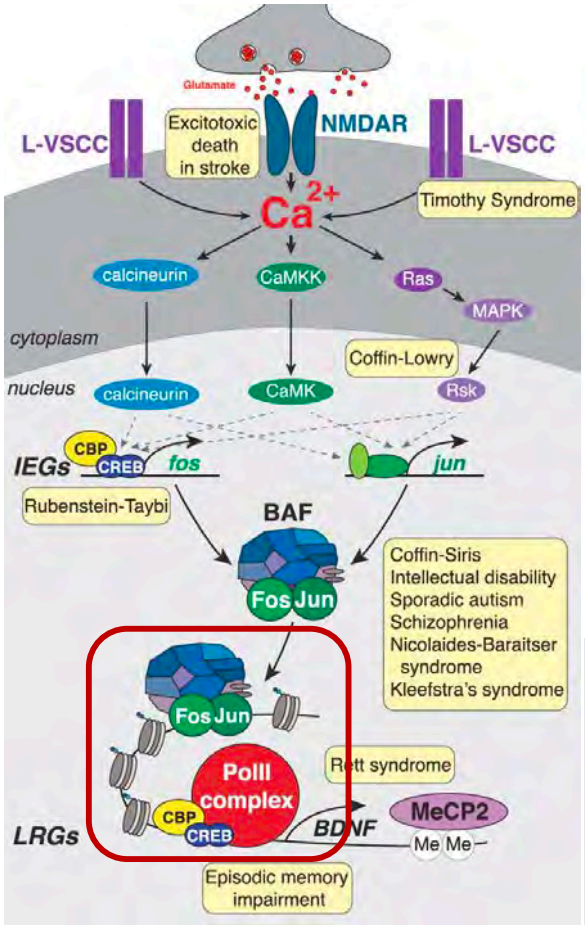
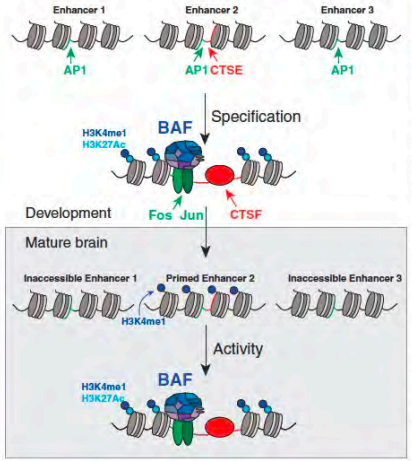
Setting up cellular memory states for later action





Chromosome architecture

Chromatin

DNA methylation

Bookmarking factors

Pioneer factors

Calcium-dependent signalling cascades lead to the activation of pre-existing transcription factors CREB, SRF/ELK, and MEF2, which regulate the expression of immediate early genes (IEGs) such as Fos.

IEG factors will in turn activate specific late response genes (LRGs) that have been pre-marked in a cell type specific way (primed) for activation.

A suivre aussi:
Longevité cerebrale
Cours 2013 et 2014
du Pr. Alain Prochiantz
https://www.college-de-france.fr/site/alain-prochiantz/course-2013-10-14-17h00.htm

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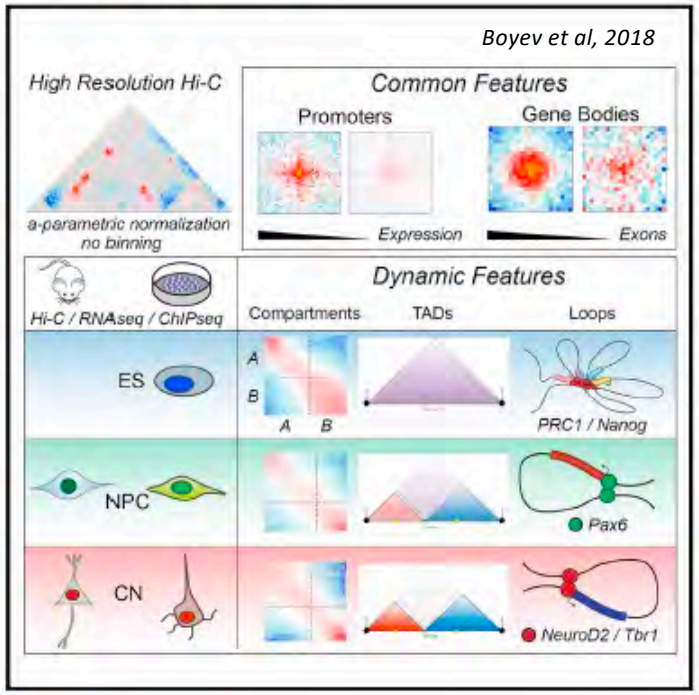
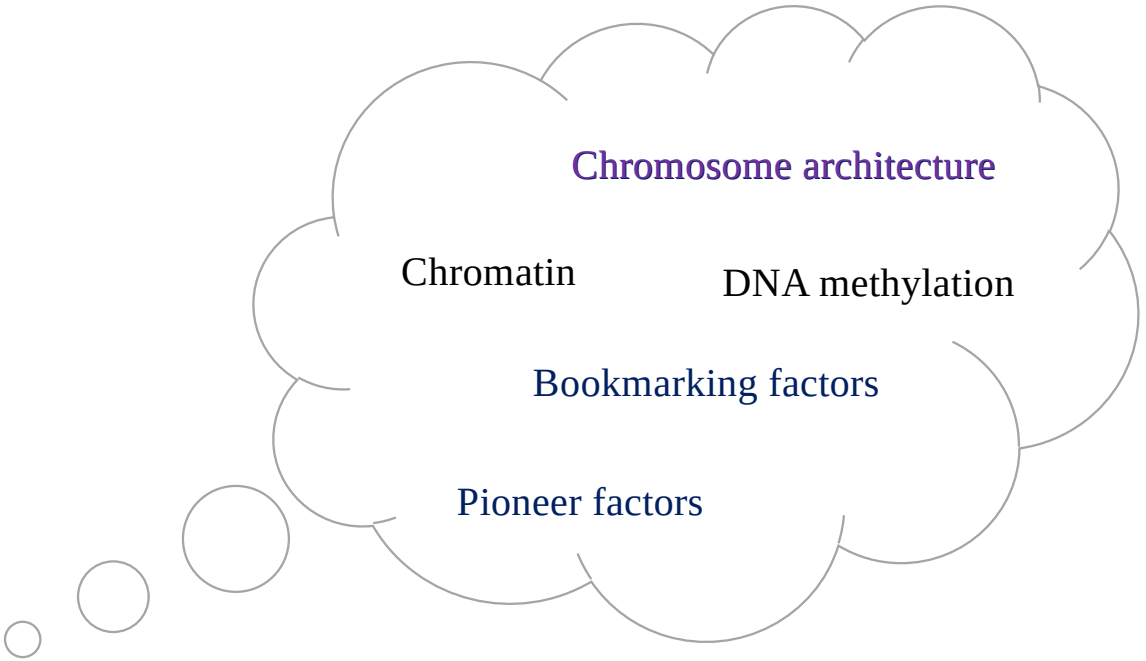
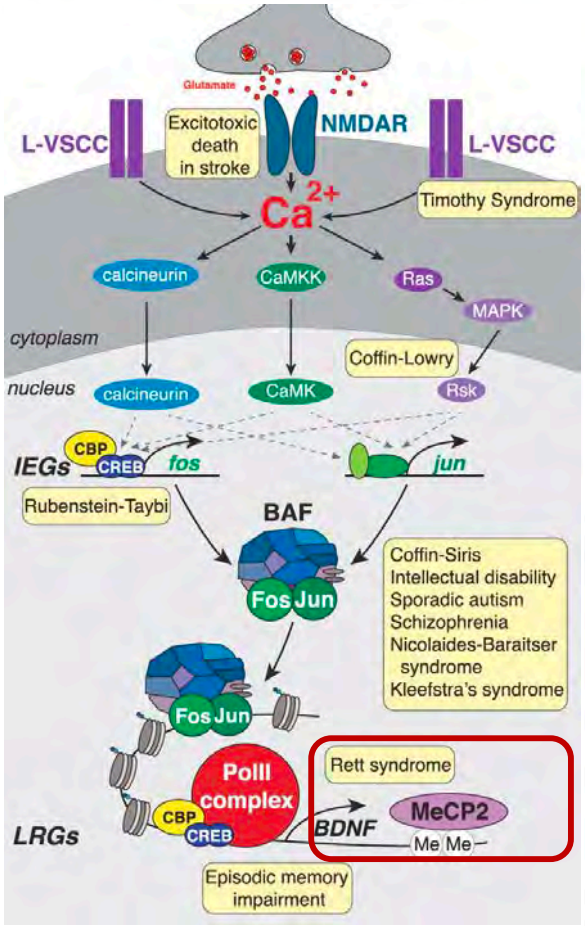
Setting up cellular memory states for later action

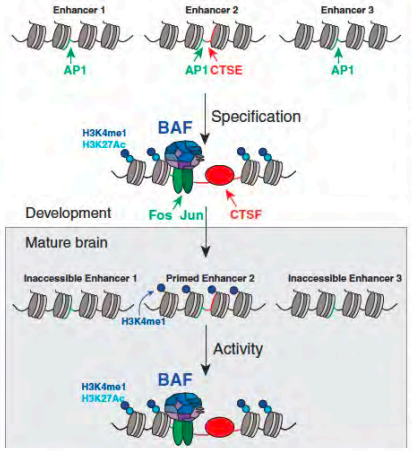


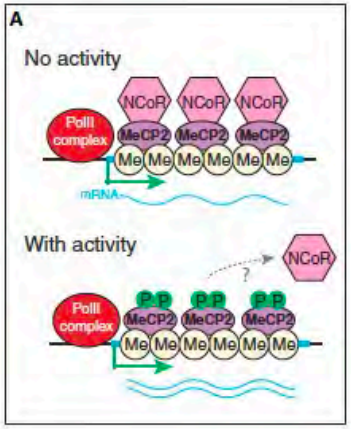


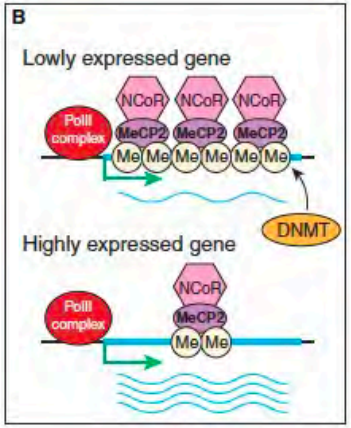
- Ultra-deep Hi-C during mouse neural differentiation, both *in vitro* and *in vivo*
- Transcription is correlated with, but not sufficient for, local chromatin insulation
- Polycomb network is disrupted, while novel contacts between neural TF sites appear
- Dynamic contacts among exon-rich gene bodies, enhancer-promoters, and TF sites

Setting up cellular memory states for later action









Chromosome architecture

Chromatin

DNA methylation

Bookmarking factors

eer factors

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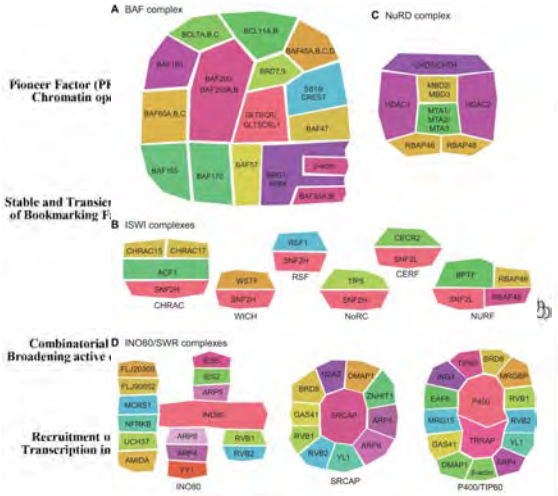
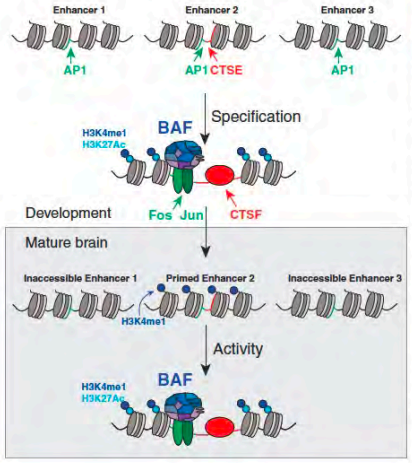
Setting up cellular memory states for later action



Hota, and Bruneau Development 2016



Chromosome architecture

Chromatin Remodelers

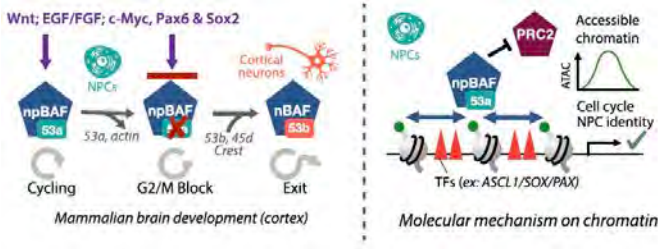
DNA methylation

Bookmarking factors

Pioneer factors

BAF subunit switching regulates chromatin accessibility to control cell cycle exit in the developing mammalian cortex

Simon M.G. Braun, Ralitsa Petrova, Jiong Tang, Andrey Krokhotin, Erik L. Miller, Yitai Tang, Georgia Panagiotakos, and Gerald R. Crabtree

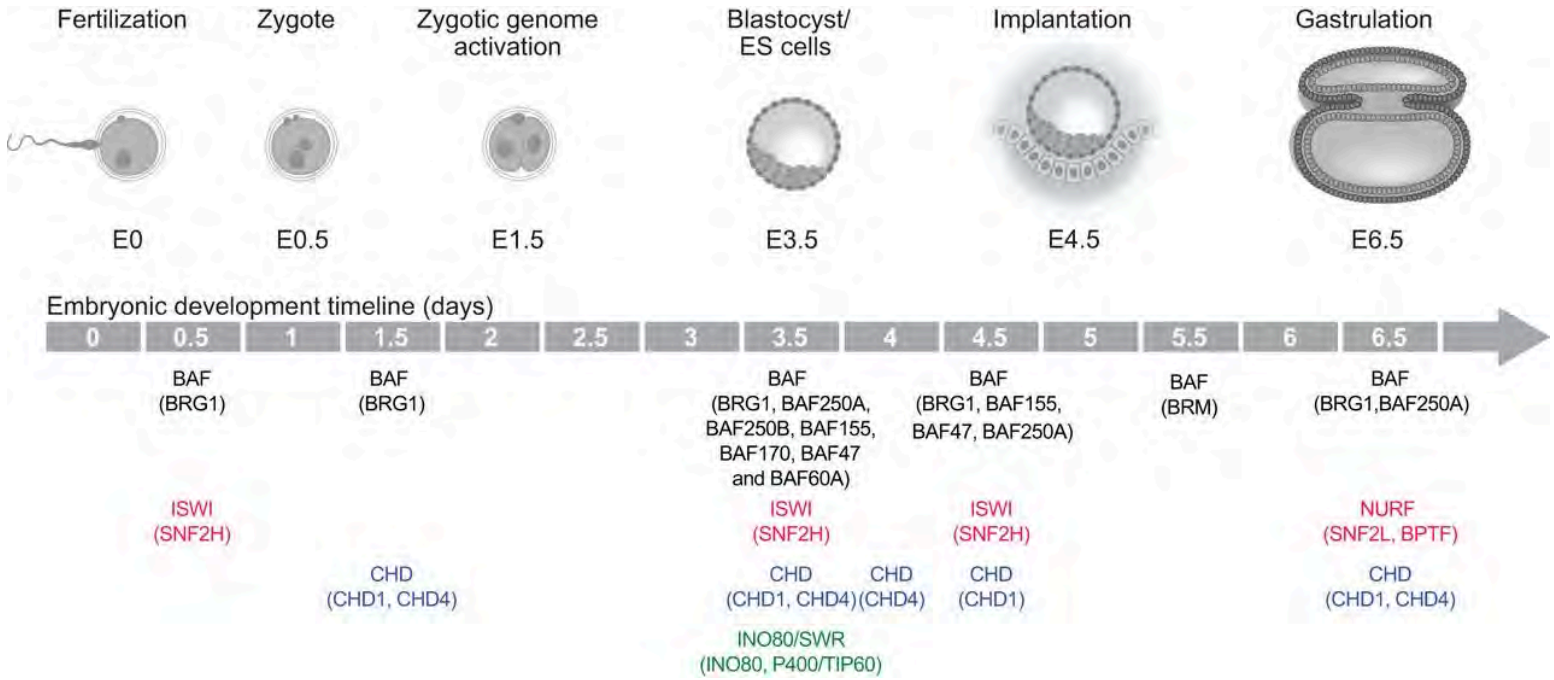


A central role for chromatin regulators in the output of NSPCs during brain development.

Antagonism between BAF and Polycomb complexes alters the balance of repressive and active chromatin states at specific TF motifs and cell cycle genes to directly regulate cell cycle exit in the developing nervous system.

Chromatin remodelers play key functions during development

Roles of chromatin remodelers in early embryogenesis



Swetansu K. Hota, and Benoit G. Bruneau Development 2016;143:2882-2897

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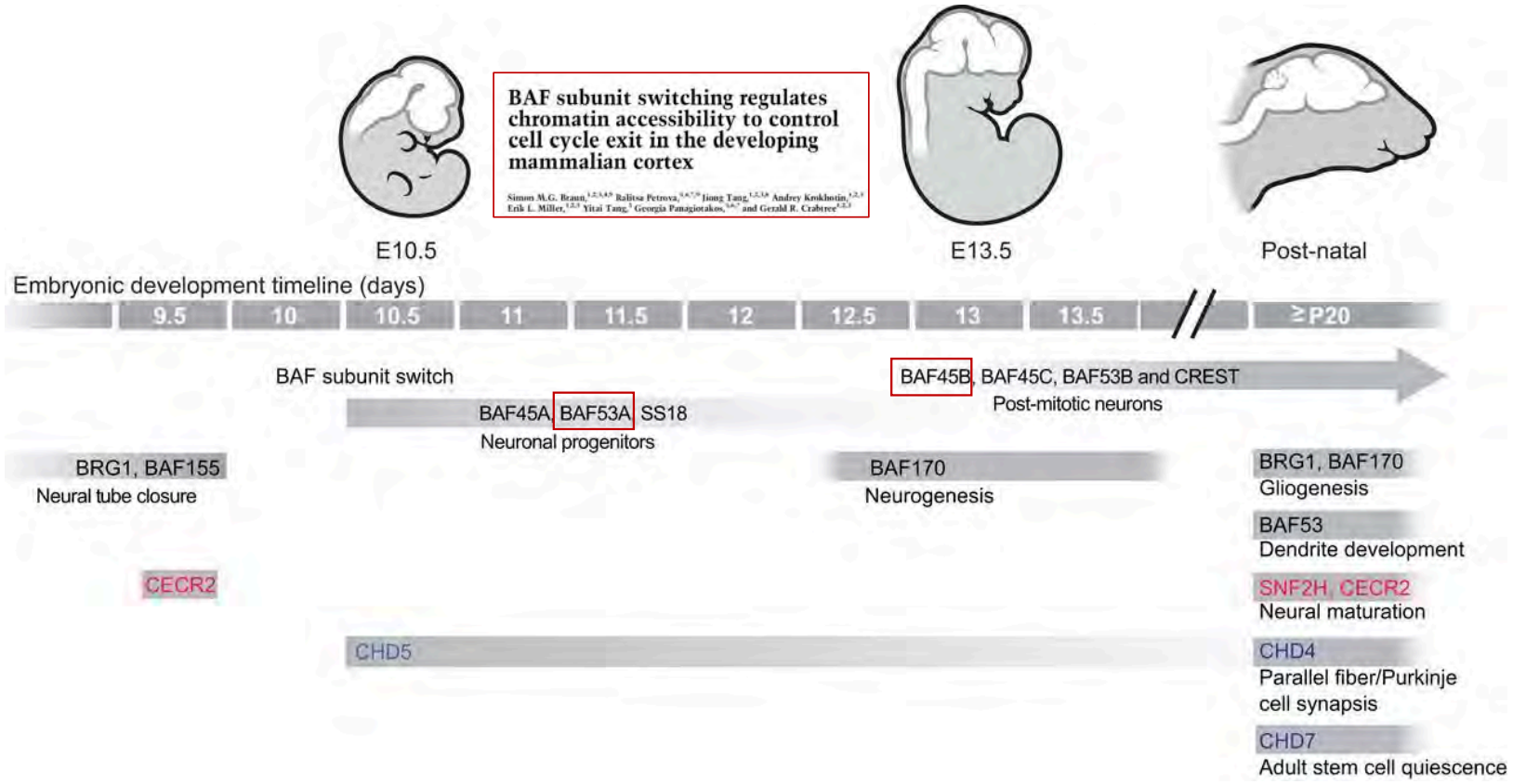
Chromatin remodelers play key functions during development

Chromatin remodeler functions and transitions in neural development



Swetansu K. Hota, and Benoit G. Bruneau Development 2016;143:2882-2897

THIS WEEK

Cell Memory in non-dividing cells and during ageing

How old are cells and proteins in adults?

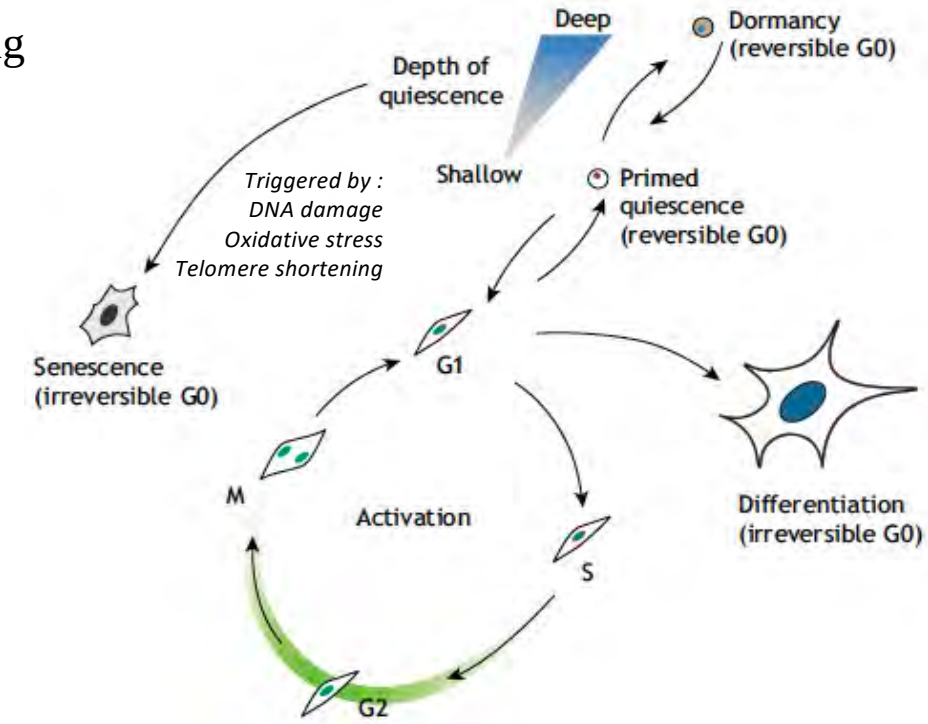
Hallmarks of quiescence

Stem cell hierarchies: classic versus modern

Stem cells and tissue homeostasis

Stem cell memory

Ageing and stem cells

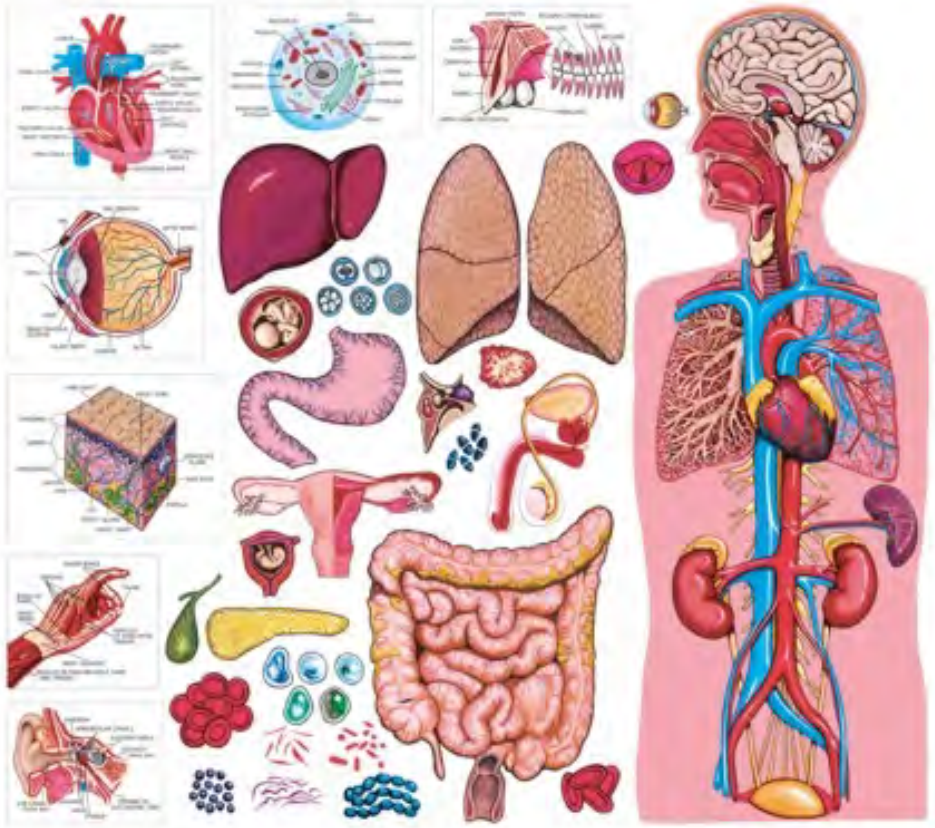


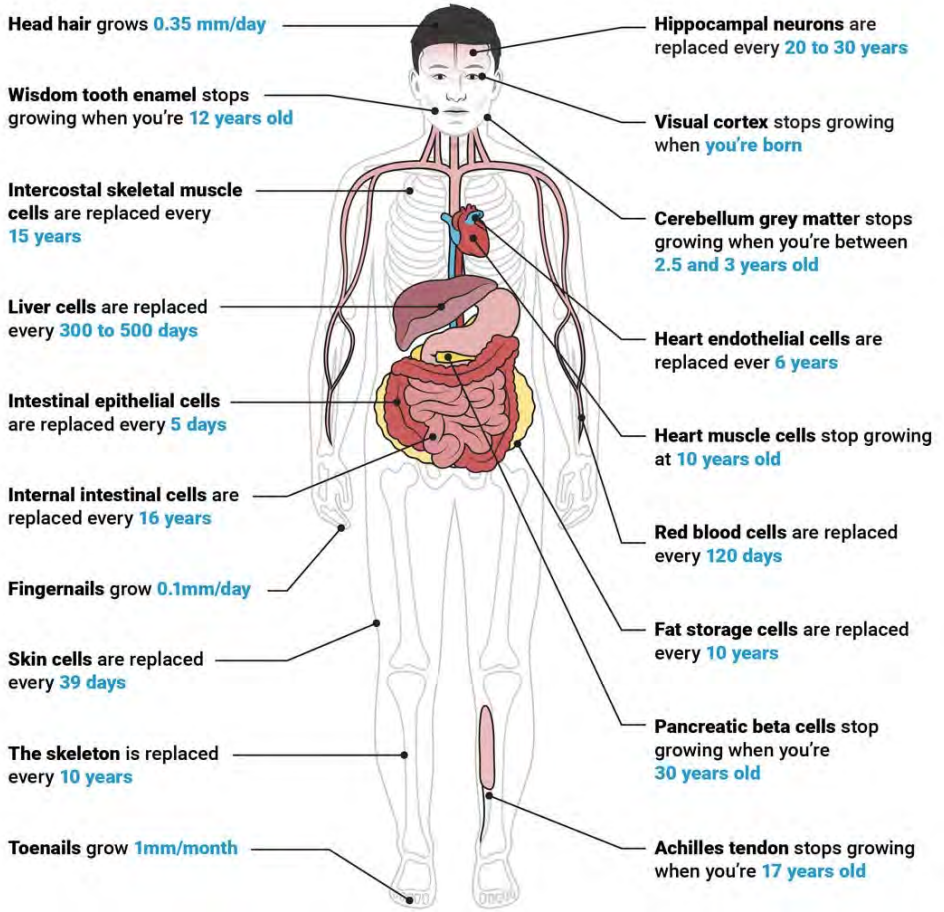
Exit from cell cycle can be reversible or irreversible

Quiescent cells (including most adult stem cells) exist in a **reversible** G0 cell cycle state

Differentiated and **senescent** cells exist in an **irreversible** G0 cell cycle state

Cellular Memory: How old are cells and proteins in adults?





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E. Heard, 22 mars, 2021

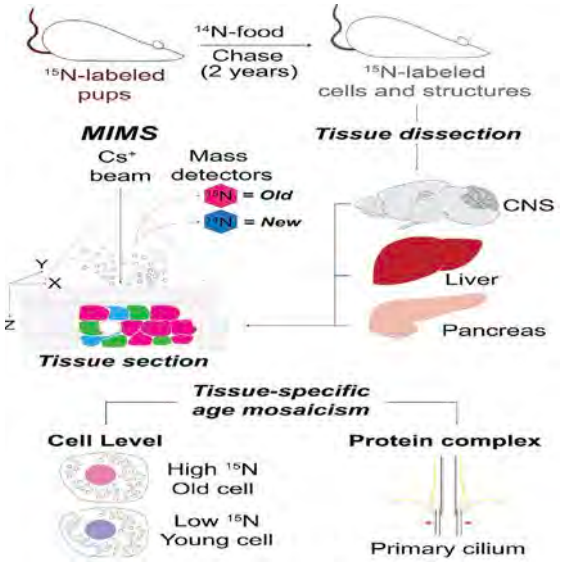
Cellular Memory: How old are cells and proteins in adults?

- The lifespan of a terminally differentiated cells is quite variable among organs: 3 to 4 days for epithelial intestinal cells, to a life time for the majority of neurons, cardiomyocytes, and all inner hear hair cells (De Anda et al., 2016; Brann and Firestein, 2014; Foglia and Poss, 2016; Steinhauser et al., 2012; Zhang et al., 2012).
- How do neurons, cardiomyocytes, or other long-lived cells (LLCs), maintain functional integrity and protein homeostasis over the span of several decades?
- As these cells are almost never replaced, they are essentially as old as the animal itself and must function properly throughout life, which in humans can be more than a century (De Anda et al., 2016).
- In some cases, somatic stem cells can respond to tissue damage and proliferate according to tissue-specific
- needs, as in the striated muscle, which can somewhat regenerate after wound because of activation of its satellite stem cells (Blau et al., 2015).
- How functionality is maintained in LLCs is important given that aging is associated with physiological impairments in these kinds of cells (e.g., neurons and cardiomyocytes) (D'Angelo et al., 2009; Mattson and Magnus, 2006).
- Proteins also have different lifespans, ranging from hours and days to years (Ori et al., 2015; Toyama et al., 2013).
- Both stressed and/or damaged cells, as well as misfolded and damaged proteins must be degraded and replaced with new and functional versions (Taylor and Dillin, 2011). **MORE NEXT WEEK COURS V**

Cellular Memory: How old are cells and proteins in adults?

- Adult tissues contain quiescent stem cells and post-mitotic cells as old as the organism
- Proteins and organelles must be protected or renewed to maintain cell homeostasis
- But just how old are cells and proteins?
- Only recently can this start to be properly estimated

- **Long lived cells and proteins in adult mice**
- **Organs are mosaics of cells that have different ages**



Isotope microscopy reveals age mosaicism of cells and specific protein complexes

Measure the age of cells and proteins using high-resolution isotope imaging and show that adult mouse organs are mosaics of cells of different ages.

Neurons in the central nervous system, pancreatic alpha beta and delta cells, and even the liver which has high turnover, contain cells as old as the animal

Cilia have differentially aged structural protein components with some lasting the lifetime of the organism

Arrojo e Drigo et al, Cell Metabolism 2019

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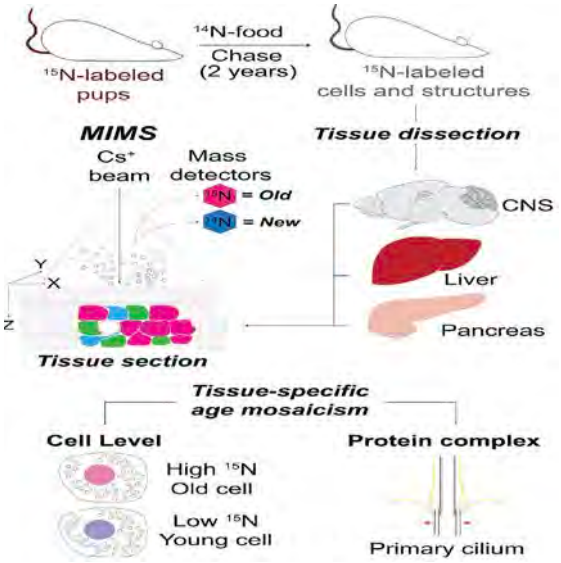

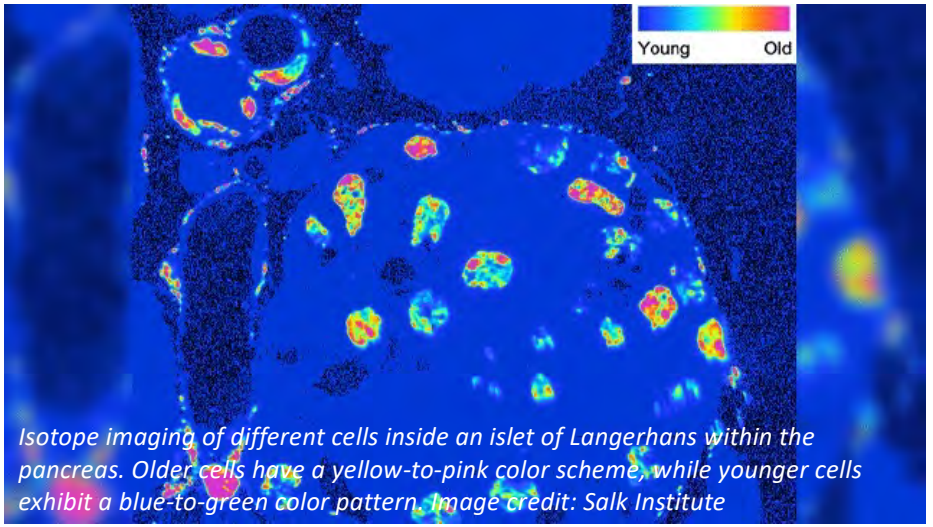

Isotope imaging of different cells inside an islet of Langerhans within the pancreas. Older cells have a yellow-to-pink color scheme, while younger cells exhibit a blue-to-green color pattern. Image credit: Salk Institute

NB caveat - MIMS-EM cannot distinguish between 15N-labeled nucleic acids and amino acids.
However regardless of the nature of the 15N signal results suggest that these cells are long-lived.

Arrojo e Drigo et al, Cell Metabolism 2019

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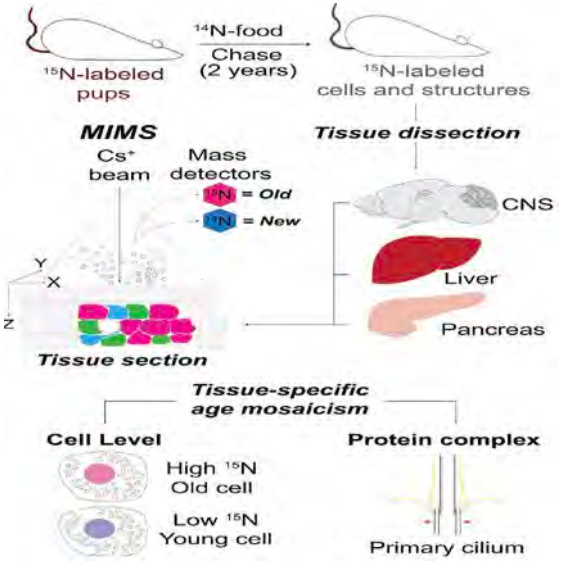

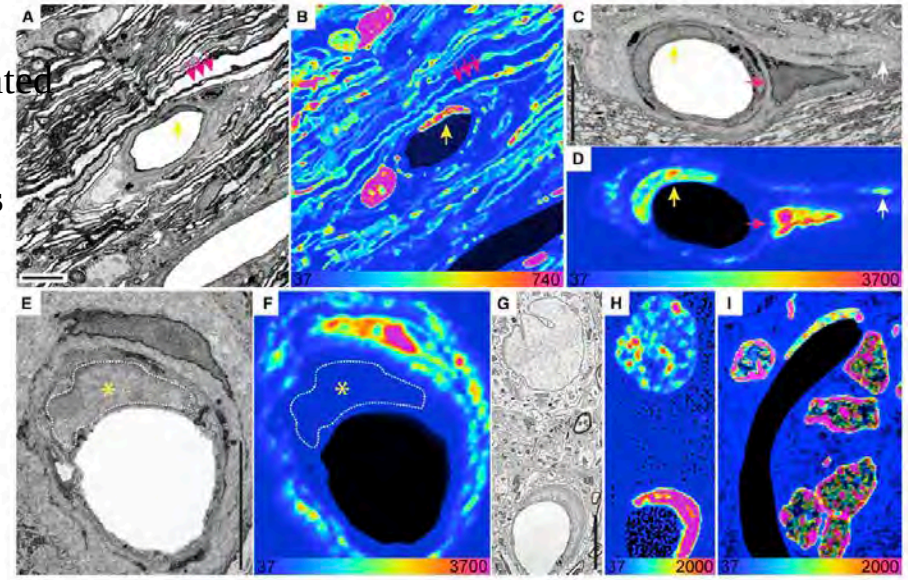

Figure 1. MIMS-EM of LLCs and Structures in the CNS

(A and B) SEM (A) and MIMS (B) of two capillaries in the optic nerve head (ONH) of a 6-month chase mouse. An endothelial cell nucleus (yellow arrow) and myelin sheaths (pink arrows) are indicated. A pericyte nucleus is visible to the left the capillary lumen.

(C and D) SEM (C) and MIMS (D) of a capillary in the ONH. An old endothelial cell nucleus (yellow arrow), a fibroblast (pink arrow) and ^{15}N-rich extracellular matrix (ECM) (white arrow) are indicated.

(E and F) SEM (E) and MIMS (F) of a capillary in the ONH, with an old fibroblast (top) and a young endothelial cell (nucleus delineated in white and indicated by the yellow asterisk).

(G and H) SEM (G) and MIMS (H) of an L2 neuron (top) and an endothelial cell.

(I) Close-up of granular cells in the rat cerebellum with an old endothelial cell visible. Full mosaic is shown in Figure S1B.

Scale bars: 5 μm (A, C, E, and G) (SEM). At the bottom of the MIMS images, the heatmap shows the $^{15}N/^{14}N \times 10^4$ and scaled with a hue saturation intensity (HSI).

Arrojo e Drigo et al, Cell Metabolism 2019

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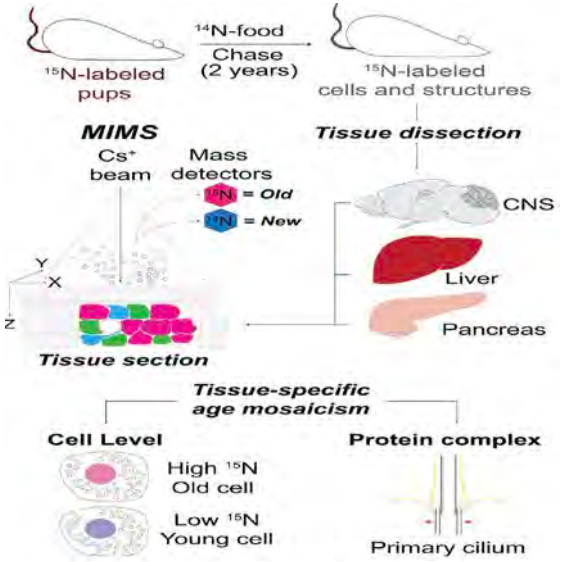

E. Heard, 22 mars, 2021

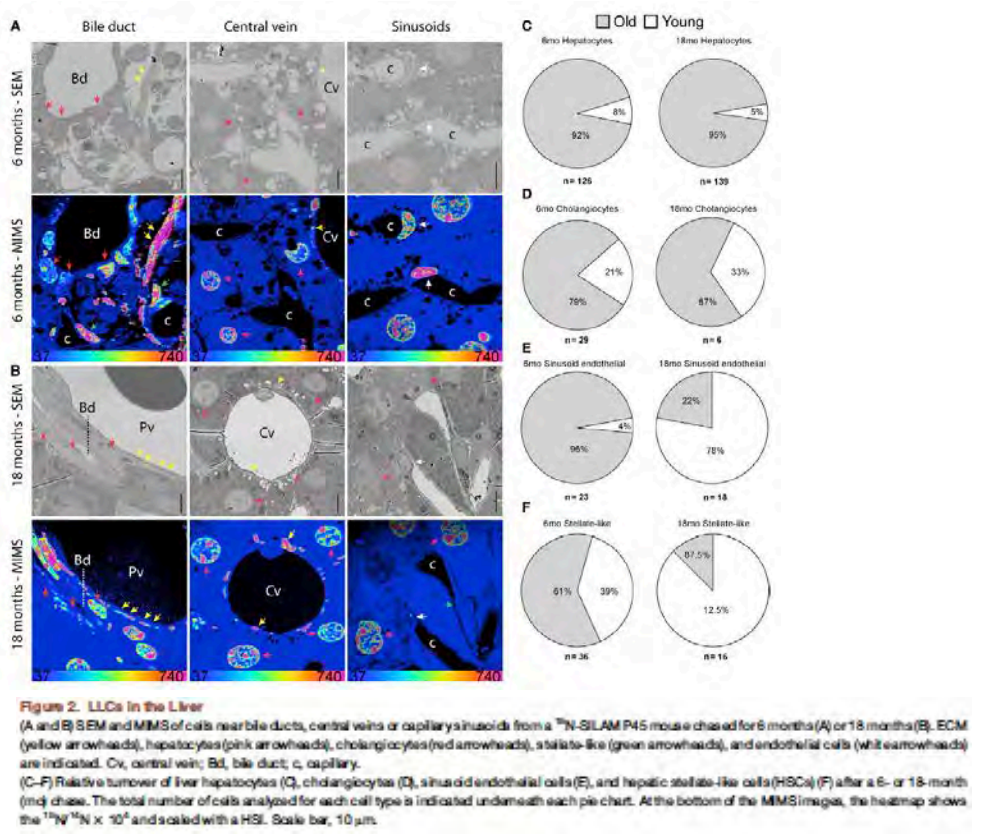

Figure 2. LLCs in the Liver

(A and B) SEM and MIMS of cells near bile ducts, central veins or capillary sinusoids from a ^{15}N-SILAM P45 mouse chased for 6 months (A) or 18 months (B). ECM (yellow arrowheads), hepatocytes (pink arrowheads), cholangiocytes (red arrowheads), stellate-like (green arrowheads), and endothelial cells (white arrowheads) are indicated. Cv, central vein; Bd, bile duct; c, capillary.

(C–F) Relative turnover of liver hepatocytes (C), cholangiocytes (D), sinusoid endothelial cells (E), and hepatic stellate-like cells (HSCs) (F) after a 6- or 18-month (mo) chase. The total number of cells analyzed for each cell type is indicated underneath each pie chart. At the bottom of the MIMS images, the heatmap shows the $^{15}N/^{14}N \times 10^4$ and scaled with a HSI. Scale bar, 10 μm.

Arrojo e Drigo et al, Cell Metabolism 2019

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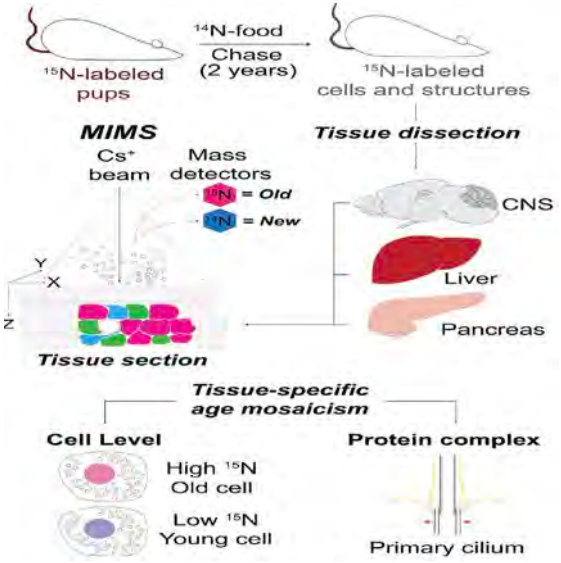

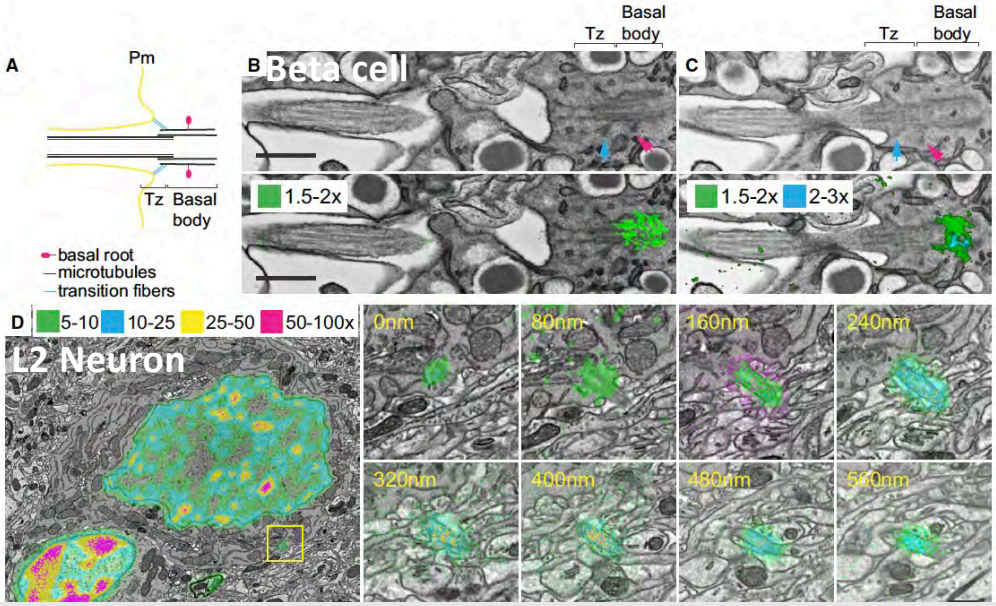

- Protein age mosaicism within a single subcellular structure
- Long-lived structures are present in the primary cilium and their turnover may be different in beta cells and neurons

Arrojo e Drigo et al, Cell Metabolism 2019

Cellular Memory: How old are cells and proteins in adults?

- Adult tissues contain quiescent stem cells and post-mitotic cells as old as the organism
- Proteins and organelles must be protected or renewed to maintain cell homeostasis
- The only organelle that does not turn over in post-mitotic cells is the nucleus
- This organelle contains the genome and the machinery that must ensure its appropriate function and expression
- Different strategies seen for nuclear pore maintenance in irreversible and reversible non-dividing cells
- See enrichment of long lived histone variants at silent loci

REPORT

Monitor the replacement of specific, long-lived components of NPCs and nucleosomes during aging in postmitotic cells.
Postmitotic cells maintain NPC proteins via a piecemeal process.
In contrast, amino acid–deprived quiescent cells are capable of removing old nuclear pores in an endosomal sorting complexes required for transport (ESC RT)–dependent manner.
Using genome-wide mapping of long-lived histones revealed specific enrichment of long-lived variants at silent gene loci
=> **age mosaicism at the level of chromatin organization**

Post-mitotic differentiated Quiescent

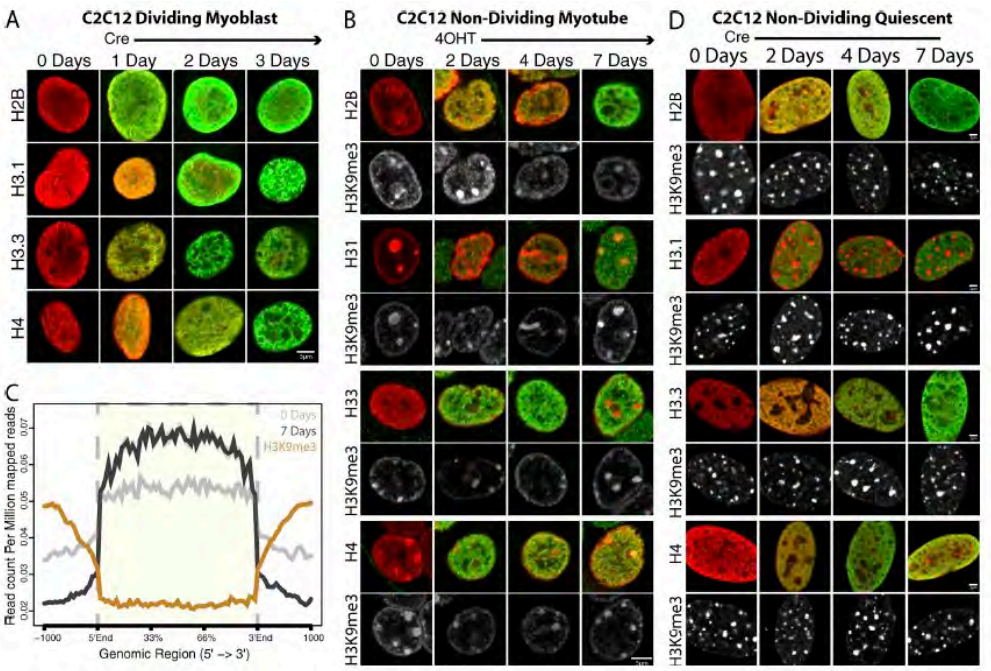

Figure 2. **Histone dynamics using the RITE system. (A)** Histone turnover in dividing cells. C2C12 cell lines stably expressing RITE-tagged (MF) H2B, H3.1, H3.3, and H4 were made, fixed, stained, and imaged using confocal microscopy before (0 Days) and 1, 2, and 3 d after tag switch was initiated in dividing cells. Representative images are displayed with Myc signal (red) overlaid with Flag signal (green). **(B)** Histone turnover in nondividing myotubes. The same cells from A were differentiated into myotubes as outlined in Fig. 1 A and fixed, stained, and imaged before (0 d) and 2, 4, and 7 d after tag switch. Upper panel of each set is the Myc (red) overlaid with Flag (green) signal. Lower panel of each set is staining of H3K9me3 in the same cells. Scale bars represent 5 µm. **(C)** ChIP-seq of RITE-tagged H3.3. RITE-tagged (MF) H3.3 C2C12 cells were differentiated into myotubes as described above and tag-switch induced. Myotube-enriched fractions were isolated from cells with no tag switch (0 d) and tag switched for 2 and 7 d. ChIP-seq was then performed on these time points using anti-Flag and anti-H3K9me3 antibodies. Regions of flag-tagged H3.3 incorporation were identified genome-wide (5' to 3' end), and H3K9me3 reads were correspondingly mapped. **(D)** Histone dynamics in quiescent cells. C2C12 cell lines from this figure were placed in quiescence, and tag switch time courses, staining, and imaging were conducted as described for B.

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Long-lived Proteins

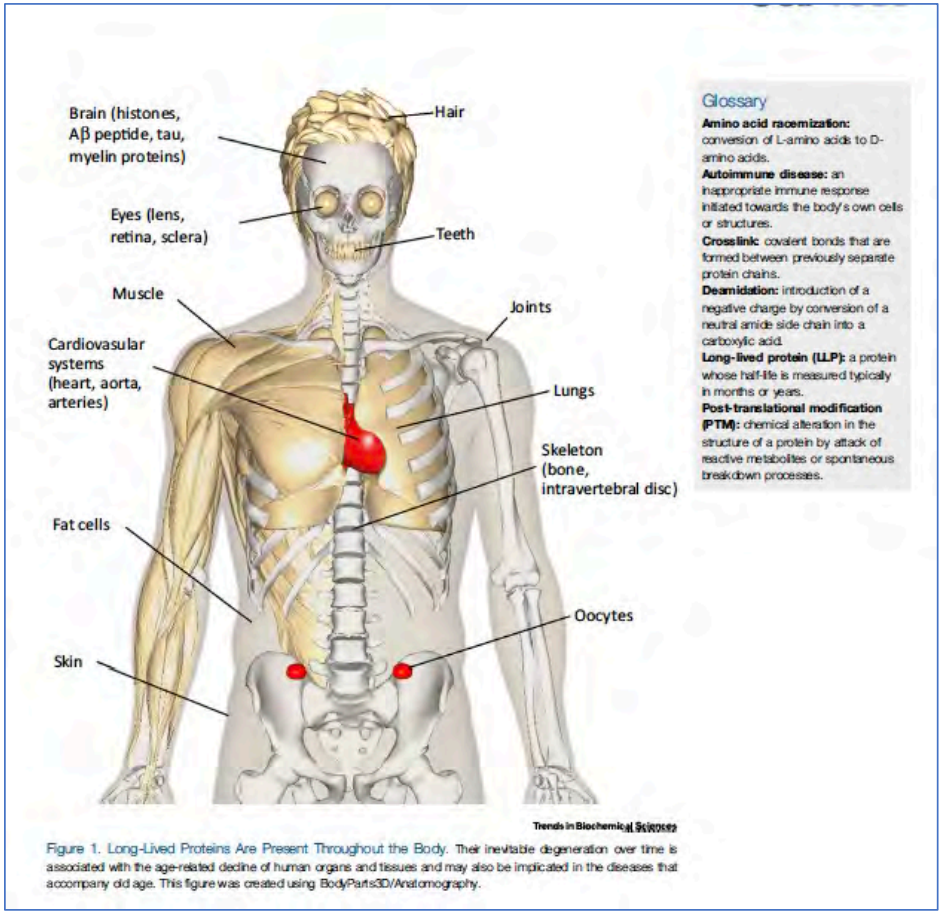

Figure 1. Long-Lived Proteins Are Present Throughout the Body. Their inevitable degeneration over time is associated with the age-related decline of human organs and tissues and may also be implicated in the diseases that accompany old age. This figure was created using BodyParts3D/Anatomography.

- LLPs decompose over time. From the time that an LLP is formed it comes under relentless attack by enzymes and reactive small molecules, resulting in degradation.
- Another source of degradation arises from spontaneous reactions take place due to the intrinsic instability of some amino acids and that are the inevitable result of heat and time.
- Spontaneous degradation appears to be the most common cause of LLP decomposition.
- When proteins decompose in this way, their structures and functions may be altered and novel epitopes can be formed that can induce an autoimmune responses.

Truscott et al, TIBs 2016

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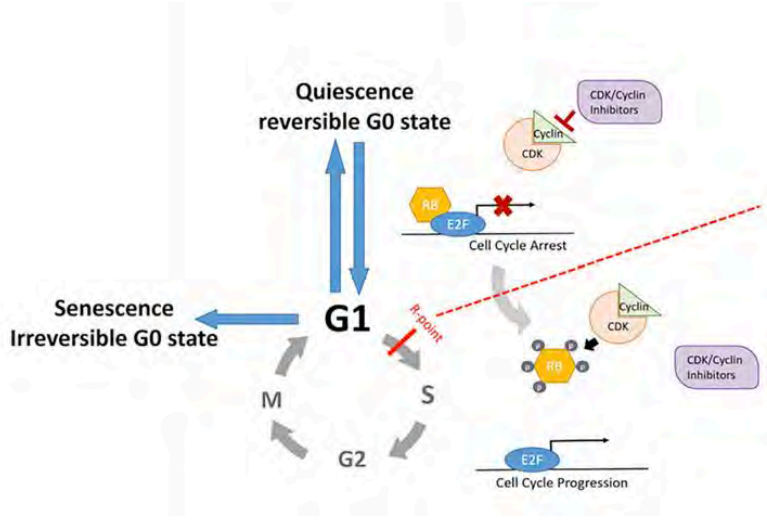
Hallmarks of Stem Cell Quiescence

- Cellular quiescence is a dormant but reversible cellular state in which cell-cycle entry and proliferation are prevented
- This state of low proliferative activity, experimentally defined by the ability to retain DNA or chromatin labels, is taken as a defining characteristic for adult stem cells.
- Stem cells are hypothesized to be slow-cycling to conserve their proliferative potential and to minimize DNA errors during replication.
- In the 1980s, investigators showed that a single pulse of tritiated thymidine does not label presumed stem cells in the epidermis and oral epithelium.



- Quiescence is a reversible G0 state, because cells retain the ability to re-enter G1 of the cell cycle after passing the restriction point (R-point) of the G1/S transition.
- Cells in G1 can also enter senescence, which is an irreversible state. E2F mediates transcription of cell-cycle genes.
- In quiescent cells, E2F is repressed by retinoblastoma (RB) binding.
- The repressive ability of RB is regulated by the CDK/cyclin complex, which in turn is controlled by CDK/cyclin inhibitors.

Adapted from Biggar and Storey (2009).

Mechanisms, Hallmarks, and Implications of Stem Cell Quiescence

Inchul J. Cho,[1,2] Prudence PokWai Lui,[1,2] Jana Obajdin,[1,2] Federica Riccio,[1,2] Wladislaw Stroukov,[1,3] Thea Louise Willis,[1,2] Francesca Spagnoli,[1] and Fiona M. Watt[1,*]
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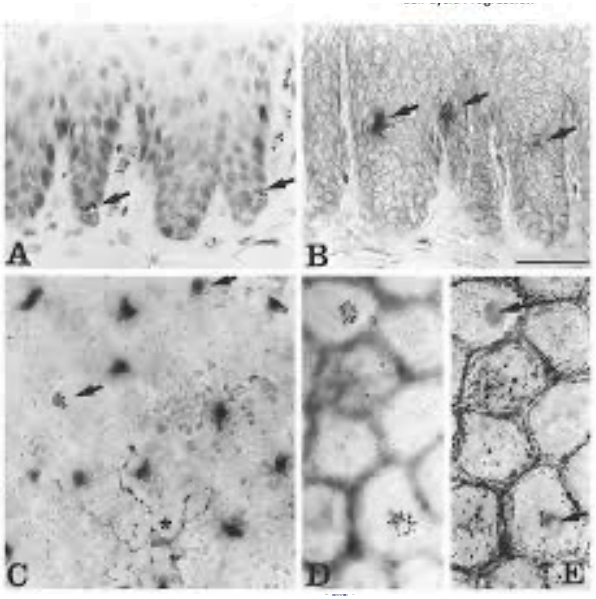
Hallmarks of Stem Cell Quiescence

- Cellular quiescence is a dormant but reversible cellular state in which cell-cycle entry and proliferation are prevented
- This state of low proliferative activity, experimentally defined by the ability to retain DNA or chromatin labels, is taken as a defining characteristic for adult stem cells.
- Stem cells are hypothesized to be slow-cycling to conserve their proliferative potential and to minimize DNA errors during replication.
- In the 1980s, investigators showed that a single pulse of tritiated thymidine does not label presumed stem cells in the epidermis and oral epithelium.
- Rather, their labeling requires prolonged administration of the DNA label. Cells that cycle slowly (the presumed stem cells) retain the isotope for an extended period of time and were termed label-retaining cells (LRCs)
- Hematopoetic Stem cells : although the majority of long-term HSCs (LTHSCs) rest in G0 at any given time yet all HSCare regularly recruited into the cycle, such that 99% of LT-HSCs divide on average every two months.

Summary. Slowly cycling cells in murine epithelia can be marked by their retention of a tritiated-thymidine nuclear label. The position and identity of such label-retaining cells in palatal and lingual epithelia and ear epidermis was examined using autoradiography and histochemistry. They were found to be either (a) basally positioned keratinocytes preferentially occupying sites within units of epithelial structure that correspond to those expected for epithelial stem cells, or (b) nonkeratinocytes of the Langerhans cell type which lie suprabasally except in the epidermis where they are present in low numbers and occupy a similar position to label-retaining keratinocytes.



Mackenzie IC, Bickenbach JR. 1985. Label-retaining keratinocytes and Langerhans cells in mouse epithelia. Cell Tissue Res. 242(3):551–56

Identifying quiescent cells

Due to the difficulty of indentifying the G0 phase, quiescent cells had remained poorly characterized.

A fusion protein consisting of mVenus and a defective mutant of CDK inhibitor, p27 (p27K2) can identify and isolate quiescent cells and effectively visualize the G0 to G1 transition.

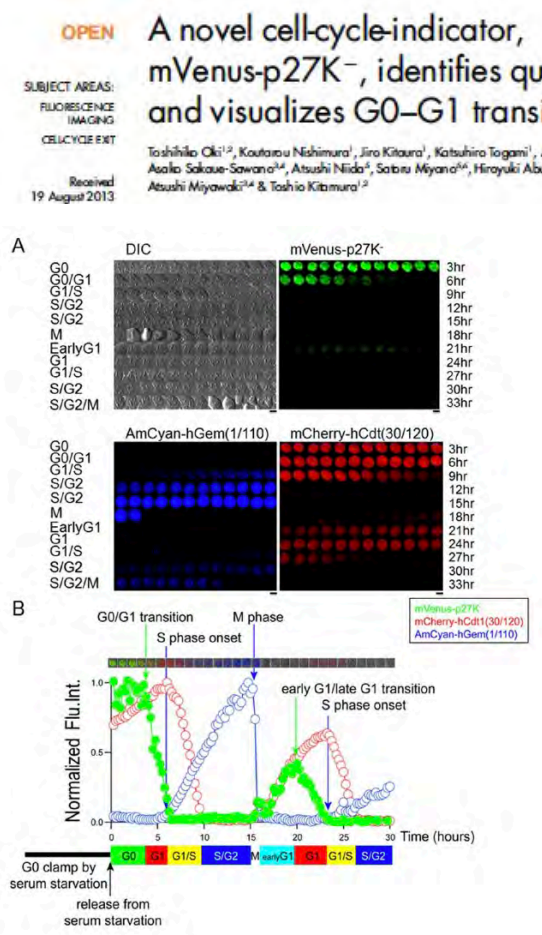
Compare expression profiles of the G0 and G1 cells defined by mVenus-p27K2: genes involved in epigenetic pathways, metabolic regulation, inflammatory responses are differentially activated in G0

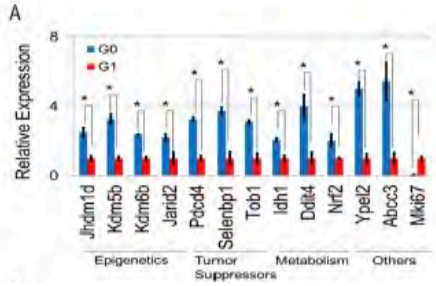
Quiescence is an important feature of many types of **stem cells** and **mVenus-p27K2-transgenic mice** enabled detection of quiescent cells with **muscle stem cell markers in muscle in vivo**.

Previous methods to identify G0 included retention of label – using markers such as bromodeoxyuridine (BrdU) staining or histone 2B-GFP (H2B-GFP) protein. Retaining such markers represents cells with a low frequency of cell division over several weeks or months but this does not explicitly identify G0 cells

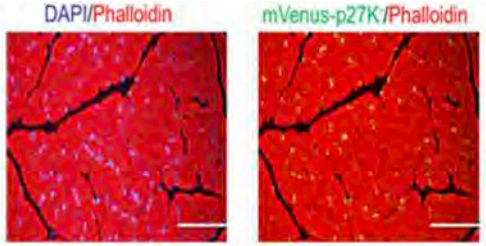
mVenus-p27K2 positive cells represent cells that did not enter the cell cycle within 4 to 10 hr.

Improved temporal resolution compared to traditional methods where retention of BrdU staining or H2B-GFP protein.





Skeletal Muscle



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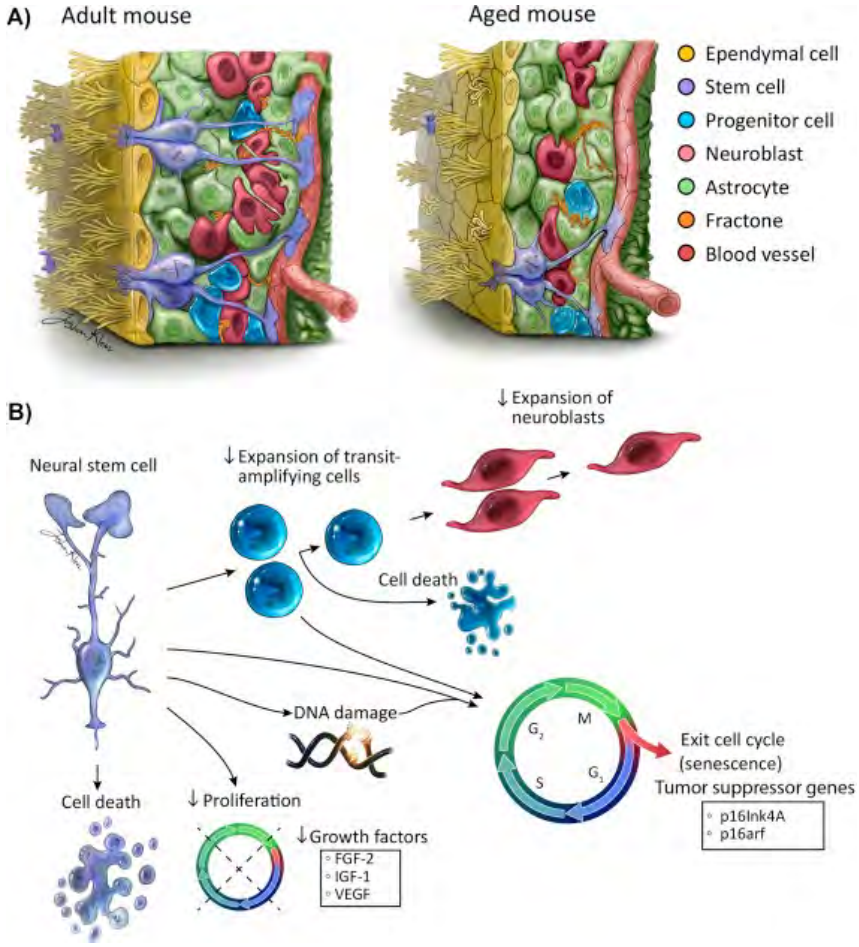
Adult Stem Cells
From Quiescence to Cell Division and Cell Fate Choices

- Adult stem cells (SCs) are key for maintenance of tissue homeostasis.
- Responsible for maintaining tissue structure and function by replacing dying cells and balancing proliferation with differentiation.
- SCs usually rare and reside in complex, specialised microenvironments (*niches*) that control SC lineage outputs depending on localized tissue needs. In their niche, SCs are connected to supporting cells, protected from harmful stimuli, and regulated by appropriate activating signals.
- SCs respond to environmental perturbations and tissue stressors in order to restore the tissue to homeostasis and to protect it from secondary assaults.
- Critical to their function are two key processes, SC lineage plasticity and SC memory.
- Ageing can lead to loss or exhaustion of SCs:intrinsic or extrinsic?



Gola and Fuchs “Environmental control of lineage plasticity and stem cell memory”. Current Opinion in Cell Biology 2021, 69:88–95

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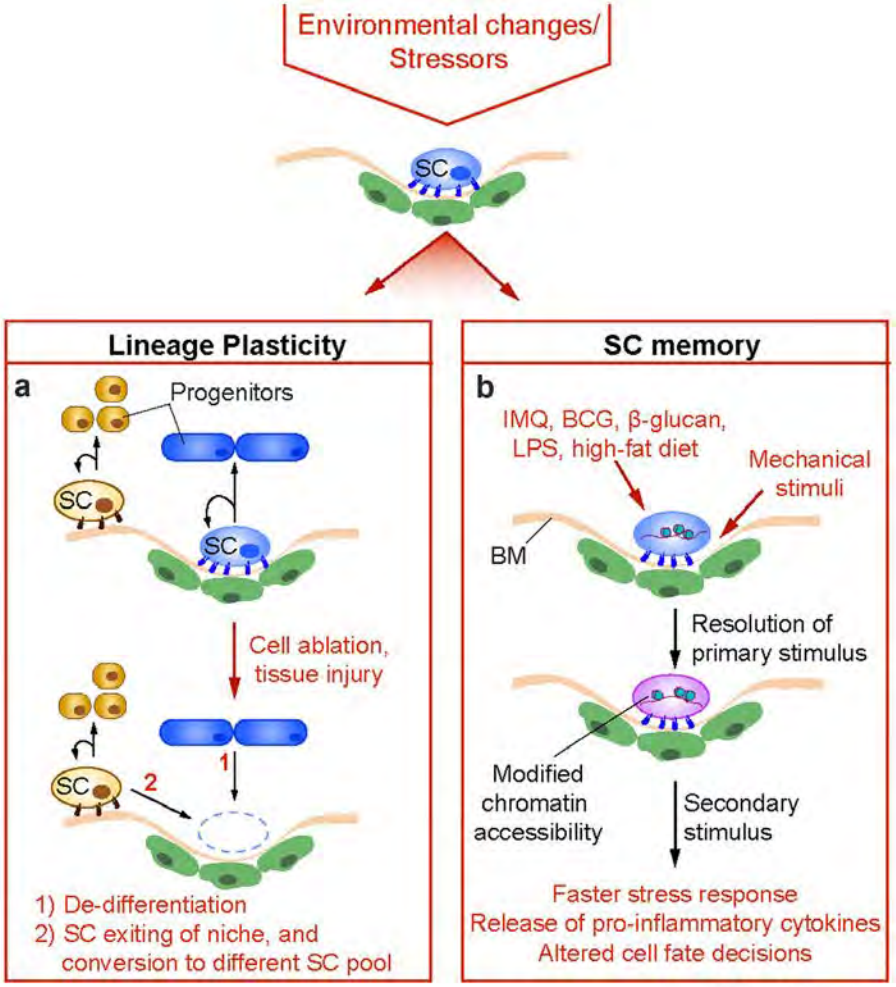

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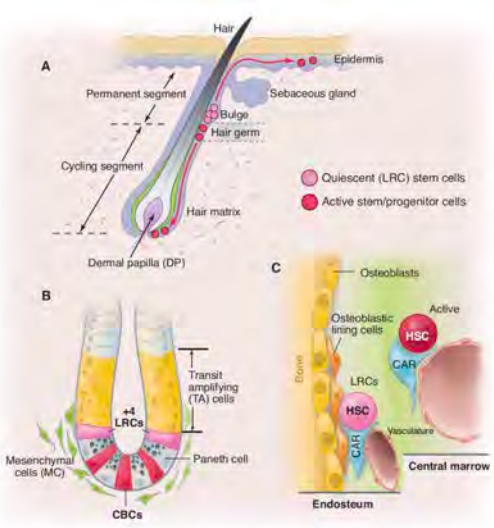
- During steady-state (homeostasis) some SCs eg epidermis, give rise to only one specific cell fate, but others eg in the hair follicle (HF), intestine, or hematopoietic system give rise to multiple lineages.
- Temporally, SC renewal can be continuous (epidermis, intestine, and lung airways), very slow (in muscle and sweat glands), or in bursts of regenerative activity (HFSCs and lactating mammary glands)



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- Temporally, SC renewal can be continuous (epidermis, intestine, and lung airways), very slow (in muscle and sweat glands), or in bursts of regenerative activity (HFSCs and lactating mammary glands)

- How do SCs replace neighbouring cells after tissue damage?
- How do they adapt to a local dynamic environment?
- Do they retain information of previous stressors to better guide cell fate decisions at later times?

- Unique markers are rare and usually not linked to function.
- Also when levels of gene expression fluctuate, molecular markers identify only subpopulation of larger stem cell pool.
- And in cycling tissues, stem cells have to function in a dynamic and noisy environment.

Gola and Fuchs “Environmental control of lineage plasticity and stem cell memory”. Current Opinion in Cell Biology 2021, 69:88–95

Identifying quiescent cells: old and new approaches

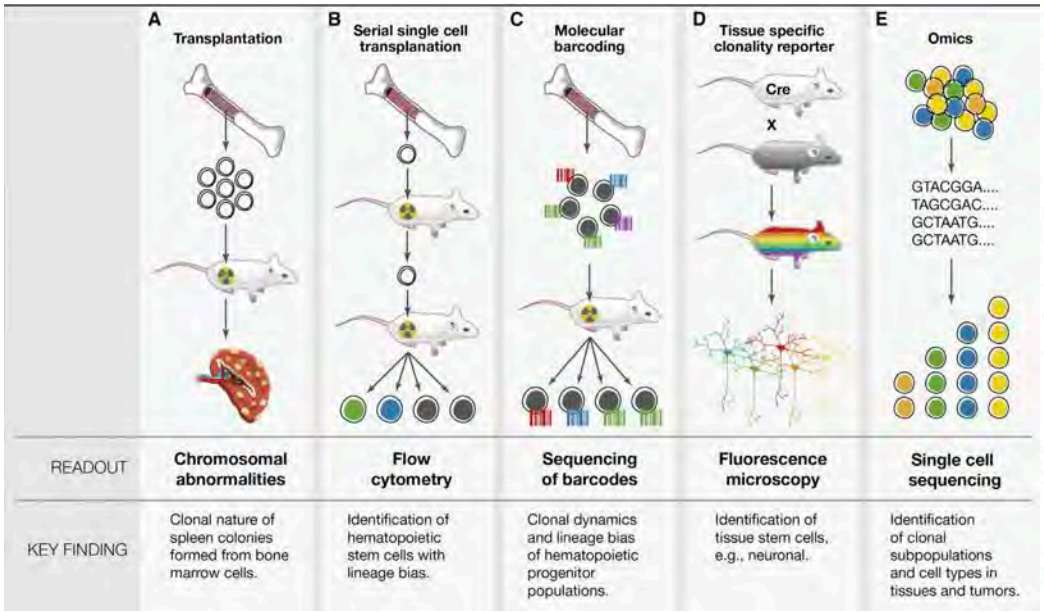


Table 1. Modalities of Single-Cell Analysis Used in Stem Cell Research

	Lineage Tracing	Time-Lapse Imaging	Molecular Profiling
Approach	flow cytometry, sequencing, microscopy	microscopy	flow cytometry, mass cytometry, polymerase chain reaction, whole-genome and transcriptome sequencing, immunohistochemistry, fluorescence in situ hybridization
Condition	in vivo, in vitro	in vivo, in vitro	ex vivo
Parameters to be measured	phenotype of progeny, proliferation	single-cell fates (in vivo only short-term), proliferation, phenotype of progeny, interactions, motility, molecular dynamics	protein, DNA, RNA
Number of markers/ molecules	1–2	1–10	1–genome-wide
Destruction of cell upon measurement	depends on readout modality	no	yes
Temporal resolution	repeated readouts, endpoint analysis	continuous observation (in vivo <12 hr) and endpoint analysis	snapshot of single time point
Identification of cellular heterogeneity	yes	yes	yes
Full lineage tree	no	yes	no
Molecular dynamics	no	yes	no
Motility	no	yes	no
Interactions	no	yes	no

- Adult SC markers: no universal gene marker – few cases where signature is really known
- Live-cell imaging with sufficient spatial and temporal resolution of adult SCs in mammals poses major technical challenges and remains restricted to extremely few specialized cases.
- The only widespread available live-cell imaging modalities for in vivo cell tracing thus are multiphoton- and confocal light-microscopy (Pittet and Weissleder, 2011; Schroeder, 2008).
- Challenge is to obtain ,meaningful in vivo single SC fate mapping because the maximum tolerable time an animal can be kept alive under anesthesia on the microscope stage is limited (usually restricted to 6–12 hr).

Etzrodt et al, Cell Stem Cell 2014

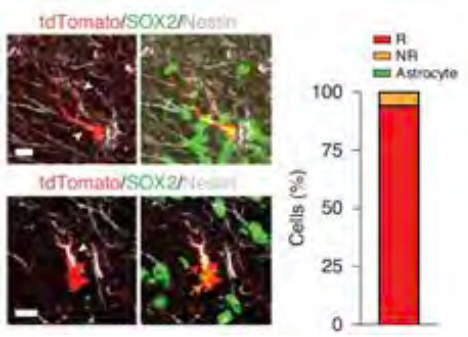
Identifying neural stem cells and their descendants

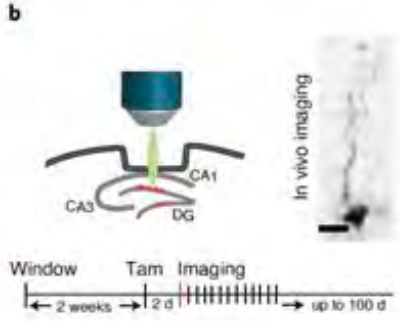
NEURODEVELOPMENT

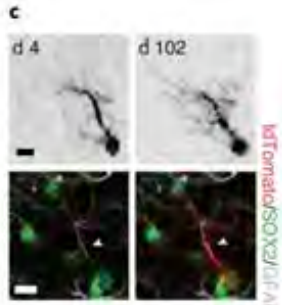
Live imaging of neurogenesis in the adult mouse hippocampus

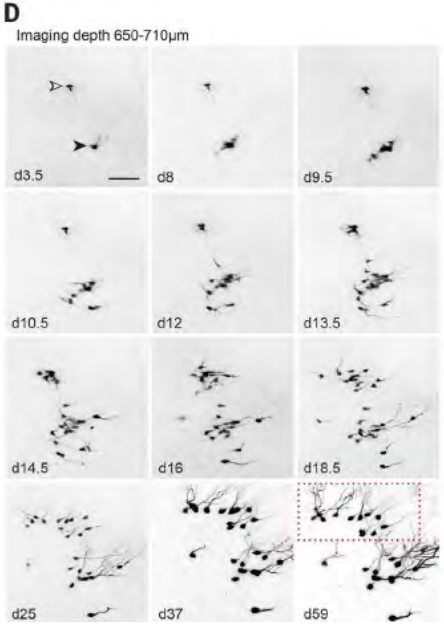
Gregor-Alexander Pilz,[1*] Sara Bottes,[1*] Marion Betizeau,[1,2] David J. Jörg,[3,4] Stefano Carta,[1,6] Benjamin D. Simons,[3,4,5] Fritjof Helmchen,[6] Sebastian Jessberger[1†]

Neural stem and progenitor cells (NSPCs) generate neurons throughout life in the mammalian hippocampus. We used chronic in vivo imaging and followed genetically labeled individual NSPCs and their progeny in the mouse hippocampus for up to 2 months. We show that NSPCs targeted by the endogenous Achaete-scute homolog 1 (Ascl1) promoter undergo limited rounds of symmetric and asymmetric divisions, eliciting a burst of neurogenic activity, after which they are lost. Further, our data reveal unexpected asymmetric divisions of nonradial glia-like NSPCs. Cell fates of Ascl1-labeled lineages suggest a developmental-like program involving a sequential transition from a proliferative to a neurogenic phase. By providing a comprehensive description of lineage relationships, from dividing NSPCs to newborn neurons integrating into the hippocampal circuitry, our data offer insight into how NSPCs support life-long hippocampal neurogenesis.









Selected imaging time points for two R cells (indicated with open and filled arrowheads) over the course of two months, showing the emergence of two neuronal clones.

Followed neural stem cells (NSCs) that were genetically labeled through conditional recombination driven by the regulatory elements of the stem cell-expressed genes GLI family zinc finger 1 (Gli1) or achaete-scute homolog 1 (Ascl1).

Intravital imaging (2-photon microscopy) of NSCs and their progeny (over several months):

- A population of Gli1-targeted NSCs was identified showing long-term self-renewal in the adult hippocampus

⇒ *Identify long-term self-renewing NSCs that contribute to the generation of new neurons in the adult hippocampus.*

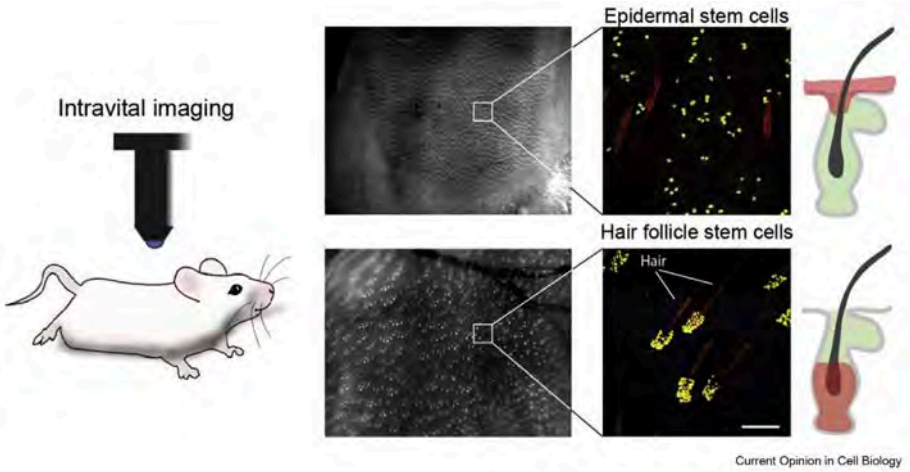
- In contrast, Ascl1-targeted NSC, once activated, undergo limited proliferative activity before they become exhausted.

Single-cell RNA sequencing: Gli1- and Ascl1-targeted cells have highly similar yet distinct transcriptional profiles, supporting the existence of heterogeneous NSC populations with diverse behavioural properties.

E. Heard, 22 mars, 2021

Pilz et al, Science 2018; Bottes et al Nature Neuroscience 2021

Identifying keratinocyte stem cells and their descendants



The identity of basal layer keratinocytes and their organization into distinct long-lived stem cell and transient progenitor populations has been the subject of intense research.

Live imaging combined with the use of inducible and light-modulated fluorescent reporters has enabled unbiased tracking of individual basal keratinocytes in vivo over multiple generations. These experiments demonstrated that the fates of epidermal daughter cells are *not predetermined*, and they appear to have lifetimes that are coupled.

Rice and Rompolas, 2021

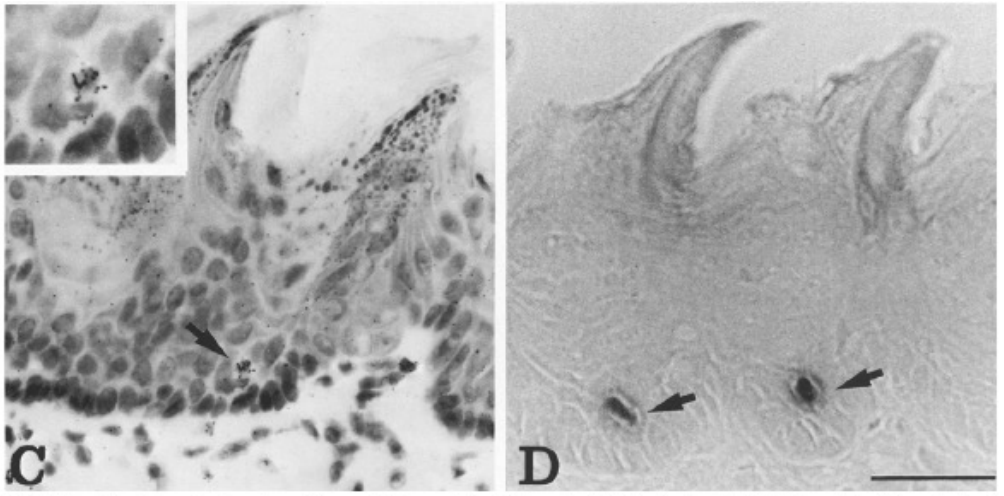
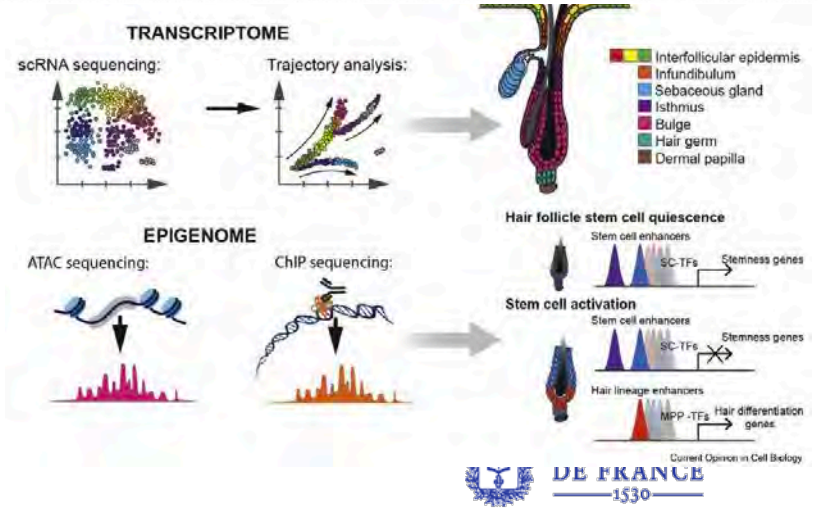
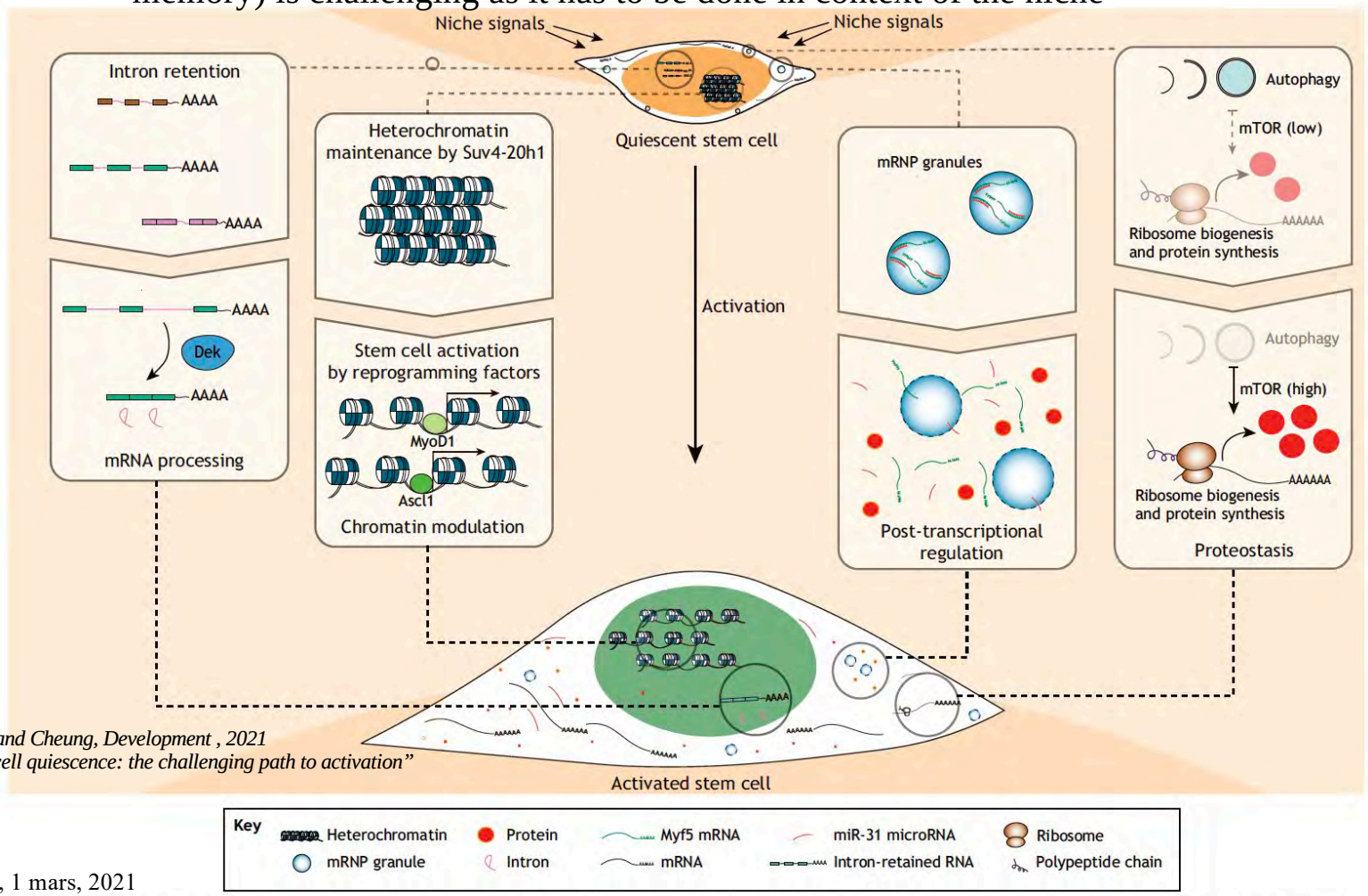


Fig. 2A–D. Autoradiograph of sections of tongue filiform papillae 30 days after labeling showing LRCs in the anterior **(A)** and posterior **(B)** stem cell positions. A suprabasal LRC is shown in **(C)** where it occupies the position typical of β-glucuronidase positive cells identified in frozen sections as illustrated in **(D)**. *Insets* show each LRC at higher magnification. (Scale – 50 μm for each micrograph)



Stem Cell Quiescence and Activation

Identification of the molecular pathways underlying stem cell activation (and memory) is challenging as it has to be done in context of the niche



Urban and Cheung, Development , 2021
"Stem cell quiescence: the challenging path to activation"

E. Heard, 1 mars, 2021

Discovery of Haematopoietic Stem Cells

XXII.

Ueber Entwickelung und Ausbildung der Erythroblasten.

(Aus dem Pathologischen Institut zu Berlin.)

Von Dr. Artur Pappenheim.

(Hierzu Taf. XIII und XIV.)

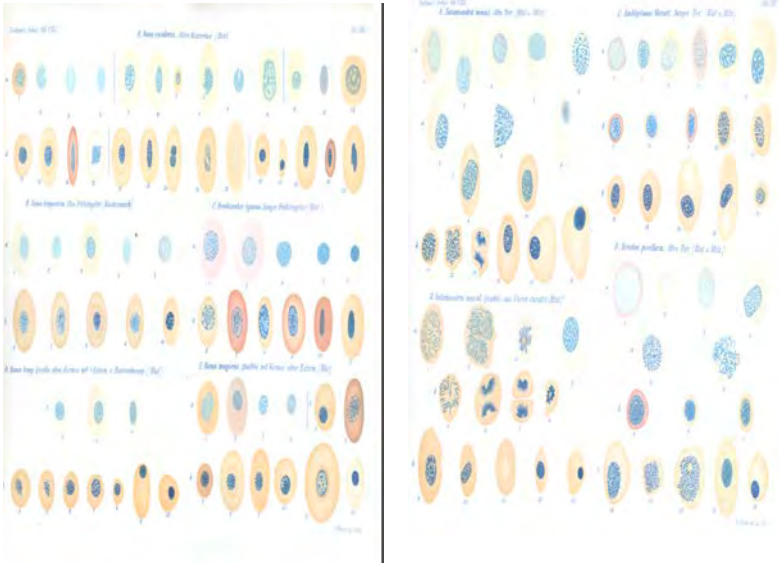
In meiner Arbeit „Die Bildung der rothen Blutscheiben“[1]) hatte ich das Problem, betreffend das Verhältniss zwischen Megaloblasten und Normoblasten, bereits kurz gestreift. Auch S. Askanazy[2]) und O. Schauman[3]) haben jüngst diese hoch interessante Frage wiederum recht eingehend erörtert, ohne indess, wie mir scheint, die Angelegenheit zu einem befriedigenden Abschluss gebracht zu haben. Rücksichtlich der grossen diagnostisch-prognostischen Bedeutung der Megaloblasten erscheint eine neue Untersuchung dieses Gegenstandes zur Kenntniss des Wesens der Anämien förderlich.

Askanazy vertritt augenblicklich die auf den ersten Blick sehr naheliegende Anschauung, als ob „die Megaloblasten die

[1]) A. Pappenheim, Diss. inaug. Berlin 1895.
[2]) S. Askanazy, Zeitschr. f. klin. Med. XXVII. 5 und 6. 1895.
[3]) O. Schauman, Zur Kenntniss der sogen. Bothriocephalus-Anämie. Berlin 1894.

The term stem cell was coined at the end of the nineteenth century by Boveri and Haecker to propose the notion of a common progenitor cell for distinct blood lineages. Pioneering work on the theory of blood stem cells was conducted by Pappenheim, Maximow and Neumann in the late 19th early 20th century.

The existence of this progenitor, called a haematopoietic stem cell (HSC), was finally proven by McCulloch and Till in the 1960s using mice – bone marrow cells injected into irradiated mice.



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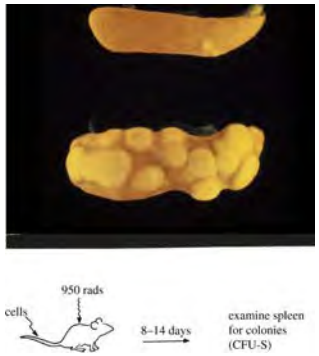
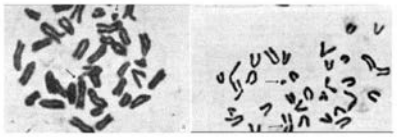
Discovery of Haematopoietic Stem Cells



Nature, Vol. 197, No. 4866, pp. 452-454, February 2, 1963

CYTOLOGICAL DEMONSTRATION OF THE CLONAL NATURE OF SPLEEN COLONIES DERIVED FROM TRANS-PLANTED MOUSE MARROW CELLS

By DR. A. J. BECKER, E. A. McCULLOCH and J. E. TILL
Department of Medical Biophysics, University of Toronto and Ontario Cancer Institute, Toronto



Experiments were performed in which the donor cells were irradiated to genetically mark each cell with random chromosome breaks to confirm that each of these different cell types in a nodule arose from a single cell.

For the "colony-forming cell" to be a true stem cell, it had to produce not only the differentiated blood cells but also more colony-forming cells. This was demonstrated by taking the nodule derived from a single genetically marked colony-forming cell and injecting the cells from this nodule into another irradiated mouse. Many spleen colonies emerged each of them having the same chromosomal arrangement as the original colony.

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The existence of this progenitor, called a haematopoietic stem cell (HSC), was finally proven by McCulloch and Till in the 1960s using mice – bone marrow cells injected into irradiated mice. The lumps observed in the spleens of the mice were linearly proportional to the number of bone marrow cells injected. They hypothesized that each lump (colony) was a clone arising from a single marrow cell (stem cell).

Siminovitch found colony-forming cells were capable of self-renewal, which is a key defining property of stem cells that Till and McCulloch had theorized

The discovery of HSCs led to the defining concept of a stem cell as a self-renewing cell positioned at the top of a hierarchy, giving rise to a range of fully differentiated, specialized cell types at the end of the hierarchy's branches.

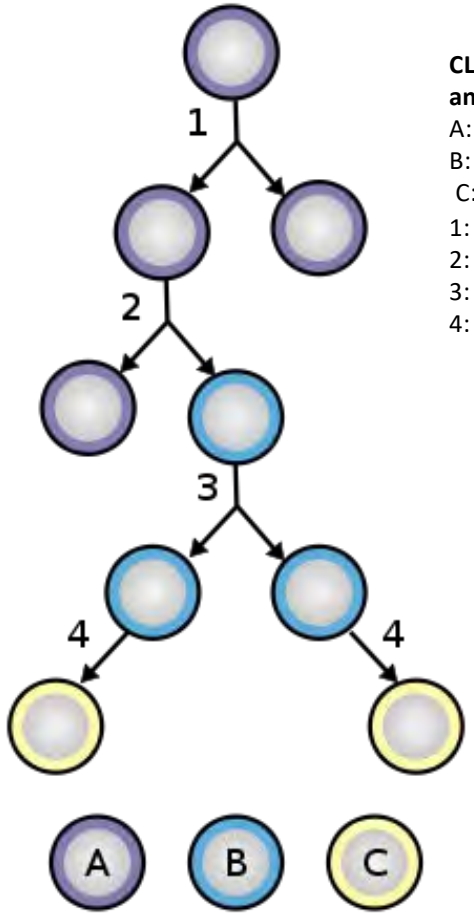
E. Heard, 22 mars, 2021

COLLÈGE DE FRANCE 1530

Haematopoietic Stem Cells

The classic HSC-type stem cell hierarchy defined since original observations in 1950's and 60's in fact seems to be rather unique

In solid tissues and organs "professional" stem cells that fuel an HSC-type stem cell hierarchy have not been found in most cases.



CLASSIC ILLUSTRATION (WIKI!) of Stem cell division and differentiation

A: stem cell;
B: progenitor cell;
C: differentiated cell;
1: symmetric stem cell division;
2: asymmetric stem cell division;
3: progenitor division;
4: terminal differentiation

The classical hematopoietic stem cell (HSC) hierarchy

The HSC is rare and quiescent, while the flow through the hierarchy is strictly unidirectional, away from the HSC.

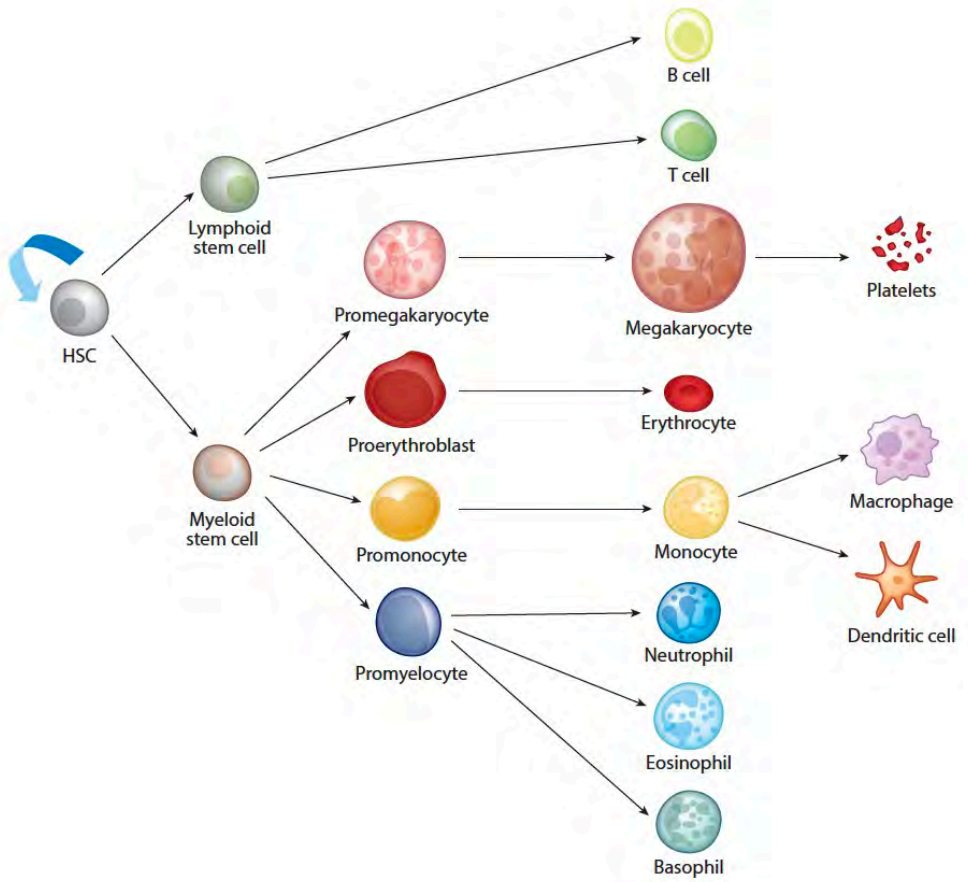
The HSC is the only cell that can act as a stem cell.

HSCs may be the exception rather than the rule.

Being our only liquid organ, it is not obvious why evolution would have come up with the same solution for the maintenance and repair of all tissues

Organs and tissues differ in size and architecture, exhibit widely divergent biological and physical properties, and are subject to different biological and physical challenges. Moreover, being our only liquid organ, the hematopoietic system appears to be rather an outlier.

What can we learn from the hematopoietic stem cell lineage in terms cellular memory?



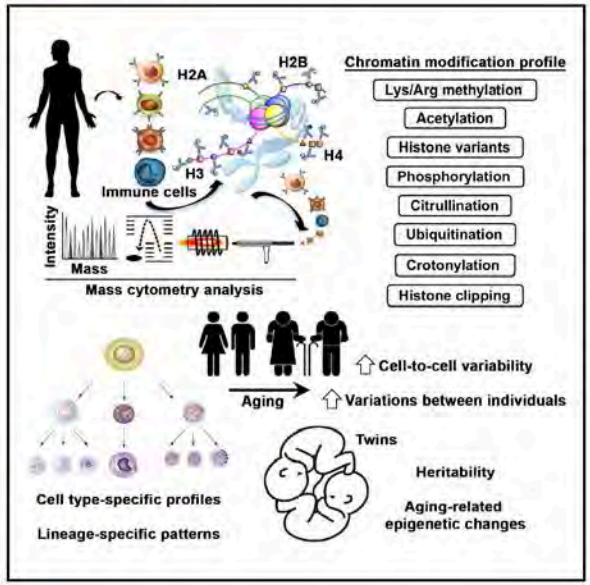
Lorenz E et al (1951) Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J. Natl. Cancer Inst. 12:197–201
Thomas ED et al (1957). Intravenous infusion of bonemarrow in patients receiving radiation and chemotherapy. N. Engl. J. Med. 257:491–96

Watt and Clevers Annu. Rev. Biochem. 2018.87:1015-1027

Hematopoietic Lineage Cellular Memory and Variation

Single-Cell Chromatin Modification Profiling Reveals Increased Epigenetic Variations with Aging

Graphical Abstract

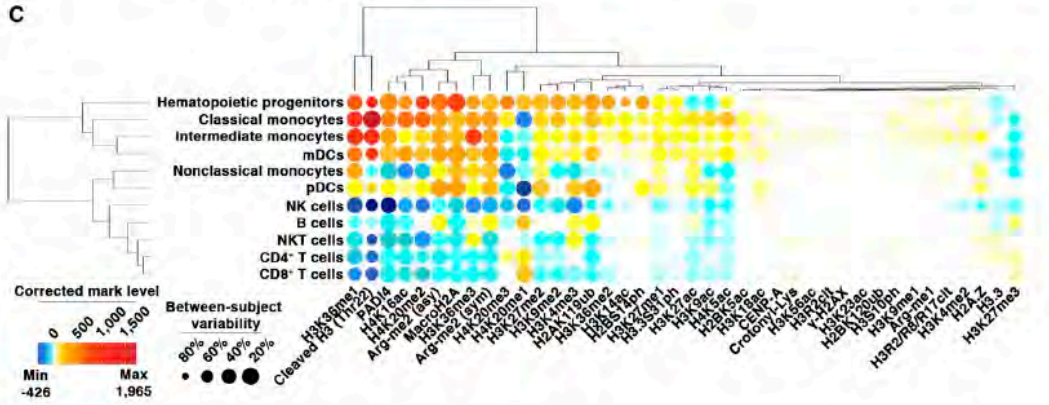


Highlights

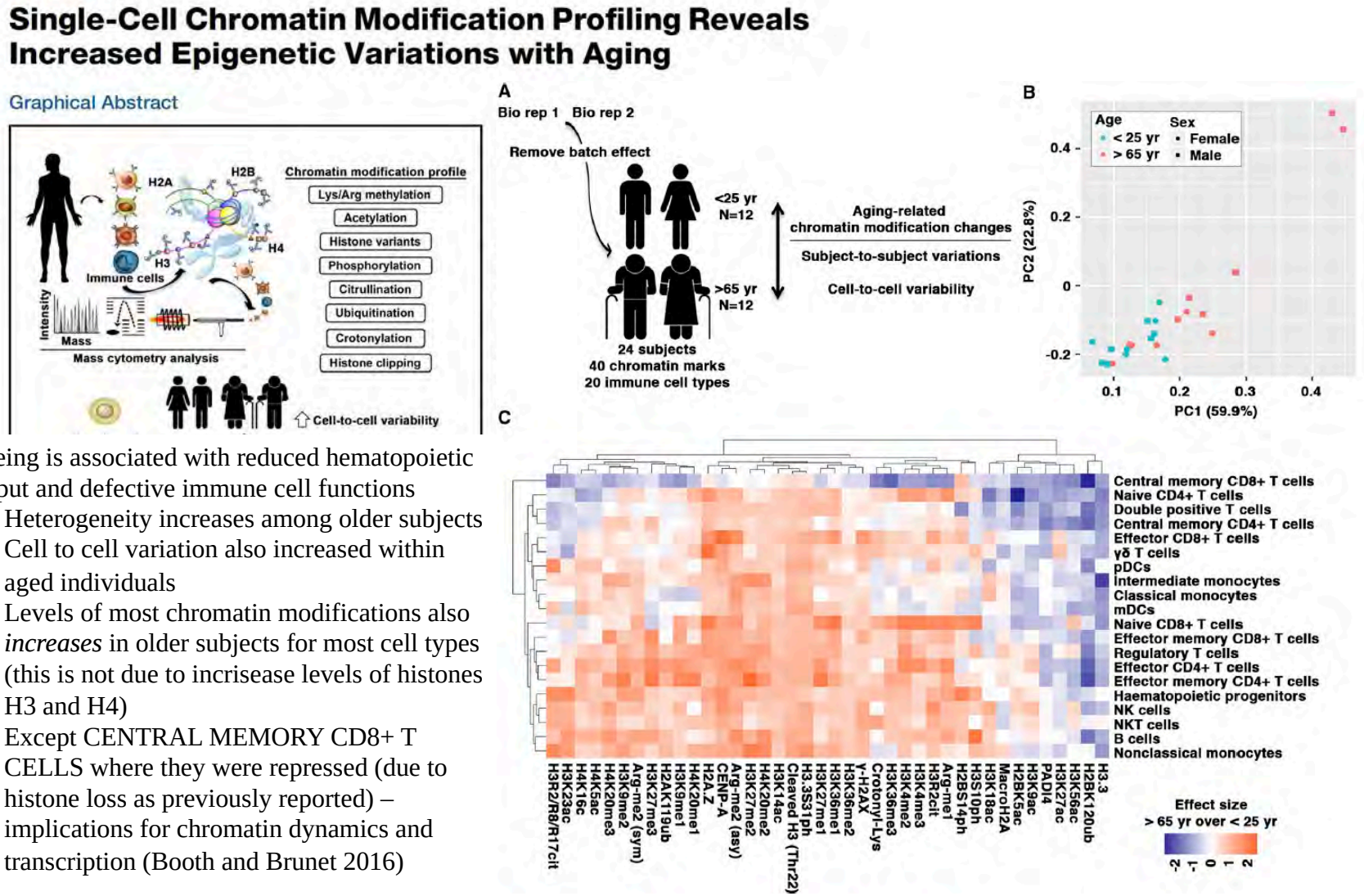
- Diverse chromatin marks in single cells are measured by mass cytometry
- Cell-type-specific profiles of chromatin marks predict immune cell identity
- Chromatin variations between individuals and single cells increase with age
- Aging-related alterations of chromatin are largely driven by non-heritable factors

Immune cell epigenetic atlas using cytometry by time-of-flight (EpiTOF)

- Degree and variability of chromatin modifications in specific human immune cells
- Employ single-cell highly multiplexed mass cytometry to profile the global levels of a broad array of chromatin modifications in primary human immune cells at the single-cell level.
- Identify markedly different cell-type- and hematopoietic-lineage-specific chromatin modification patterns: sets of chromatin marks can act as signatures for cell types
- Differential analysis between younger and older adults shows that aging is associated with increased heterogeneity between individuals and elevated cell-to-cell variability in chromatin modifications.
- Analysis of a twin cohort (10 Dizygotic and 9 Monozygotic twins) unveils heritability of chromatin modifications (young MZ twins show higher similarity than DZ twins)
- Also demonstrates that aging-related chromatin alterations are predominantly driven by n heritable influences (both MZ and DZ twins show similar variation to random individuals



Aging and Epigenetic changes



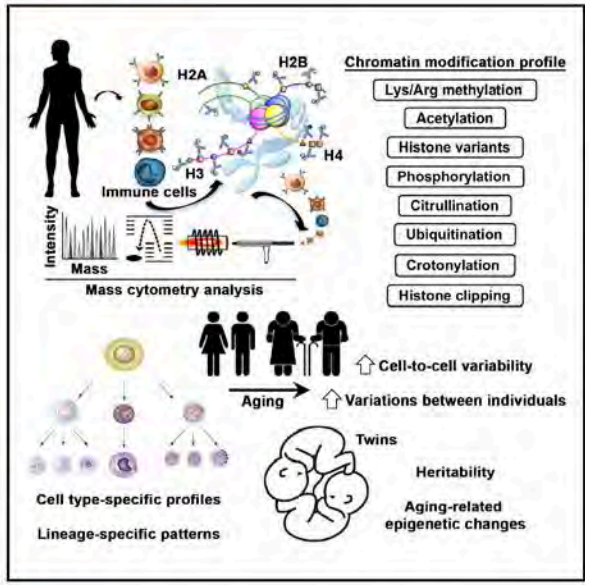
Ageing is associated with reduced hematopoietic output and defective immune cell functions

- Heterogeneity increases among older subjects
- Cell to cell variation also increased within aged individuals
- Levels of most chromatin modifications also *increases* in older subjects for most cell types (this is not due to incrisease levels of histones H3 and H4)
- Except CENTRAL MEMORY CD8+ T CELLS where they were repressed (due to histone loss as previously reported) – implications for chromatin dynamics and transcription (Booth and Brunet 2016)

Aging and Epigenetic changes

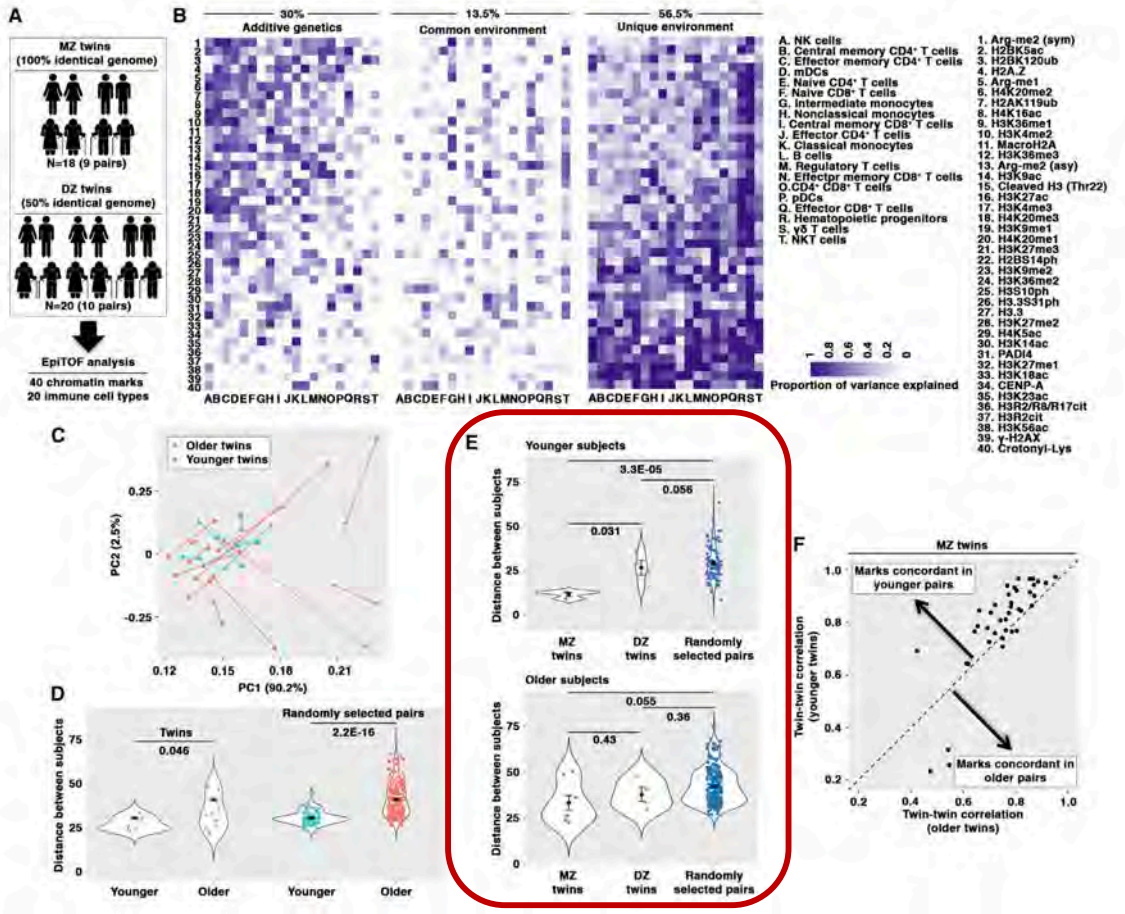
Single-Cell Chromatin Modification Profiling Reveals Increased Epigenetic Variations with Aging

Graphical Abstract



Highlights

- Diverse chromatin marks in single cells are measured by mass cytometry
- Cell-type-specific profiles of chromatin marks predict immune cell identity
- Chromatin variations between individuals and single cells increase with age
- Aging-related alterations of chromatin are largely driven by non-heritable factors



The Epigenetic control of Stemness in CD8+ T Cell Fate Commitment

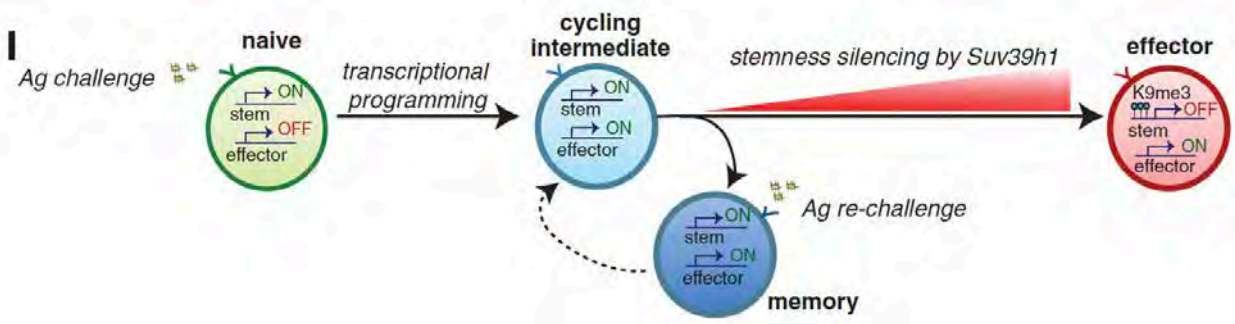
RESEARCH ARTICLE

IMMUNOLOGY

The epigenetic control of stemness in CD8$^+$ T cell fate commitment

Luigia Pace,[1,2,3]*† Christel Goudot,[1,2] Elina Zueva,[1,2] Paul Gueguen,[1,2] Nina Burgdorf,[1,2] Joshua J. Waterfall,[1,4,5] Jean-Pierre Quivy,[1,6,7] Geneviève Almouzni,[1,6,7]* Sebastian Amigorena[1,2]*

After priming, naïve CD8$^+$ T lymphocytes establish specific heritable transcription programs that define progression to long-lasting memory cells or to short-lived effector cells. Although lineage specification is critical for protection, it remains unclear how chromatin dynamics contributes to the control of gene expression programs. We explored the role of gene silencing by the histone methyltransferase Suv39h1. In murine CD8$^+$ T cells activated after *Listeria monocytogenes* infection, Suv39h1-dependent trimethylation of histone H3 lysine 9 controls the expression of a set of stem cell–related memory genes. Single-cell RNA sequencing revealed a defect in silencing of stem/memory genes selectively in *Suv39h1*-defective T cell effectors. As a result, *Suv39h1*-defective CD8$^+$ T cells show sustained survival and increased long-term memory reprogramming capacity. Thus, Suv39h1 plays a critical role in marking chromatin to silence stem/memory genes during CD8$^+$ T effector terminal differentiation.



Memory T lymphocytes provide lifelong protection against pathogens and cancer.

Unlike naïve and effector T cells, memory cells possess unique properties of “stemness,” enabling long term survival and plasticity to replenish effector pools after renewed antigen challenges

Stem cell–like/memory genes are expressed by memory precursors and cycling CD8+ T intermediates, and silenced by Suv39h1 in terminal effectors.

After priming, cycling CD8+ T lymphocytes reprogram both self-renewing and effector gene expression profiles.

These cycling cells may represent bipotent intermediates, which would then repress either the effector or stem cell/memory programs while they differentiate to memory precursors or effectors, respectively

The silencing of the stem cell/memory gene expression program is under the control of Suv39h1 by imposing the H3K9me3 modification on chromatin at the corresponding loci. In doing so, Suv39h1/H3K9me3 would establish an epigenetic barrier on the stem/memory gene expression program, preventing effector reprograming into memory cells.

Pace et al., Science 359, 177–186 (2018)

COLLÈGE DE FRANCE 1530

Solid Tissue Stem Cells and Alternative Stem Cell Hierarchies

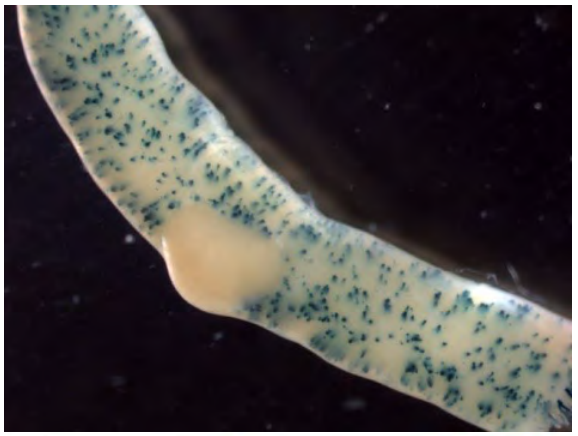
From a stem cell perspective, solid tissues come in two types:

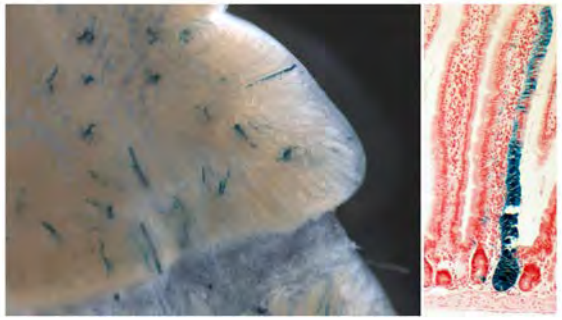
1. Some (like **liver**, **pancreas**, or **lung**) display very little proliferative activity in their steady state but undergo bursts of proliferation upon damage.

2. Others (like the **epidermis**, **testis**, or **epithelia** along the length of the **intestinal tract**) are constantly self-renewing with tissue replacement rates in the order of days, weeks, or months. These resemble the bone marrow in their continuous generation of daughter cells.

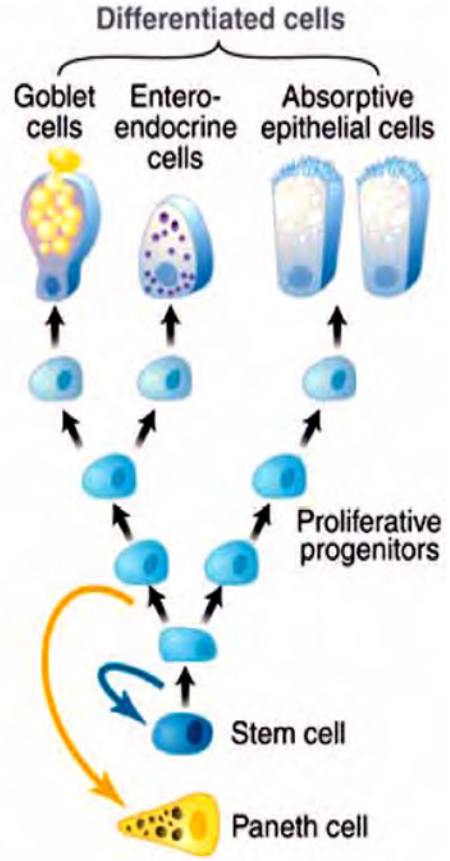
Hans Clevers
Hubrecht Institute for Developmental Biology and Stem Cell Research
Utrecht, Netherlands

The intestinal crypt





Courtesy of Hans Clevers



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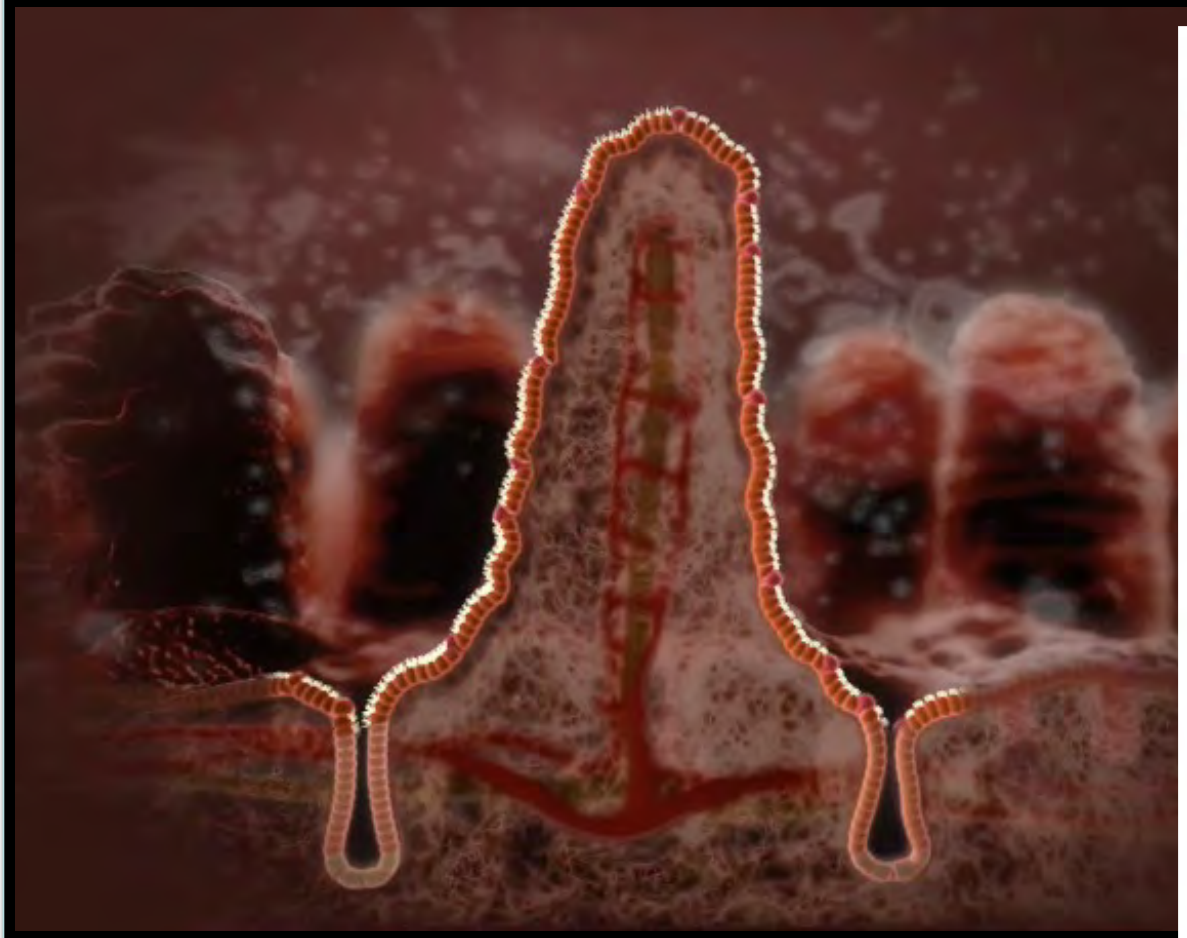
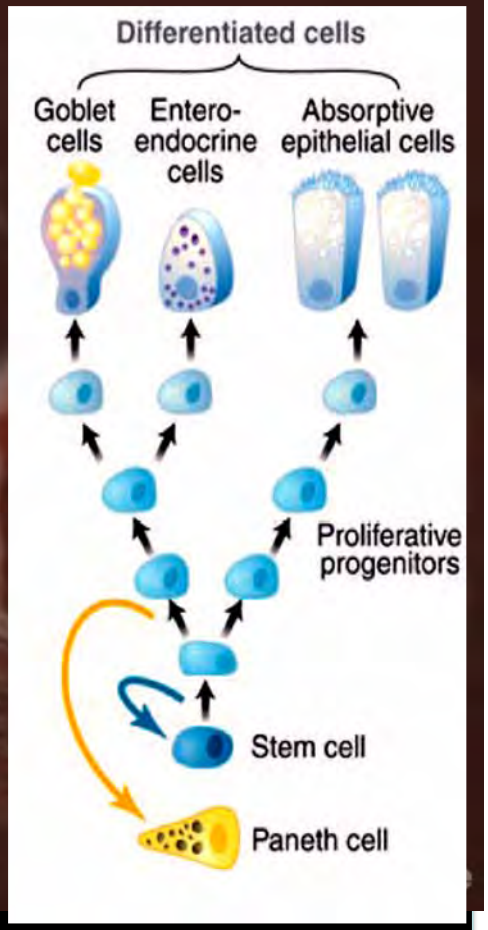




The ‘champion’ of all adult stem cells

Courtesy of Hans Clevers

Alternative Stem Cell Hierarchies
Other models of Adult stem cells: the intestinal crypt

In the intestinal crypt, a continuously proliferative stem cell type marked by Lgr5 gene expression is located at the base, while a quiescent stem cell type has been identified directly above Lgr5 stem cells at the position +4.

Genetic lineage tracing demonstrated that the constantly cycling Lgr5 stem cells persist throughout life

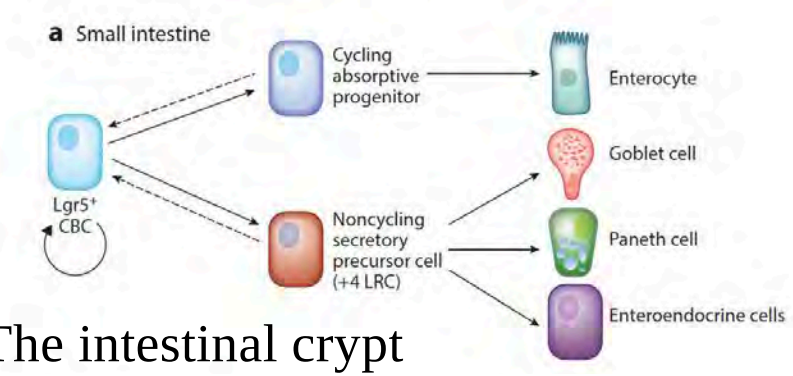
These stem cells go through as many as 1,000 cell divisions in the lifetime of a mouse

Originally assumed that these quiescent +4 cells would occupy the apex of the crypt stem cell hierarchy to give rise to the proliferative Lgr5 cells

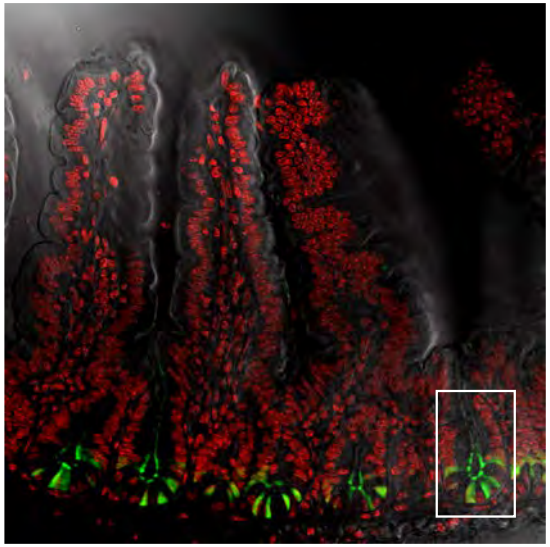
However genetic tracing revealed that these nondividing +4 cells actually represent lineage-restricted daughters that have exited the cell cycle in preparation for terminal differentiation into a variety of secretory cell types.

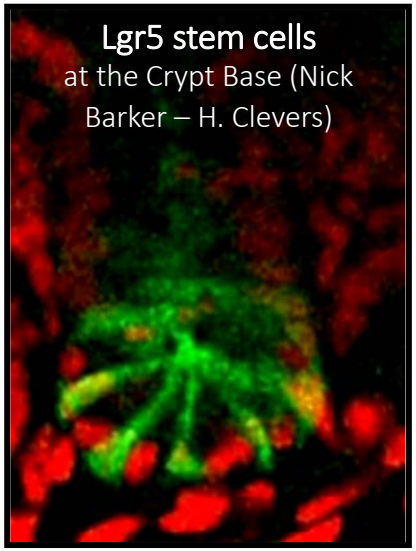
Hans Clevers
Hubrecht Institute for Developmental Biology and Stem Cell Research
Utrecht, Netherlands

E. Heard, 22 mars, 2021



The intestinal crypt





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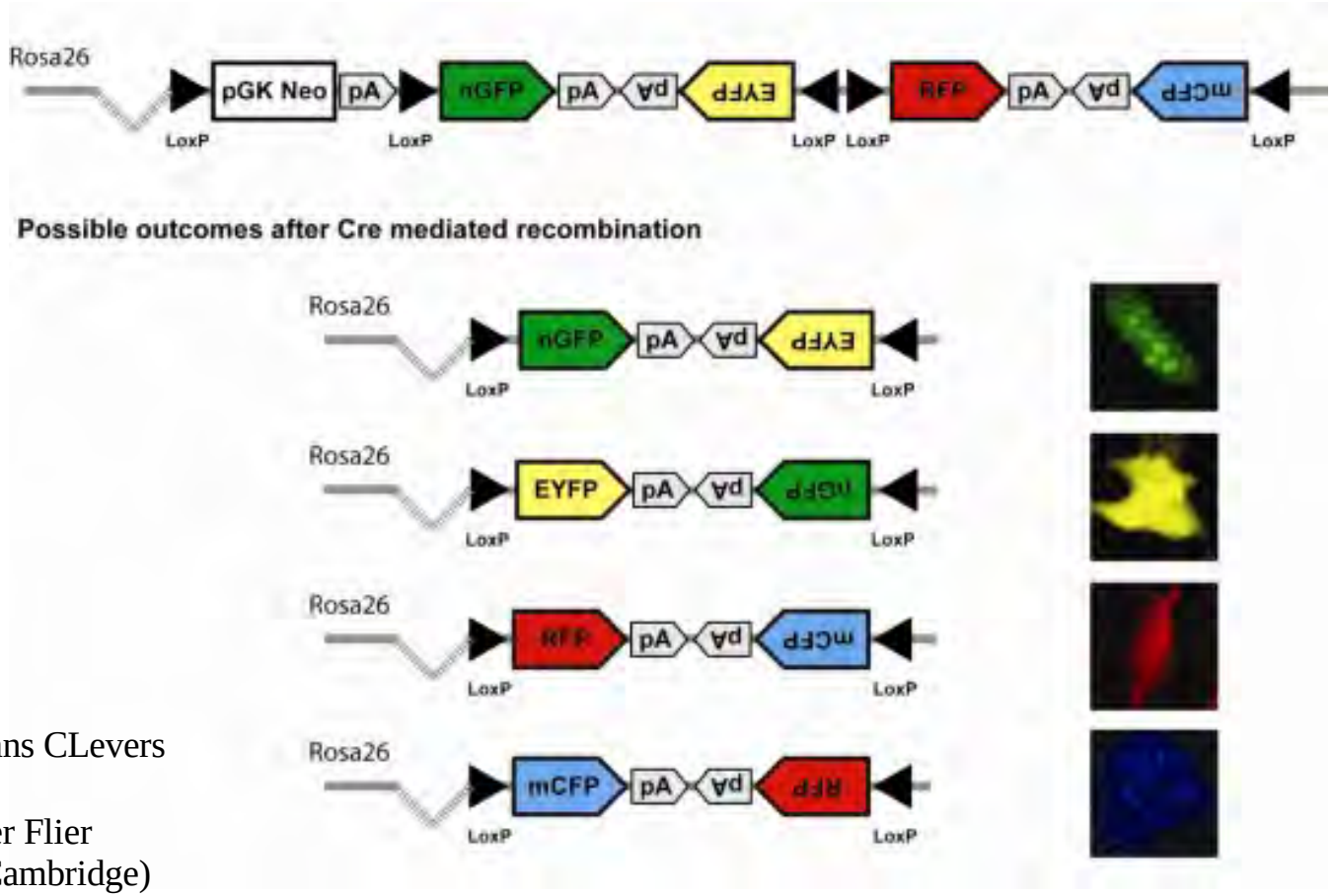
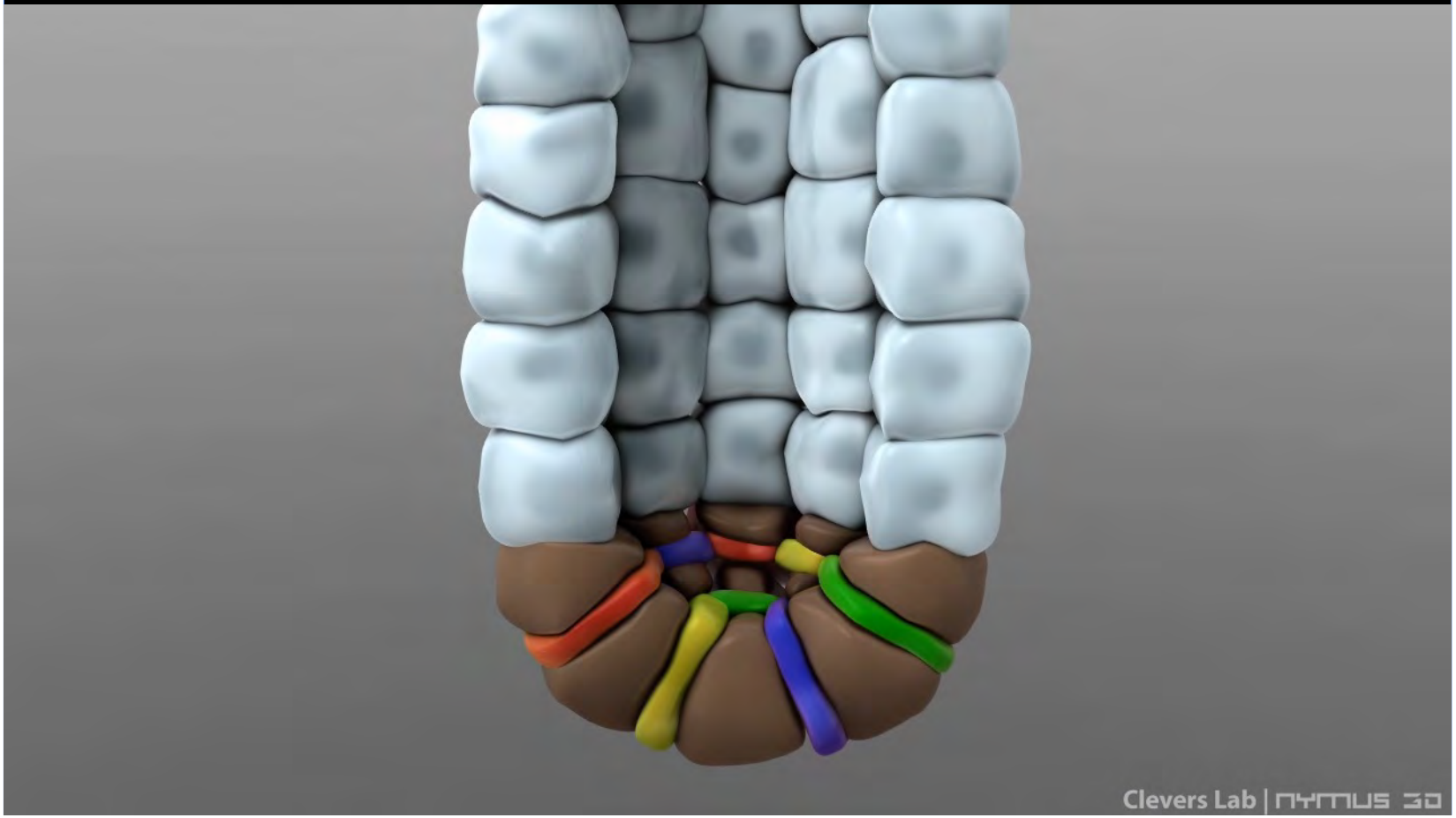
Rosa-Confetti
Multicolor lineage tracing based on Brainbow
(J.Lichtman)



Courtesy of Hans CLevers
Hugo Snippert
Laurens van der Flier
Ben Simons (Cambridge)

Fifteen Lgr5 stem cells in each crypt divide every day. They neutrally compete for space



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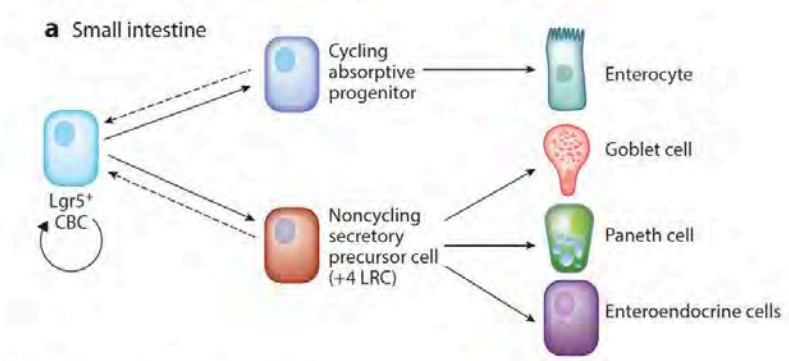
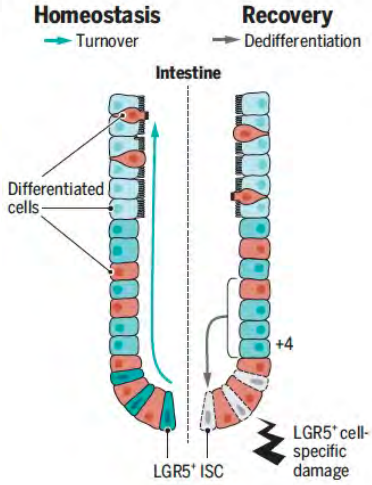
However genetic tracing revealed that these nondividing +4 cells actually represent lineage-restricted daughters that have exited the cell cycle in preparation for terminal differentiation into a variety of secretory cell types.

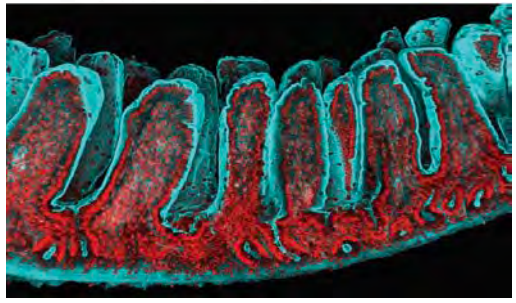
Acute loss of the Lgr5 stem cells can coax these cells to literally take a U-turn, settle at the base of the crypts, and reacquire a multipotent Lgr5 stem cell phenotype. The notion that short-lived progenitors can replace lost Lgr5 stem cells was confirmed for the secretory lineage as well as for the more abundant enterocyte lineage (Tetteh et al, 2006)).

Individually, these quiescent +4 cells are short-lived, as they progress to full differentiation and die. Yet, in their intermediary state, these cells functionally build a persistent reserve stem cell pool.

The +4 cells act as reserve of stem cells => violating the rule that the flow through a stem cell hierarchy should be unidirectional.







A cross section of mouse small intestine, showing intestinal crypts and villi, is visualized with immunofluorescence microscopy (nuclei in red, and F-actin, which marks the cytoskeleton, in blue). Intestinal stem cells reside at the base of crypts, where they maintain cell turnover.

STEM CELLS

Tissue regeneration: Reserve or reverse?

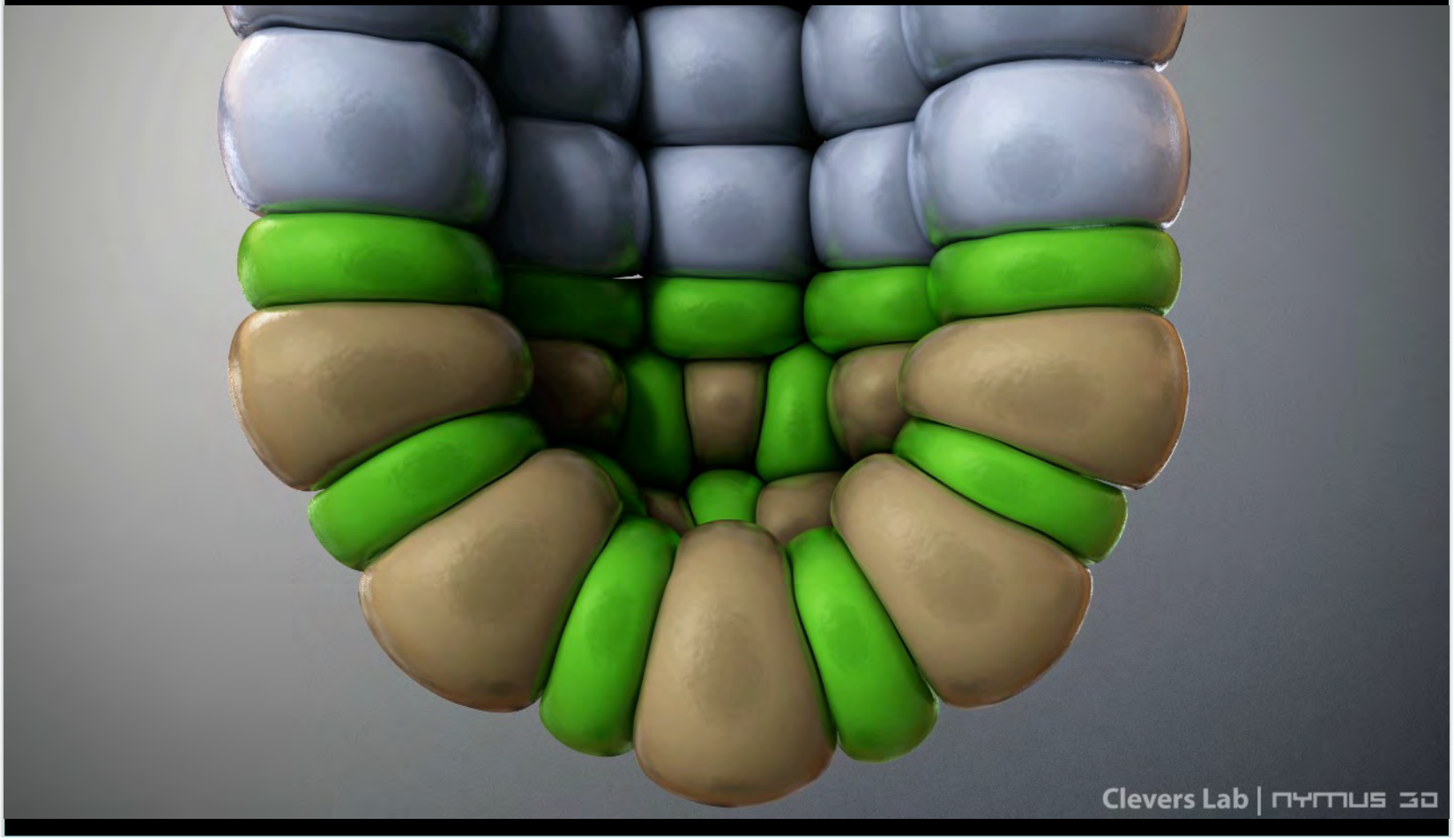
Stem cells recover from injury by tissue dedifferentiation, not from dedicated reserves

By Ramesh A. Shivdasani[2,3], Hans Clevers[4,5,6], Frederic J. de Sauvage[7]

because all tissues experience some cell attrition over a lifetime, and knowing how

Watt and Clevers Annu. Rev. Biochem. 2018.87:1015-1027

Plasticity in the crypt:
Daughter cells can revert to Lgr5 stem cells upon damage



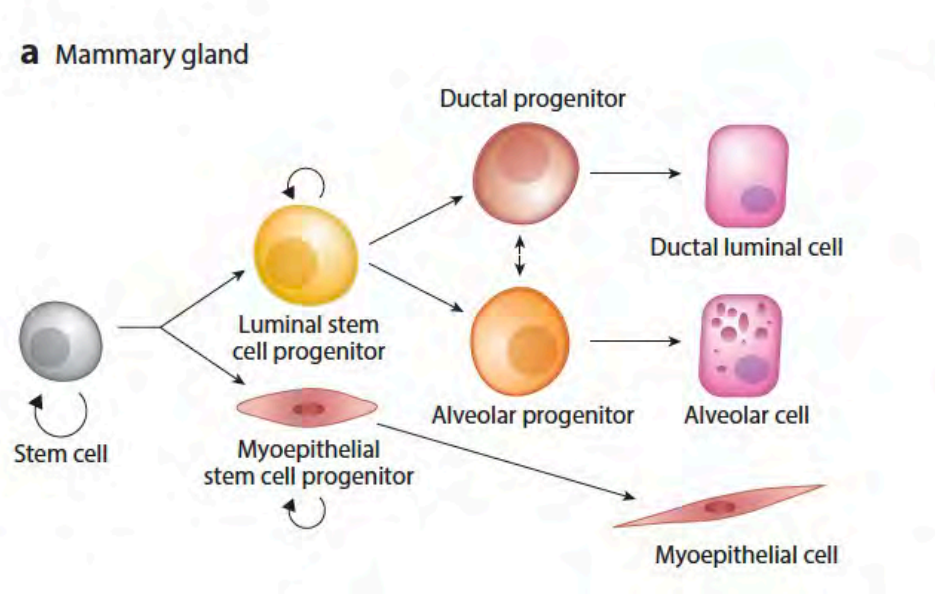
Other models of Adult stem cells: mammary gland

MULTIPLE STEM CELL TYPES IN ONE TISSUE

Two classical studies on mammary stem cells have followed the HSC paradigm. Transplantation of single sorted mammary epithelial cells into their niche, the fat pad, has identified rare cells that—on their own—can build a complete, functional mammary gland comprising two main layers: the basal (or myoepithelial) layer and the luminal layer

Recent studies using genetic marking in vivo have convincingly confirmed the presence of such multipotent stem cells and yet another study has convincingly demonstrated that the basal and luminal lineages are maintained long-term by independent stem cells.

Does not fit HSC-like stem cell hierarchy



Renewal strategies of the mammary gland epithelium. Basal cells sit at the top of the hierarchy. Yet, the luminal and myoepithelial lineages are maintained largely independently, driven by stem cell–like progenitors

Watt and Clevers Annu. Rev. Biochem. 2018.87:1015-1027

Other models of Adult stem cells: Liver

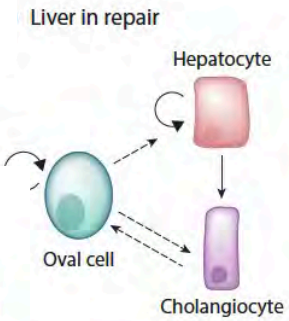
Liver is the champion of tissue regeneration following injury.

Only two cell types make up the liver proper: hepatocytes and cholangiocytes (all other cell types are developmental immigrants).
Upon hemihepatectomy, the hepatocytes in the remaining half of the liver rapidly and massively enter the cell cycle, without any significant sign of dedifferentiation.

Within weeks, original liver mass and function are restored, and all cells within the organ return to their non-proliferative state.
This surgical procedure can be performed multiple times, yet the liver will grow back to its original size each time.

Nusse and colleagues recently demonstrated that fully functional hepatocytes located around the central vein serve as a source of new hepatocytes in a healthy liver, generating a flow of hepatocytes through the liver lobule to its periphery

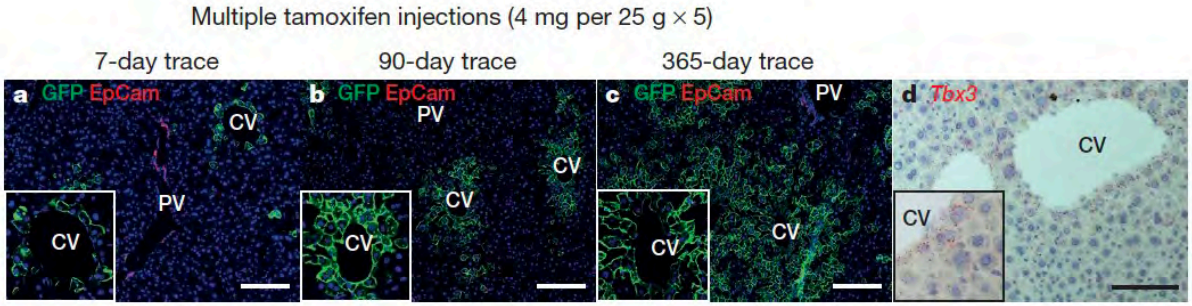
Few would use the term self-renewal for the process of producing new hepatocytes from existing hepatocytes in tissue homeostasis or upon tissue loss.



Self-renewing diploid Axin2^{+} cells fuel homeostatic renewal of the liver

Bruce Wang[1,2], Ludan Zhao[1], Matt Fish[1], Carriena Y. Logan[1] & Roel Nusse[1]

The source of new hepatocytes in the uninjured liver has remained an open question. By lineage tracing using the Wnt-responsive gene Axin2 in mice, we identify a population of proliferating and self-renewing cells adjacent to the central vein in the liver lobule. These pericentral cells express the early liver progenitor marker Tbx3, are diploid, and thereby differ from mature hepatocytes, which are mostly polyploid. The descendants of pericentral cells differentiate into Tbx3-negative, polyploid hepatocytes, and can replace all hepatocytes along the liver lobule during homeostatic renewal. Adjacent central vein endothelial cells provide Wnt signals that maintain the pericentral cells, thereby constituting the niche. Thus, we identify a cell population in the liver that subserves homeostatic hepatocyte renewal, characterize its anatomical niche, and identify molecular signals that regulate its activity.



Watt and Clevers Annu. Rev. Biochem. 2018.87:1015-1027

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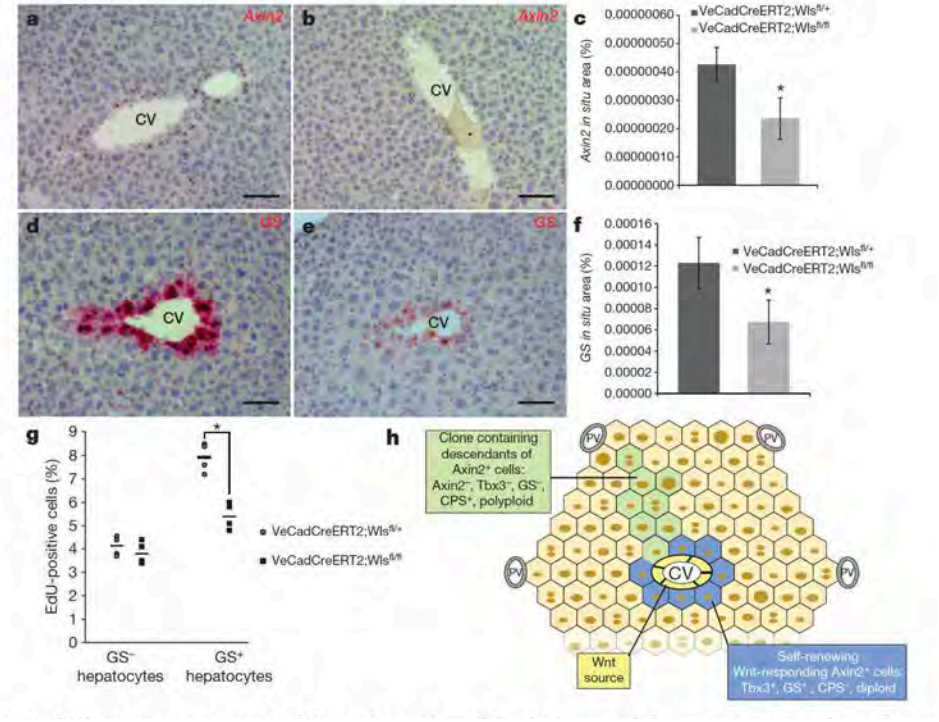
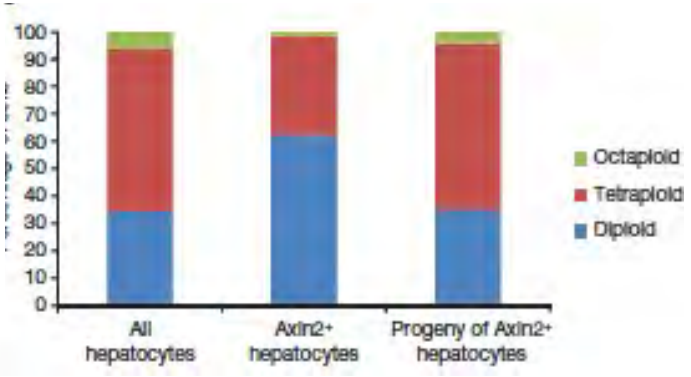
Other models of Adult stem cells: Liver

- Axin2 pericentral cells generate expanding clone
- Axin2 cells self-renew
- Axin2 cells proliferate faster than other hepatocytes
- Axin2 cells are mostly diploid unlike most other hepatocytes
- Central vein endothelium acts as a Wnt-producing niche
- Wnt signals are required for pericentral cell proliferation





Liver is known to regenerate efficiently after injuries such as partial hepatectomy or chemical insult. It has been reported that during regeneration after chemical damage, a Wnt-responsive population of cells near the portal vein can be labelled by the Lgr5 receptor gene.

These cells, unlike pericentral Axin2 cells, do not express hepatocyte genes, but subsequently differentiate into bile duct epithelial cells and hepatocytes and thus could be similar to injury-induced oval cells.

Clearly, Lgr5/oval cells are distinct from the Axin2 cells, as pericentral cells maintain hepatocyte homeostasis in the uninjured liver while Lgr51/oval cells have only been reported after injury.

Other models of Adult stem cells: Neuronal stem cells

Neural stem cells (NSCs) generate neurons throughout life in the mammalian hippocampus. However the potential for long-term self-renewal of individual NSCs within the adult brain has been controversial.

By intravital imaging (2-photon microscopy) of NSCs and their progeny:

- A population of **Gli1**-targeted NSCs was identified showing long-term self-renewal in the adult hippocampus

⇒ Identify long-term self-renewing NSCs that contribute to the **generation of new neurons in the adult** hippocampus.

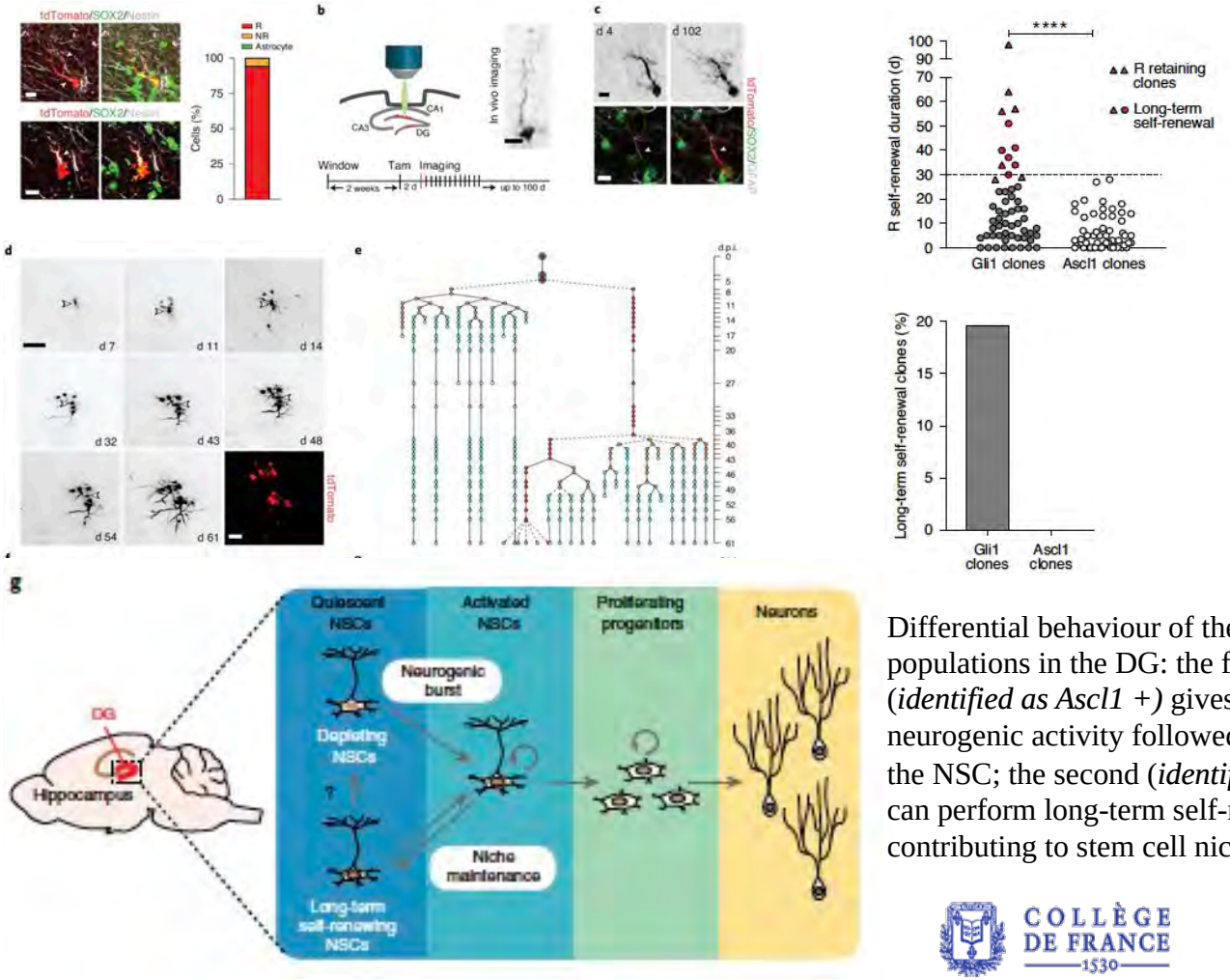
- In contrast, **Ascl1**-targeted NSC, once activated, undergo l**imited proliferative activity** before they become exhausted.

Single-cell RNA sequencing: Gli1- and Ascl1-targeted cells have highly similar yet distinct transcriptional profiles, supporting the existence of **heterogeneous NSC populations with diverse behavioural properties.**

Impact of cellular history/memory on capacities of these adult NSCs?

Mechanisms that regulate neural stem cells (NSCs) during aging?

E. Heard, 22 mars, 2021



Differential behaviour of the two NSC populations in the DG: the first one (*identified as Ascl1 +)* gives rise to a burst of neurogenic activity followed by depletion of the NSC; the second (*identified as GliI +)* can perform long-term self-renewal contributing to stem cell niche maintenance.

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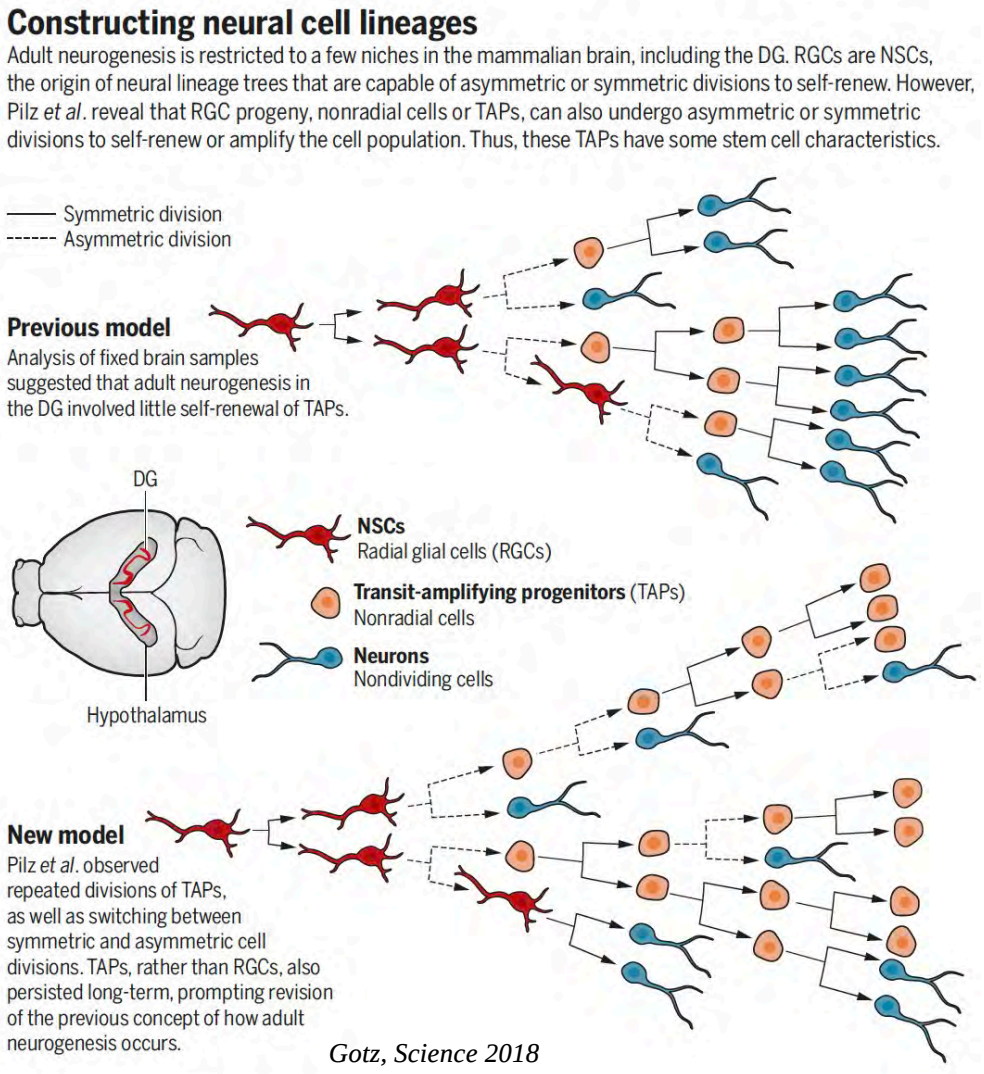
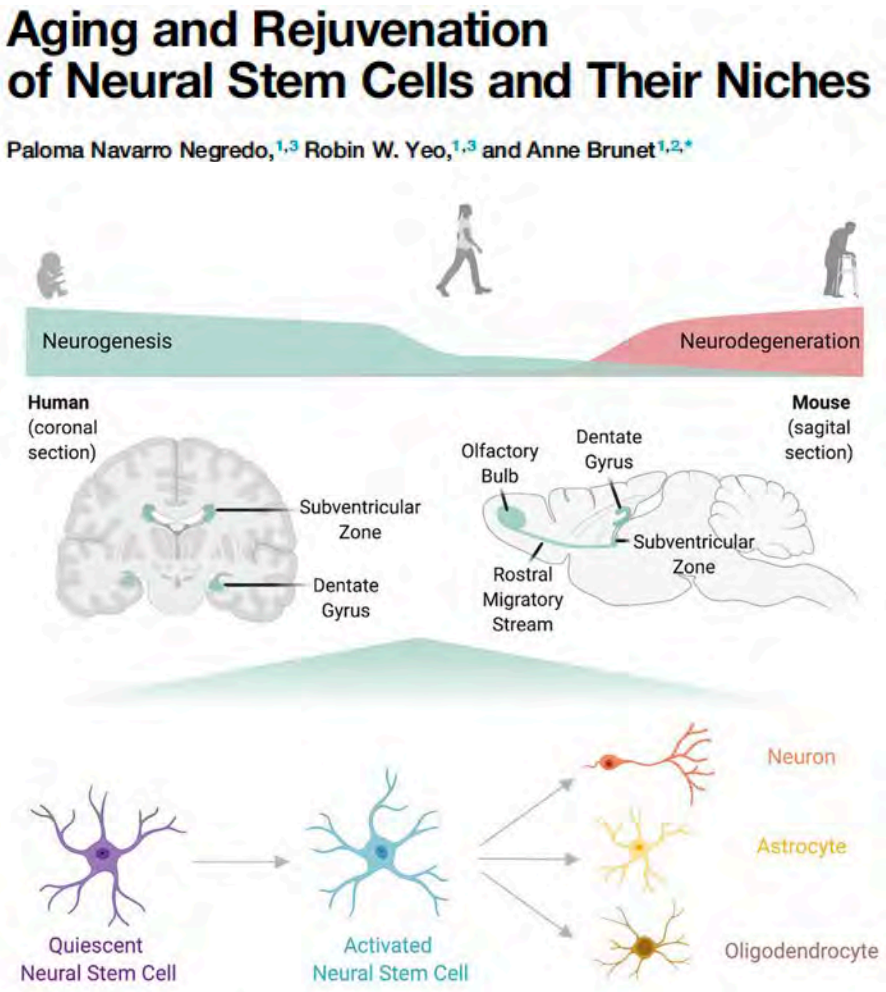
New model for Neuronal stem cell Lineages



Gotz, Science 2018
(News and Viewas about Pilz et al, Science 2018)

Neural Stem Cell Aging



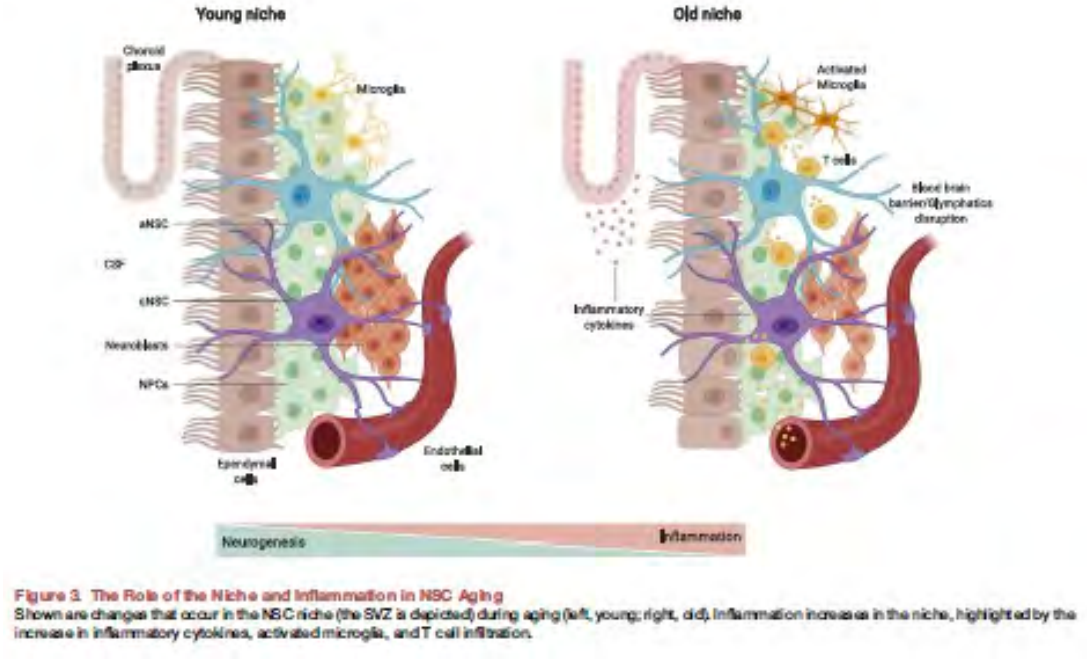
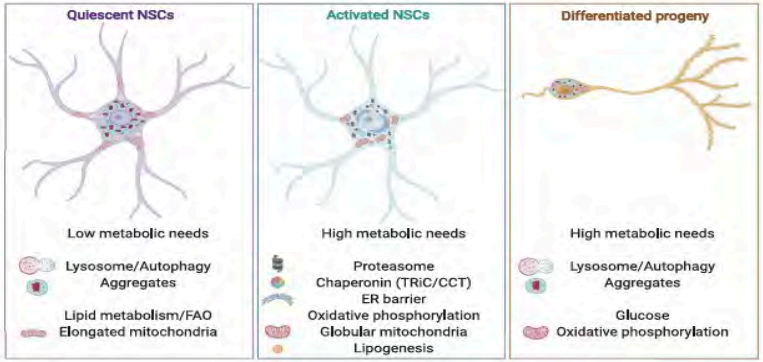


Figure 3. The Role of the Niche and Inflammation in NSC Aging
Shown are changes that occur in the NSC niche (the SVZ is depicted) during aging (left, young; right, old). Inflammation increases in the niche, highlighted by the increase in inflammatory cytokines, activated microglia, and T cell infiltration.



E. Heard, 22 mars, 2021

SUMMARY

Cell Memory in non-dividing cells

Somatic cells and proteins can be extremely long-lived in adults:

- Implications for protein and tissue homeostasis – and pathologies (next week)

Hallmarks of quiescence

- Scarcity and dynamics, need to capture SC in their tissue and niche
- New tools to identify quiescent and activated stem cells
- Live cell imaging and genetic marking
- Combined with single cell approaches

Stem cell hierarchies: classic versus modern

- Stem cell function may—in some tissues—be embodied in HSC-like, hardwired, professional stem cells.
- However in solid tissues it may be executed in a diffuse fashion by much larger populations of undifferentiated cells
- Or by facultative stem cells: proliferative, undifferentiated cells that are opportunistically recruited from committed
- Or even from fully differentiated cellular compartments upon tissue damage eg liver

Stem cells and tissue homeostasis

Stem cell memory

Ageing and stem cells

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

COURS 1 (lundi 1er mars 10h-12h)
Introduction

COURS 2 (lundi 8 mars 10h-12h)
Stabilité et plasticité au cours du développement
Stability and plasticity during embryonic development

COURS 3 (lundi 15 mars 10h-12h)
Maintien de l'identité cellulaire dans les cellules non-prolifératives
Maintaining cellular identity in non-dividing cells

COURS 4 (lundi 22 mars 10h-12h)
Stabilité génétique et épigénétique au cours du vieillissement
Genetic and epigenetic stability during ageing

COURS 5 (lundi 29 juin 10h-12h)
Perte d'idéntité cellulaire au cours de la reprogrammation et dans des pathologies
Losing cellular identity during reprogramming and in disease