Denis Duboule

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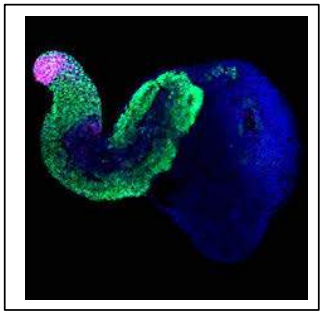

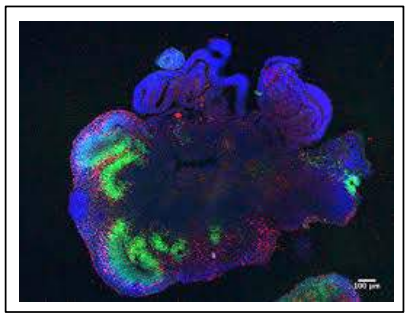

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2018-2019
Organoïdes, embryoïdes: de cultures en trois dimensions aux modèles de développement et de pathologie

Cours 3

21 mai 2019

Résumé de l'épisode précédent
Organoïdes humains
(importance potentielle et état des lieux actuels; exemple: rétine)
Organoïdes intestinaux
(histoire, recherche fondamentale, recherche médicale..)

Résumé de l'épisode précédent (2/6)

**Principe d'auto-organisation (Holtfreter, Gierer..)*

**La fabrication des organoïdes (supports, milieux..)*

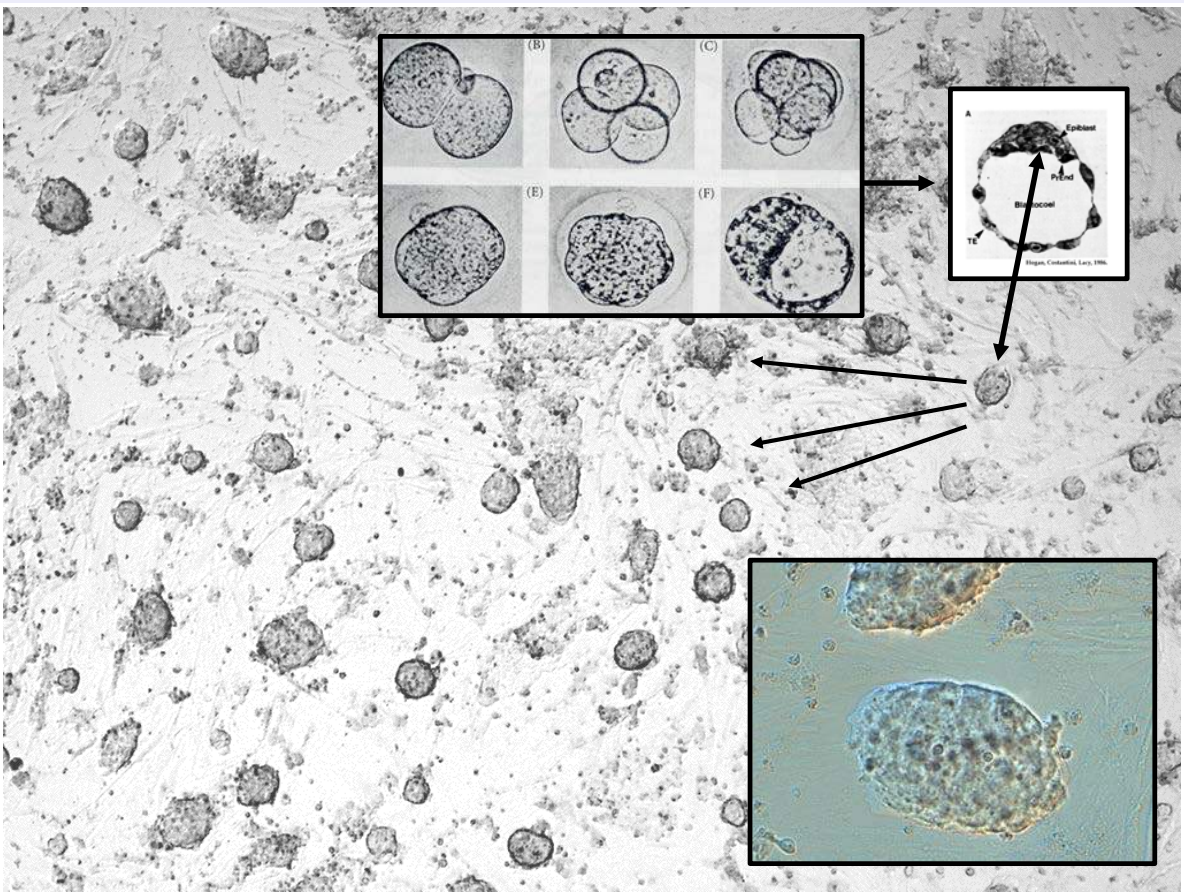
**Différents types de cultures*

**Signaux endogènes et exogènes ('symmetry breaking')*

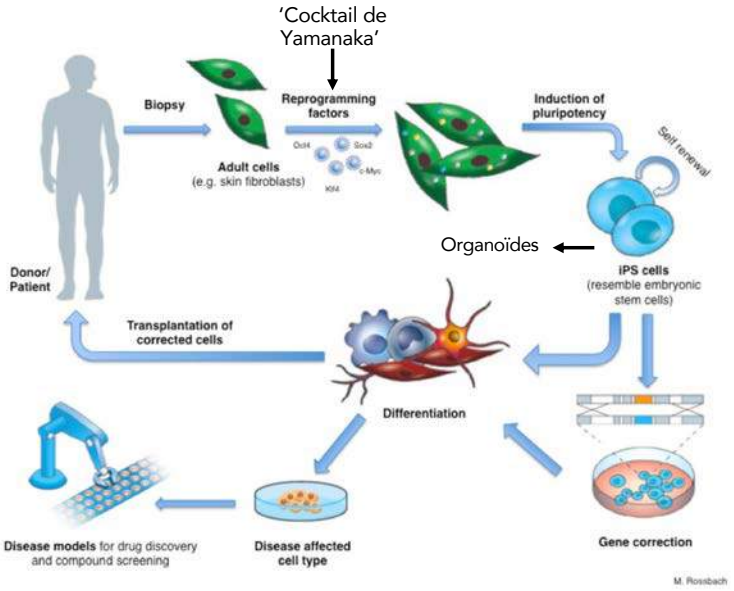
**Origines des cellules (fœtale, pancréas)*

**Cellules ES et iPS*

Cellules souches embryonnaires (ES)



Cellules souches embryonnaires induites (iPS)



https://www.eurostemcell.org/ips-cells-and-reprogramming-turn-any-cell-body-stem-cell

Organoïdes humains

**Importance pour étudier l'embryon humain*
**Transfert potentiel à la clinique*

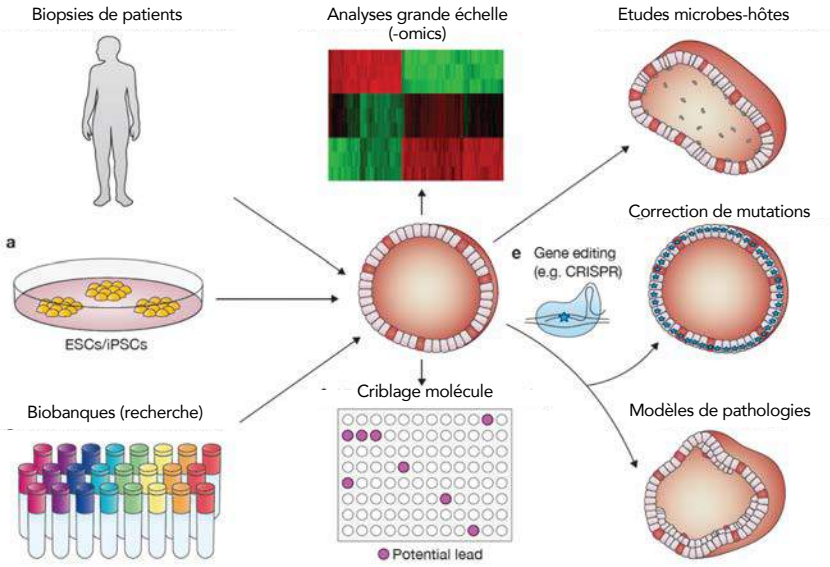
Organoïdes humains

Organoids as an *in vitro* model of human development and disease

Aliya Fatehullah[1], Si Hui Tan[1] and Nick Barker[1,2,3,*]

The *in vitro* organoid model is a major technological breakthrough that has already been established as an essential tool in many basic biology and clinical applications. This near-physiological 3D model facilitates an accurate study of a range of *in vivo* biological processes including tissue renewal, stem cell/niche functions and tissue responses to drugs, mutation or damage. In this Review, we discuss the current achievements, challenges and potential applications of this technique.

SERIES ON STEM CELL BIOLOGY REVIEW



Organoïdes de rétines humaines

RESEARCH

RESEARCH ARTICLE SUMMARY

NEURODEVELOPMENT

Thyroid hormone signaling specifies cone subtypes in human retinal organoids

Kiara C. Eldred, Sarah E. Hadyniak, Katarzyna A. Hussey, Boris Brenerman, Ping-Wu Zhang, Xitiz Chamling, Valentin M. Sluch, Derek S. Welsbie, Samer Hattar, James Taylor, Karl Wahlin, Donald J. Zack, Robert J. Johnston Jr.*

University John Hopkins, Baltimore USA

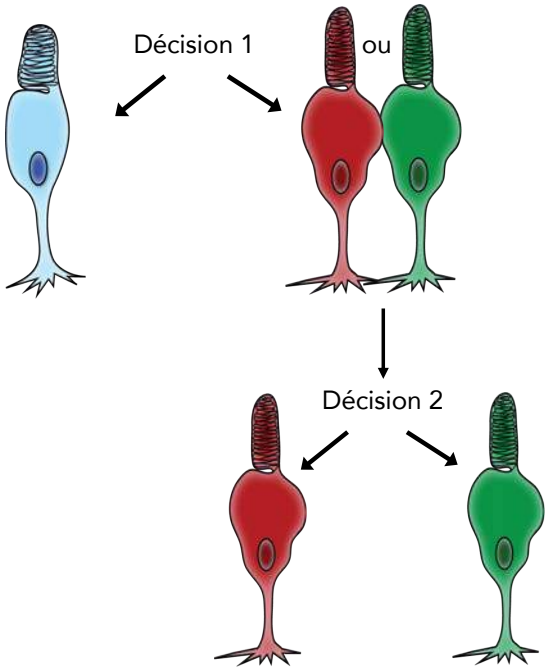
Science
Vol 362, Issue 6411
12 October 2018

*Origine des sous-types de photorécepteurs (cônes) humains

*Les cônes sont situés dans la rétine et transforment le signal électromagnétique de la lumière en signal nerveux. Les cônes permettent une vision diurne en couleurs (vision nocturne par les bâtonnets).

*Il y a trois types de cônes avec des sensibilités spectrales différentes; les cônes S (sensibles à la lumière bleue S-opsin -400nm-), les cônes M (lumière verte, M-opsin -450nm -) et les cônes L (lumière rouge, L-opsin -700nm).

Organoïdes de rétines humaines



*La différenciation des types de cônes se passent en deux étapes distinctes

*Comment étudier ce mécanisme (essentiel pour comprendre de nombreuses pathologies) in vitro?

*Organoïdes de rétines

From: Eldred et al., Science, 362, 12 octobre 2018

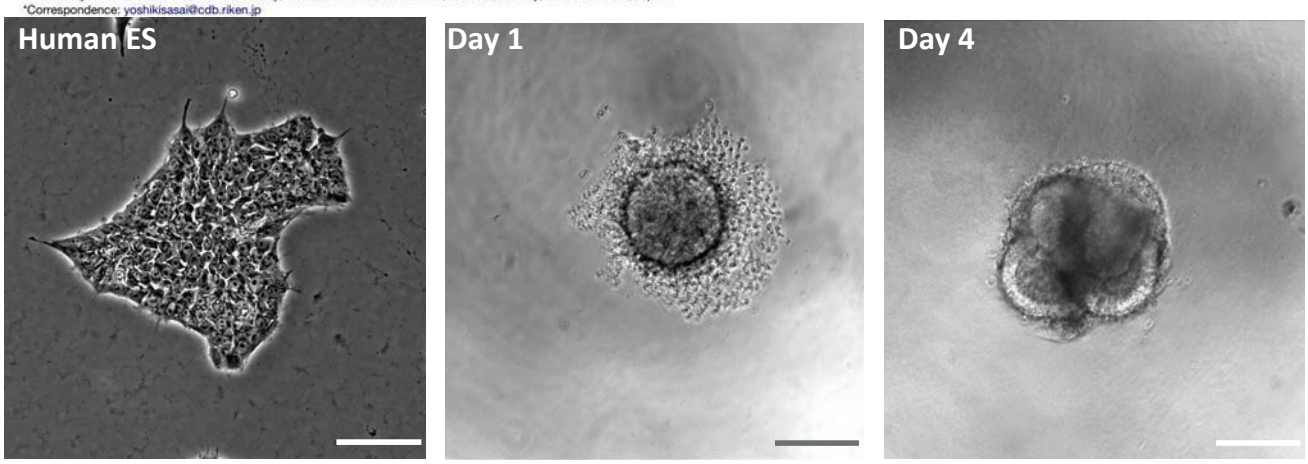
Organoïdes de rétines humaines

Cell Stem Cell
Article

Self-Formation of Optic Cups and Storable Stratified Neural Retina from Human ESCs

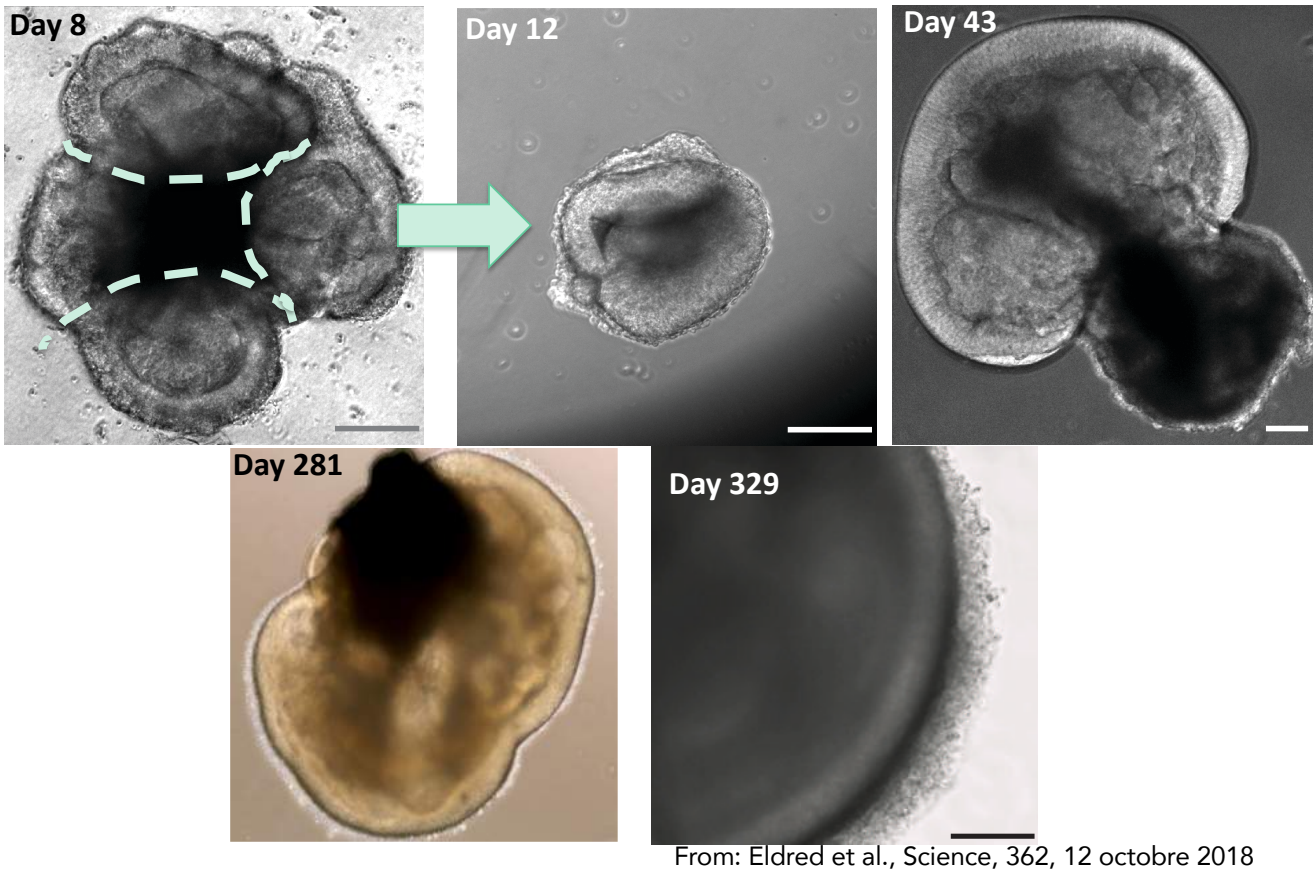
Tokushige Nakano,[1,2,4,5] Satoshi Ando,[1,2,4] Nozomu Takata,[1] Masako Kawada,[1] Keiko Muguruma,[1] Kiyotoshi Sekiguchi,[6] Koichi Saito,[4] Shigenobu Yonemura,[3] Mototsugu Eiraku,[1,2] and Yoshiki Sasai[1,2,5,*]
[1]Organogenesis and Neurogenesis Group
[2]Division of Human Stem Cell Technology
[3]Electron Microscopy Laboratory
RIKEN Center for Developmental Biology, Kobe 650-0047, Japan
[4]Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan
[5]Department of Medical Embryology, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan
[6]Laboratory of Extracellular Matrix Biochemistry, Institute for Protein Research, Osaka University, Suita 565-0871, Japan
*Correspondence: yoshikisasai@cdb.riken.jp

Yoshiki Sasai

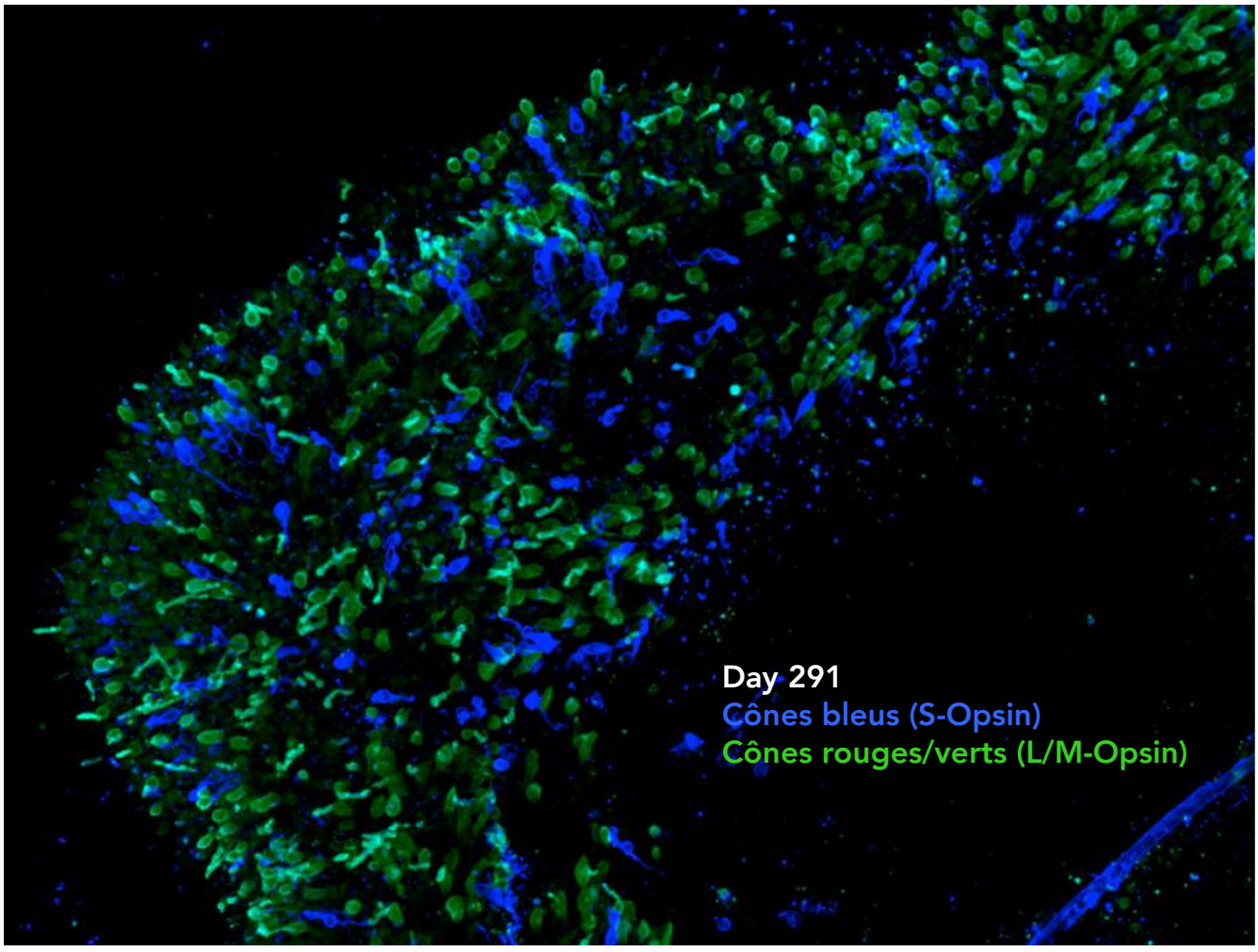


From: Eldred et al., Science, 362, 12 octobre 2018

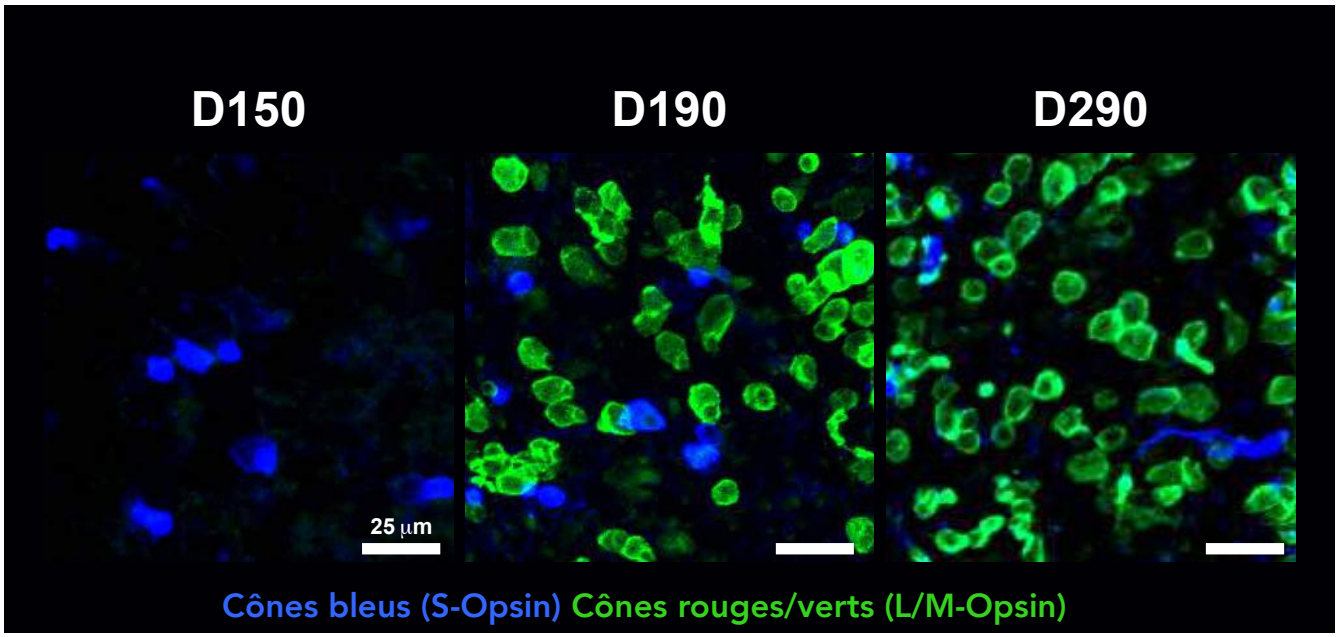
Organoïdes de rétines humaines



From: Eldred et al., Science, 362, 12 octobre 2018



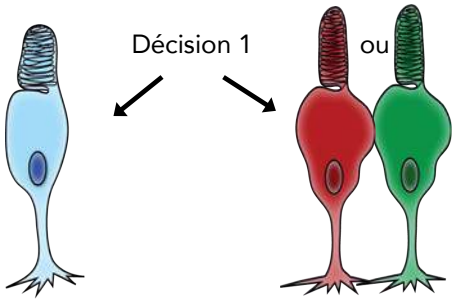
Organoïdes de rétines humaines



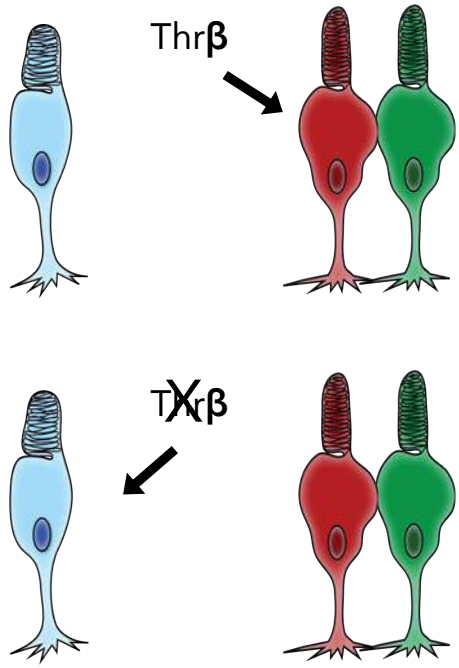
From: Eldred et al., Science, 362, 12 octobre 2018

Organoïdes de rétines humaines

En présence du récepteur nucléaire béta aux hormones thyroïdiennes (*Thrbéta*) la différenciation s'oriente vers les cônes rouges/verts..



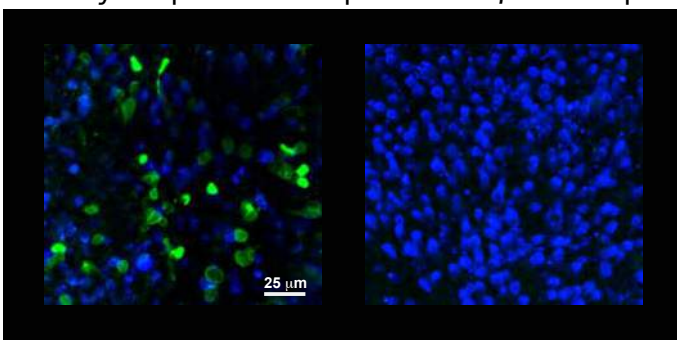
Approche par 'Perte de fonction'
Approche par 'Gain de fonction'

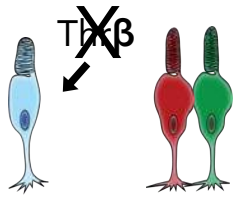


From: Eldred et al., Science, 362, 12 octobre 2018

Organoïdes de rétines humaines *(Eldred et al.Science, 2018)*

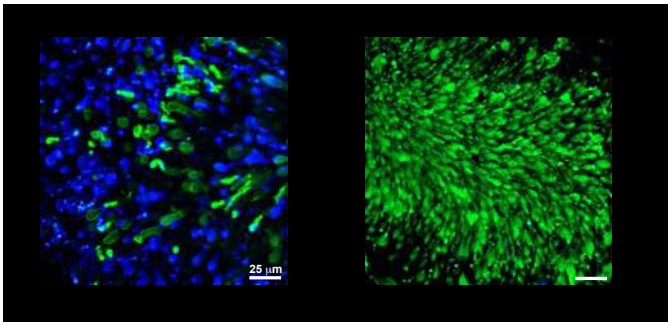
Les organoïdes n'ayant pas de récepteurs Thrβ n'ont que des cones bleus

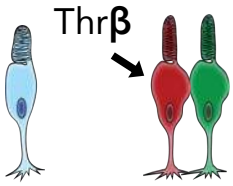




Témoin Thrβ mutant

En présence d'hormone T3 (triiodothyronine) les cônes sont tous rouges/verts





Organoïdes humains

Origine des cellules

Interactions hôte-pathogène

Modèles de pathologies

Cancers

BRAIN
ESC/iPSC organoids
Model for host-pathogen interaction
ZIKA virus
Disease modelling
Patient-derived:
Microcephaly
Autism
Genome engineered:
Aicardi Goutieres syndrome (TREX-1 gene)
Tumor modelling
Patient-derived-biopsies

BREAST
ASC organoids
Tumor modelling
Patient-derived-biopsies

LUNGS
ASCs and ESC/iPSC organoids
Model for host-pathogen interaction
Cryptosporidium
Disease modelling
Patient-derived:
CF (CFTR gene)

KIDNEY
ESC/iPSC organoids
Disease modelling
Genome engineered:
Nephrotic syndrome (PODXL gene)
Polycystic kidney disease (PKD gene)

BLADDER
ASC organoids
Tumor modelling
Patient-derived-biopsies

PROSTATE
ASC organoids
Tumor modelling
Patient-derived-biopsies

EYES
ESC/iPSC organoids
Gene correction
Retinite pigmentosa (RPGR gene)

INNER EAR
ESC organoids

PANCREAS
ASC and iPSC organoids
Tumor modelling
Patient-derived-biopsies

ESOPHAGUS
ASC organoids
Disease modelling
Patient derived:
Barrett's esophagus

STOMACH
ASCs and ESC/iPSC organoids
Model for host-pathogen interaction
H.pylori

INTESTINE and COLON
ASCs and ESC/iPSC organoids
Model for host-pathogen interaction
Rotavirus
Norovirus
Cryptosporidium
Disease modelling
Patient-derived:
CF (CFTR gene)
Intestinal Atresia (TTC7A gene)
Microvillus inclusion disease (STX3 gene)
Tumor modelling
Patient-derived biopsies
Targeted gene mutations
Gene correction
Cystic fibrosis (CFTR gene)
Dyskeratosis congenital (DKC1 gene)
Reporter generation
LGR5
KRT20
ASCL2

OVARY
ASC organoids

LIVER
ASCs and ESC/iPSC organoids
Disease modelling
Patient-derived:
Alagille syndrome
A1AT-deficiency
Genome engineered:
Alagille syndrome (JAG1 gene)
Tumor modelling
Patient-derived-biopsies

Artegiani and Clevers (2018)

Human Molecular Genetics, Volume 27, Issue R2, 01 August 2018, Pages R99–R107, https://doi.org/10.1093/hmg/ddy187

Avantages, limitations, solutions..

Organoids as an *in vitro* model of human development and disease

Aliya Fatehullah[1], Si Hui Tan[1] and Nick Barker[1,2,3,*]

The *in vitro* organoid model is a major technological breakthrough that has already been established as an essential tool in many basic biology and clinical applications. This near-physiological 3D model facilitates an accurate study of a range of *in vivo* biological processes including tissue renewal, stem cell/niche functions and tissue responses to drugs, mutation or damage. In this Review, we discuss the current achievements, challenges and potential applications of this technique.

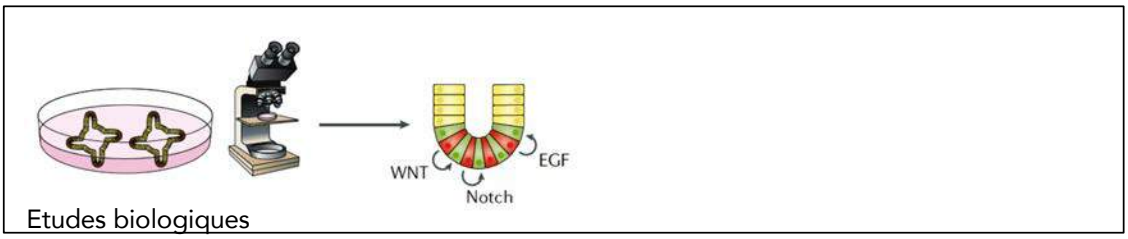
SERIES ON STEM CELL BIOLOGY REVIEW

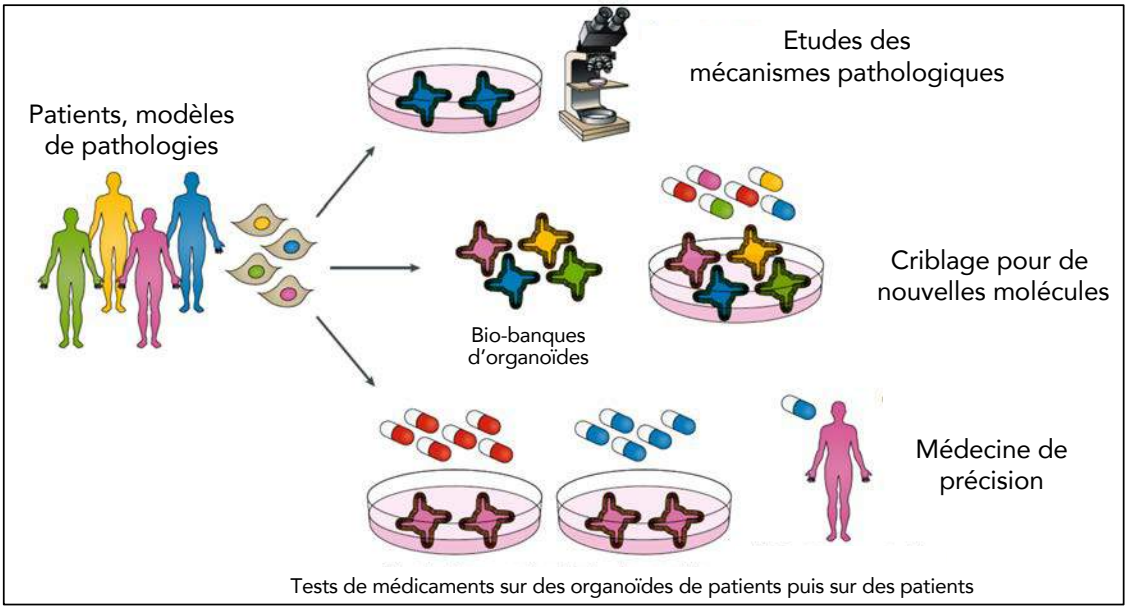
Table 1 Advantages, limitations and possible solutions to challenges faced with organoid cultures

Advantages
Near-physiological model system for studying adult stem cells and tissues in a variety of contexts
Adult stem cells can be propagated in organoids, and specific tissue lineages can be cultured in high purity with minimal contributions from other cell types (for example, fibroblasts and endothelial cells)
Can be propagated for a long time (years) without genomic alterations
Amenable to a wide variety of established experimental techniques
Can be derived from multiple sources: adult and foetal tissues, ESCs and iPSCs
Can generate organoids encompassing a broad range of tissues
Limited amounts of starting material can be expanded for numerous applications
Human diseases that are difficult to model in animals can be studied with patient-derived organoids
Possibility of generating isogenic adult tissue for transplantation in regenerative procedures

Limitations	Possible solution(s)
Lack of native microenvironment precludes studies about interaction of stem cells with their niches, immune cells, etc.	Complement with organotypic culture system, or co-culture with other cell types such as stromal cells[15] or immune cells
Limited use in modelling inflammatory responses to infection or drugs due to absence of immune cells in culture system	
Unable to mimic *in vivo* growth factor/signalling gradients in Matrigel matrix	Apply microfluidic technologies to generate concentration gradients
Unable to mimic biomechanical forces that stem cells encounter *in vivo*	Novel substrates and ECM factors are being identified to model such interactions *in vitro*[50–52]
Relatively rigid ECM could limit drug penetration, hence hampering the use of organoids in drug screens	Devise ways to vary physical attributes of ECM such as composition, porosity and stiffness
Challenging to culture organoids from tissues whose niche factors are not well understood (for example, the ovary)	Screen for small-molecule modulators of key signalling pathways and specific hormones as potential culture components
Organoids in the same culture are heterogeneous in terms of viability, size and shape, impeding phenotype screens	Organoids can be tracked individually by live or time-lapse imaging
Organoid cultures depend on mouse-sarcoma-derived Matrigel, which precludes transplantation of organoids into humans	More defined ECMs that support organoid growth are being developed to comply with regulations for transplantation into humans

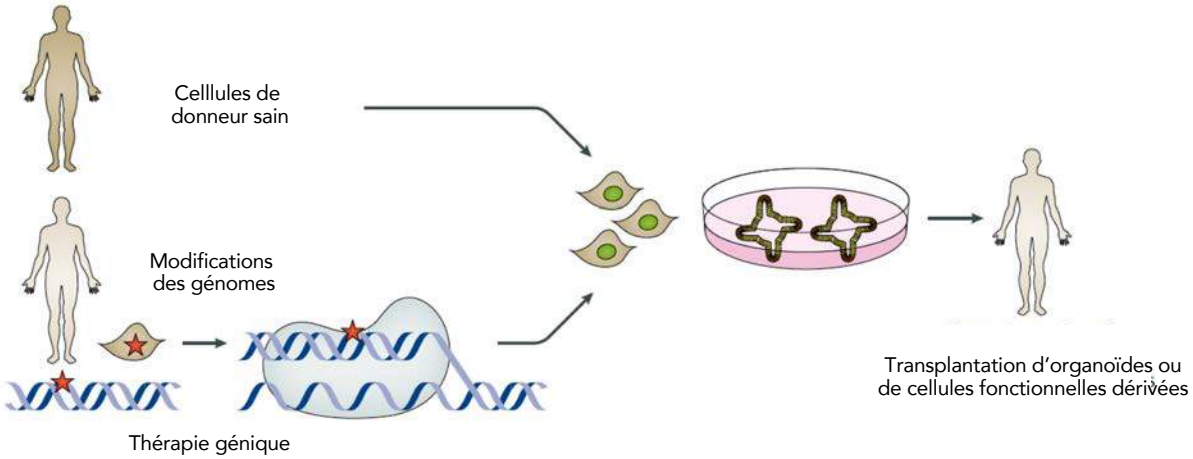
Organoïdes: Potentiel thérapeutique





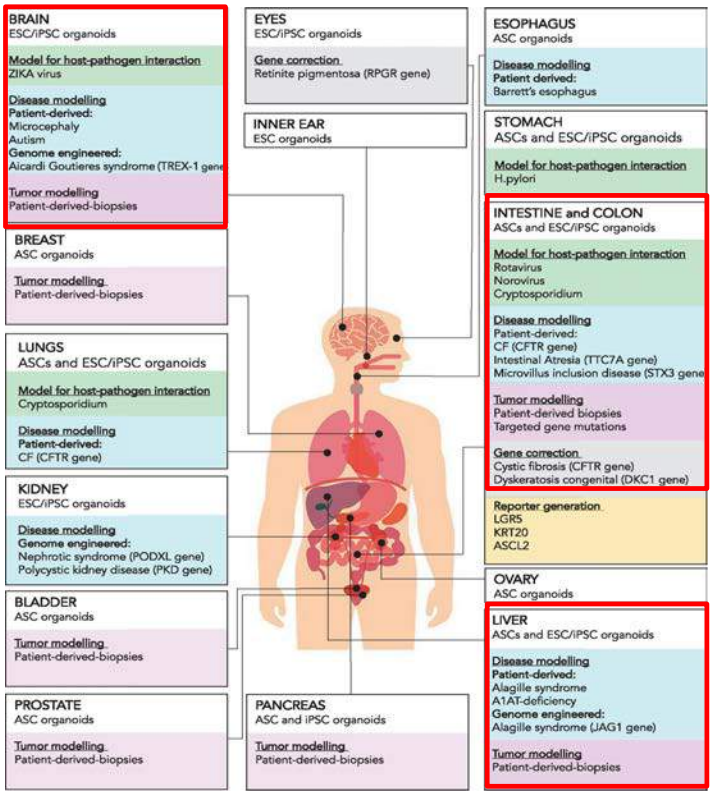
Organoïdes: Potentiel thérapeutique

Médecine régénérative



Organoïdes: Recherches récentes

Organoïdes
de cerveau



Organoïdes
intestinaux

Organoïdes
hépatiques

Artegiani and Clevers (2018)

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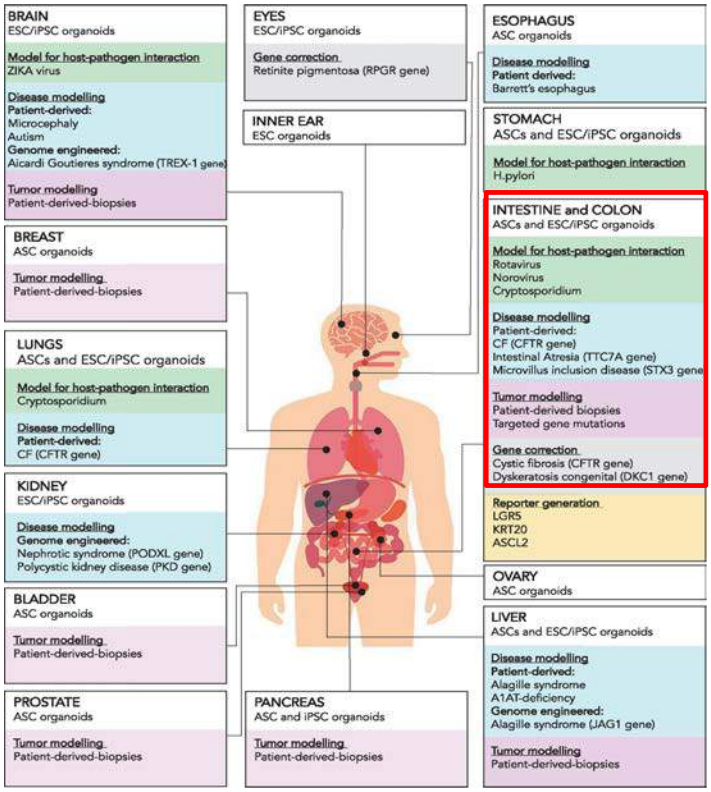
Organoïdes: Recherches récentes



Organoïdes intestinaux

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OXFORD UNIVERSITY PRESS

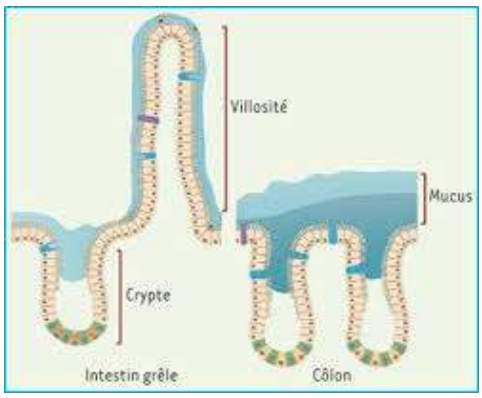
Organoïdes

Organoïdes intestinaux

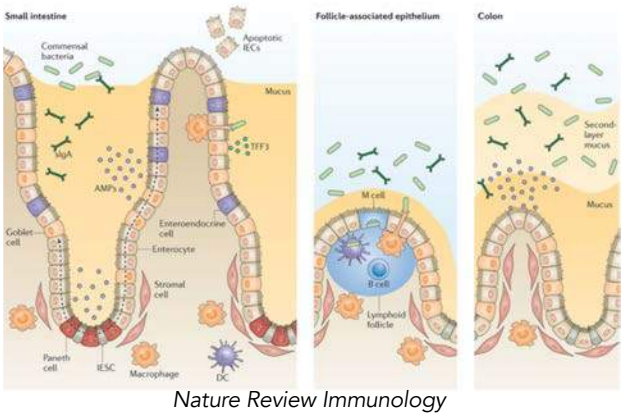
**Origine*
**Utilisation en recherche fondamentale*
**Utilisation en recherche médicale*

Organoïdes intestinaux

Structure de l'épithélium intestinal



https://www.irsd.fr/cellules-souches-intestinales.html



Nature Review Immunology

Cellules de Paneth: Organisatrices des cryptes

The Intestinal Crypt, A Prototype Stem Cell Compartment

Hans Clevers[1,2,*]
[1]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, 3584 CT Utrecht, the Netherlands
[2]University Medical Center, 3584 CX Utrecht, the Netherlands
*Correspondence: h.clevers@hubrecht.eu
http://dx.doi.org/10.1016/j.cell.2013.07.004

Cell, 2013

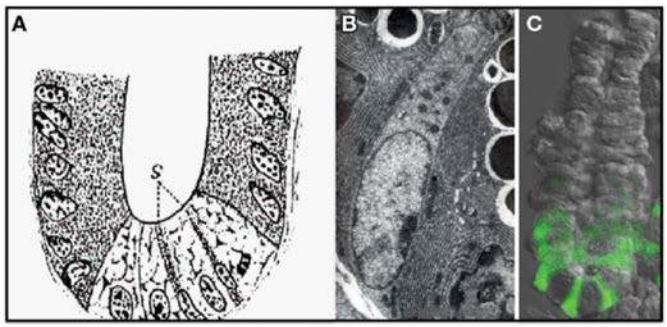


Figure 3. Paneth Cells and Crypt Base Columnar Cells
(A) Hand-drawn crypt (Paneth, 1887). The large white cells are Paneth cells. s, "schmale Zellen" (small/narrow cells).
(B) First electro-microscopic image of a crypt base columnar (CBC) cell, flanked by two Paneth cells with large black granules (from Cheng and Leblond, 1974a).
(C) Confocal image of Lgr5-GFP CBC cells in green, separated by dark, large Paneth cells.

**'Crypt base columnar cells' (CBC) détectées par EM en 1974*

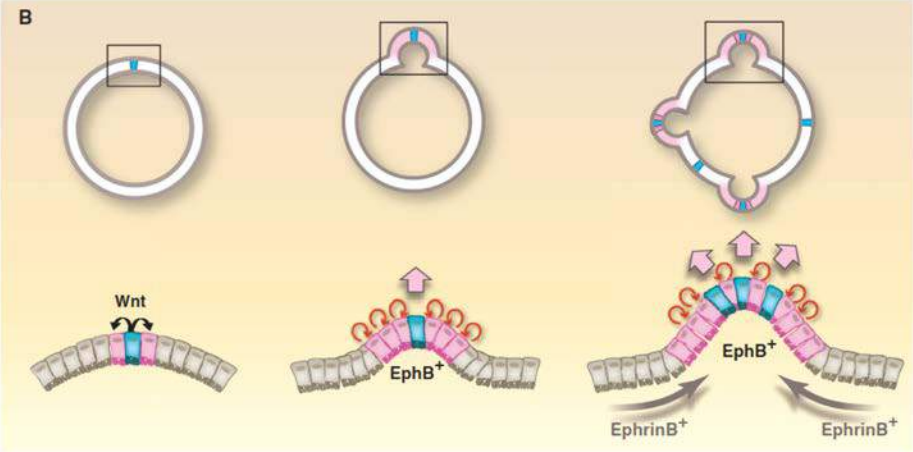
**Cellules CBC sont positives pour LGR5*

**Les cellules LGR5⁺ sont détectées pendant toute la vie de la souris et donnent toutes les cellules de l'intestin. Ce sont des 'cellules souches'*

**App 15 cellules LGR5 par crypte. Division en 24 heures.*

Cellules de Paneth: Organisatrices des cryptes

Initiation et développement des cryptes; cellules de Paneth (*Wnt*+)



Sato and Clevers, Science, 2013

Wnt ⟶ *EphB* ⊣ *Ephrin*

↓

Bourgeonnement et formation des cryptes

Cellules de Paneth: Organisatrices des cryptes

**Wnt, EGF et Delta proviennent des cellules de Paneth.*

**BMP a une action Inhibitrice*

**LGR4/5 signal est nécessaire pour l'activation par Wnt*

Morphogenesis

REVIEW

Growing Self-Organizing Mini-Guts from a Single Intestinal Stem Cell: Mechanism and Applications

Toshiro Sato[1*] and Hans Clevers[2*]

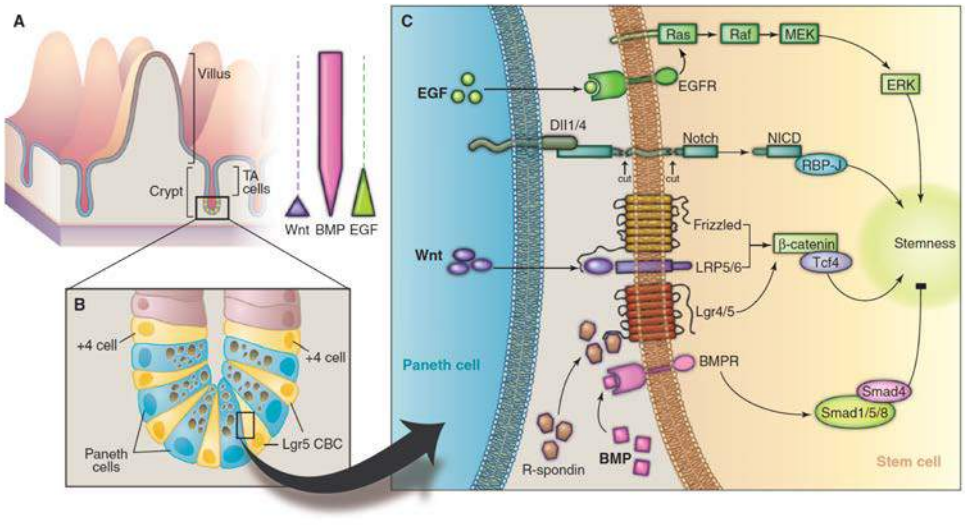


Fig. 1. Histological location and biological interaction of intestinal stem cells and their niche. (**A**) Scheme of intestinal epithelial structure and stem cells. Spatial gradients of Wnt, BMP, and EGF signals are formed along the crypt axis. (**B**) Cartoon of the stem cell niche. Lgr5$^+$ intestinal CBC cells intimately adhere to Paneth cells and receive signals for stem cell maintenance. (**C**) Three signals (EGF, Notch, and Wnt) are essential for intestinal epithelial stemness, whereas BMP negatively regulates stemness. For full Wnt activation in the intestinal epithelium, R-spondin–Lgr4/5 signal is required. Currently, the source of R-spondin is unknown.

Organoïdes intestinaux (étude originale)

nature Vol 459|14 May 2009 doi:10.1038/nature07935

LETTERS

Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

Toshiro Sato[1], Robert G. Vries[1], Hugo J. Snippert[1], Marc van de Wetering[1], Nick Barker[1], Daniel E. Stange[1], Johan H. van Es[1], Arie Abo[2], Pekka Kujala[3], Peter J. Peters[3] & Hans Clevers[1]

Figure 1

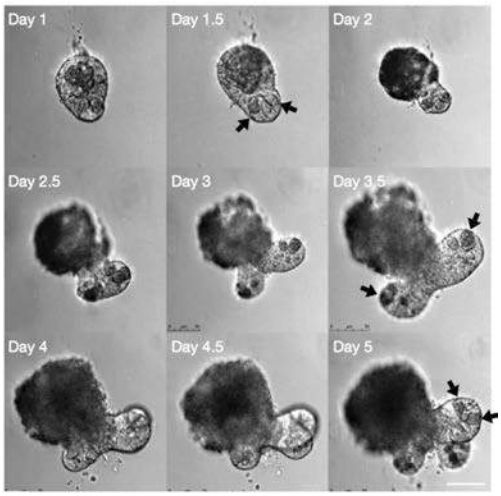
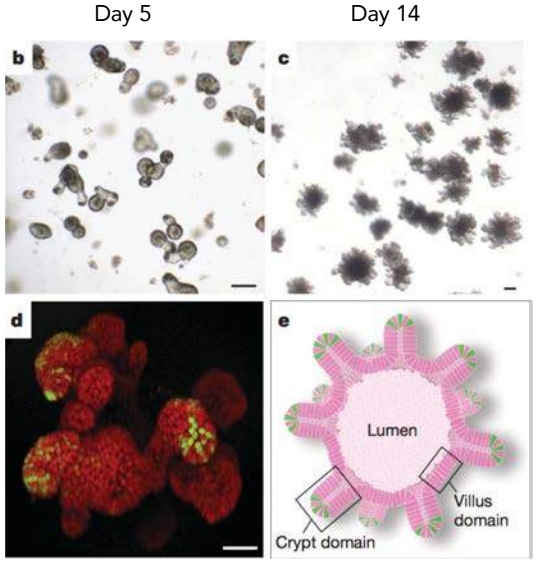


Figure 1 | Establishment of intestinal crypt culture system. a, Time course of an isolated single crypt growth. Differential interference contrast image reveals granule-containing Paneth cells at crypt bottoms (arrows).



. b, c, Single isolated crypts efficiently form large crypt organoids within 14 days; **b,** on day 5; **c,** on day 14. **d,** Three-dimensional reconstructed confocal image after 3 weeks in culture. Lgr5–GFP^{+} stem cells (green) are localized at the tip of crypt-like domains. Counterstain, ToPro-3 (red). **e,** Schematic representation of a crypt organoid, consisting of a central lumen lined by villus-like epithelium and several surrounding crypt-like domains. Scale bar, 50 μm.

Organoïdes intestinaux (étude originale)

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LETTERS

Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

Toshiro Sato[1], Robert G. Vries[1], Hugo J. Snippert[1], Marc van de Wetering[1], Nick Barker[1], Daniel E. Stange[1], Johan H. van Es[1], Arie Abo[2], Pekka Kujala[3], Peter J. Peters[3] & Hans Clevers[1]

Figure 2

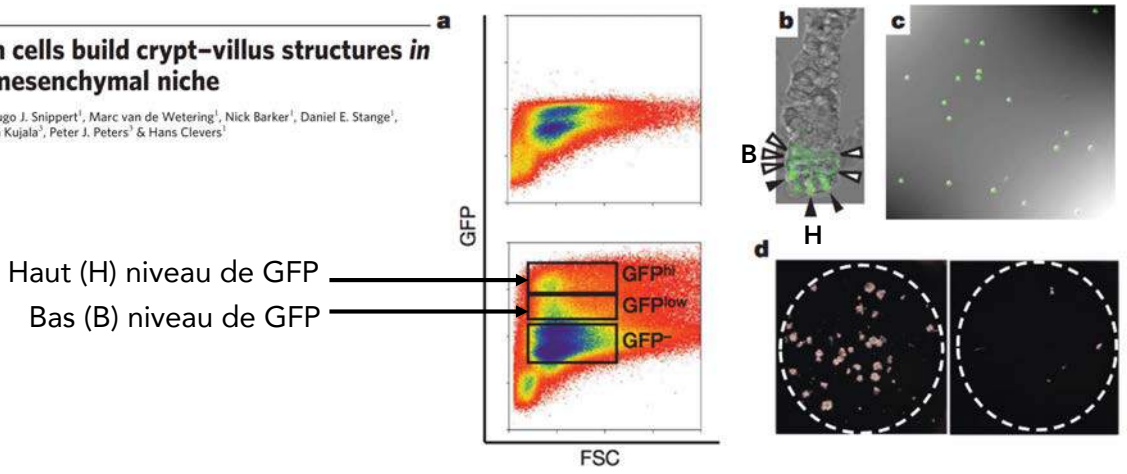
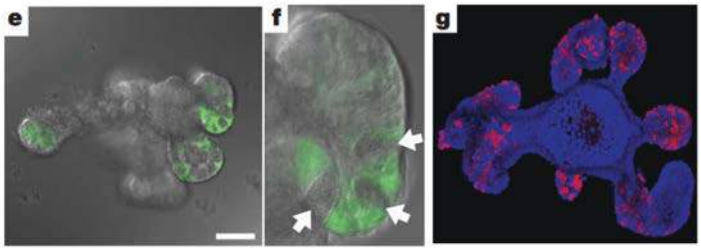


Figure 2 | Single Lgr5^{+} cells generate crypt–villus structures. **a,** Lgr5–GFP^{+} cells from an *Lgr5–EGFP–ires–CreERT2* intestine (bottom); wild-type cells (top). Two positive populations, GFP^{hi} and GFP^{low}, are discriminated. FSC, forward scatter. **b,** Confocal analysis of a freshly isolated crypt. Black arrowheads, GFP^{hi}; white arrowheads, GFP^{low}. **c,** Sorted GFP^{hi} cells. **d,** 1,000 sorted GFP^{hi} cells (left) and GFP^{low} cells (right) after 14 days in culture. **e, f,** Fourteen days after sorting, single GFP^{hi} cells form crypt organoids, with Lgr5–GFP^{+} cells and Paneth cells (white arrows) located at crypt bottoms. Scale bar, 50 μm. **f,** Higher magnification of **e. g,** Organoids cultured with the thymidine analogue EdU (red) for 1 h. Note that only crypt domains incorporate EdU. Counterstain, 4,6-diamidino-2-phenylindole (DAPI; blue).



14 jours en culture de cellules uniques EdU

Organoïdes intestinaux (étude originale)

nature Vol 459|14 May 2009|doi:10.1038/nature07935

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Figure 3

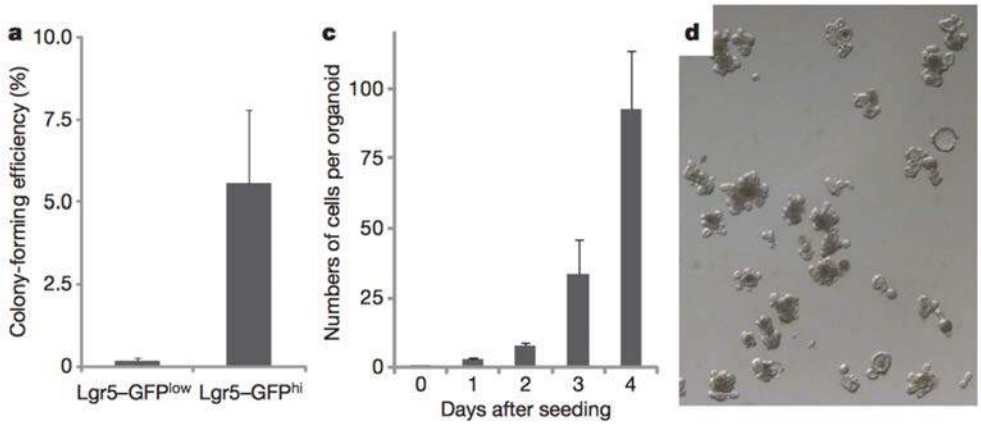

Figure 3 | Colony-forming efficiency of single cells sorted in individual wells. **a**, Colony-forming efficiency was calculated from 100 single sorted GFP^{hi} cells. **b**, An example of a successfully growing single GFP^{hi} cell. Numbers above the images are the days of growth. **c**, Numbers of cells per single organoid averaged for five growing organoids. **d**, A single-cell suspension derived from a single-cell-derived-organoid was replated and grown for 2 weeks. Error bars in **c** and **d** indicate s.e.m. Original magnifications in **b**: days 0–4, ×40; days 5–7, ×20; days 8–11, ×10; days 12 and 13, ×4.

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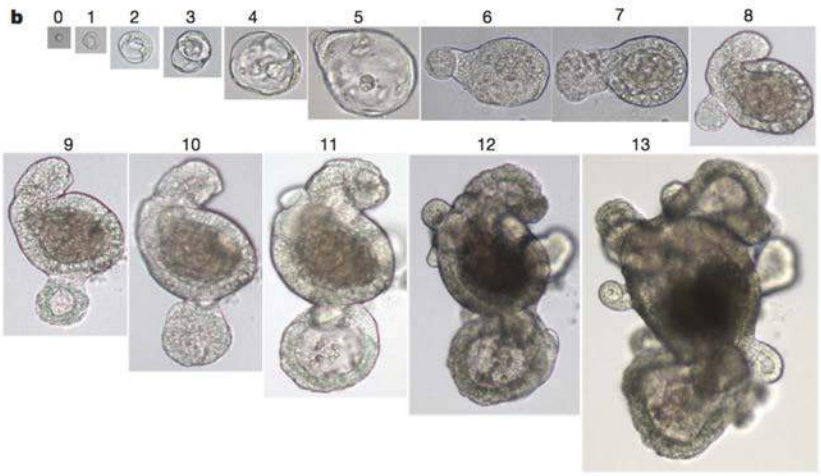

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Organoïdes intestinaux (*étude originale*)

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Présence de la plupart des types cellulaires dans les organoïdes intestinaux

Figure 4

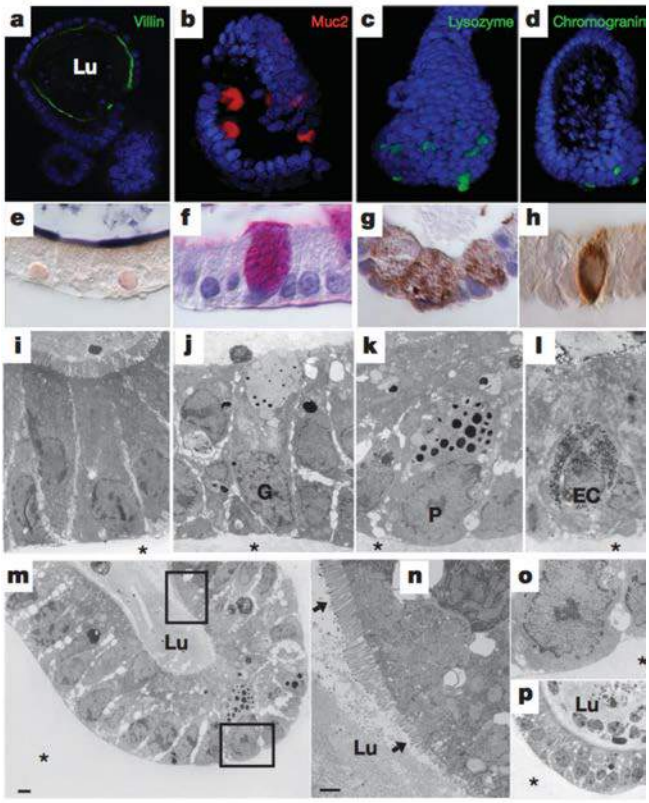


Figure 4 | Composition of single stem cell-derived organoids. **a–d**, Confocal image for villin (**a**, green, enterocytes), Muc2 (**b**, red, goblet cells), lysozyme (**c**, green, Paneth cells) and chromogranin A (**d**, green, enteroendocrine cells). Counterstain, DAPI (blue). **e–h**, Paraffin sections stained for alkaline phosphatase (**e**, green, enterocytes), periodic acid-Schiff (**f**, red, goblet cells), lysozyme (**g**, brown, Paneth cells) and synaptophysin (**h**, brown, enteroendocrine cells). **i–p**, Electron microscopy demonstrates enterocytes (**i**), goblet cells (**j**), Paneth cells (**k**) and enteroendocrine cells (**l**). **m–o**, Low-power crypt images. **n**, **o**, Higher magnifications of **m**. **n**, Maturation of brush border (black arrows). **p**, Low-power villus domain image. Lu, lumen with apoptotic bodies, lined by polarized enterocytes. G, goblet cells; EC, enteroendocrine cells; P, Paneth cells; *, Matrigel. Scale bars, 5 μm (m, p) and 1 μm (n, o).

Organoïdes intestinaux (*étude originale*)

Sato et al., 2009 Nature

Noggin antagonise la signalisation par BMP

Jagged-1 est un ligand de *Notch*

Y-27632 est un Inhibiteur de la *Rho*-associated kinase (ROCK, régulatrice du cytosquelette et de l'actine etc)

METHODS SUMMARY

Mice. Outbred mice 6–12 weeks old were used. Generation and genotyping of the *Lgr5–EGFP–Ires–CreERT2* allele[1] has been described previously[3]. Rosa26–lacZ or YFP–Cre reporter mice were obtained from Jackson Labs.

Crypt isolation, cell dissociation and cell culture. Crypts were released from murine small intestine by incubation for 30 min at 4 °C in PBS containing 2 mM EDTA (Supplementary Methods). Isolated crypts were counted and pelleted. A total of 500 crypts were mixed with 50 μl of Matrigel (BD Bioscience) and plated in 24-well plates. After polymerization of Matrigel, 500 μl of crypt culture medium (Advanced DMEM/F12 (Invitrogen)) containing growth factors (10–50 ng ml^{-1} EGF (Peprotech), 500 ng ml^{-1} R-spondin 1 (ref. 11) and 100 ng ml^{-1} Noggin (Peprotech)) was added. For sorting experiments, isolated crypts were incubated in culture medium for 45 min at 37 °C, followed by trituration with a glass pipette. Dissociated cells were passed through cell strainer with a pore size of 20 μm. GFP^{hi}, GFP^{low} and GFP^{-} cells were sorted by flow cytometry (MoFlo; Dako). Single viable epithelial cells were gated by forward scatter, side scatter and pulse-width parameter, and by negative staining for propidium iodide. Sorted cells were collected in crypt culture medium and embedded in Matrigel containing Jagged-1 peptide (1 μM; AnaSpec) at 1 cell per well (in 96-well plates, 5 μl Matrigel). Crypt culture medium (250 μl for 48-well plates, 100 μl for 96-well plates) containing Y-27632 (10 μM) was overlaid. Growth factors were added every other day and the entire medium was changed every 4 days. For passage, organoids were removed from Matrigel and mechanically dissociated into single-crypt domains, and then transferred to fresh Matrigel. Passage was performed every 1–2 weeks with a 1:5 split ratio.

Organoïdes intestinaux (*Résumé et usage dans la régénération*)

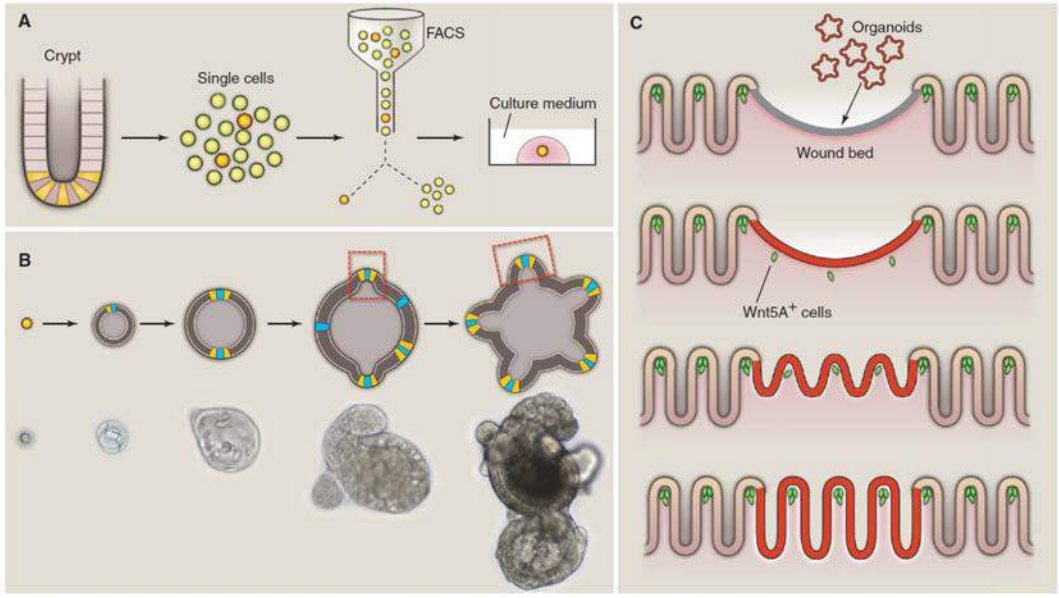


Fig. 2. Mini-gut culture system. (**A**) Lgr5$^+$ CBC cells genetically labeled by EGFP are sorted and embedded in Matrigel. The culture medium consists of EGF, Noggin, and R-spondin. FACS, fluorescence-activated cell sorting. (**B**) Time course of organoid growth. A single stem cell forms a symmetric cyst structure. The symmetry is broken by bud formation. The budding structure resembles a crypt. Lgr5$^+$ CBC cells are depicted in yellow, and Paneth cells are shown in blue. (**C**) Scheme showing the engraftment of intestinal oganoids. Organoids adhere to a de-epithelialized wound bed. Organoids form a flat epithelial layer, followed by crypt reconstruction. Wnt-5A$^+$ mesenchymal cells support crypt structure formation in the damaged area. Donor organoids are depicted in red.

www.sciencemag.org **SCIENCE** VOL 340 7 JUNE 2013

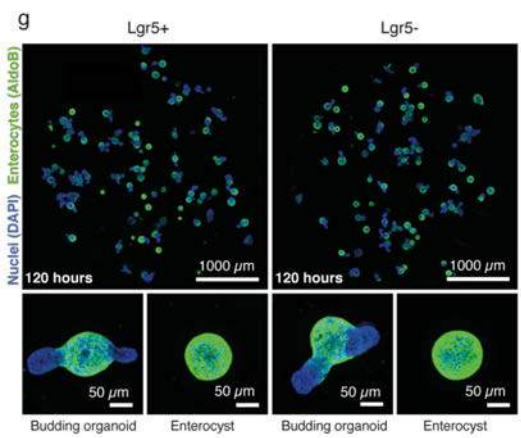
Organoïdes intestinaux (*dernières avancées*)

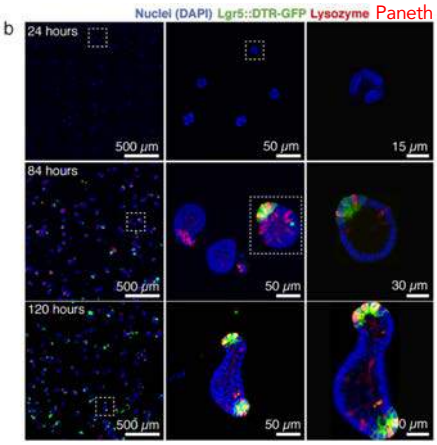
''*Self-organization and symmetry breaking in intestinal organoid development*''
Serra, Mayr, Boni, ….and Prisca Liberali (Institut FM, Bâle). Nature, sous presse.

*__En présence de Wnt3A__, les cellules Lgr5$^-$ sont également capables de faire des organoïdes (Van Es et al. *Nat. Cell. Biol.* (2016); Yan et al., *Cell Stem Cell* (2017)

*Pour comparer les organoïdes: production d'organoïdes Lgr5$^+$ après triage GFP et production d'organoïdes Lgr5$^-$ dérivés de souris Lgr5::DTR-EGFP (les cellules exprimant La DTR -récepteur à la toxine diphtérique- sous le contrôle du promoteur LGR5 meurent en présence de la toxine).

*Imagerie automatique de milliers d'organoïdes avec des algorithmes appropriés.





Organoïdes intestinaux *(dernières avancées)*

''*Self-organization and symmetry breaking in intestinal organoid development*''
Serra, Mayr, Boni, ….and Prisca Liberali (Institut FM, Bâle). Sous presse.

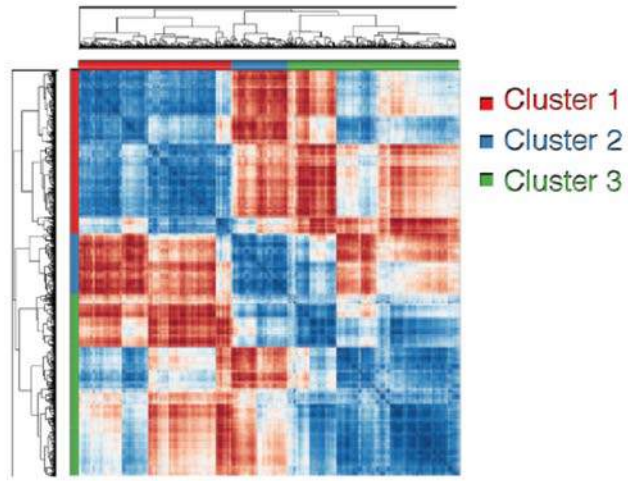
*Un événement d'interruption de symétrie se passe dans les premiers 3 jours avec la différentiation de cellules de Paneth (*Wnt*). En absence de ce processus, l'organoïde se développe en 'entérocyste'.

*Séquences des ARNs des cellules uniques après triage dans les deux cas: 3 clusters

*Cluster 1: Expression ↘ jusqu'à D3 puis ↗. Enrichi en RNAs marqueurs souche (re-programming?) puis diff.

*Cluster 2: Expression ↗ jusqu'à D3 puis ↘. Enrichi en RNAs ECM, cytosquelette, cycle cellulaire (croissance)

*Cluster 3: Expression ↗. Enrichi en RNAs enzymes, métabolisme



Organoïdes intestinaux *(dernières avancées)*

''*Self-organization and symmetry breaking in intestinal organoid development*''
Serra, Mayr, Boni, ….and Prisca Liberali (Institut FM, Bâle). Sous presse.

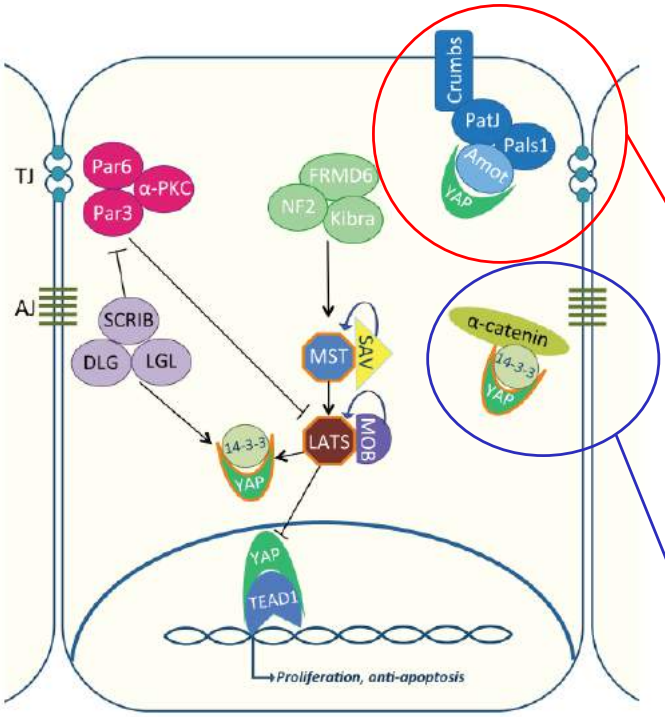
Pour cibler les évènements précoces, collection des promoteurs des gènes qui augmentent leurs ARNs à un jour de développement et examen des séquences répondantes à des facteurs de transcription (quels sont les régulateurs?)

Motifs prépondérants: Liaisons à FOS-like 1 et aux facteurs à domaine TEA (Tead1) et Tead4), qui tous nécessitent la protéine 'Yes-activated protein (Yap1) comme élément co-activateur.

*Yap1 est une protéine nucléaire mécanosensible qui est un effecteur de la voie de signalisation 'Hippo', une voie qui est impliquée dans la régulation de la croissance des organes, la régénération et le processus tumoral.

*Yap1 est également impliquée dans la régénération de l'intestin en inhibant la différenciation des cryptes (cellules de Paneth..).

La voie de transduction Hippo



*Une chaine d'activation (kinases) phosphoryle YAP qui est retenue dans le cytoplasme

*En absence d'activation, YAP est transloquée dans le noyau, se lie à TEAD1 qui active la transcription de gènes cibles

*Les complexes qui agissent en amont de la voie sont localisés soit aux 'tight junctions' soit aux 'adherens junctions' et sont donc liés à la physiologie de ces jonctions cellulaires

Sarah Al Haddadh
https://archive-ouverte.unige.ch/unige:115409

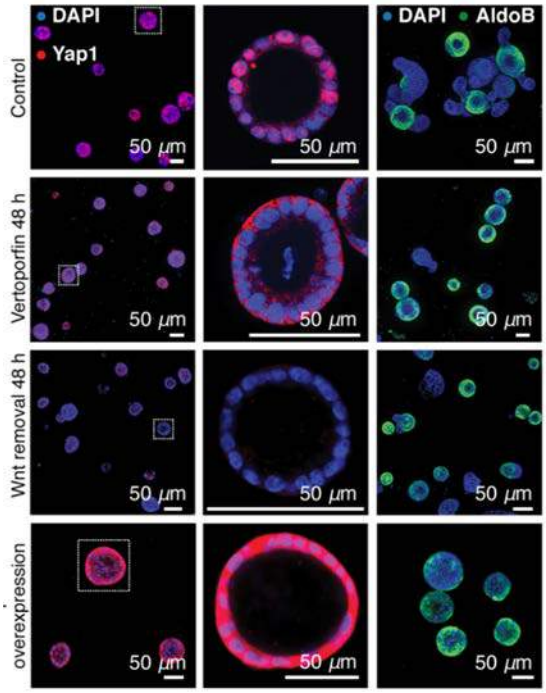
Organoïdes intestinaux (dernières avancées)

''*Self-organization and symmetry breaking in intestinal organoid development*''
Serra, Mayr, Boni, ….and Prisca Liberali (Institut FM, Bâle). Sous presse.

*Yap1 RNA est stable et donc vraisemblablement une différence post-transcription qui rend la protéine active

*Quantité de Yap1 variable au niveau cellulaire

*Inactivation de Yap1 (*vetreprofin*) ou sur-expression de Yap1 donnent une majorité de sphères d'entérocytes (plus de *rupture de symétrie*).



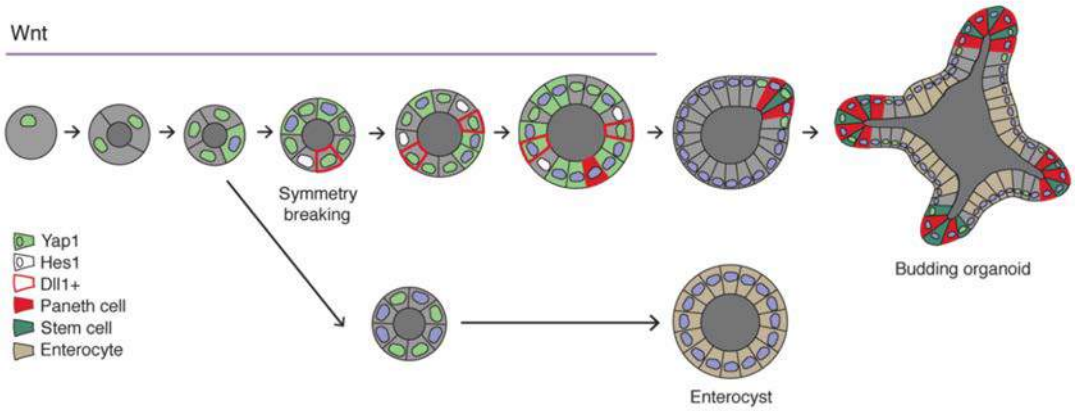
Organoïdes intestinaux (dernières avancées)

''Self-organization and symmetry breaking in intestinal organoid development''
Serra, Mayr, Boni, ….and Prisca Liberali (Institut FM, Bâle). Sous presse.

Comment se fait la rupture de symétrie?

*Variations précoces de Yap1 induisent une variabilité dans l'expression des ligands de Notch
*Si Dll1 est élevé, Notch est inhibé: cellules coliciforme (ou autres cellules sécrétrices)
*Les cellules adjacentes basses en Dll1 active Notch (Hes) et deviennent entérocytes.

La symétrie est rompue par les variations de Yap1 et leur effet sur Dll1 et Notch



Organoïdes intestinaux (dernières avancées)

LETTER 15 mai 2019 (ahead of print)
https://doi.org/10.1038/s41586-019-1212-5

Tracing the origin of adult intestinal stem cells

Jordi Guiu[1,14], Edouard Hannezo[2,3,14], Shiro Yui[3,13], Samuel Demharter[1], Svetlana Ulyanchenko[1], Martti Maimets[1], Anne Jørgensen[4], Signe Perlman[5], Lene Lundvall[5], Linn Salto Mamsen[6], Agnete Larsen[7], Rasmus H. Olesen[7], Claus Yding Andersen[6], Lea Langhoff Thuesen[8], Kristine Juul Hare[8], Tune H. Pers[9], Konstantin Khodosevich[1], Benjamin D. Simons[2,10,11] & Kim B. Jensen[1,12]✉

Adult intestinal stem cells are located at the bottom of crypts of Lieberkühn, where they express markers such as LGR5[1,2] and fuel the constant replenishment of the intestinal epithelium[1]. Although fetal LGR5-expressing cells can give rise to adult intestinal stem cells[3,4], it remains unclear whether this population in the patterned epithelium represents unique intestinal stem-cell precursors. Here we show, using unbiased quantitative lineage-tracing approaches, biophysical modelling and intestinal transplantation, that all cells of the mouse intestinal epithelium—irrespective of their location and pattern of LGR5 expression in the fetal gut tube—contribute actively to the adult intestinal stem cell pool. Using 3D imaging, we find that during fetal development the villus undergoes gross remodelling and fission. This brings epithelial cells from the non-proliferative villus into the proliferative intervillus region, which enables them to contribute to the adult stem-cell niche. Our results demonstrate that large-scale remodelling of the intestinal wall and cell-fate specification are closely linked. Moreover, these findings provide a direct link between the observed plasticity and cellular reprogramming of differentiating cells in adult tissues following damage[5–9], revealing that stem-cell identity is an induced rather than a hardwired property.

!

Flexibilité architecturale des cryptes intestinales

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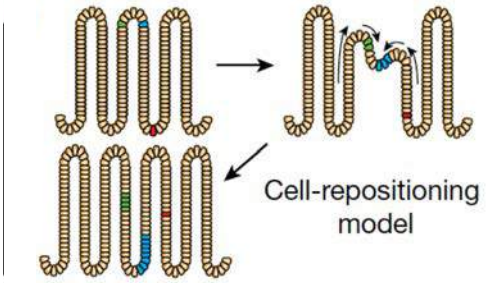


Fig. 3 | Villus fission is required to explain epithelial expansion.

d, Model for cell repositioning based on villi emerging from the intervillus regions or through villus fission. Clones from villi can recolonize the intervillus region and clones from the intervillus region can recolonize villi.

Organoïdes intestinaux; recherches et applications

Outils de recherche, mais également diagnostiques et thérapeutiques

Sato and Clevers, Science, 2013

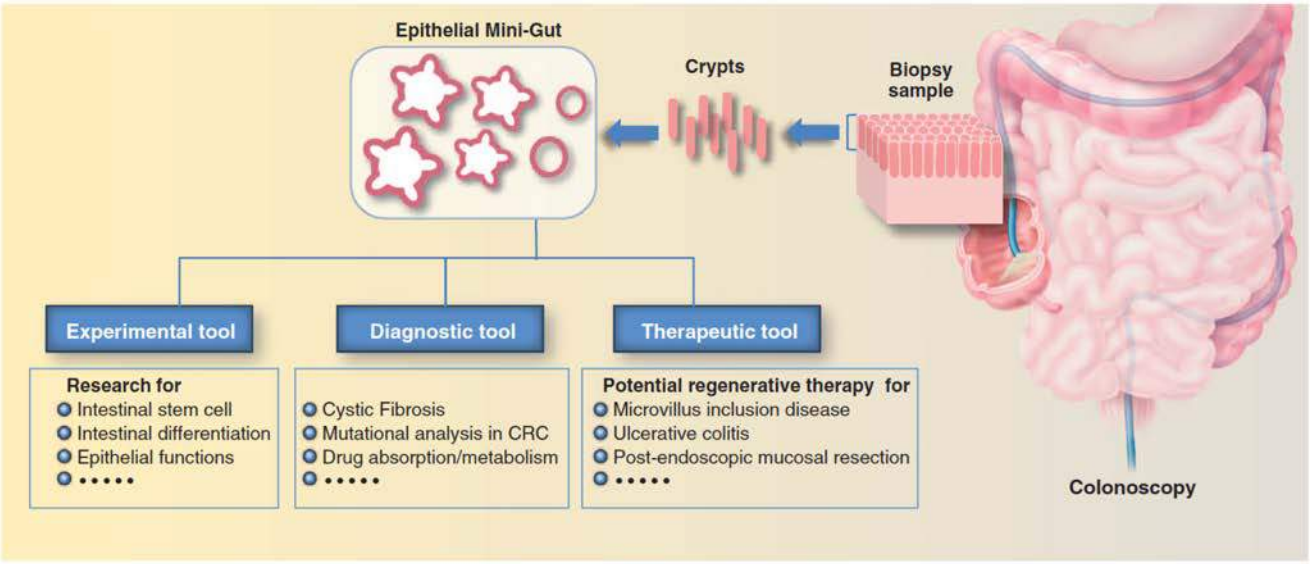


Fig. 4. Basic and clinical applications of an epithelial mini-gut. An epithelial mini-gut is efficiently established from a single (3 to 5 mm^2) endoscopic biopsy sample. EDTA chelation releases ~3000 crypts from a biopsy sample. An epithelial mini-gut grows logarithmically and expands 1000-fold within a month. Three applications of epithelial mini-guts are as follows: (i) As an experimental tool. Genetic manipulation, gene expression analysis, live imaging, and other standard biological analyses can be employed for normal and patient-derived epithelial mini-guts. (ii) As a diagnostic tool. Patient-derived epithelial mini-guts recapitulate in vivo intestinal epithelial functions and genetic signatures. Efficient expansion of pure epithelial cells provides a high-quality source for deep sequencing or functional assays. (iii) As a therapeutic tool. Epithelial mini-gut transplantation may become a feasible regenerative therapy.

Organoïdes intestinaux normaux ou pathologiques

Outils de recherche, mais également diagnostiques et thérapeutiques

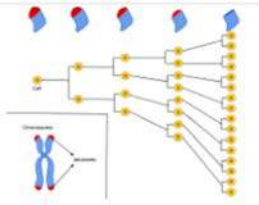
Gastroenterology. 2011 Nov;141(5):1762-72. doi: 10.1053/j.gastro.2011.07.050. Epub 2011 Sep 2.

Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium.

Sato T[1], Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H.

CONCLUSIONS: We developed a technology that can be used to study infected, inflammatory, or neoplastic tissues from the human gastrointestinal tract. These tools might have applications in regenerative biology through ex vivo expansion of the intestinal epithelia. Studies of these cultures indicate that there is no inherent restriction in the replicative potential of adult stem cells (or a Hayflick limit) ex vivo.

*La limite de *Hayflick* ou phénomène de *Hayflick* reflète le nombre de fois qu'une population de cellules humaines 'normales' se divisera avant d'arrêter toute division cellulaire



Wikipedia

Organoïdes intestinaux normaux ou pathologiques

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Cultures de cryptes dérivées du colon

**Souris: addition de Wnt3A au milieu utilisé pour cultiver les cryptes de l'intestin permet une pousse illimitée dans le temps.*

**Humain: addition nécessaire de nicotinamide et de molécules inhibitrices de Alk et de p38 pour faire pousser des cryptes de colon.*

**Ces conditions permettent également de dériver et de maintenir des cultures de cancers du colon (adénomes de souris et adénocarcinomes humain et de l'épithélium métaplasique de l'oesophage de Barrett (œsophage avec un épithélium intestinal..). Les cellules de cancers du colon ne nécessitent pas de traitement avec la R-spondin ou Noggin*

Organoïdes intestinaux normaux ou pathologiques

Outils de recherche, mais également diagnostiques et thérapeutiques *Figure 2*

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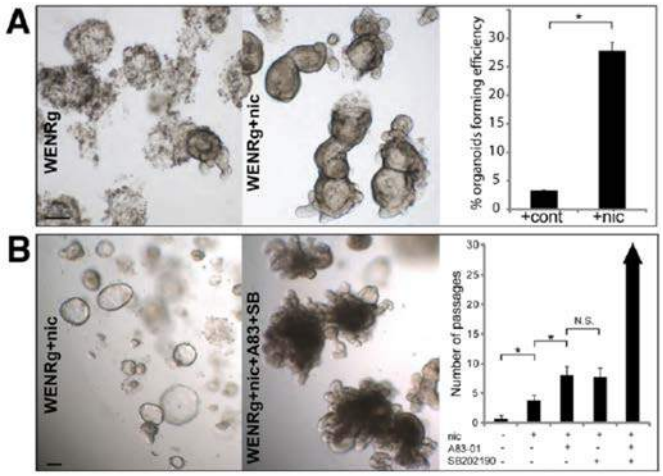
-nicotinamide +nicotinamide



+nicotinamid (3-4 weeks..)

+nicotinamide +Alk and p38 inh.

*Nicotinamide: Vitamine B, anti-inflammatoire..?

*Alk: Tyrosine kinase impliquées dans de nombreux cancers.

*p38: MAP kinase (MAPK) impliquée dans la différentiation, apoptose en réponse au stress etc..

Organoïdes intestinaux normaux ou pathologiques

Outils de recherche, mais également diagnostiques et thérapeutiques *Figure 3*

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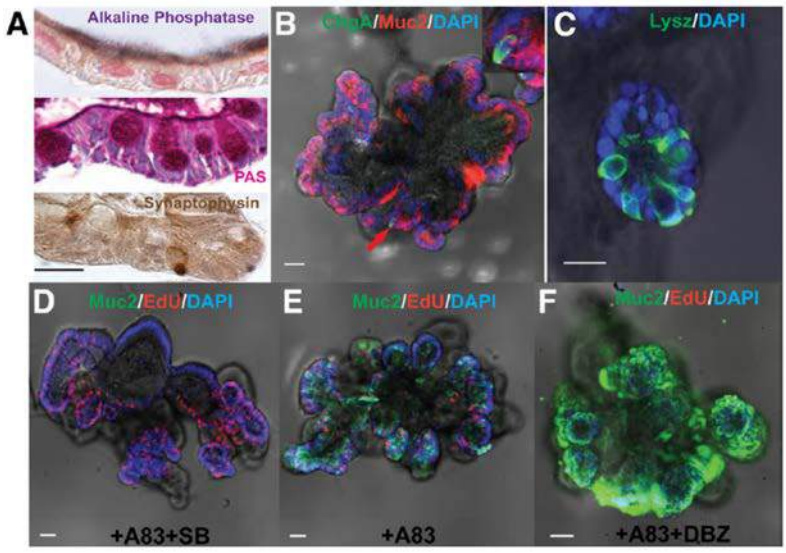
entérocytes (top) cellules caliciformes (goblet) cellules de Paneth



*Différenciation des types cellulaires en absence de nicotinamide et p38 inh.

*Alk: Tyrosine kinase impliquées dans de nombreux cancers (inh: A83)

*p38: MAP kinase (MAPK) impliquée dans la différentiation, apoptose en réponse au stress etc.. (inh: SB)

*DBZ: dibenzazepine, un inhibiteur de la signalisation *Notch* stoppe la prolifération et induit la différenciation de cellules caliciformes

Organoïdes intestinaux normaux ou pathologiques

Outils de recherche, mais également diagnostiques et thérapeutiques

Gastroenterology. 2011 Nov;141(5):1762-72. doi: 10.1053/j.gastro.2011.07.050. Epub 2011 Sep 2.

Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium.

Sato T[1], Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H.

L'œsophage de Barrett

États précancéreux de l'œsophage

L'œsophage de Barrett est l'état précancéreux de l'œsophage le plus fréquent. Les cellules normales qui tapissent l'œsophage sont remplacées par des cellules qui ressemblent à celles du revêtement de l'intestin ou de l'estomac. Le processus par lequel les cellules normales se transforment en cellules anormales est appelé métaplasie intestinale. La métaplasie intestinale apparaît habituellement dans la partie inférieure de l'œsophage, près de l'endroit où il se joint à l'estomac (jonction œsophago-gastrique, ou OG).

https://www.cancer.ca (société canadienne du cancer)

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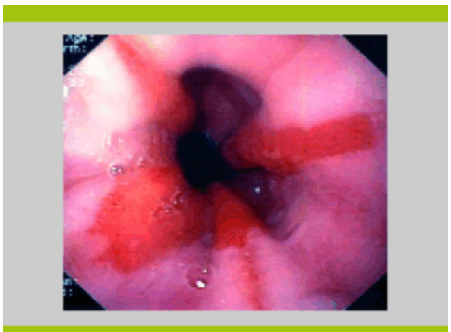

Figure 1. Languettes de muqueuse de Barrett (en rouge foncé) observées lors d'une endoscopie de routine chez un patient souffrant d'un reflux chronique

https://www.revmed.ch/RMS/2005/RMS-31/30614

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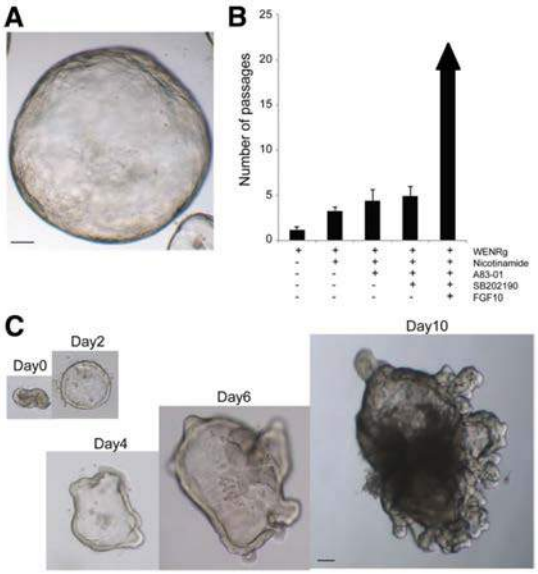

A. Epithélium isolé (7 jours)

B. Addition de Fgf10. Effet sur le nombre de passages

C. Organoïde d'œsophage de Barrett