



COLLÈGE
DE FRANCE
—1530—

Collège de France; Chaire:
Evolution du Développement et des Génomes

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Cours 2024: La Fabrique des 'Embryons'



Fœtus humain
'stade Carnegie' 23
(app 60 jours, 20 mm)

Embryon et embryotide
de souris à 8,5 jours
Amadei et al. Nature 2021

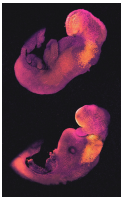


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La Fabrique des Embryons



Cours 2024: La Fabrique des 'Embryons'

Déroulé du cours

- **Cours #4:** 4 juin 2024. Les embryons intégrés; définition, caractérisation et comparaisons souris/humains. Potentiel expérimental (et quelques questions éthiques et sociales).

Les embryons intégrés; fabrication et potentiel futur

Différents types de pseudo-embryons humains

Developmental Biology 509 (2024) 43-50



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Developmental Biology

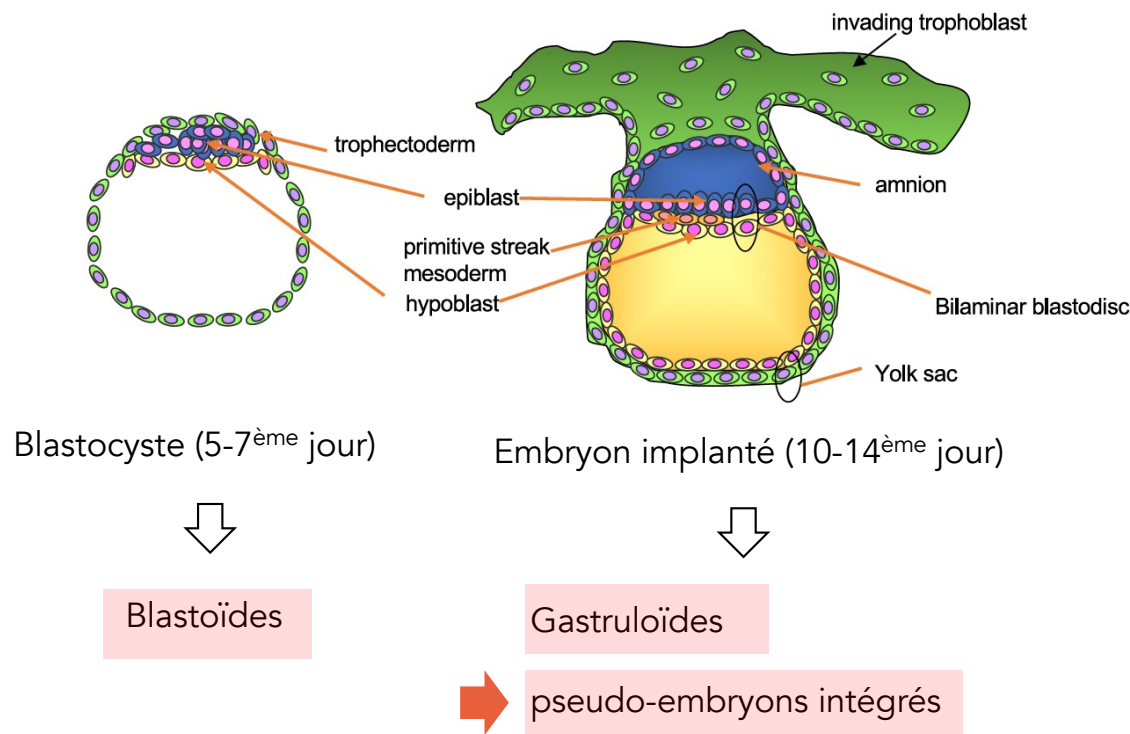
journal homepage: www.elsevier.com/locate/developmentalbiology



Why study human embryo development?

Janet Rossant

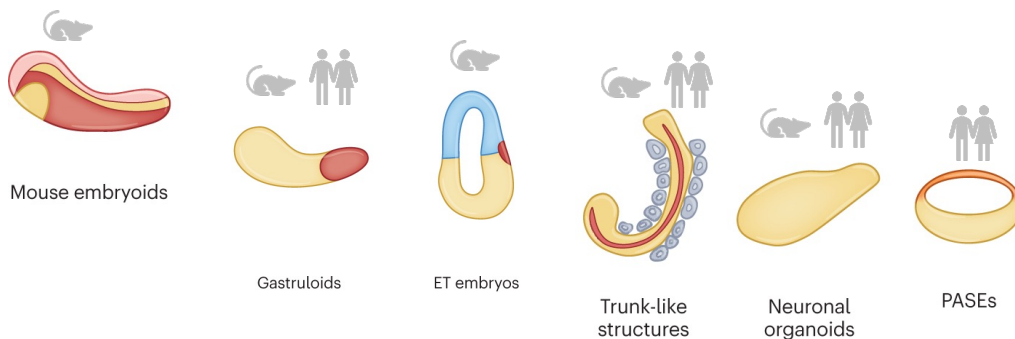
Différents types de pseudo-embryons en fonction des étapes du développement qu'ils sont sensés représenter



Catégoriser les modèles d'embryons

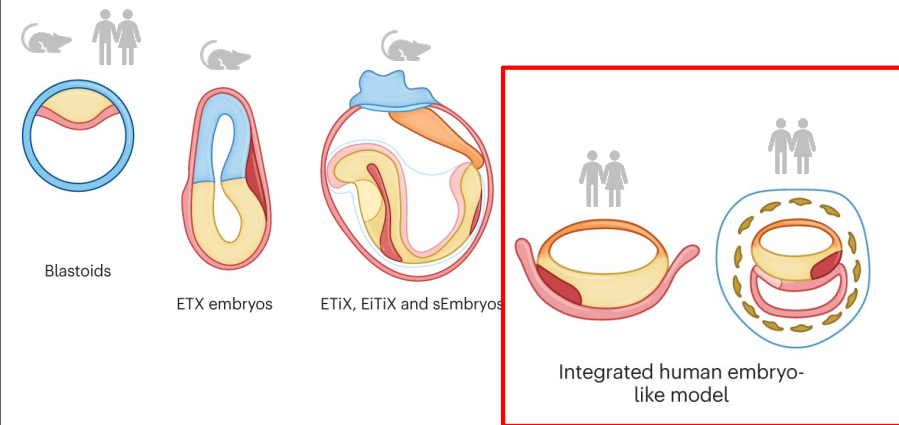
Non-Intégrés

- Contiennent un sous-ensemble des tissus embryonnaires
- Organisation similaire ou non à l'embryon
- Pas de développement ultérieur possible



Intégrés

- Contiennent tous les tissus 'embryonnaires'
- Organisation similaire à l'embryon
- Développement ultérieur possible



Adapté de Zernicka-Goetz et al. (2023)

Pseudo-embryons humains 'intégrés'



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La Fabrique des Embryons

> [Nature](#). 2023 Oct;622(7983):584–593. doi: 10.1038/s41586-023-06368-y. Epub 2023 Jun 27.

Pluripotent stem cell-derived model of the post-implantation human embryo

Bailey A T Weatherbee # 1, Carlos W Gantner # 1, Lisa K Iwamoto-Stohl 1, Riza M Daza 2, Nobuhiko Hamazaki 2, Jay Shendure 2 3 4 5, Magdalena Zernicka-Goetz 6 7 8

> [Nature](#). 2023 Oct;622(7983):574–583. doi: 10.1038/s41586-023-06354-4. Epub 2023 Jun 27.

Self-patterning of human stem cells into post-implantation lineages

Monique Pedroza # 1, Seher Ipek Gassaloglu # 1, Nicolas Dias 1 2, Liangwen Zhong 1, Tien-Chi Jason Hou 1 2, Helene Kretzmer 3, Zachary D Smith 1 2, Berna Sozen 4 5 6

> [Nature](#). 2023 Oct;622(7983):562–573. doi: 10.1038/s41586-023-06604-5. Epub 2023 Sep 6.

Complete human day 14 post-implantation embryo models from naive ES cells

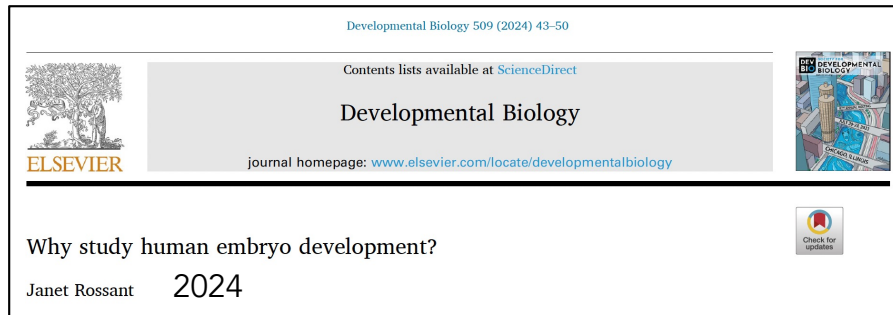
Bernardo Oldak # 1, Emilie Wildschutz # 1, Vladyslav Bondarenko # 1, Mehmet-Yunus Comar 1, Cheng Zhao 2 3, Alejandro Aguilera-Castrejon 1, Shadi Tarazi 1, Sergey Viukov 1, Thi Xuan Ai Pham 4, Shahd Ashoukhi 1, Dmitry Lokshtanov 1, Francesco Roncato 1, Eitan Ariel 1, Max Rose 1, Nir Livnat 1, Tom Shani 1, Carine Joubran 1, Roni Cohen 1, Yoseph Addadi 5, Muriel Chemla 5, Merav Kedmi 5, Hadas Keren-Shaul 5, Vincent Pasque 4, Sophie Petropoulos 2 3 6 7, Fredrik Lanner 2 3 8, Noa Novershtern 1, Jacob H Hanna 9

> [Nature](#). 2024 Feb;626(7998):367–376. doi: 10.1038/s41586-023-06914-8. Epub 2023 Dec 13.

Modelling post-implantation human development to yolk sac blood emergence

Joshua Hislop 1 2 3, Qi Song # 4 5, Kamyar Keshavarz F # 1 2 3, Amir Alavi # 4 5, Rayna Schoenberger 1 2 3, Ryan LeGraw 2 3, Jeremy J Velazquez 2 3, Tahere Mokhtari 1 2 3, Mohammad Naser Taheri 1 2 3, Matthew Rytel 2 3, Susana M Chuva de Sousa Lopes 6, Simon Watkins 7 8, Donna Stolz 7 8, Samira Kiani 1 2 3 9, Berna Sozen 10, Ziv Bar-Joseph 4 5, Mo R Ebrahimkhani 11 12 13 14

Pourquoi étudier l'embryon humain et ses ersatz?



2. Why study human development?

Given the limited access to human embryos, the limitations of current stem cell models and the ongoing ethical and political debates around human embryo research (Rugg-Gunn et al., 2023; Rivron et al., 2023), it is important to demonstrate the potential benefits of this research. There are many-some realized now and some to be realized in the future.

1. Fundamental understanding of what it means to be human. There is an intrinsic curiosity about the very beginnings of human development. How does the single cell, the fertilized egg, develop into the miracle of life-the baby? Parents the world over have marvelled at this. In fact, of course, many of the events that we are discussing here-implantation, gastrulation, early neural development, take place usually before pregnancy is even detected. This is the true 'black box' stage of human development and there is great intrinsic interest in being able to peek inside these stages. Studies in model organisms, from flies to fish to mice, have provided many of the key molecular pathways that underly all developmental processes, but the details of morphogenesis and exact identity of genes involved do vary across species, and even across mammals. Mouse studies laid the

1/13 raisons

Une curiosité intrinsèque à comprendre notre origine en tant qu'humain. Ce que signifie 'être un humain' du point de vue du développement embryonnaire.

Les principes généraux sont partagés entre les animaux (*a fortiori* entre les mammifères) mais beaucoup de caractéristiques spécifiques aux humains/primates restent à découvrir.

Pseudo-embryons humains 'intégrés'

> [Nature](#). 2023 Oct;622(7983):584–593. doi: 10.1038/s41586-023-06368-y. Epub 2023 Jun 27.

Pluripotent stem cell-derived model of the post-implantation human embryo

... modular, tractable, integrated³ model of the human embryo that will enable us to probe key questions of human post-implantation development, a critical window during which substantial numbers of pregnancies fail.

> [Nature](#). 2023 Oct;622(7983):574–583. doi: 10.1038/s41586-023-06354-4. Epub 2023 Jun 27.

Self-patterning of human stem cells into post-implantation lineages

...reproducible, tractable and scalable experimental platform to understand the basic cellular and molecular mechanisms that underlie human development, including new opportunities to dissect congenital pathologies with high throughput.

> [Nature](#). 2023 Oct;622(7983):562–573. doi: 10.1038/s41586-023-06604-5. Epub 2023 Sep 6.

Complete human day 14 post-implantation embryo models from naive ES cells

SEM: Stem-cells based Embryo Models 

... This SEM platform will probably enable the experimental investigation of previously inaccessible windows of human early post implantation up to peri-gastrulation development.

> [Nature](#). 2024 Feb;626(7998):367–376. doi: 10.1038/s41586-023-06914-8. Epub 2023 Dec 13.

Modelling post-implantation human development to yolk sac blood emergence

... an easy-to-use, high-throughput, reproducible and scalable platform to probe multifaceted aspects of human development and blood formation at the early post-implantation stage. It will provide a tractable human-based model for drug testing and disease modelling.

Pseudo-embryons humains 'intégrés'

Article

Complete human day 14 post-implantation embryo models from naive ES cells

<https://doi.org/10.1038/s41586-023-06604-5>

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Bernardo Oldak^{1,9}, Emilie Wildschutz^{1,9}, Vladyslav Bondarenko^{1,9}, Mehmet-Yunus Comar¹, Cheng Zhao^{2,3}, Alejandro Aguilera-Castrejon¹, Shadi Tarazi¹, Sergey Viukov¹, Thi Xuan Ai Pham⁴, Shahd Ashoukhi¹, Dmitry Lokshtanov¹, Francesco Roncato¹, Eitan Ariel¹, Max Rose¹, Nir Livnat¹, Tom Shani¹, Carine Joubran¹, Roni Cohen¹, Yoseph Addadi², Muriel Chemla⁵, Merav Kedmi⁶, Hadas Keren-Shaul⁵, Vincent Pasque⁴, Sophie Petropoulos^{2,3,6,7}, Fredrik Lanner^{2,3,8}, Noa Novershtern¹ & Jacob H. Hanna^{1,9}

Nature | Vol 622 | 19 October 2023 | 563

The ability to study human post-implantation development remains limited owing to ethical and technical challenges associated with intrauterine development after implantation¹. Embryo-like models with spatially organized morphogenesis and structure of all defining embryonic and extra-embryonic tissues of the post-implantation human conceptus (that is, the embryonic disc, the bilaminar disc, the yolk sac, the chorionic sac and the surrounding trophoblast layer) remain lacking^{1,2}. Mouse naive embryonic stem cells have recently been shown to give rise to embryonic and extra-embryonic stem cells capable of self-assembling into post-gastrulation structured stem-cell-based embryo models with spatially organized morphogenesis (called SEMs)³. Here we extend those findings to humans using only genetically unmodified human naive embryonic stem cells (cultured in human enhanced naive stem cell medium conditions)⁴. Such human fully integrated and complete SEMs recapitulate the organization of nearly all known lineages and compartments of post-implantation human embryos, including the epiblast, the hypoblast, the extra-embryonic mesoderm and the trophoblast layer surrounding the latter compartments. These human complete SEMs demonstrated developmental growth dynamics that resemble key hallmarks of post-implantation stage embryogenesis up to 13–14 days after fertilization (Carnegie stage 6a). These include embryonic disc and bilaminar disc formation, epiblast lumenogenesis, polarized amniogenesis, anterior–posterior symmetry breaking, primordial germ-cell specification, polarized yolk sac with visceral and parietal endoderm formation, extra-embryonic mesoderm expansion that defines a chorionic cavity and a connecting stalk, and a trophoblast-surrounding compartment demonstrating syncytium and lacunae formation. This SEM platform will probably enable the experimental investigation of previously inaccessible windows of human early post implantation up to peri-gastrulation development.



*Les auteurs s'inspirent de leur étude précédente montrant que des cellules ESC naïves de souris peuvent donner des embryons ayant des signes de post-gastrulation (Tarazi et al., 2022, embryons SEM 'Synthetic whole Embryo Model').



*Ils adaptent ces observations à des cellules ESC humaines, qui sont capables de donner des embryons SEM complets, ayant pratiquement toutes les marques d'un embryon humain post-implantation de stade Carnegie 6 (13-14 jours)

Synthetic whole embryo models (SEMs)



Cell

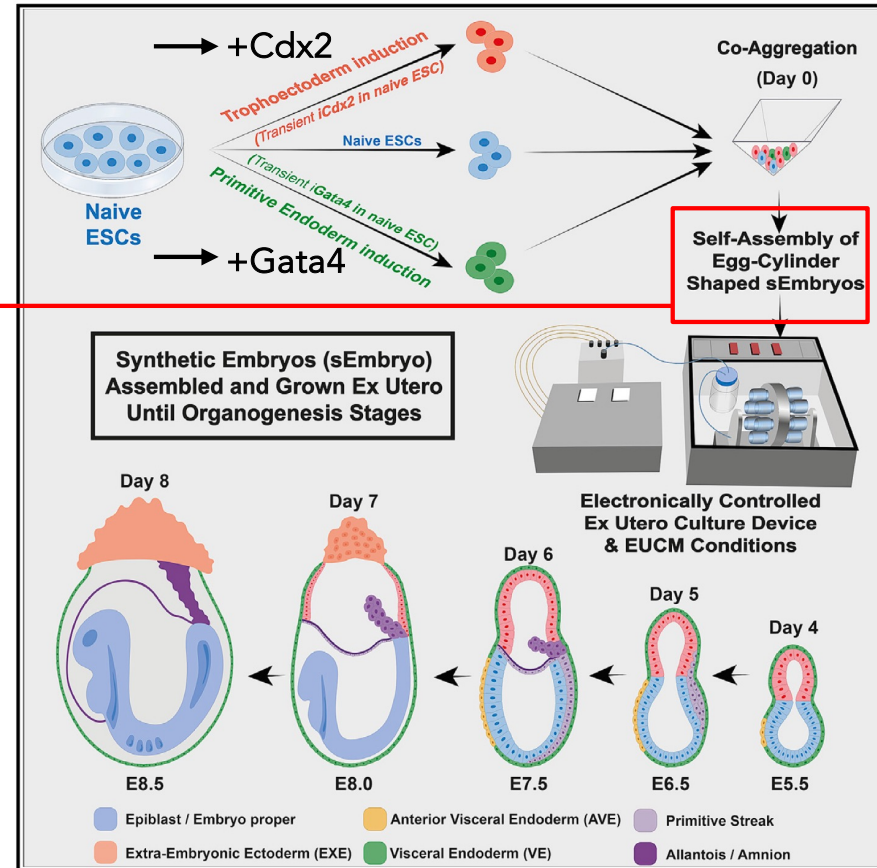
Article

Post-gastrulation synthetic embryos generated *ex utero* from mouse naive ESCs

Shadi Tarazi,^{1,8,*} Alejandro Aguilera-Castrejon,^{1,8,10,*} Carine Joubran,^{1,8} Nadir Ghanem,² Shahd Ashoukhi,¹ Francesco Roncato,¹ Emilie Wildschutz,¹ Montaser Haddad,³ Bernardo Oldak,¹ Elidet Gomez-Cesar,¹ Nir Livnat,¹ Sergey Viukov,¹ Dmitry Lokshantov,¹ Segev Naveh-Tassa,¹ Max Rose,¹ Suhair Hanna,⁴ Calanit Raanan,⁵ Ori Brenner,⁵ Merav Kedmi,⁶ Hadas Keren-Shaul,⁶ Tsvee Lapidot,³ Itay Maza,^{7,9,*} Noa Novershtern,^{1,9,10,*} and Jacob H. Hanna^{1,10,11,*}

Tarazi et al., 2022, Cell 185, 3290–3306

- Advanced synthetic embryos (sEmbryos) self-assembled from ESCs in an *ex utero* setup
- Naive ESCs give rise to all embryonic and extraembryonic compartments in sEmbryos
- Post-gastrulation stem cell derived sEmbryos develop organ-specific progenitors
- Extraembryonic compartments adequately develop in post-gastrulation whole sEmbryos



Mélange de trois types cellulaires, comme dans le protocole de Sozen et al. 2018 (discuté au cours DD de 2018-2019), mais là les trois types de cellules dérivent de la même lignée ESC naïve, plutôt que de cultures indépendantes



nature
cell biology

TECHNICAL REPORT

<https://doi.org/10.1038/s41556-018-0147-7>

Corrected: Publisher Correction

Self-assembly of embryonic and two extra-embryonic stem cell types into gastrulating embryo-like structures

Berna Sozen^{1,2,10}, Gianluca Amadei^{1,10}, Andy Cox¹, Ran Wang³, Ellen Na⁴, Sylwia Czukiewska¹, Lia Chappell⁵, Thierry Voet^{5,6}, Geert Michel⁷, Naihe Jing^{3,8}, David M. Glover⁹ and Magdalena Zernicka-Goetz^{1*}

NATURE CELL BIOLOGY | VOL 20 | AUGUST 2018 | 979–989 | www.nature.com/naturecellbiology

$Esc + TSc + XENc = Embryons ETX$

Types cellulaires à produire pour l'agrégation

Developmental biology

A peek into the black box of human embryology

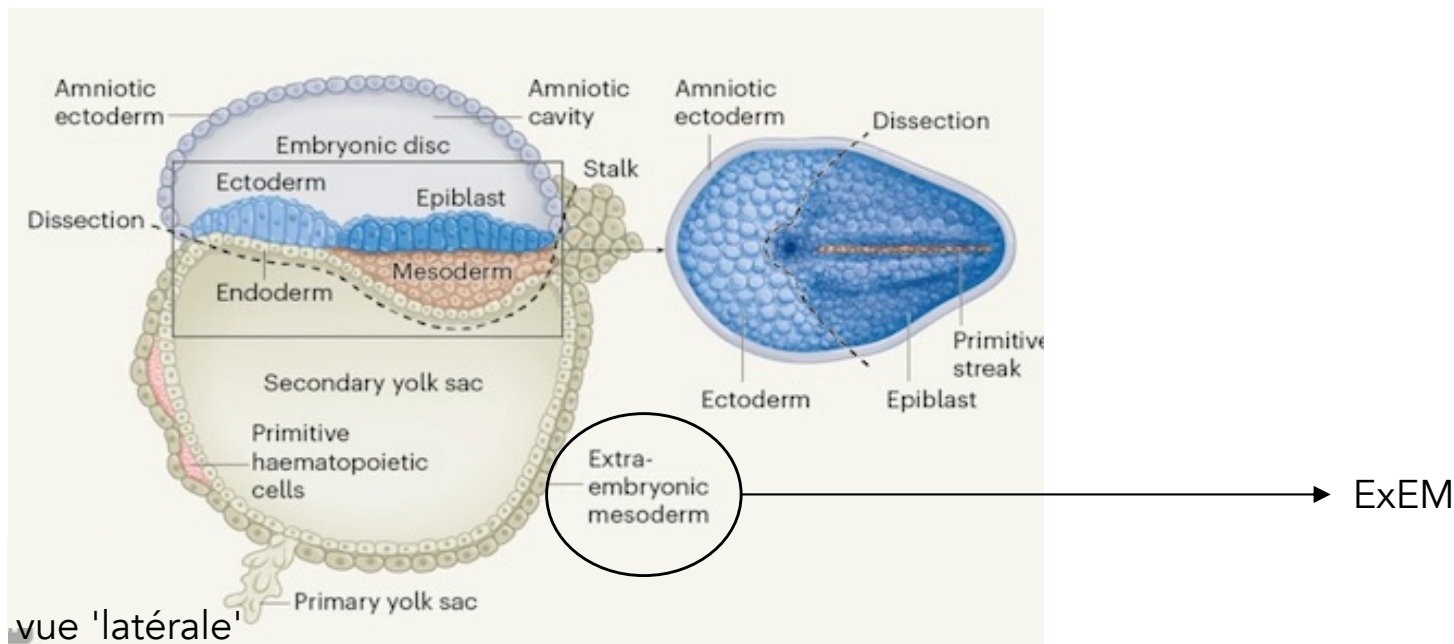
Alexander Goedel & Fredrik Lanner

The molecular mechanisms involved in human gastrulation, a crucial stage in early embryonic development, have been largely elusive. Gene-expression data from a gastrulating human embryo shed light on this process. See p.285

Nature | Vol 600 | 9 December 2021 | 223

*Contrairement à la souris, l'embryon humain post-implantation et pré-gastrulation contient du mésoderme extra-embryonnaire (ExEM) en plus de l'endoderme extra-embryonnaire (primitive endoderme (PrE) et du linéage trophoctoderme-like (TE). Donc tentative de production des 4 types cellulaires à partir de la même lignée de cellules ESC naïve.

embryon en gastrulation'



La fabrication d'embryons humains 'SEMs'

Article

Complete human day 14 post-implantation embryo models from naive ES cells

<https://doi.org/10.1038/s41586-023-06604-5> Bernardo Oldak^{1,9}, Emilie Wildschutz^{1,9}, Vladyslav Bondarenko^{1,9}, Mehmet-Yunus Comar¹, Cheng Zhao^{2,3}, Alejandro Aguilera-Castrejon¹, Shadi Tarazi¹, Sergey Viukov¹, Thi Xuan Ai Pham⁴, Shahd Ashoukhi¹, Dmitry Lokshtanov¹, Francesco Roncato¹, Eitan Ariel¹, Max Rose¹, Nir Livnat¹, Tom Shani¹, Carine Joubran¹, Roni Cohen¹, Yoseph Addadi⁵, Muriel Chemla⁶, Merav Kedmi⁶, Hadas Keren-Shaul⁶, Vincent Pasque⁴, Sophie Petropoulos^{2,3,6,7}, Fredrik Lanner^{2,3,8}, Noa Novershtern¹ & Jacob H. Hanna^{1,8}

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Cellules ES inductibles (doxycycline*) pour:

GATA4 : PrE

GATA6: ExEM

Readout: expression de PDGFRA*, qui est un marqueur de ces deux linéages

Il s'agit de trouver les meilleures conditions de culture pour produire le plus de PDGFRA possible, comme un indicateur de différenciation en PrE et ExEM

Optimisation des conditions de différenciation de cellules humaines naïves en linéages extra-embryonnaires pour produire des embryons SEMs.

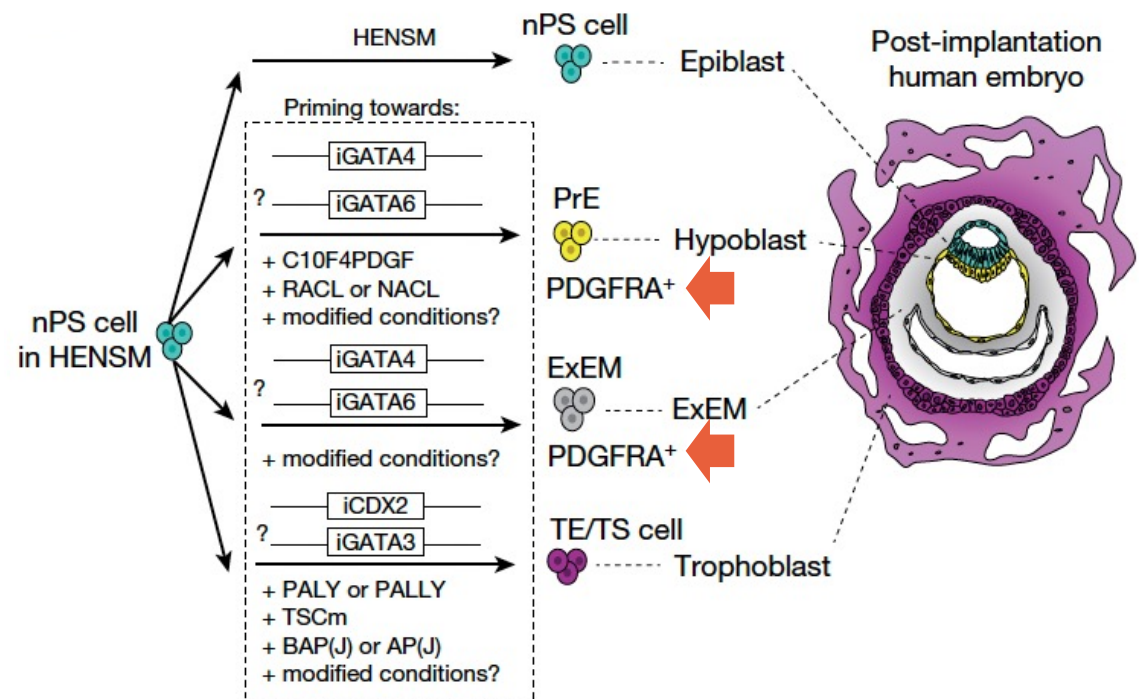
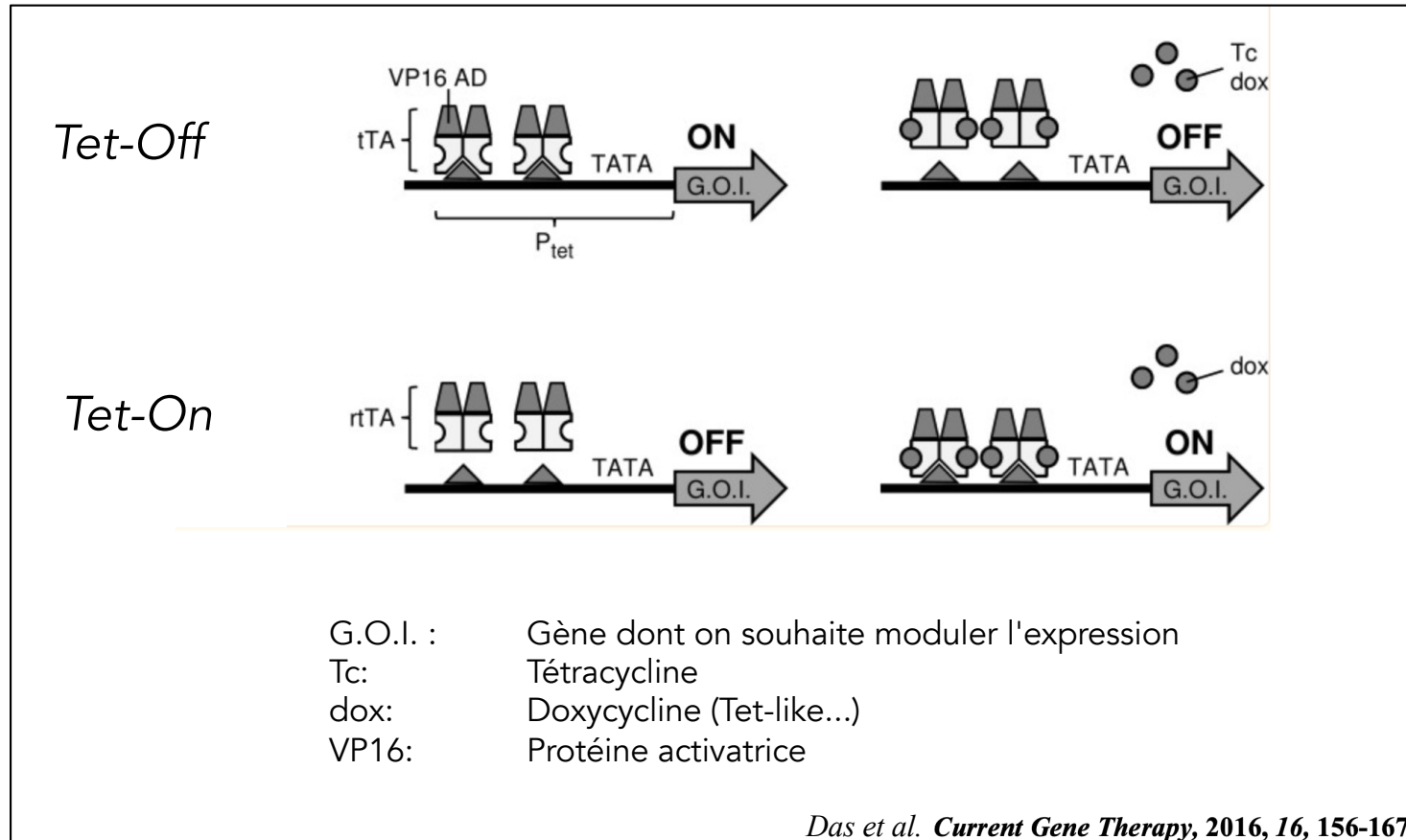


Fig. 1 | Optimizing human naive ES cell differentiation towards extra-embryonic lineages competent for early post-implantation SEM generation.

Systemes Tet-Off et Tet-On



L'addition de la doxycycline soit empêche (Tet-Off), soit favorise (Tet-On) la liaison du complexe activateur en amont du gène que l'on souhaite réguler (GATA4 et GATA6 dans ce cas particulier)

PDGF Récepteur alpha

Platelet-derived growth factor receptor A

Article [Talk](#)

From Wikipedia, the free encyclopedia

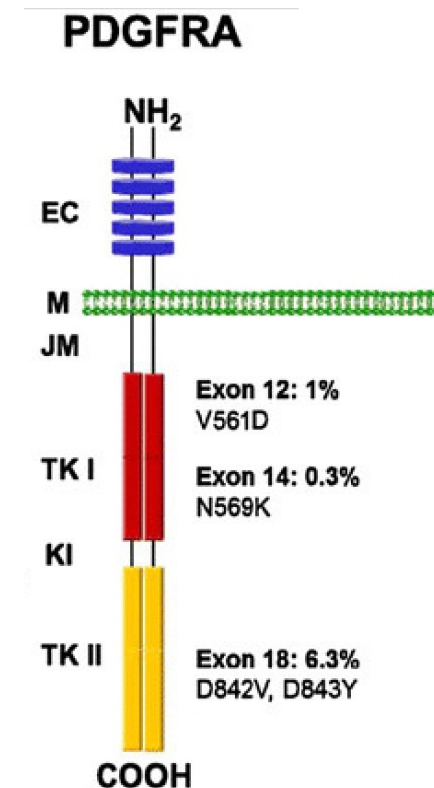
Platelet-derived growth factor receptor A, also termed **CD140a**, is a [receptor](#) located on the surface of a wide range of cell types. The protein is encoded in the human by the *PDGFRA* [gene](#). This receptor binds to certain [isoforms](#) of [platelet-derived growth factors](#) (PDGFs) and thereby becomes active in stimulating [cell signaling](#) pathways that elicit responses such as [cellular growth](#) and [differentiation](#). The receptor is critical for the [embryonic development](#) of certain tissues and organs, and for their maintenance, particularly [hematologic](#) tissues, throughout life. [Mutations](#) in *PDGFRA*, are associated with an array of clinically significant [neoplasms](#), notably ones of the [clonal hypereosinophilia](#) class of malignancies, as well as [gastrointestinal stromal tumors](#) (GISTs).

Overall structure [\[edit \]](#)

This gene encodes a typical receptor tyrosine kinase, which is a transmembrane protein consisting of an extracellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase domain. The molecular mass of the mature, glycosylated PDGFR α protein is approximately 170 kDa. cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family.

https://en.wikipedia.org/wiki/Platelet_derived_growth_factor_receptor_A

Ligand: Platelet-derived growth factor (important dans le développement et dans un grand nombre de cancers). Signalisation par des cascades de phosphorylations (Tyrosine-kinase)



Production des types cellulaires (PrE et ExEM)

Article

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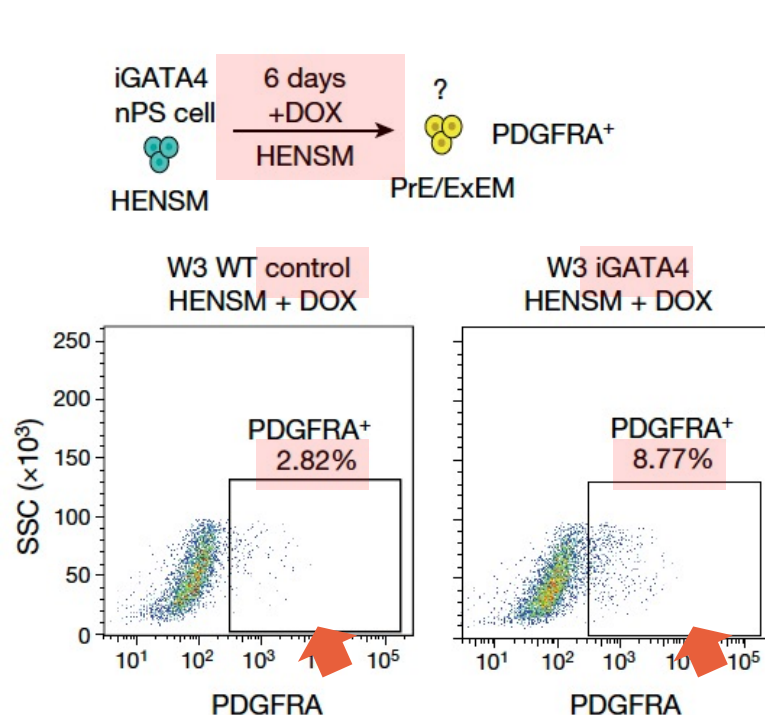
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'Read out'

L'efficacité de ces traitements est estimée en triant les cellules qui produisent suffisamment de PDGFRA (cell sorter), exprimé en % du total des cellules.

Les résultats montrent que ces transgenes seuls ne sont pas vraiment efficaces... (moins de 10%).

Donc:

Changement pour un milieu de culture 'enrichi' RACL: Milieu de base contenant en plus de l'Activine (TGFbeta-like); du Chiron (Wnt agonist) et du LIF.

Production des types cellulaires (PrE et ExEM)

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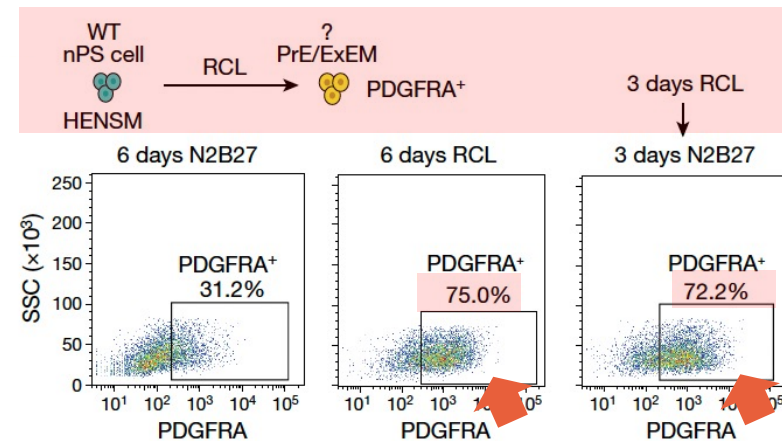
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RACL

induction medium (RPMI-based medium supplemented with activin A, CHIR99021 and LIF), which has been used to prime human naive ES cells towards the PrE and ExEM states¹⁰, or NACL medium (based on DMEM/F12, neurobasal and N2B27 media) that stabilizes naive endoderm cells generated in RACL conditions¹⁰, also led to low levels of the PDGFRA⁺ fraction (Fig. 1c and Extended Data Fig. 1c).

Because activin A inhibits the *in vitro* differentiation of human naive ES cells into ExEM cells⁹, we omitted it from RACL medium (called RCL here). RCL medium resulted in PDGFRA induction in the majority (>50%) of cells in iGATA4 and iGATA6 cells (Fig. 1c). However, high efficiencies of PDGFRA⁺ cell formation was evident in RCL conditions from isogenic wild-type (WT) cells without exogenous expression of GATA4 or GATA6 (Fig. 1d), which indicated that transient transgene expression is not required for efficient PDGFRA⁺ induction in human naive HENSMEs cells. Further optimization showed that 3 days of induction in the RCL condition followed by 3 days of incubation in basal N2B27 conditions produced comparable results (Fig. 1d and Extended Data Fig. 1d). Notably, incubating naive ES cells in N2B27 medium also produced PDGFRA⁺ cells, but at significantly 2.5-fold lower levels than RCL medium (Fig. 1d and Extended Data Fig. 1c). As we preferred to use genetically unmodified cells, we focused on using RCL conditions on WT non-transgenic cells for further characterization.



Milieu RACL moins le 'A' (activine qui semble inhiber la différenciation des cellules ES en ExEM). Donc utilisation d'un milieu RCL (milieu de base avec Chiron et LIF), sur des cellules iGATA4 et 6. Bonne stimulation de PDGFRA, mais une stimulation identique est observée dans des cellules ES WT... (plus de 50% des cellules.), indiquant que les transgènes GATA ne sont pas nécessaires dans ces conditions.

Optimisation d'un protocole final pour le PrE et le ExEM sans la présence de transgènes.

Les cellules PrE et ExEM sont alors triées/purifiées en utilisant des marqueurs appropriés et leurs identités vérifiées par scRNAseq en comparant avec des datasets produits avec des protocoles de différenciation à traitements 'séparés'.



Production des types cellulaires (Trophectoderme)



Denis Duboule/2024
La Fabrique des Embryons

Article

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*Cellules ES naïves testées avec dox CDX2 (CDX2 qui donne des cellules TS chez souris). Cependant, les cellules TE obtenues ne se comportent pas comme telles quand elles sont agrégées avec des cellules PrE, ExEM et ES. Elles ne recouvrent pas l'agrégat comme le font les cellules du TE.

*Essai de différentes conditions de culture et, là encore, l'utilisation d'un milieu particulier conduit à ce résultat. Mais, les cellules contrôles WT (sans transgènes) produisent les cellules TE souhaitées...

*Milieu BAP(J): DMEM/F12 basal + Alk4, 5 et 7 inhibiteur A83-01, + ERK et MEK inhibiteur PD0325901 + BMP4 pour 24h remplacé par un inhibiteur de JAK pour 48h.

*ALK: Récepteur tyrosine Kinase qui fonctionne par dimérisation induite par le ligand (petits peptides..?) . Signalisation importante pour la survie, la prolifération, la migration des cellules (Kinase du Lymphome Anaplasique).

Production des types cellulaires (Trophectoderme)

Article

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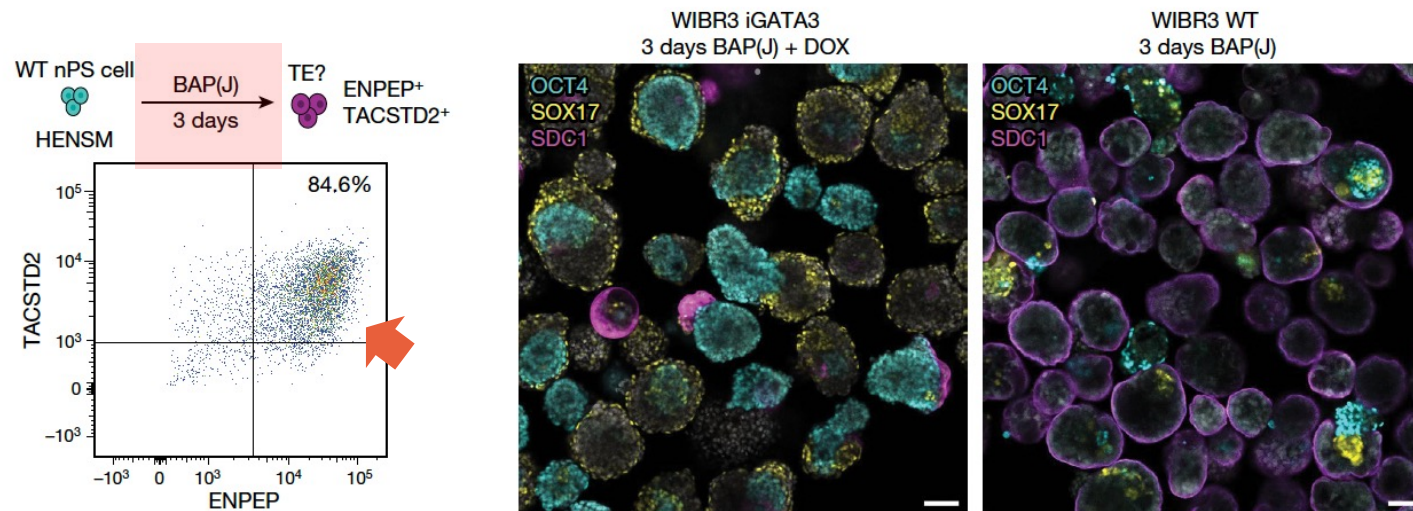
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*Ces cellules entourent les agrégats, comme le montre la coloration avec SDC1 (marqueur du trophoblaste et du placenta et de plusieurs pathologies associées (Syndecan-1).



*Cytométrie en flux avec TACSTD2 (cellules TE précoces et tardives) et ENPEP (TE tardif seulement) montre 85% de cellules double-positives

*L'identité TE de ces cellules est alors vérifiée par scRNAseq.

Article

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Nature | Vol 622 | 19 October 2023 | **563**

The ability to derive relevant extra-embryonic lineages from genetically unmodified WT human naive ES cells without the need for transgene overexpression is in line with recent studies demonstrating that human naive pluripotent cells can be more easily coaxed to give rise to early progenitors of PrE-like, amnion-like, ExEM-like and TE-like cells when compared to mouse naive ES cells, which require the overexpression of ectopic transcription factors^{4,9,14,15}. This is consistent with our observation that enhancers of key TE and PrE regulators (GATA3, GATA6 and GATA4) are accessible in human but not in mouse naive ES cells while being transcriptionally inactive in both (Extended Data Fig. 4a). The latter might render human naive ES cells to be relatively more responsive to the addition or omission of signalling cues to activate endogenous *GATA3*, *GATA6* and *GATA4* without the additional obligatory need for their ectopic expression to induce naive ES cell fates towards extra-embryonic lineages.



*Linéages extra-embryonnaires obtenus sans transgènes dans les cellules humaines: Sans doute lié à l'état d'avancement des cellules ES humaines comparé à la souris où les transgènes sont nécessaires.

*Consistant avec le fait que les enhancers trouvés en amont de gènes régulateurs clés du TE et du PrE (GATA3, 6 et 4) sont accessibles (ATAC-seq) dans les cellules humaines (pas chez la souris) alors même qu'ils ne sont pas activés...Probablement plus 'responsive' chez les humains et donc 'mieux' déclanchés, sans avoir besoin de compenser par des gains de fonction de gènes introduits.

ATAC-seq (cours #2 DD CdF 2023, 23 mai)

ATAC-seq: Mesure de l'accessibilité de la chromatine par l'effet (ou non) d'une activité enzymatique couplée au transposome Tn5 coupant l'ADN... Le transposome fragmente l'ADN accessible et y transfère ses 'étiquettes'... utilisée pour l'amplification et la séquence.

L'approche ATAC globale (X milliers-ions de cellules)

**CURRENT
PROTOCOLS**
A Wiley Brand

in Molecular Biology

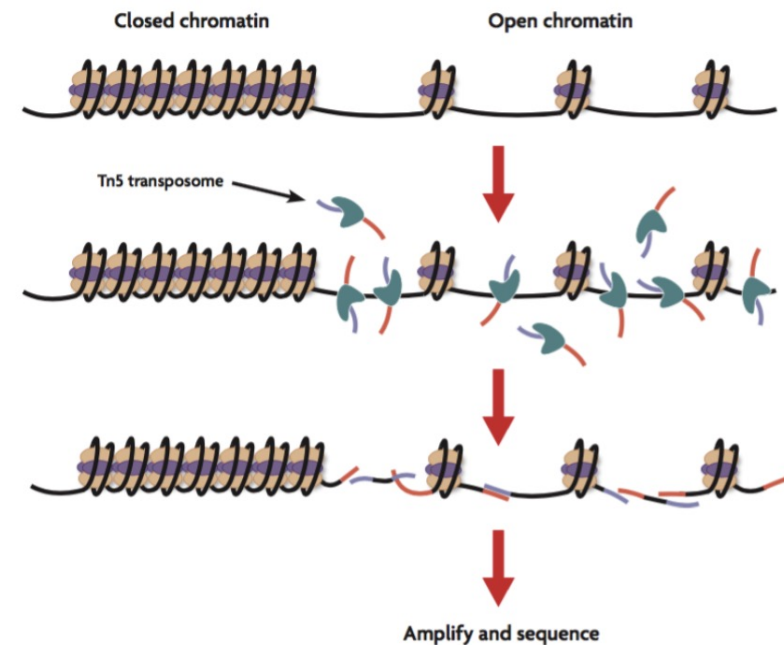
2015

UNIT

**ATAC-seq: A Method for Assaying Chromatin Accessibility
Genome-Wide**

Jason D. Buenrostro, Beijing Wu, Howard Y. Chang, William J. Greenleaf

First published: 05 January 2015 | <https://doi.org/10.1002/0471142727.mb2129s109> | Citations: 1,512



L'approche **ATACseq** mesure l'accessibilité à la chromatine, donc les endroits 'actifs' (là où des facteurs sont déposés, ou où la chromatine est modifiée etc..)

Production des embryons SEMs (intégrés)

Article

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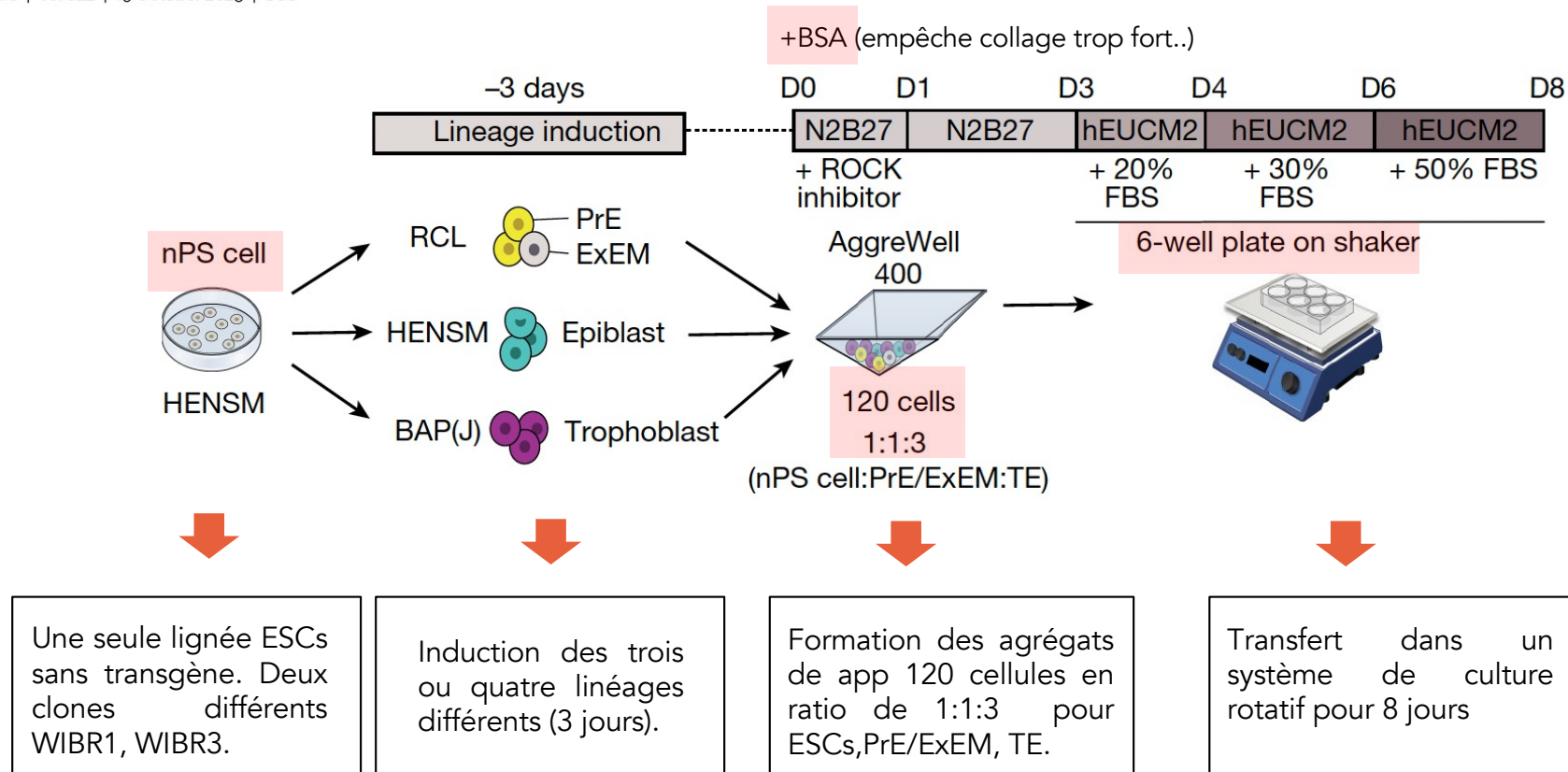
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Recette:



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Morphologies attendues (embryons et annexes embryonnaires):

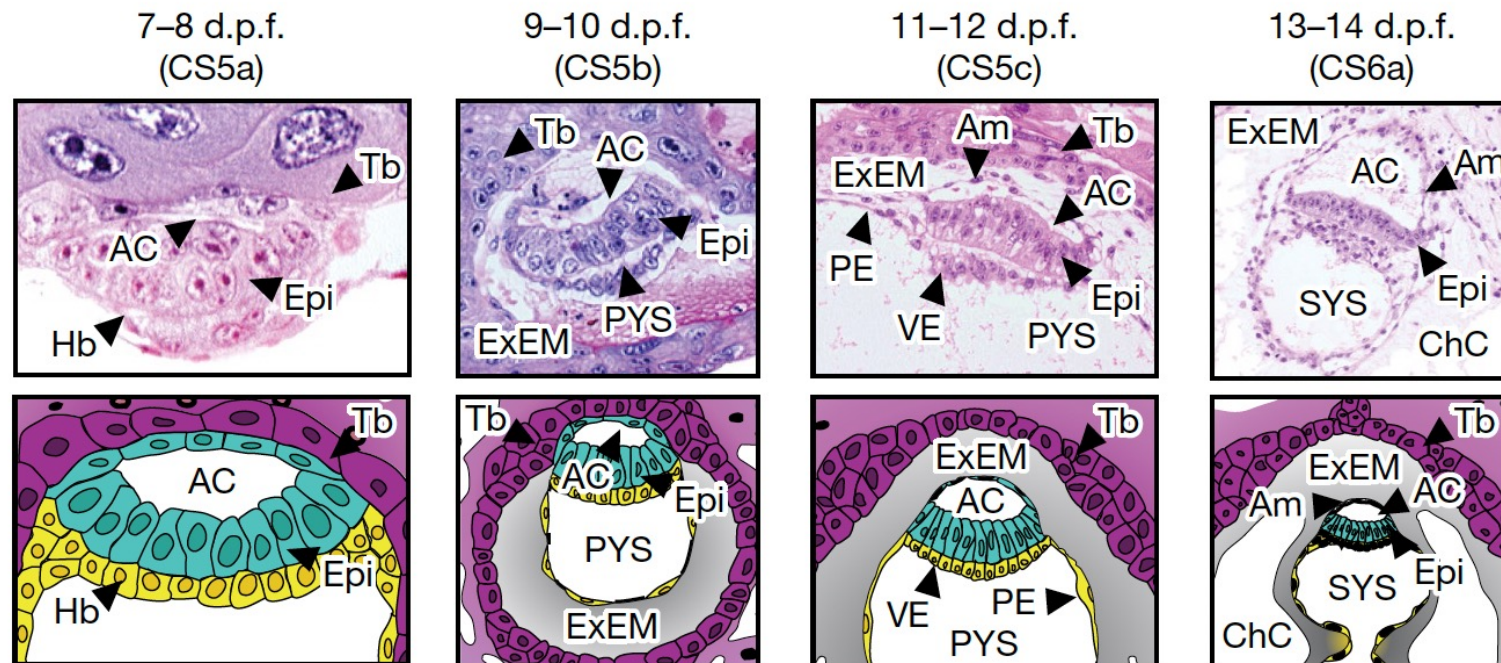
Stades

Jours: d.p.f.
Carnegie: CS

Histologie

 'Virtual Human Embryo'

Schémas



Epi: Epiblaste
Hb: Hypoblaste
AC: Cavité amniotique
Tb: Trophoblaste

ExEM: mésoderme extra-embryonnaire.
PYS: Sac vitellin primaire
SYS: Sac vitellin secondaire

Am: Amnios
VE: Endoderme viscéral
PE: Endoderme pariétal

ChC: Cavité du Chorion

Développement des embryons SEMs (intégrés)

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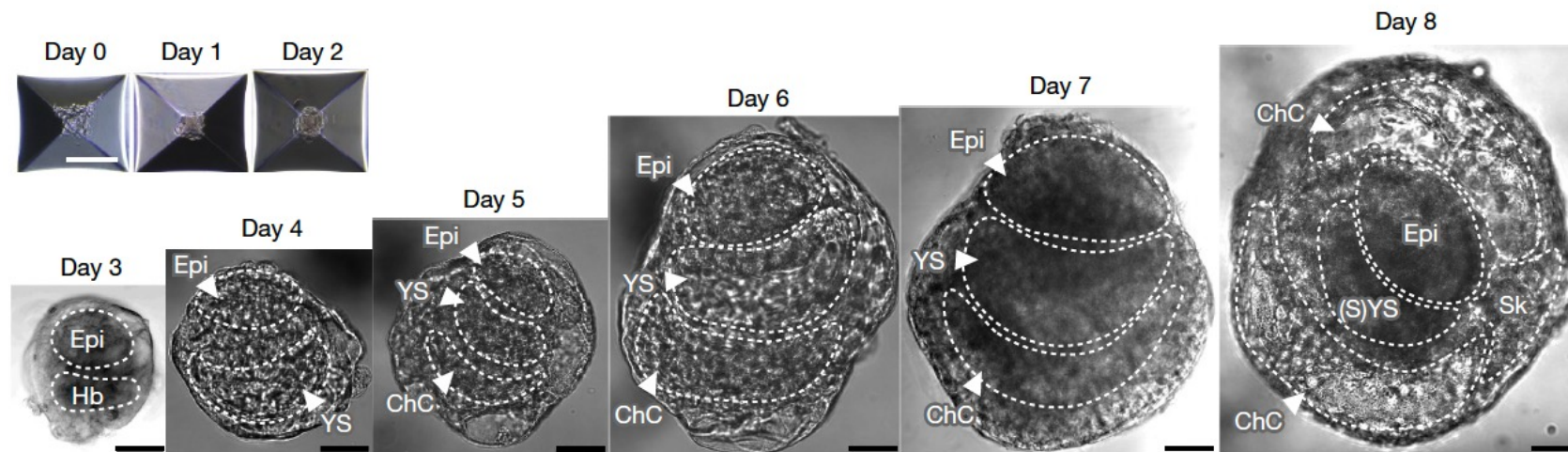
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Développement des annexes embryonnaires:
(images représentatives)



Epi: Epiblaste
Hb: Hypoblaste

YS: Sac vitellin primaire
SYS: Sac vitellin secondaire

Sk: Stalk (attache)
ChC: Cavité du Chorion

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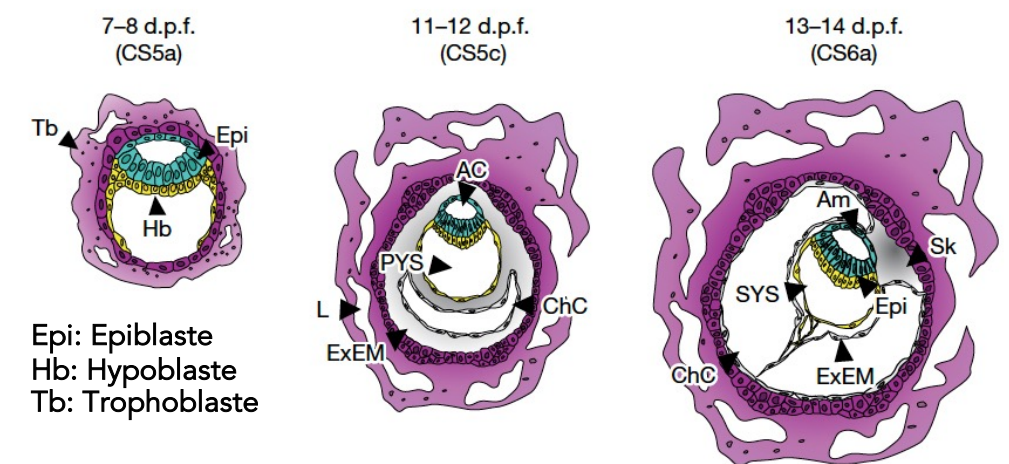
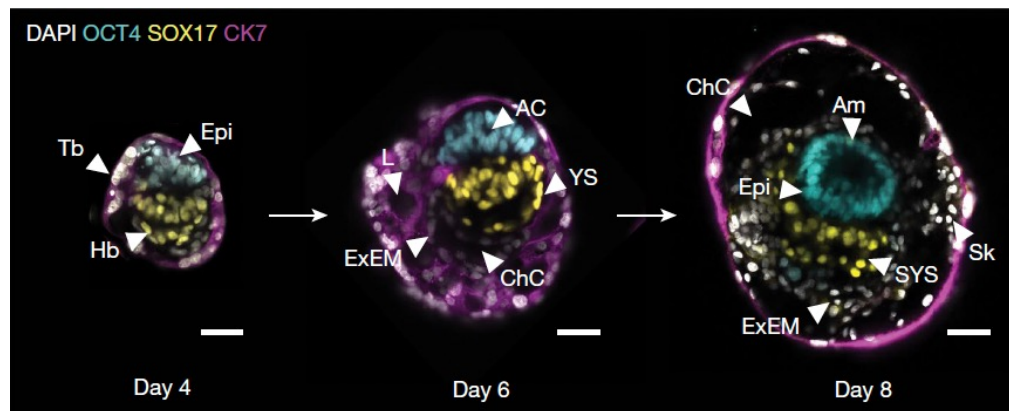
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Développement des annexes embryonnaires:
(Marqueurs fluorescents et schémas)

Dapi: Marquage nucléaire SOX17: Hypoblaste
OCT4: Epiblaste CK7: Trophoblaste-like



*Pas de cavité blastocystique (ni de blastocyste-ICM-)...(d'ailleurs, des blastoïdes humains ne se développent pas plus loin dans ce protocole...).

*Passage direct à une organisation en lignées, avec ségrégation spatio-temporelle des trois premiers tissus.

Emergence des annexes embryonnaires, avec une organisation correcte. Apparition de l'amnios dans l'épiblaste, d'un 'sac vitellin' dans l'hypoblaste. Formation de ExEM qui remodèle une cavité chorionique avec un point d'attachement (stalk, futur nombril...). Vascularisation...

YS: Sac vitellin primaire
SYS: Sac vitellin secondaire

Sk: Stalk (attache)
ChC: Cavité du Chorion

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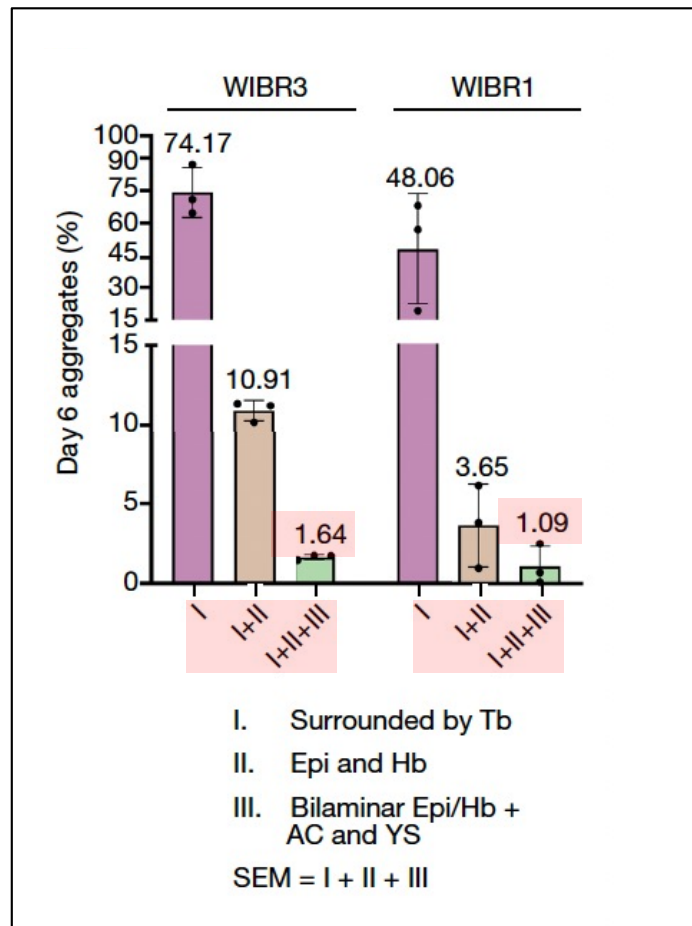
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Efficacité du développement de tels SEMs (Stem cells-based Embryo Models).

% observés à jour 6 post-agrégation, avec deux clones différents (WIBR 3 et 2):

I: Agrégat entouré par du trophoblaste

II: Présence d'épi- et d'hypoblaste

III: Structure complète et organisée ('SEM')

Conclusion:

Clone WIBR3: 1,64%
Clone WIBR1: 1,09%

*C'est peu en terme relatif de succès par agrégation
*Mais la production est quasiment illimitée...
*Marqueurs prédictifs de succès...??? (expériences...)

Polarisation des embryons SEMs (intégrés)

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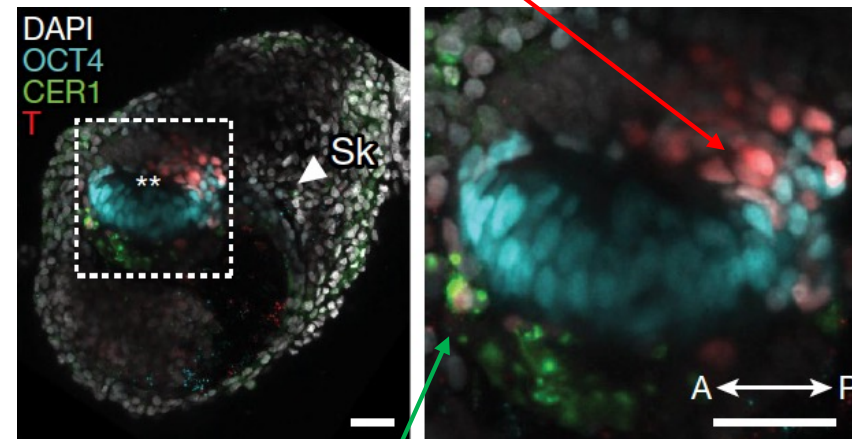
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Early emergence of the anterior–posterior axis is prevalent in mammals when a proportion of epiblast cells initiates T expression at the prospective posterior side of the embryonic disc. Therefore, we checked for the expression of T in human SEMs and identified a T⁺ population of epiblast-like cells that marked the posterior part of the SEM epiblast (Fig. 3f and Extended Data Fig. 8c). In parallel, the emergence of the anterior visceral endoderm (AVE)-like compartment, which constitutes the anterior signalling centre for epiblast patterning, was seen by the expression of CER1 in the epiblast-adjacent part of the visceral endoderm (VE) from day 6 (Extended Data Fig. 8d). The vesicular localization of CER1 was evident in human SEMs (Extended Data Fig. 8d). Co-immunostaining for these markers further supported the establishment of the anterior–posterior axis and symmetry breaking starting from day 6, with an efficiency of 1.02% (Extended Data Fig. 8e), at which T⁺ epiblast-like cells could be found in the region opposite to CER1⁺ AVE-like cells in the Hb (Fig. 3f).

Polarisation correcte de l'axe antéro-postérieur, jugée par l'apparition précoce des marqueurs T et CER1.

Expression de **T (Brachyury)** comme marqueur des cellules de l'épiblaste (**OCT4**) les plus 'postérieures'



Expression de **CER1** comme marqueur des cellules de l'endoderme viscéral antérieur AVE, qui contribue à différencier l'épiblaste antérieur.

Développement du compartiment trophoblastique

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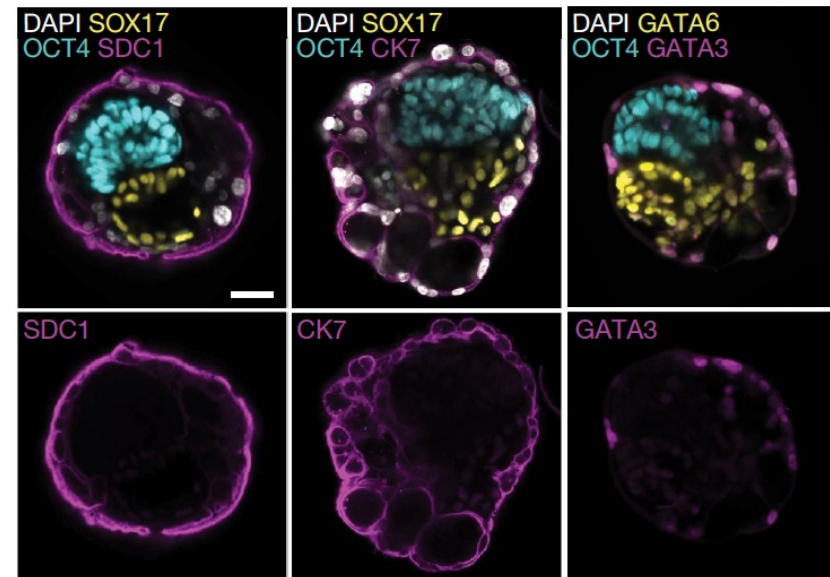
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In utero, the human embryo develops surrounded by the trophoblast layer, which is essential for truly integrated experimental models of early post-implantation development. Immunofluorescence analysis showed that the majority of SEM aggregates were surrounded by trophoblast-like cells, with an efficiency of 48–74%, and expressed multiple trophoblast markers such as GATA3, CK7 and SDC1 (ref. 14) (Fig. 5a,b and Extended Data Fig. 10a, b). Marker expression and cell morphology further indicated that the outer-most trophoblast-like layer was formed by syncytiotrophoblast-like cells, thereby confirming the development of the post-implantation trophoblast in human SEMs (Fig. 5a and Extended Data Fig. 10a–c). Notably, SDC1 was not expressed on the starting TE-like cells following BAP(J) induction before the aggregation step, which indicated that maturation of the TE-like cells occurs in the aggregates (Extended Data Fig. 3d).

Développement et maturation du compartiment trophoblastique.



Dapi: Marquage nucléaire
SOX17, GATA6: Hypoblaste, ExEM-like
OCT4: Epiblaste
SDC1, CK7, GATA3: Trophoblaste-like

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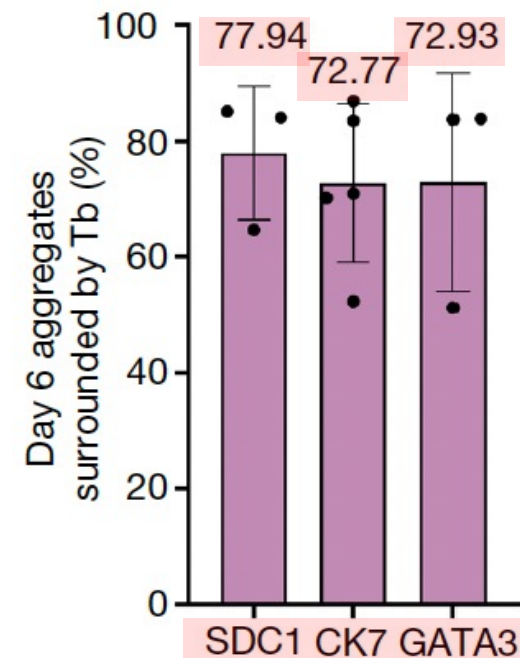
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Le marqueur SDC1 n'est pas exprimé initialement dans les cellules TE avant agrégation. Son activation tardive illustre la maturation du compartiment trophoblastique dans les SEMs.

Fréquence (à jour 6) élevée du développement et de la maturation du compartiment trophoblastique dans les SEMs.



Développement d'un trophoblaste syncytial

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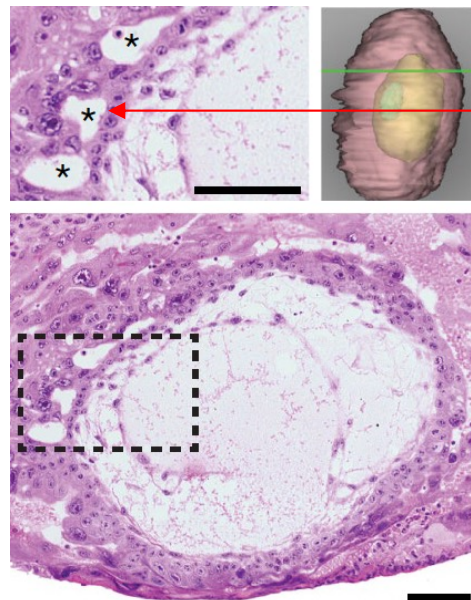
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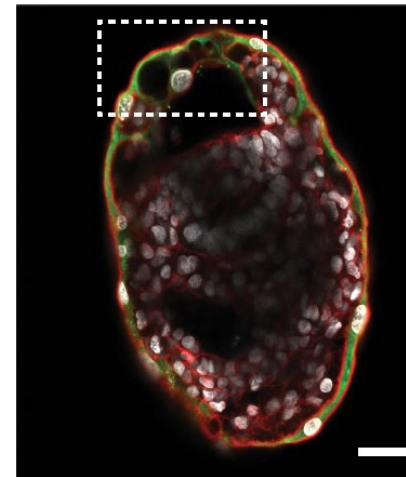
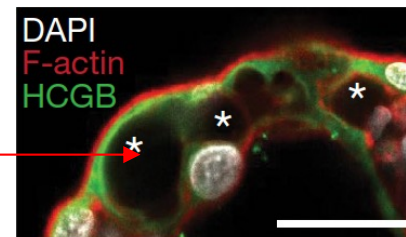
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Lacunae



Dapi: Marquage nucléaire.

F-actin : membranes cellulaires:

On voit que les cellules sont multinucléées.

bêtaHCG: Hormone chorionique gonadotrope bêta (gonadotropine chorionique).

Hormone produite par le trophoblaste syncytial (placenta), essentielle pour le maintien du *corpus luteum* ovarien et donc pour la sécrétion de progestérone qui assure l'état de la muqueuse utérine etc.



Histologie d'un embryon humain (Carnegie collection CS5c –app 9 jours-) avec des lacunes dans le trophoblaste syncytial (*).

SEMs à jour 6 avec des lacunes dans le trophoblaste syncytial (*).

Validation et alignement avec l'embryon humain



Denis Duboule/2024
La Fabrique des Embryons

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It was previously shown that conventional single-cell transcriptomic analysis (scRNA-seq), which lacks spatial information, is insufficient on its own to indicate whether a stem-cell-derived aggregate has embryo-like structure and compartmental organization, as it does not distinguish between correctly organized mouse SEMs and disorganized aggregates^{3,25}. Thus, such an scRNA-seq approach is necessary but not sufficient and should be used to reconfirm a cell-type presence within aggregates after the initial critical microscopy and immunostaining-based tests have been conducted, to unequivocally prove the structural integrity of aggregates as SEMs, as was done above.



L'analyse des mARNs n'est en elle-même pas suffisante car elle ne donne pas d'indication sur l'organisation spatiale de l'objet biologique. Elle ne renseigne que sur la présence ou l'absence d'un type cellulaire, caractérisé par son contenu en ARNs.

Donc nécessité de combiner cette analyse avec des analyses spatio-temporelles de la localisation de ces populations cellulaires (anticorps, fluorescence, voir avant).

Validation des SEMs par l'utilisation de séquences des ARNs de cellules uniques 'scRNAseq' et comparaison avec l'embryon humain.

Validation et alignement avec l'embryon humain

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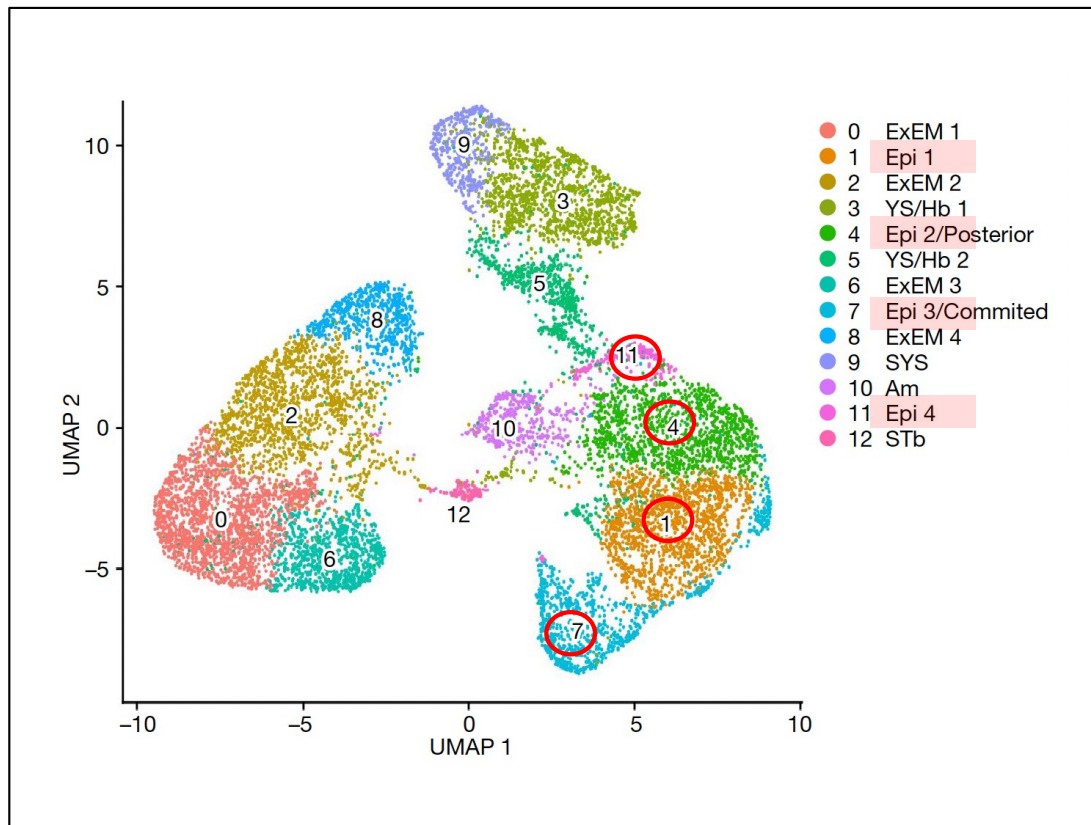
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Validation des SEMs par l'utilisation de séquences des ARNs de cellules uniques 'scRNAseq' et comparaison avec l'embryon humain.



Matériel de départ:

SEMs de 4 jours: ca. 80

SEMs de 6 jours: ca. 50

SEMs de 8 jours: ca. 50

Le UMAP mélange les trois types de SEMs



Identification de 13 'clusters' (0-12), reflétant des types cellulaires différents, regroupés par leurs 'similarités'..

Nombre de cellules / clusters

(Methods). Cluster sizes: 1,963 (cluster 0); 1,483 (cluster 1); 1,431 (cluster 2); 1,344 (cluster 3); 1,265 (cluster 4); 957 (cluster 5); 905 (cluster 6); 898 (cluster 7); 662 (cluster 8); 448 (cluster 9); 441 (cluster 10); 265 (cluster 11); and 128 (cluster 12).

Validation et alignement avec l'embryon humain

Article

Complete human day 14 post-implantation embryo models from naive ES cells

<https://doi.org/10.1038/s41586-023-06604-5>

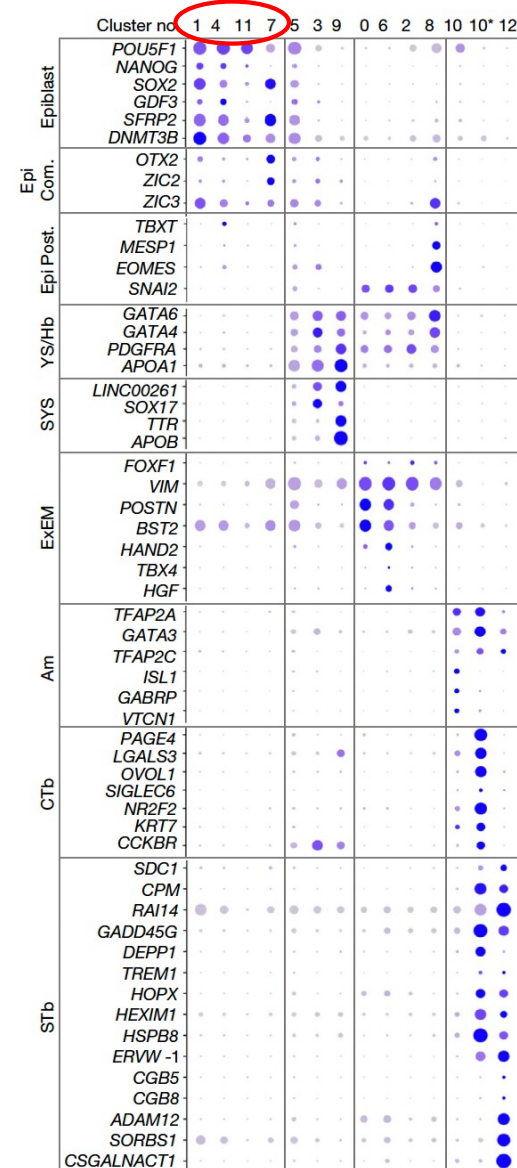
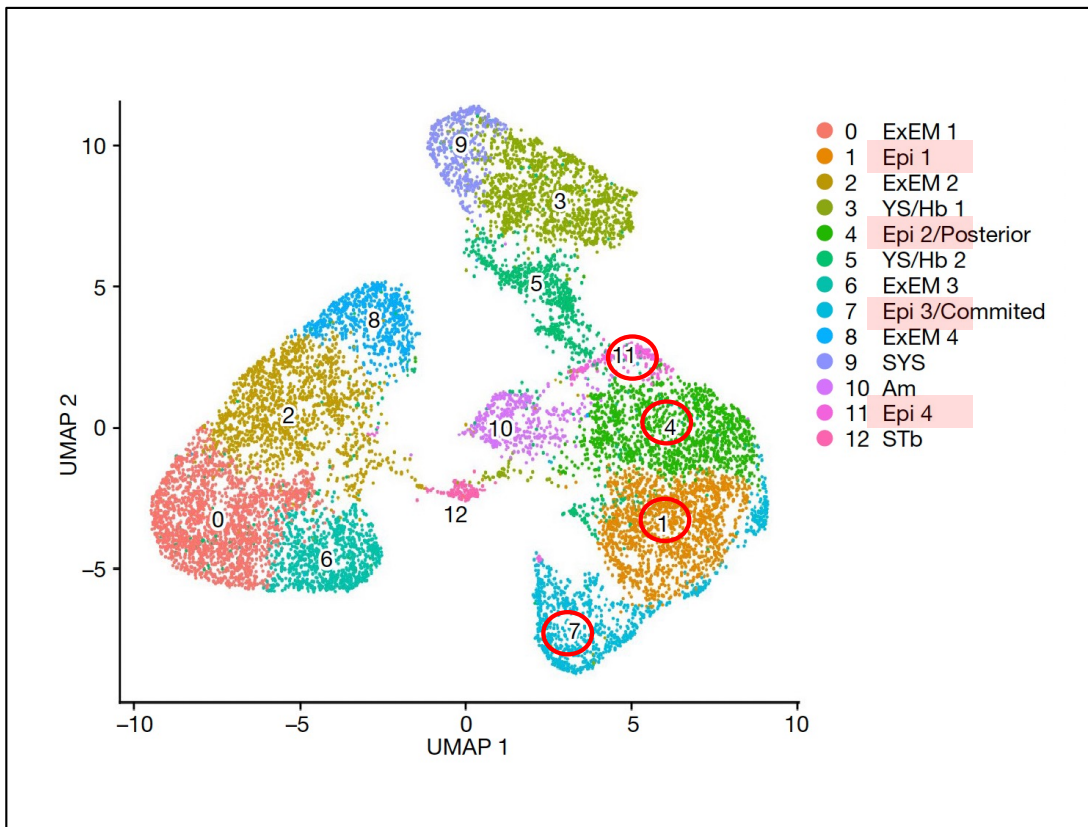
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*Les clusters sont définis par un ensemble d'ARNs présents.

*Différents types cellulaires mettent en œuvre des programmes génétiques différents.

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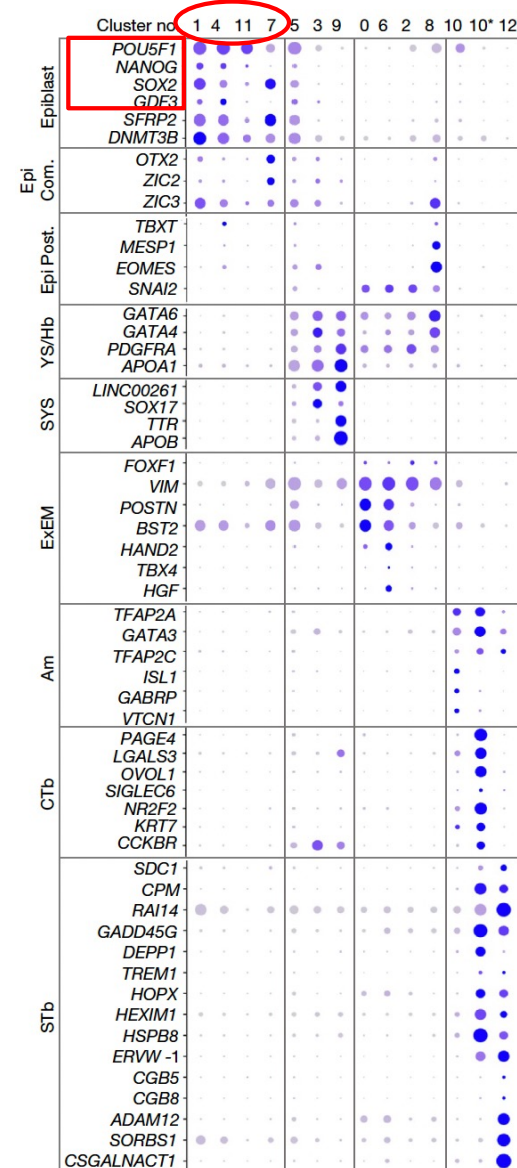
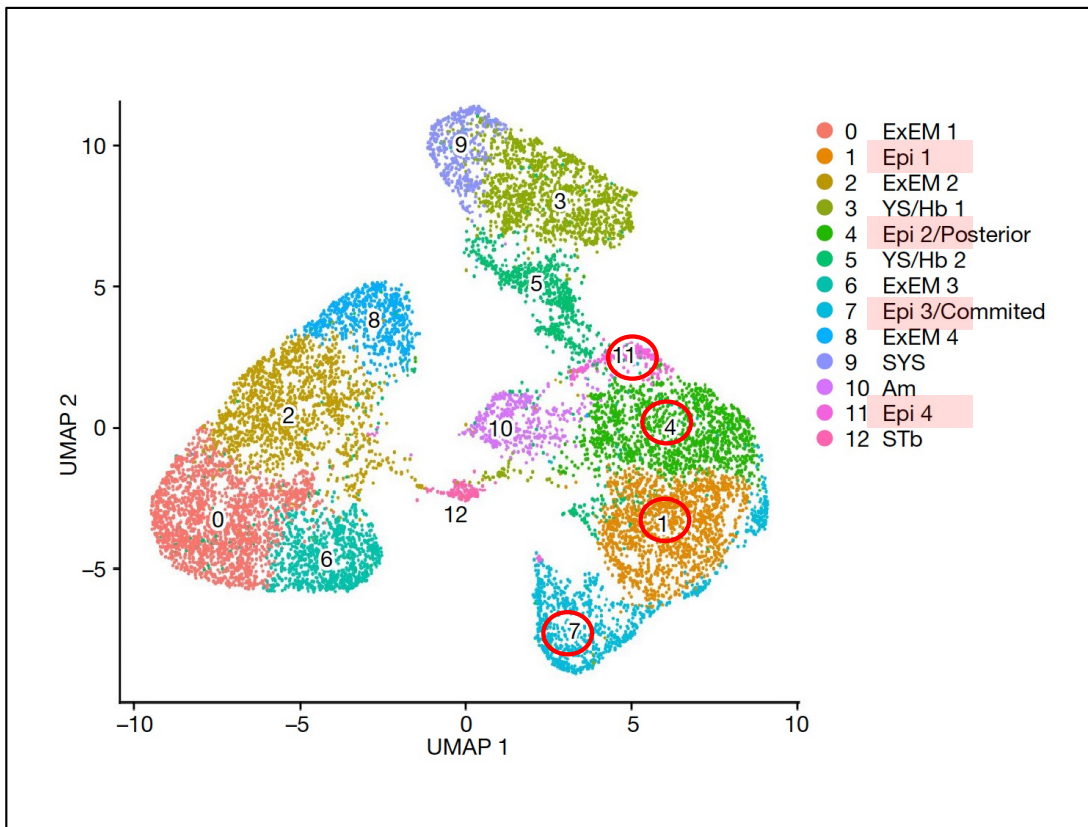
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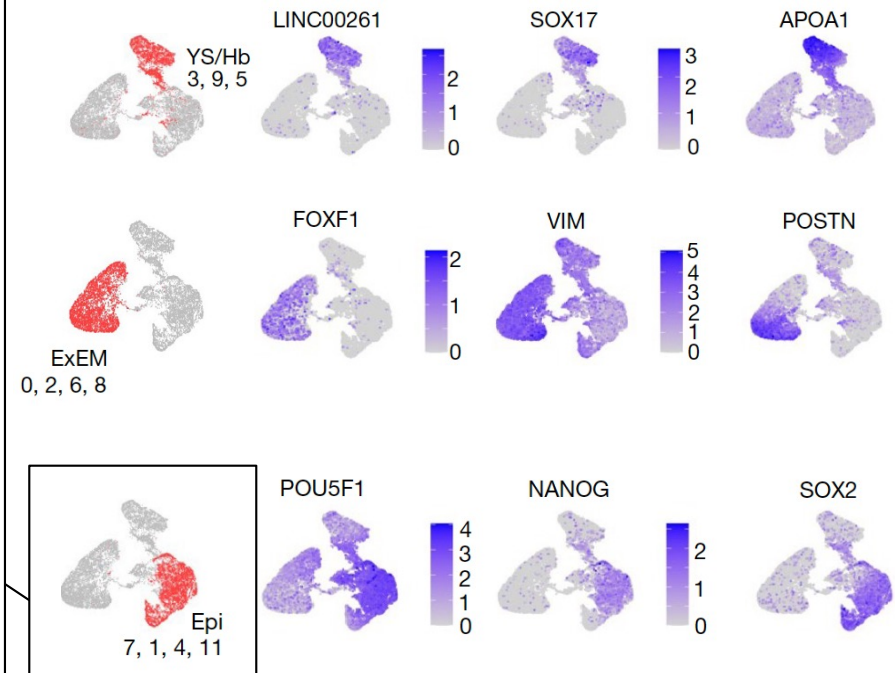
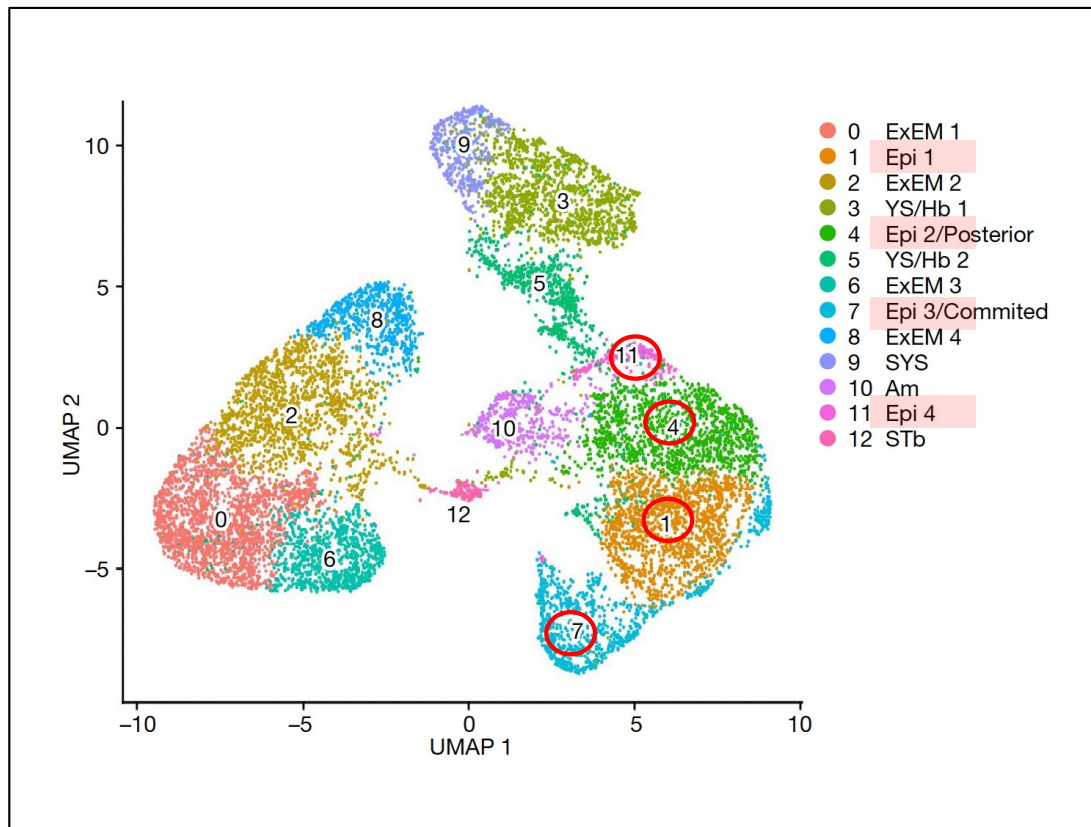
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*Exemples de la présence d'ARNs de gènes ayant été analysés par des approches *in vivo* microscopiques. Ces ARNs sont à la base du regroupement des clusters dans le UMAP



Présence de cellules germinales

Article

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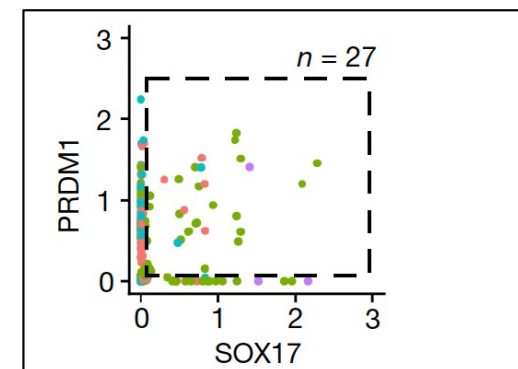
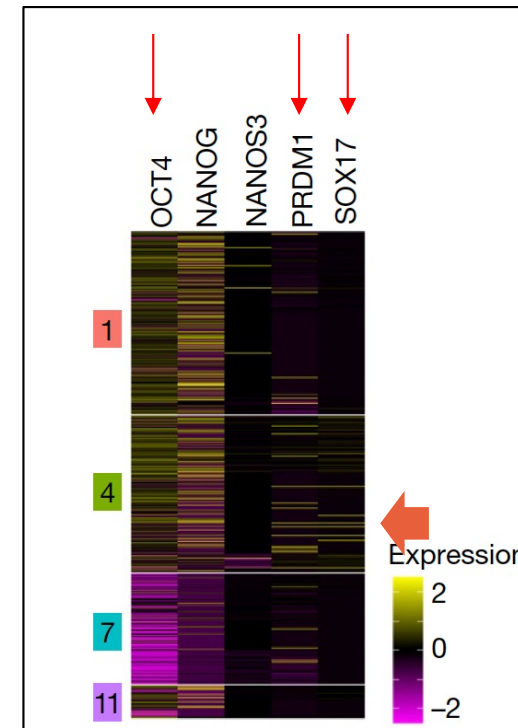
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PGC-like cells ($OCT4^+PRDM1^+SOX17^+$, a human definitive PGC marker combination²⁶) ($n = 27$) could be also identified in SEMs within epiblast clusters, with most of them ($n = 19$) in cluster 4 (Fig. 6d). Rare cells expressing $CD34^+TALI^+ERG^+$ could also be detected, which corresponded to early blood progenitors (Extended Data Fig. 12a,b).

*A l'intérieur du cluster #4, quelques cellules expriment à la fois **OCT4**, **PRDM1** et **SOX17**, une combinaison unique pour le marquage des PGCs humaines.

27 cellules de ce type sont identifiées (positives pour ces trois marqueurs) dont 19 (en vert) sont dans le cluster #4.

*Présence de cellules germinales primordiales (PGCs) et de cellules progénitrices de cellules sanguines.



Présence de cellules progénitrices sanguines

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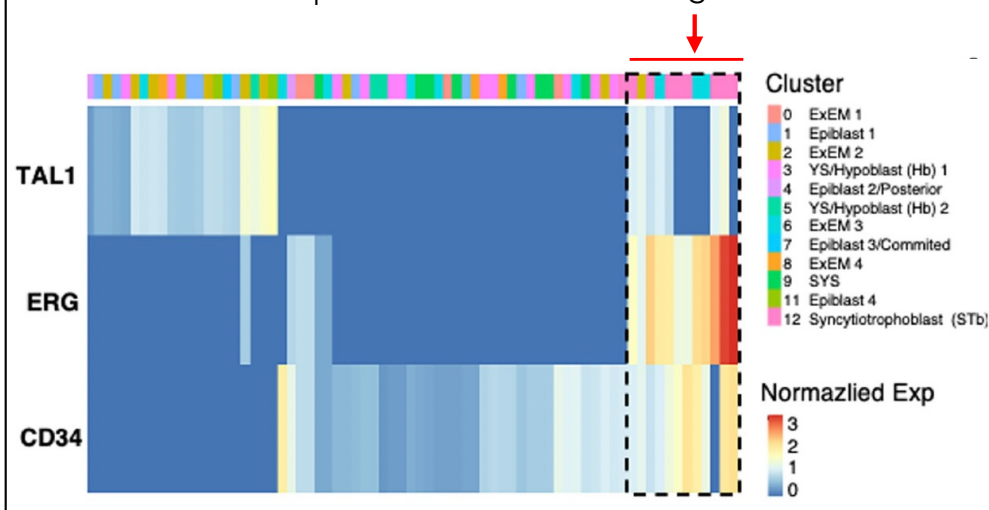
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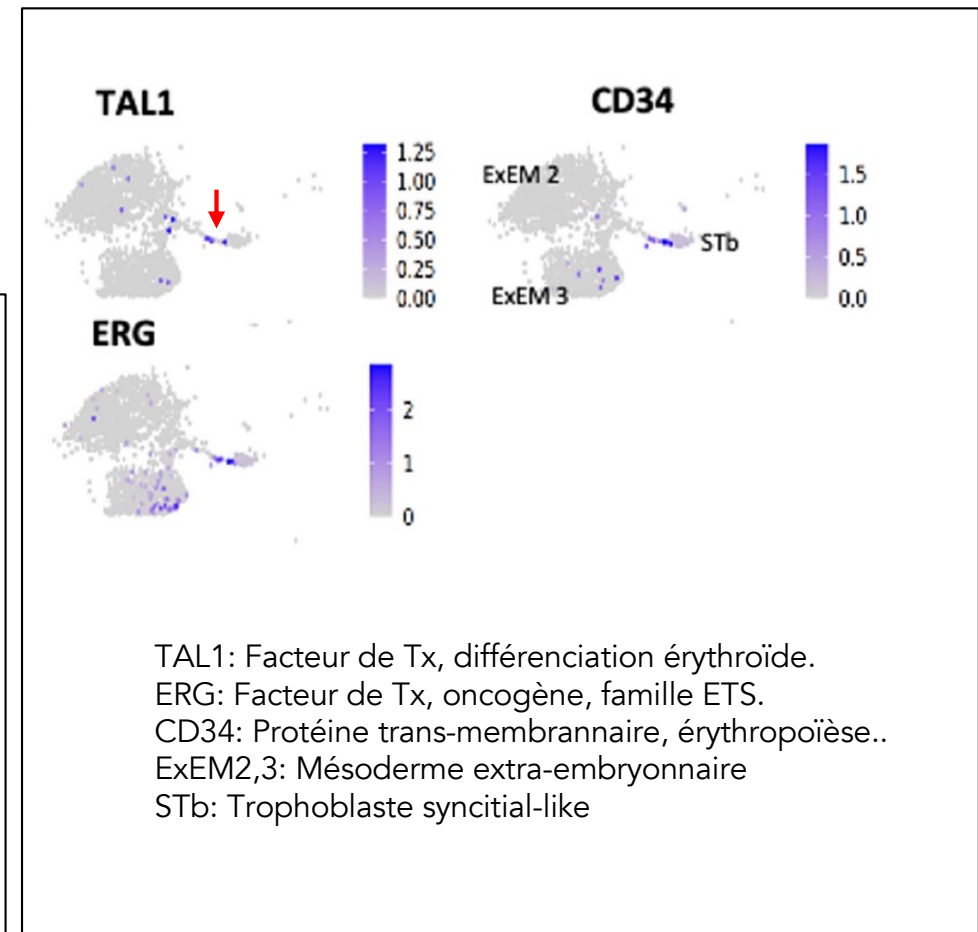
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*Dans cette partie hachurée (clusters #4 et #7), 6 cellules (six) expriment les trois marqueurs, définissant des précurseurs de cellules sanguines.



*Présence de cellules germinales primordiales (PGCs) et de cellules progénitrices de cellules sanguines.



Comparaison avec des scARN-seq d'embryons

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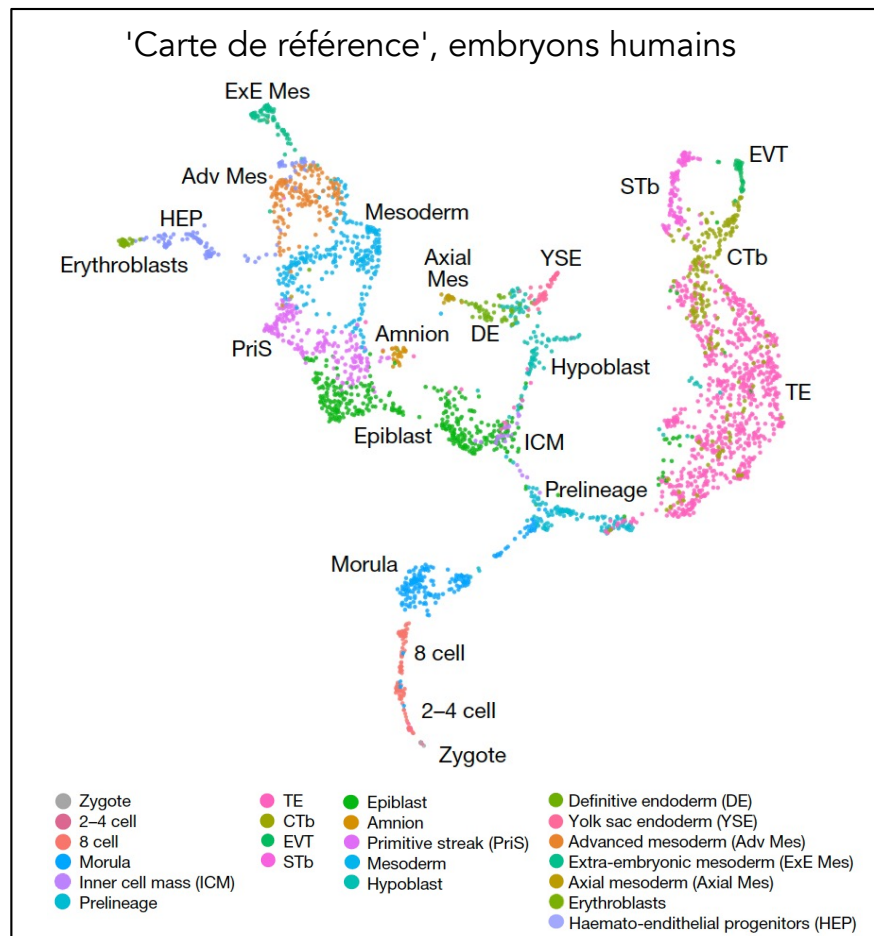
Comparaison entre les scARNseq des SEMs et ceux obtenus avec des embryons humains. Les data sont cumulés à partir de 6 datasets différents recouvrant:

*Zygotes, cultivés en clivages..

*Blastocystes

*Embryons pré-gastrulation

*CS7 (16-19 jours post-fertilisation)



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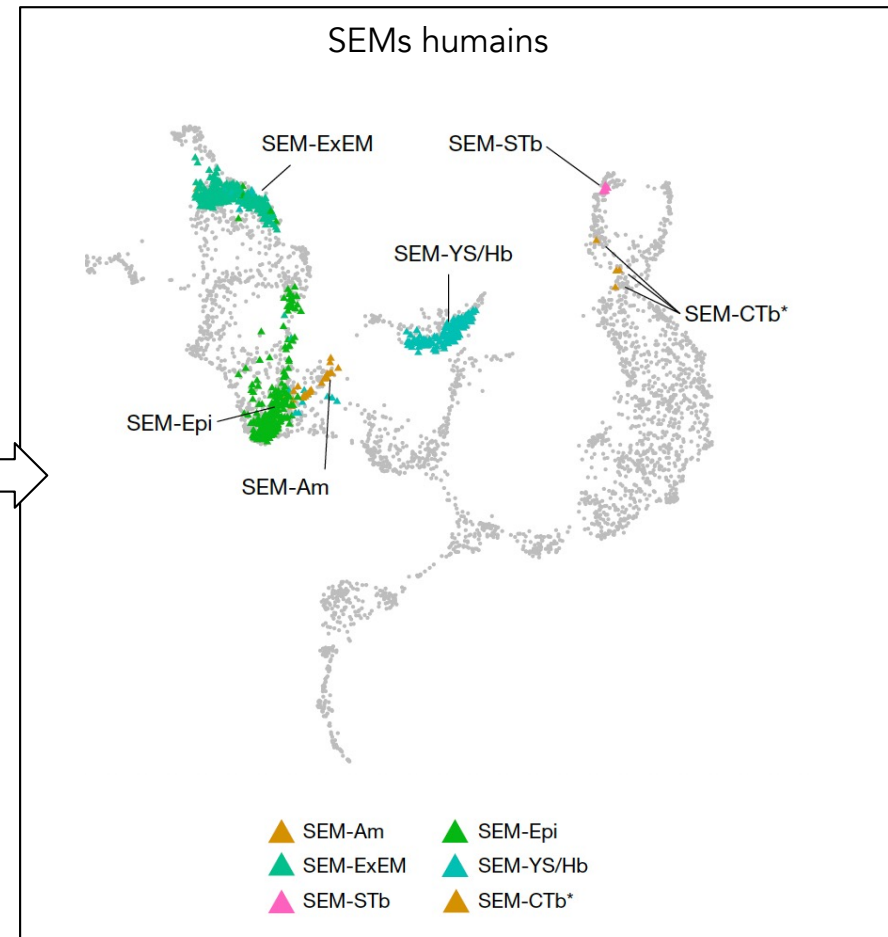
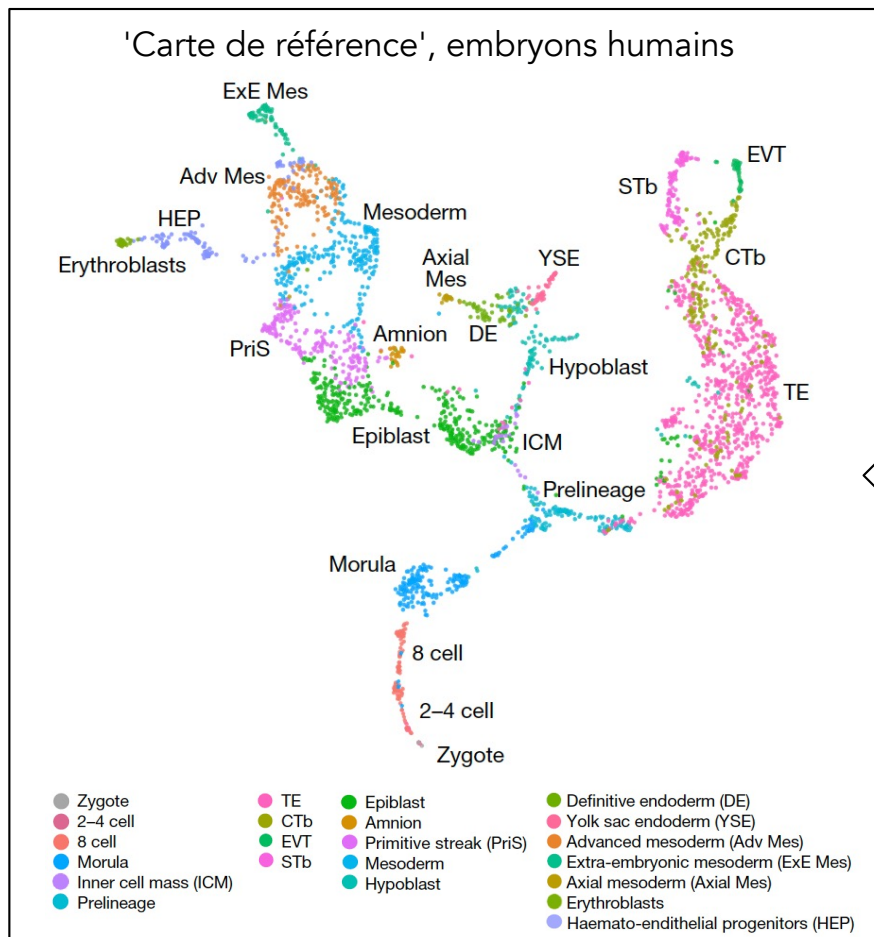
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Matériel de départ:

SEMs de 4 jours: ca. 80

SEMs de 6 jours: ca. 50

SEMs de 8 jours: ca. 50

Le UMAP mélange les trois types de SEMs

Conclusion de la comparaison SEMs-Embryons:

Les cellules identifiées (par leur contenu en ARNs) dans les SEMs correspondent chez l'embryon à des cellules de stades post-implantation et ne correspondent pas à des cellules de stades pré-implantation

Les SEMs progressent donc bien dans une direction de développement comparable à celle des embryons humains.



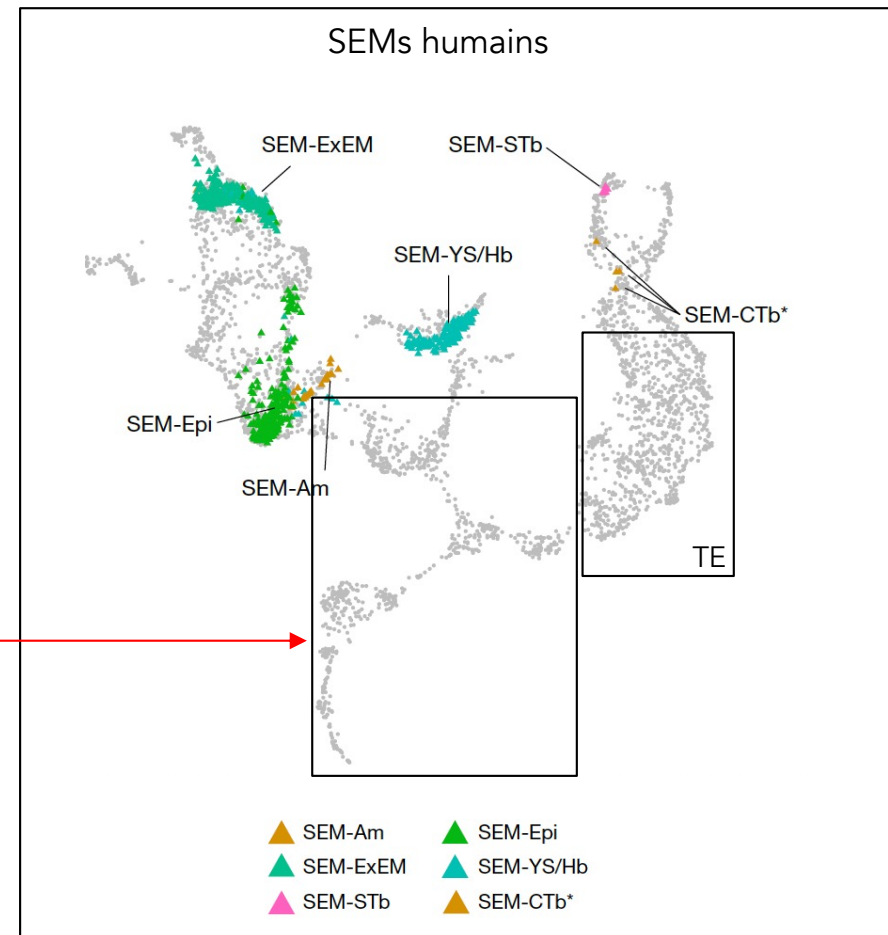
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Discussion et conclusions de l'étude



Denis Duboule/2024
La Fabrique des Embryons

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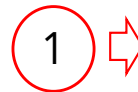
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Here we built on our recently described approach in mice³ to generate self-organizing complete post-implantation SEMs solely from naive ES cells, without the need to derive and utilize extra-embryonic cell lines from embryo tissue samples³. The latter technical finding is of great importance, as it suggested that only establishing naive PS cells may be sufficient to enable the generation of advanced embryo-like structures ex utero from other mammals, including humans³. Remarkably, the protocol developed here to generate complete human SEMs relies on starting solely from naive PS cells and does not even require the genetic modification or overexpression of exogenous lineage factors for priming the naive ES cells towards the three different extra-embryonic lineages prevalent at these developmental stages, contrary to what is currently still required in mouse SEM derivation protocols³. The latter further underscores the importance of devising



Discussion et conclusions de l'étude:

Un SEM complet et autoorganisé 'correspondant' à un embryon post-implantation peut être produit à partir de cellules ES naïves, sans utiliser des cellules de type extra-embryonnaires produites en parallèle. Ce protocole ne requiert pas non plus de modification génétique des cellules ES afin de 'primer' les cellules naïves (tout est dans la mise au point d'un protocole de culture et des milieux). C'est donc plus efficace que les protocoles courant pour faire des SEMs chez la souris.

We observed proper spatial allocation of cell lineages into defined and correctly positioned embryonic and extra-embryonic compartments in the complete absence of fertilization or interaction with maternal tissues and without the need to provide external directed signalling pathway induction during the self-organization of the aggregated cells.



La localisation des tissus embryonnaires et extra-embryonnaires est spatialement correcte, ceci en complète absence d'interactions avec des tissus maternels, sans fertilisation et sans induction extérieure directe de voies de signalisation.

Discussion et conclusions de l'étude



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At the structural level, our human SEM highly resembles, but not identical, to the in utero situation.



D'un point de vue structurel, ces SEMs humains ressemblent -mais ne sont pas identiques- à des embryons.

The current low efficiency and developmental-stage variability observed during the formation of our human SEMs are limiting factors that need to be overcome to facilitate the use of such platforms for certain experimental set-ups. Nevertheless, the emergence of well-defined complete structures suggests that this will probably be possible in the future (Supplementary Information). It will be of interest to further



L'efficacité très basse de ce protocole est un facteur limitant qui devra être résolu à l'avenir si l'on veut vraiment utiliser cette plateforme pour étudier différentes questions.

Finally, testing whether human SEMs described here can develop further towards completing gastrulation and advancing through organogenesis, as recently achieved with mouse SEMs exclusively made from naive ES cells³, may be of experimental importance and will offer insights into previously inaccessible windows of early human development and might contain authentic differentiated somatic cell types that could be useful for cell-transplantation therapy and tissue regeneration.



Vérifier que ces SEMs peuvent se développer plus tardivement, terminer leur gastrulation et avancer dans l'organogenèse (comme récemment montré chez la souris à partir de cellules ES naïves) pourrait être important d'un point de vue expérimental, pour comprendre certains phénomènes et pour certaines thérapies (transplantation, régénération..).

Questions et cadres éthiques (à l'interne...)

Debate ethics of embryo models from stem cells

By [Nicolas Rivron](#), [Martin Pera](#), [Janet Rossant](#), [Alfonso Martinez Arias](#), [Magdalena Zernicka-Goetz](#), [Jianping Fu](#), [Susanne van den Brink](#), [Annelien Bredenoord](#), [Wybo Dondorp](#), [Guido de Wert](#), [Insoo Hyun](#), [Megan Munsie](#) & [Rosario Isasi](#)

Stem Cell Reports Perspective



OPEN ACCESS

Human embryo research, stem cell-derived embryo models and *in vitro* gametogenesis: Considerations leading to the revised ISSCR guidelines

Amander T. Clark,^{1,*} Ali Brivanlou,² Jianping Fu,³ Kazuto Kato,⁴ Debra Mathews,⁵ Kathy K. Niakan,⁶ Nicolas Rivron,⁷ Mitinori Saitou,⁸ Azim Surani,⁹ Fuchou Tang,¹⁰ and Janet Rossant¹¹

‘Ceci n’est pas un embryon?’ The ethics of human embryo model research

[Nienke de Graeff](#), [Lien De Proost](#) & [Megan Munsie](#)

Changing the public perception of human embryology

[Nicolas C. Rivron](#), [Alfonso Martinez-Arias](#), [Karen Sermon](#), [Christine Mummery](#), [Hans R. Schöler](#), [James Wells](#), [Jenny Nichols](#), [Anna-Katerina Hadjantonakis](#), [Madeline A. Lancaster](#), [Naomi Moris](#), [Jianping Fu](#), [Roger G. Sturmeijer](#), [Kathy Niakan](#), [Janet Rossant](#) & [Kazuto Kato](#)

Modelling human embryogenesis: embryo-like structures spark ethical and policy debate

Ana M. Pereira Daoud^{1,2,3,†}, Mina Popovic^{4,†}, Wybo J. Dondorp^{1,3,5,6}, Marc Trani Bustos^{4,7}, Annelien L. Bredenoord², Susana M. Chuva de Sousa Lopes^{4,8}, Susanne C. van den Brink⁷, Bernard A. J. Roelen⁹, Guido M. W. R. de Wert^{1,3,5,†}, and Björn Heindryckx^{4,*†}

Opportunities and challenges with stem cell-based embryo models

[Janet Rossant](#)¹, [Patrick P.L. Tam](#)²

Human stem cell-derived embryo models: Toward ethically appropriate regulations and policies

[Alessandro Blasimme](#) • [Jeremy Sugarman](#)

Perspective

An ethical framework for human embryology with embryo models

Nicolas C. Rivron,^{1,*} Alfonso Martinez Arias,² Martin F. Pera,³ Naomi Moris,⁴ and Hafez Ismaili M'hamdi⁵

Forum

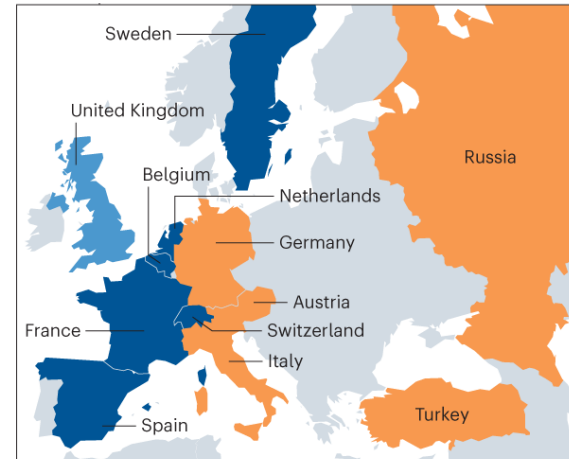
Human embryo models made from pluripotent stem cells are not synthetic; they aren't embryos, either

Hannah L. Landecker^{1,2,3,*} and Amander T. Clark^{3,4,5,*}

Des cadres légaux disparates

Culture d'embryons

- Légale jusqu'à 14 jours
- Légale jusqu'à 14 jours ou apparition de la ligne primitive
- Légale jusqu'à l'apparition de la ligne primitive
- Illégale



Définition d'un embryon

- Pas de définition
- La définition inclut la notion de potentialité
- La définition inclut la fertilisation d'un ovocyte
- La définition inclut n'importe quelle méthode de création



Adapté de de Graeff et. al. (2023)

Quelques questions ouvertes...

Technologie:

- *Quels vrais niveaux de **similarité** entre ces modèles et des embryons ?
- *Quels **stades** du développement peuvent être atteints en principe ?
- *Peut-on **implanter** ces embryons ?

Cadre éthique:

- *Est-il acceptable/désirable de **produire** ces modèles d'embryons ?
- *Est-il acceptable/désirable de les utiliser à des fins de **recherche** ?
- *Serait-il acceptable/désirable de les **implanter in vivo*** ?

Cadre légal:

- *La législation **actuelle** s'appliquent-elle aux modèles d'embryons ?
- *Les lois actuelles fournissent-elles le cadre légal souhaitable ?
- *Quel besoin de **légiférer** ou de mieux surveiller ?

*soit temporairement, soit à terme dans le cas très hypothétique de risque zéro....