COLLEGE ED BIOLOGIE DOCTORAL SANTE PAYS DE LA LOIRE

Year 20 /20

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin: ANR + Region + Kinetomics Please contact Karine.cantele@univ-nantes.fr for further details**
Thesis title: **Mechanism of AVE Differentiation in Anterior-Posterior Axis Determination in Human Development**		3 keywords: Anterior Visceral Endoderm (AVE), Symmetry breaking, Human embryogenesis
Unit / team: **UMR1064 Team2**		
Supervisor's name: **Thomas Fréour**		Phone number: +33 240084370 Email address: thomas.freour@chu-nantes.fr

Socio-economic and scientific context (approximately 10 lines):

The induction of asymmetry during embryonic development is crucial for the proper formation of body axes. In mammals, the anterior-posterior axis is the first to form and is determined by the site of gastrulation induction. The anterior visceral endoderm (AVE), derived from the primitive endoderm (PrE) of extra-embryonic tissues, exhibits asymmetry before gastrulation and acts as a tissue organizer that controls the induction of the anterior-posterior axis. However, ethical constraints on using human embryos for research have limited the understanding of the differentiation mechanism of human AVE. This research aims to elucidate the mechanisms underlying AVE differentiation, which is key to understanding anterior-posterior axis determination in human development. Understanding these mechanisms has significant implications not only for fundamental questions about the symmetry-breaking process in developmental biology but also for improving assisted reproductive technologies and therapeutic interventions for developmental disorders.

Working hypothesis and aims (approximately 8 lines):

The research project aims to uncover the mechanisms of anterior visceral endoderm (AVE) patterning to understand the symmetry-breaking process during anterior-posterior axis formation in human embryonic development. We hypothesize that AVE fate is uniformly induced in primitive endoderm (PrE) cells, followed by spatial confinement to the anterior region through intrinsic self-patterning mechanisms, as AVE is the first lineage to establish symmetry breaking along the anterior-posterior axis. The project first aims to decipher the differentiation mechanisms from PrE to AVE. Then, we will investigate spatial AVE patterning, focusing on cell fate decisions and migration regulation using stem cell-based modeling technology.

Main milestones of the thesis (approximately 12 lines):

1. Identify the Stage-Specific AVE Marker: Utilize publicly available single-cell RNA sequencing data from embryos to identify stage-specific AVE markers and potential differentiation regulators.
2. Identify Differentiation Conditions from Naive PSC to AVE: Optimize published differentiation protocols to transition naive pluripotent stem cells (PSCs) to primitive endoderm (PrE) and further differentiate them into AVE, utilizing the AVE markers identified in the first milestone.
3. Establish AVE Local Regulation Model: Develop a model to study the local regulation of AVE patterning using micropatterning technology with the differentiation protocol established in the second milestone, focusing on spatial confinement and self-patterning mechanisms.
4. Investigate Spatial AVE Patterning: Examine the spatial regulation of AVE patterning by investigating cell fate decisions and migration regulation using live-cell imaging and spatial transcriptomics.
5. Validation of Findings in 3D Post-Implantation Development Model: Use post-implantation culture of stem cell based blastocyst model to validate that the mechanisms found in the 2D model play a role in the 3D context under the influence of other cell lineages.

Scientific and technical skills required by the candidate (2 lines):

Stem cell culture especially human naive pluripotent stem cells.
Imaging and image analysis

3 publications from the team related to the topic (last 5 years):

1. **Onfray, C., Chevolleau, S., Moinard, E., Girard, O., Mahadik, K., Allsop, R., Georgolopoulos, G., Lavigne, R., Renoult, O., Aksoy, I., Lemaitre, E., Hulin, P., Ouimette, J. F., Fréour, T., Pecqueur, C., Pineau, C., Pasque, V., Rougeulle, C., & David, L. (2024). Unraveling hallmark suitability for staging pre- and post-implantation stem cell models. Cell reports, 43(5), 114232.**
2. **Kagawa H, Javali A, Khoei HH, Sommer TM, Sestini G, Novatchkova M, Scholte Op Reimer Y, Castel G, Bruneau A, Maenhoudt N, Lammers J, Loubersac S, Freour T, Vankelecom H, David L, Rivron N. Human blastoids model blastocyst development and implantation. Nature. 2022 Jan;601(7894):600-605.**
3. **Meistermann D, Bruneau A, Loubersac S, Reignier A, Firmin J, François-Campion V, Kilens S, Lelièvre Y, Lammers J, Feyeux M, [...], Soumillon M, Mikkelsen T, Barrière P, Chazaud C, Chappell J, Pasque V, Bourdon J, Fréour T, David L*. Integrated pseudotime analysis of human pre-implantation embryo single-cell transcriptomes reveals the dynamics of lineage specification. Cell Stem Cell. 2021 Sep 2;28(9):1625-1640.e6.**

National and international collaborations:

Claire Chazaud GReD (France)
Pierre Osteil (France)

Nicolas Rivron IMBA (Austria)