Organelle dynamics in cellular fate determination

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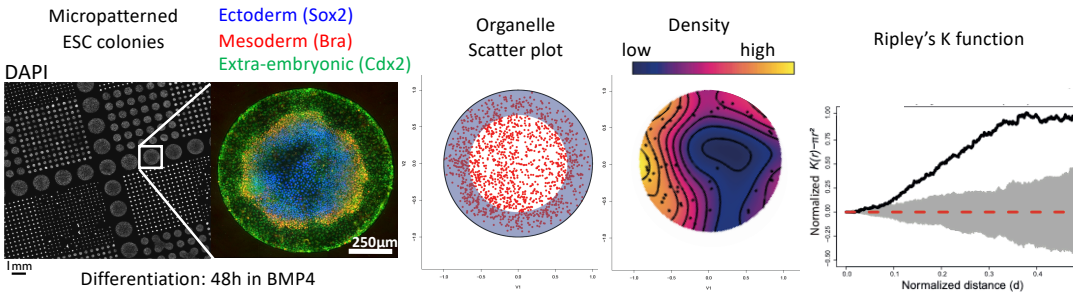
Ce stage peut être poursuivi en thèse : OUI
Si oui, la thèse est-elle financée : NON

Sujet du stage:

The presence of intracellular organelles is the major characteristic of eukaryotic cells. Organelles permit the compartmentalization of diverse cellular functions, but also contribute to the complexity of eukaryotic cell behavior. We study the landscape and dynamics of different organelles in a minimal system. Using cell culture on bioengineered surfaces that allow controlled cell geometry we have found that organelles adopt a characteristic and highly reproducible positioning at the single cell level (Schauer et al, 2012). Our micropatterned cells mimic reproducible intracellular landscapes typically found in tissue however are much easier to study and lack the complexity of the tissue environment. Dramatic changes in the intracellular landscape are taking place during cell differentiation, however, the changes on the organelle level are poorly characterized. Novel *in vitro* approaches employing micropatterning of embryonic stem cell (ESC) colonies can be used to replicate early differentiation during embryonic gastrulation (Warmflash et al, 2014). ESCs confined to circular disks comparable in size to mammalian embryos and treated with the gastrulation inducing signal BMP4 differentiate to an outer trophectoderm-like ring followed by the three embryonic germ layers. This technology allows the investigation of organelle alterations during embryonic patterning in the human system.

In this M2 project, we want to quantify organelle landscapes during embryonic patterning and compare the distribution and dynamics of different organelles (mitochondria, lysosomes, nucleus) between the trophectoderm-like ring and the three embryonic germ layers throughout differentiation. The intern will prepare micropatterned substrates, grow ESC cultures on them, image intracellular organelles in fixed samples and by live cell imaging, perform image analysis based on segmentation, and analyze organelle distribution employing a tool box of spatial statistics as in (Lachuer et al. 2020). The internship will be performed between two teams, the *in vitro* growth of ESC colonies will be performed in the SORRE team and the spatial analysis will be done in the SCHAUER team. For imaging, sophisticated high-end microscopes will be used in the imaging facility of Institut Curie. The goal of the project is to set-up a pipeline to analyze organelles in a multicellular ESC colony and to obtain first quantitative data about organelle dynamics during differentiation.



Warmflash A*, **Sorre B*,** et al. *Nature Methods* (2014)

Lachuer H, et al. *JoVE* (2020)

Fig1: Pipeline to analyze organelles in a micropatterned colony of hESCs: Organelles in the embryonic germ layers will be images and segmented organelle coordinates will be analyzed with different statistical tools in order to obtain quantitative data about organelle dynamics during differentiation.

1. Warmflash A, **Sorre B**, et al. A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. Nat Methods. 2014 Aug;11(8):847-54.
2. **Schauer K**, et al. Probabilistic density maps to study global endomembrane organization. Nat Methods. 2010 Jul;7(7):560-6.
3. Lachuer H, Mathur P, Bleakley K, **Schauer K**. Quantifying Spatiotemporal Parameters of Cellular Exocytosis in Micropatterned Cells. J Vis Exp. 2020 Sep 16;(163).