Microfluidic gradient chip to understand early human embryonic patterning

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Equipe: Dynamic Control of Signaling and gene Expression
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One striking property of embryonic tissues is their ability to self-organize in order to form well-defined patterns that prefigure the body plan. One such early morphogenetic event is gastrulation, during which the cells of the pluripotent epiblast are allocated into three germ layers that derive from it: the ectoderm, the mesoderm and the endoderm.

Assessing the relative contribution of parameters controlling this process (morphogen gradients, mechanics…) in a quantitative manner requires at the same time to record fates and movement with single cell resolution but also to be able to apply well defined perturbations to the system, which is usually not possible in live embryos, especially for species developing *in utero*, like human. Thus, to address these issues in a quantitative manner, we are developing tool that allow *in vitro* recapitulation of early embryonic patterning and morphogenetic processes (Warmflash et al. Nat Methods 2014).

Recently, we have designed a microfluidic chip to cultivate human Embryonic Stem Cells (hESC) for several days under stimulation of fully controllable gradients of morphogens. Using this unique device, we have studied how the tissue patterning depend on the maximum concentration, and the shape (steepness, linear vs parabolic) of a BMP gradient. Cell identity can be recorded live thanks to fluorescent reporters (Fig1A-C).

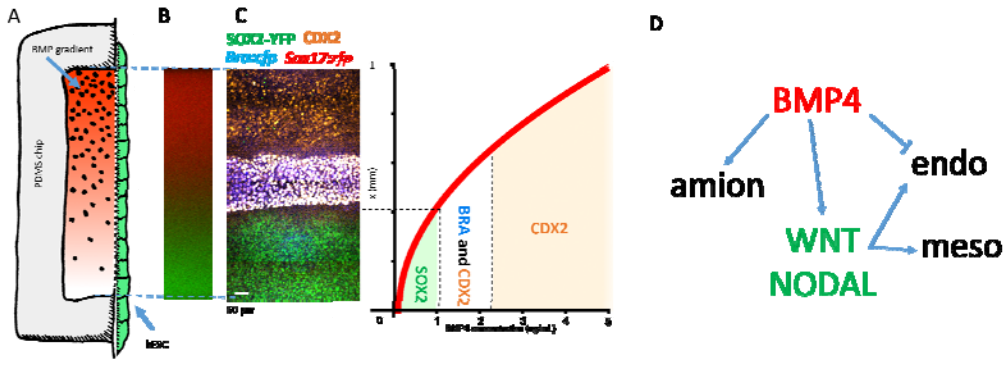

*Fig1: **Antiparallel gradient chip**. A: sketch showing the principle of the chip B: Proof of principle of hESC differentiation in the gradient chip. after 48h of differentiation in a parabolic gradient of BMP. Cells get an extra-embryonic identity at the high end of the BMP gradient (CDX+2, amnion) at intermediate dose cells adopt a mesodermal fate (BRACHYURY+) and stay pluripotent under a certain threshold (SOX2+). (D) Model for the patterning to be tested. BMP4 induces the amnion identity directly and also induces the production of secreted diffusible factors (WNT, NODAL) necessary for formation of endoderm and mesoderm. BMP4 prevents formation of endo.*

Our preliminary results show that in a parabolic gradient of BMP4, some cell types (amnion-like cells, mesoderm) are generated in a concentration dependent manner, in perfect agreement with the classical "French flag model" (Wolpert, 1969). However, endoderm cells are not generated in those parabolic BMP gradients. They were observed however in steeper profiles of BMP concentration, such as step function. These observations suggest a model for patterning of the embryo in which the tradeoff between the spatial profile of BMP and the diffusion properties of mesendoderm inducing molecules (WNT, NODAL) plays a crucial role in the tissue patterning (fig1D). The goal of this internship is to investigate how the patterning evolves when other morphogens are added in order to test and constraint this model that defines the rules for the spatial organization of cell identities present in the gastrula.

During this internship, you will learn: making and operating advanced hybrid 2-layer microfluidic chips with porous membranes, culture and characterization of human embryonic stem cells, confocal microscopy and image analysis.