Mechacompa Publishable Final Summary (including Graphics)

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During pre-implantation development, the mammalian embryo self-organizes into the blastocyst consisting of an epithelial layer encapsulating the inner-cell mass (ICM), which gives rise to all embryonic tissues (Wennekamp et al., 2013). The positioning of blastomeres at the surface or inside the embryo is a pre-requisite to their differentiation into trophectoderm or ICM, respectively (Chazaud and Yamanaka, 2016). Indeed, cell-cell contacts promote the expression of pluripotency genes while apical membrane, exposed to the outside medium, promotes trophectoderm genes (Cockburn et al., 2013; Hirate et al., 2013). How cells acquire their position within the mammalian embryos remains poorly understood.

Following the discovery of periodic contractions in 8-cell stage blastomeres (Maître et al., 2015), I observed that, after division to the 16-cell stage, only blastomeres destined to form the inner cell mass would show prominent periodic contractions. This difference in behavior between sister cells could arise from asymmetric division of components of the contractile apparatus. However, after inspecting the inheritance of actin and myosin, I could not observe any asymmetry between sister cells immediately after division. That said, during the 16-cell stage, within a few hours, increasing levels of actin and myosin in the blastomeres destined to be internalized create an asymmetry between the sister cells. Previous studies showed that apical material is asymmetrically distributed during the 8- to 16-cell stage division (Johnson and Ziomek, 1981). Close inspection of the apical domain at the 8- and 16-cell stage reveals that it contains reduced levels of actin and myosin. Moreover, at the 8-cell stage, periodic contractions present lower amplitudes at the apical domain as compared to the non-apical surface of the blastomere. To test the functional relationship between apical proteins and contractility, I used double knockout embryos, which lack the zeta and iota isoforms of aPKC, an essential protein of the apical domain (Hirate et al., 2013). These embryos showed high levels of actin and myosin in lieu of the apical domain. Therefore, the apical domain is a domain of low contractility, which, when inherited by one daughter cell only, generates heterogeneities in the contractility of blastomeres.

As cells adopt different contractile behaviors during the 16-cell stage, the strongly contracting blastomeres progressively internalize. Cell sorting can result from differences in contractility (Krieg et al., 2008; Maître et al., 2012). To test whether differences in contractility are responsible for the positioning of the precursors to the inner cell mass, I mixed blastomeres engineered to have different contractility. First, I identified Myh9 among the 3 isoforms of the non-muscle myosin heavy chain present in the mouse genome as the isoform responsible for contractility during pre-implantation development. Then, I generated chimaeras using wild-type (WT) and maternal Myh9 knockout embryos (mMyh9). When placed at the surface of mMyh9 embryos, WT blastomeres often pull themselves to the inside of host embryos (Figure). On the contrary, mMyh9 blastomeres always stay at the surface of WT embryos. Therefore, differences in contractility are sufficient to control the positioning of cells within the pre-implantation embryo.

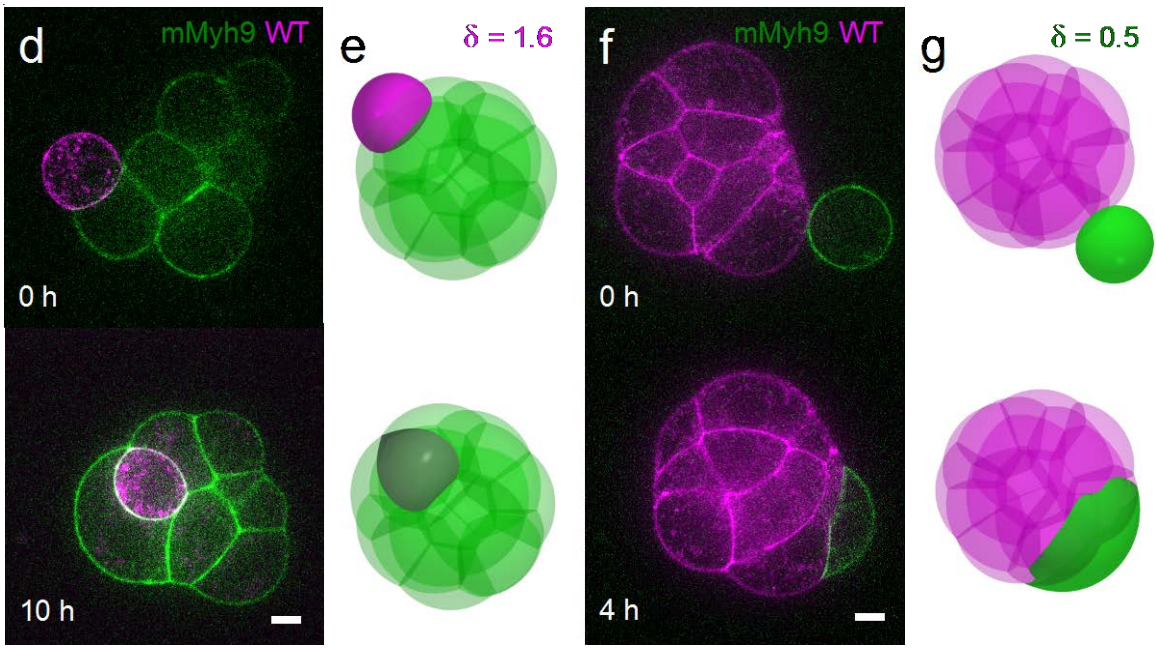

Figure: Experiments (d, f) with chimeric embryos consisting of maternal myosin knockout (mMyh9, green) and wild-type (WT, magenta) blastomeres. Accompanying simulations (e, g) mimic the experiments by giving WT blastomeres a tension permitting internalization (δ = 1.6) and mMyh9 blastomeres one that does not (δ = 0.5). Taken from (Maître et al., 2016).

To understand the precise mechanisms controlling cell sorting within the pre-implantation embryo, I teamed up with Hervé Turlier, a theoretical physicist who is also a Marie Curie fellow. Hervé Turlier developed a three-dimensional mesh-based model to simulate 16 cells with different tensions, which are controlled by contractility. Simulations successfully recapitulated the shapes and movements observed in experiments. Importantly, the model predicted a threshold value for the internalization of cells to occur. With the degree of compaction observed in the mouse embryo (Maître et al., 2015), the model predicts that internalization occurs whenever the tension of a blastomere exceeds 1.5 times the one of neighboring blastomeres (Figure). To test this quantitative prediction, I mapped the tensions of the blastomeres before internalization using micropipette aspiration. Indeed, tension mapping reveals that no blastomeres at the surface of the embryo exceed the threshold value of 1.5, which suggests that any cell having passed the threshold has already internalized and is inaccessible to the micropipette. Moreover, tension measurements of mMyh9 embryos confirm that these mutant blastomeres have tensions lower than the internalization threshold. The theoretical model together with experimental validations give us a quantitative understanding of the conditions and mechanisms for the formation of the inner cell mass.

Controlling the positioning of blastomeres should allow me to control their fate (Chazaud and Yamanaka, 2016). When disturbing contractility, cell positioning is affected and upon inspection, fate specification is also affected, indicating that contractility is essential for normal specification of the external extra-embryonic and internal embryonic tissue. However, upon closer inspection, I realized that although cells fail to internalize when contractility is impaired, the fate they adopt is not of extra-embryonic tissue, as expected for external cells, but instead of the embryonic one. In other words, when contractility is defective, blastomeres fail to internalize but also adopt the incorrect fate. One possible explanation lies

in the mechanisms by which mouse blastomeres differentiate into embryonic or extra-embryonic tissue. Indeed, the Yap transcriptional co-activator, which controls this initial lineage specification in the mouse embryo, is thought to respond to mechanical cues (Dupont et al., 2011). Such mechanosensing would explain how contractility couples the positioning and fate specification of the blastomeres during pre-implantation development.

Altogether, this study elucidates the mechanics of the positioning of the precursors to the mammalian fetus and reveals unexpected mechanisms controlling fate specification.

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