Four simple rules that are sufficient to generate the mammalian blastocyst

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***Short Abstract* — Early mammalian development is a fascinating example of how deterministic spatiotemporal patterns emerge at the level of cell populations from highly stochastic regulatory components, but why is this process so successful, and what ensures the stability of the blastocyst? With four simple rules, we get cavity formation, salt and pepper pattern, and three distinct lineages. In addition, we get most phenotypes by eliminating one rule at a time. Finally, we show that time from post-fertilization regulates the cells' competence of FGF. This could provide the robustness necessary for the evolutionary diversification of the preimplantation gene regulatory network.**

***Keywords* — Early embryonic development, blastocysts, cell polarity, cell communication, fibroblast growth factor (FGF), segregation, differential adhesion, apoptosis.**

I. Purpose

EARLY mammalian development is both highly regulative and self-organizing [1]. It involves the interplay of cell position, gene regulatory networks, and environmental interactions to generate the physical arrangement of the blastocyst with precise timing [2-3]. However, this process occurs in the absence of maternal information and in the presence of transcriptional stochasticity [4-5].

How does the preimplantation embryo ensure robust, reproducible development in this context? It utilizes a versatile toolbox that includes complex intracellular networks coupled to cell-cell communication, segregation by differential adhesion, and apoptosis.

Here, we ask whether a minimal set of developmental rules based on this toolbox is sufficient for successful blastocyst development, and to what extent these rules can explain mutant and experimental phenotypes.

II. Results

We implemented experimentally reported mechanisms for polarity, cell-cell signaling, adhesion, and apoptosis as a set of four developmental rules in an agent-based *in silico* model of physically interacting cells.

First rule, surface cells develop polarity at E3.0. Second rule, inner cells surrounded by too many cells of the same type, switch fate. Third rule, primitive endoderm (PrE) progenitors get less adhesion. Fourth and final rule, PrE progenitors in a wrong position undergo apoptosis.

We find that this model quantitatively reproduces specific mutant phenotypes and provides an explanation for the emergence of heterogeneity without requiring any initial transcriptional variation [6].

It also suggests that a fixed time point for the cells' competence of fibroblast growth factor (FGF)/extracellular signal-regulated kinase (ERK) sets an embryonic clock that enables certain scaling phenomena, a concept that we evaluate quantitatively by manipulating embryos *in vitro*.

Embryos were obtained at 8-cell stage and placed in pairs or triplets to make aggregates. Controls were cultured in KSOM only, while a Mek inhibitor was added to delay experiments [7]. At E4.5, embryos were fixed and stained, and analyzed with a custom-built MATLAB script.

III. Conclusion

Based on these observations, we conclude that the minimal set of rules enables the embryo to experiment with stochastic gene expression and could provide the robustness necessary for the evolutionary diversification of the preimplantation gene regulatory network.

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