Mechanochemical symmetry breaking in gastruloids

Torregrosa G.[2]*, Oriola D.[1]*, Trivedi V.[1]†, and Garcia-Ojalvo J.[2]†
[1]European Molecular Biology Laboratory (EMBL), Barcelona Biomedical Research Park (PRBB), Spain
[2]Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park (PRBB), Spain

Multicellular animals are a paradigm of self-organization, especially during their developmental stages. The sophistication of this self-organization has led to the proposal of multiple alternative mechanisms to achieve the timing, precision, and patterning required for developing their complex structures. These mechanisms range from communication through chemical components [1] to mechanical interactions [2], both with other cells and with their environment. Disentangling the spontaneous self-organization mechanisms used during development is still a challenge [1].

In recent years, gastruloids have been developed as a novel *in vitro* model to study self-organization during development [5]. In contrast with *in vitro* cell cultures, these biological models offer the possibility of studying self-organization in 3D: gastruloids are closer to the actual topology in which multicellular organisms develop. Moreover, they are easier to control experimentally than embryos.

In this work, we study theoretically and experimentally the process of symmetry breaking in the expression of a gene, specifically the mesodermal transcription factor Brachyury, in mouse gastruloids. This process establishes the initial coordinate axis that defines the anterior-posterior organization of the future embryo (head-tail). A quantitative study of single-cell RNA expression of the gastruloid cells provides information of the transcriptional paths at play during the decision. In this process, the cells start to maturate from the pluripotent state, expressing specific genetic pathways to a new fate marked by the gene Brachyury (Figure 1). This transition occurs during the initial 24h of development. We model the process by a master equation describing the jumps between metastable states:

$$\frac{dp_i}{dt} = f_i(p_i; \{p_j\}_{j\neq i}), \tag{1}$$

where $i \in 1, 2, 3$ are the metastable states defined by the transcriptomics data (Figure 1). The model considers that the transition function f_i depends on the existence of interaction between populations, supporting the idea that cell-cell communication is essential to control the proportion between cell types throughout the differentiation process.

Although the master equation approach explains the communication and proportions of cells at the different stages, additional candidate mechanisms for the observed long-range ordering are required. Candidates could be the long-range communication of diffusible chemicals, such as the ones underlying Turing patterning or wave pinning [1], or the aggregating effect of differential adhesion [2]. Lacking evidence of highly diffusible compounds and given the experimental evidence of different adhesion properties of the cell aggregates, we model the segregation with an agent-based model that takes into account differential adhesion between cells (Figure 2).

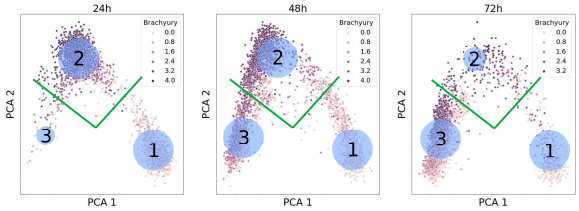

Fig. 1. Principal component projection of the single-cell RNA transcriptome. 1) Pluripotent state 2) Brachyury state 3) Posterior stages.

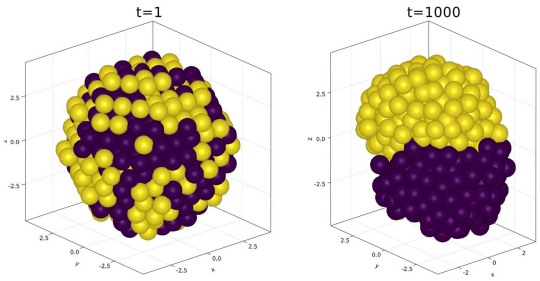

Fig. 2. Agent-based simulation exhibiting phase separation between cells in an *in silico* gastruloid, driven by active forces and differential adhesion between two populations.

The combination of the chemical communication and mechanical segregation recapitulates the generation of the Brachyury pole in gastruloids. These results pave the way towards understanding the processes underlying symmetry breaking in multicellular organisms.

[1] Green, J. B., & Sharpe, J. (2015). Development, 142(7), 1203-1211.

[2] Muncie, J. M., Ayad, N. M., Lakins, J. N., Xue, X., Fu, J., & Weaver, V. M. (2020). Developmental cell, 55(6), 679-694.

[3] Flenner, E., Janosi, L., Barz, B., Neagu, A., Forgacs, G., & Kosztin, I. (2012). Physical Review E, 85(3), 031907.

[4] Stumpf, P. S., Smith, R. C., Lenz, M., Schuppert, A., Mller, F. J., Babtie, A., ... & MacArthur, B. D. (2017). Cell Systems, 5(3), 268-282.

[5] Van den Brink, S. C., Baillie-Johnson, P., Balayo, T., Hadjantonakis, A. K., Nowotschin, S., Turner, D. A., & Martinez Arias, A. (2014). Development, 141(22), 4231-4242.

* Equal contribution.
† Equal contribution.