of Self-Organization Biological Systems Lab

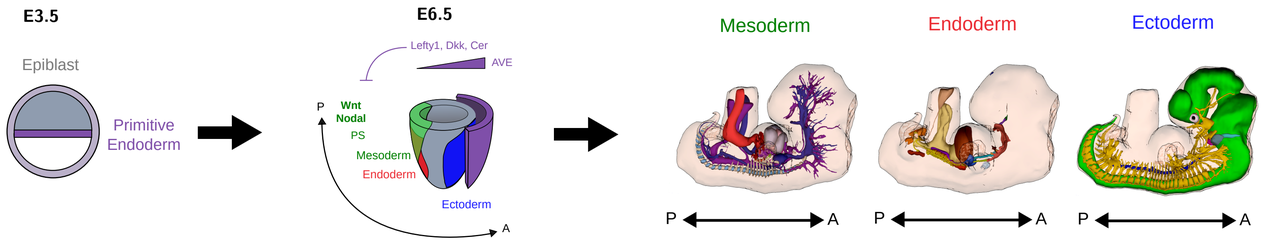
Centro Andaluz de Biología del Desarrollo

Scaling and variability of embryoid symmetry breaking

Rosana Cáceres Carrillo, María Lobo, Jelena Raspopovic, Luciano Marcon

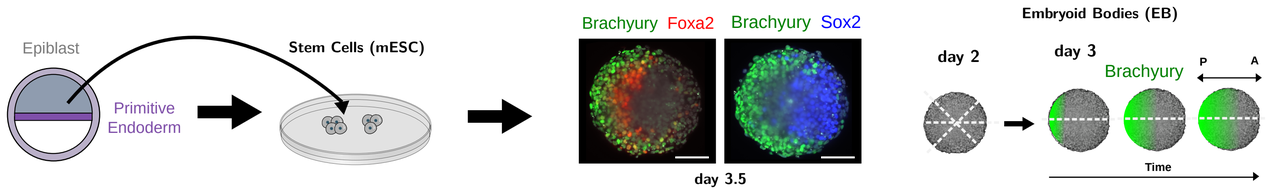
Introduction

Mouse development



During early mouse development, epiblast (gray) of the blastocyst forms the gastrula, a hollow cone surrounded by primitive Endoderm (PE, purple). Inhibitors coming from the anterior part of the PE (AVE), restrict Nodal and Wnt signaling to the posterior part of the epiblast to form the primitive streak (PS) marked by Brachyury. Cells ingress through the PS to form the Mesoderm and Endoderm. These two tissues together with the Ectoderm constitute the three germ layers.

Embryoid bodies

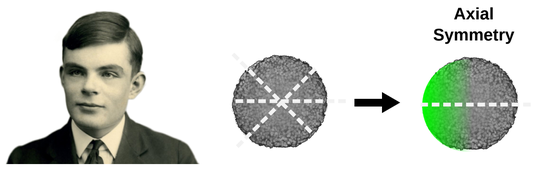


When mouse embryonic stem cells (mESC) are cultured in suspension, they form Embryoid Bodies (EBs) that can spontaneously form an anterior-posterior axis after 3.5 days in the absence of the PE, as shown by the expression of germ layer markers: Brachyury (Mesoderm), Foxa2 (Endoderm) and Sox2 (Ectoderm). The self-organization of the anterior-posterior axis is marked by by a moving front of mesendodermal fates (Bry) that originates on one side of the EBs.

Modeling

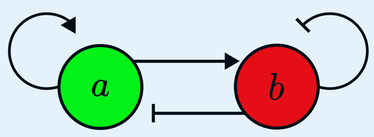
Turing reaction-diffusion model

Symmetry Breaking



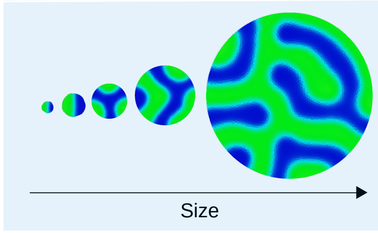
Alan Turing

Turing proposed that the initial symmetry of embryos could be broken by two diffusing and interacting substances, which in the presence of noise-dependent fluctuations form periodic spatial concentration profiles.



$$\frac{\partial a}{\partial t} = k_1\, a - k_2\, b - k_a\, a^3 + D_a \nabla^2 a$$
$$\frac{\partial b}{\partial t} = k_3\, a - k_4\, b + D_b \nabla^2 b$$

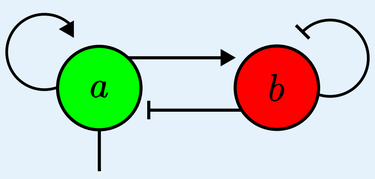
Simulations



Classic Turing systems predict an increasing number of concentration peaks as the size of the spatial domain increases. However, our data shows that self-organizing axis formation is a size-independent process.
In addition, Turing self-organizing peaks appear simultaneously across the whole tissue and cannot recapitulate the progressive expansion of Brachyury observed in EBs.

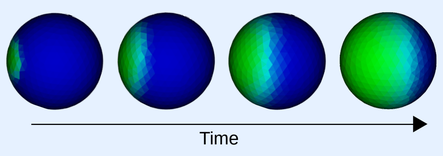
Bistable reaction-diffusion model

The propagation of moving wave fronts is a well-known property of bistable reaction-diffusion systems (excitable systems).

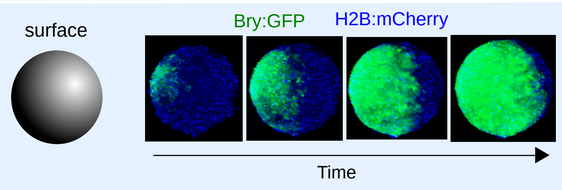


$$\frac{\partial \mathrm{WN}}{\partial t} = \gamma(\omega \frac{\mathrm{WN}^2}{1+\mathrm{WN}^2} - \mathrm{DL})$$
$$\frac{\partial \mathrm{DL}}{\partial t} = \gamma(\omega \frac{\mathrm{WN}^2}{1+\mathrm{WN}^2} - \mathrm{DL})$$

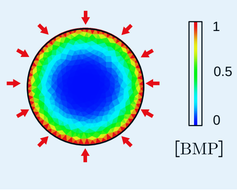
Simulations

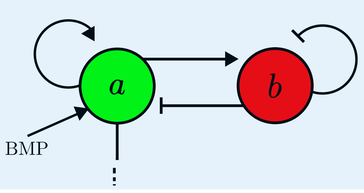


Experimental



Analysis of expression patterns in 3D shows that germ layer markers are expressed in a crescent shaped three-dimensional domain that expands along surface of the EB but does NOT reach its internal core. These expression patterns suggest that the mechanisms that underlie axis self-organization in EBs is under the influence of signals from the boundary, so to explore the effect of boundary conditions on the symmetry breaking process, we extended the bistable reaction-diffusion model by adding a third component BMP that diffuses from the boundary and decays homogeneously.





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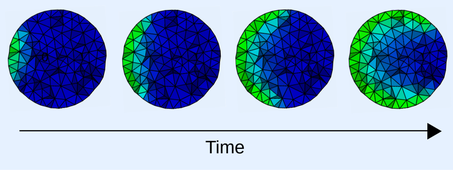
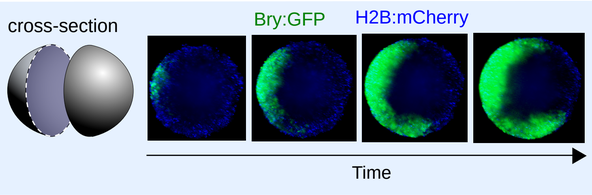
Simulations



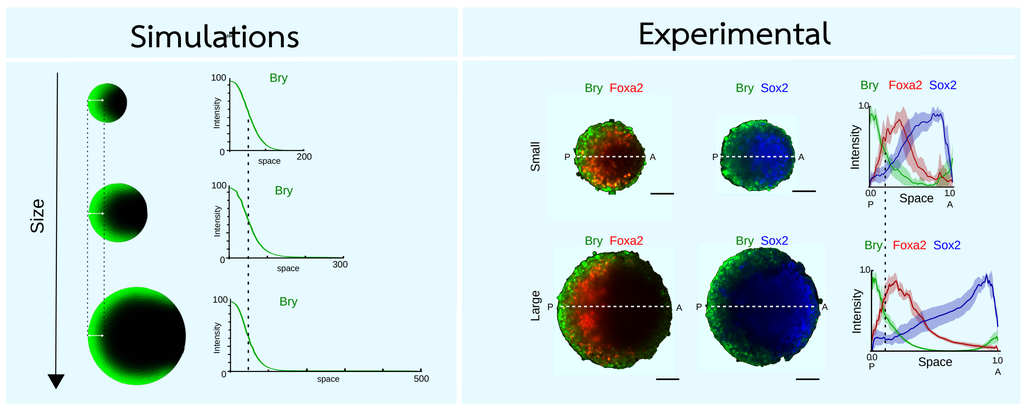
Experimental



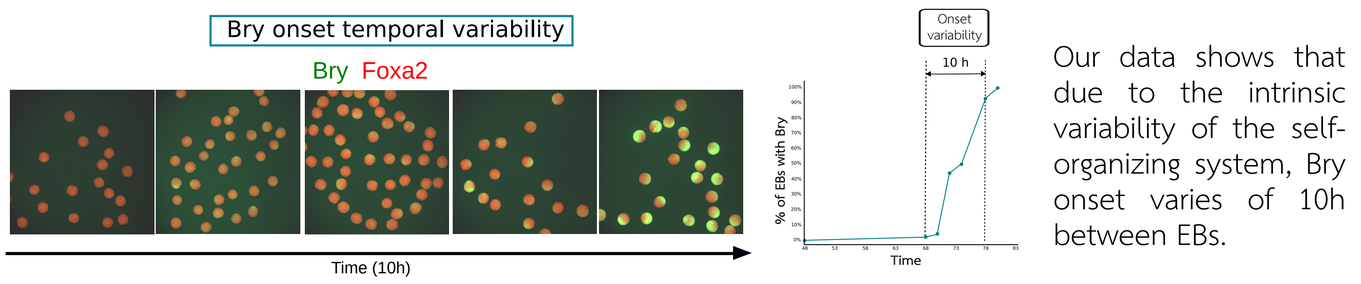
Quantification

Scaling

The bistable reaction-diffusion system can generate an embryonic axis independently of size. EBs of different sizes with 300 μm or 500 μm diameter show no sign of multiple peaks of gene expression of germ layer markers, confirming that only one AP-axis is generated irrespective of size. However, in agreement wit the model, the influence of the boundary has a fixed length scale i.e. the extension of Bry from the outer shell to the internal core does not scale with size (dashed line).

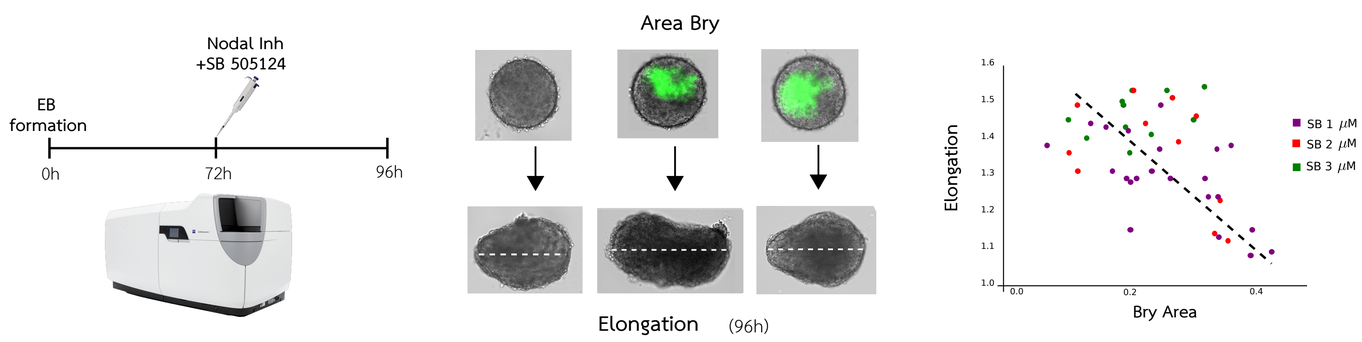


Variability



Our data shows that due to the intrinsic variability of the self-organizing system, Bry onset varies of 10h between EBs.

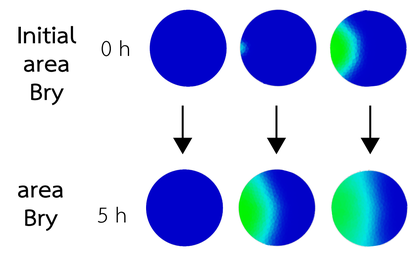
When a Nodal signaling inhibitor (SB-505124) is added to the media the expansion of Bry stops and the embryoid Bodies (EBs) elongate with an additional morphological variability. The data shows that EB elongation depends on the the area of Bry.



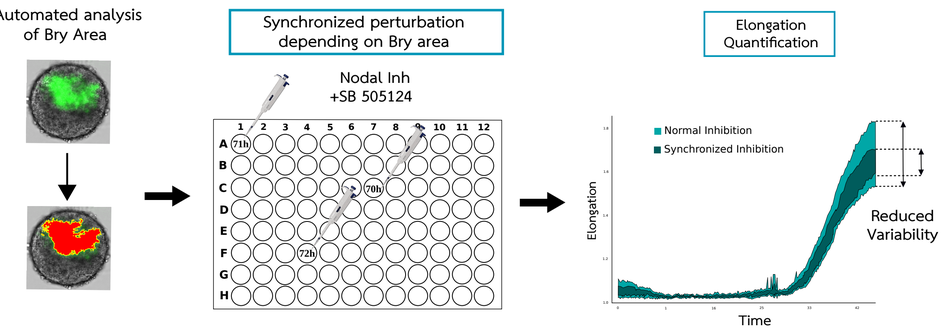
In the model, Nodal inhibition is implemented with the reduction of omega.

$$\frac{\partial \mathrm{WN}}{\partial t} = \gamma(\mathrm{BMP} \downarrow\omega \frac{\mathrm{WN}^2}{1+\mathrm{WN}^2} - \mathrm{DL})$$
$$\frac{\partial \mathrm{DL}}{\partial t} = \gamma(\mathrm{BMP} \downarrow\omega \frac{\mathrm{WN}^2}{1+\mathrm{WN}^2} - \mathrm{DL})$$

When the inhibition is applied at the same time, the model predicts that the final area of Bry depends on the initial area of Bry.



We are devising a system to apply the inhibitory molecule depending on the initial Bry area in order to reduce the intrinsic variability of elongation.



References: L. Marcon, J. Raspopovic, M. Langegger, R. Cáceres, P. Müller. A multi-stable Turing mechanism for size-independent symmetry breaking of mouse embryoid bodies (in preparation)

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less". M. Curie

Acknowledgments: M. Lobo, J. Serrano J. Raspopovic.