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Special Issue: Singularity Biology and Beyond

Commentary and Perspective (Invited)

Analysis of the singularity cells controlling the pattern formation in multi-cellular systems

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Developmental pattern formations even from unorganized initial conditions are excellent models of singularity phenomena. It is hypothesized that rare but crucial cells (singularity cells) govern the behavior of the entire cell population during the emergence of population-scale orders. However, conventional studies often average the cellular dynamics over the population, so the existence and importance of rare cells have been misevaluated. To elucidate the functional importance of rare cells, a technical breakthrough such as trans-scale analysis of the whole system dynamics at the single cell resolution would be needed.

In the Singularity Biology A03-2 group (Table 1), we tried to perform a comprehensive analysis of the multicellular pattern formation in a simple biological model by developing and utilizing a trans-scale scope, analytical strategy, and mathematical modeling. As an experimental model, we focused on the developmental pattern formation of social amoeba, *Dictyostelium discoideum*, that self-organizes wave-like aggregation streams. Under fed conditions, social amoebae proliferate as single cells, but under starvation, they spontaneously form multicellular bodies composed of thousands of cells by self-organizing chemotactic aggregation using cyclic adenosine monophosphate (cAMP) as a chemoattractant. Past studies showed that cells sensing environmental cAMP synthesize and release cAMP sequentially, enabling long-distance signal transmission over several centimeters in a chain reaction involving thousands of cells [1]. However, at the earliest stage of the development, it remained unclear whether the signal relay is initiated by a large or small number of cells.

Table 1 A03-2 group composition and collaborators in the Singularity Biology A03-2 group

Principal Investigator	Kazuki Horikawa (Tokushima University)
Co-Investigator (CI)	Tatsuya Takemoto (Tokushima University)
Collaborators in the Singularity Biology	
A01-2 PI	Takeharu Nagai (Osaka University)
A01 PI	Akihito Harada (Kyushu University)
A02-2 PI	Tamiki Komatsuzaki (Hokkaido University)
A03-3 CI	Takanobu Nakazawa (Tokyo University of Agriculture)
A03-4 PI	Taku Okazaki (University of Tokyo)

To visualize chemotactic signals, we established cells expressing a fluorescent probe for cAMP [2]. While no cell showed cAMP signals in the first few hours of starvation, cells gradually began to produce cAMP, spreading to all cells

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by 10 hours post-starvation. The entire cell population aggregates in the form of rotating spiral waves of cAMP signaling at intervals of 6-10 minutes. To directly observe the emergence and growth of cAMP signal waves, we utilized a newly developed transscale scope, named AMATERAS (Aspired Multimodal/Multifunctional Analytical Tools for Every Rare Activities in Singularity) [3]. This allowed a large-scale and high-resolution observation at an exceptionally large wider field of view (1.5 cm^2 containing 130,000 cells) with a single-cell resolution and a high enough sampling rate for signal waves (every 30 seconds for 10 hours). Huge data-sets were analyzed in a high-throughput manner by implementing semi-automated single-cell tracking assisted by machine learning. Specifically, a detailed analysis of signal dynamics of 4,000 cells revealed the presence of leaders and followers, which functioned as a signal initiator and passive amplifier of the cAMP wave, respectively [3,4]. The rarity of leader and follower was as small as 0.2% and 10%, suggesting that initial patterns were controlled by these rare cells. Indeed, local wave propagation around the leader showed non-linear development over time, whose bursting was regulated by the critical density of followers [4].

The spatially biased presence of these rare cells further brings a spatially heterogeneous development in the ability of cAMP signaling yielding a patchy distribution of signaling clusters each consisting of ~1,000 cells. Interestingly, these signaling clusters were found to switch the propagation of the cAMP wave from a simple ring wave to a rotating one. Such a drastic transition of wave propagation has been known as a reentry, being an important wave dynamic generating a spiral wave in signal relaying systems. Although the importance of reentry in cardiac tissue has been well understood, mechanisms for reentry itself have remained a long-lasting mystery. Furthermore, the involvement of reentry dynamics has not been suggested for the development of spiral waves in other systems, including the amoeba population. We here thought that the rare presence of singularity cells would account for the self-organization of cAMP waves in the amoeba population. To test this idea, we performed numerical simulations considering the presence of a small number of leader and follower cells. As a result, the mesoscale heterogeneity developed by leader and follower was essential to bring about reentry that finally converted to spiral waves [4], demonstrating the functional importance of singularity cells in the self-organized pattern formation.

In summary, we investigated the developmental pattern formation of the amoebae population and found the presence and function of rare leader and follower cells which control the whole system dynamics of cAMP signaling. Developed methods to discover [3-5], manipulate [6,7], and analyze singularity cells [8,9] would no doubt benefit future studies of biological singularities in diverse systems such as embryonic pattern formation [10] and immune systems [11].

References

[1] Ohta, Y., Furuta, T., Nagai, T., Horikawa, K. Red fluorescent cAMP indicator with increased affinity and expanded dynamic range. Sci. Rep. 8, 1866 (2018). https://doi.org/10.1038/s41598-018-20251-1

[2] Okada, H., Arai, S., Inoue, T., Kitaguchi, T. Genetically-encoded yellow fluorescent cAMP indicator with an expanded dynamic range for dual-color imaging. PLoS One 9, e100252 (2014). https://doi.org/10.1371/journal.pone.0100252

[3] Ichimura, T., Kakizuka, T., Horikawa, K., Seiriki, K., Kasai, A., Hashimoto, H., et al. Exploring rare cellular activity in more than one million cells by a transscale scope. Sci Rep. 11, 16539 (2021). https://doi.org/10.1038/s41598-021-95930-7

[4] Kakizuka, T., Hara, Y., Ohta, Y., Mukai, A., Ichiraku, A., Arai, Y., et. al. Cellular logics bringing the symmetry breaking in spiral nucleation revealed by trans-scale imaging. bioRxiv (2020). https://doi.org/10.1101/2020.06.29.176891

[5] Horikawa, K., Nagai, T. Live imaging of cAMP signaling in D. discoideum based on a bioluminescent indicator, nano- lantern (cAMP). Methods Mol. Biol. 2483, 231-240 (2022). https://doi.org/10.1007/978-1-0716-2245-2_14

[6] Suzuki, A., Sakano, T., Sasaki, H., Watahiki, R., Sone, M., Horikawa, K., et. al. Design and synthesis of gene-directed caged cyclic nucleotides exhibiting cell type selectivity. Chem. Commun. 57, 5630-5633 (2021). https://doi.org/10.1039/d1cc01405f

[7] Takemoto, T. Zygote electroporation for CRISPR/Cas9 delivery to generate genetically modified mice. Methods Mol. Biol. 2050, 121-126 (2019). https://doi.org/10.1007/978-1-4939-9740-4_13

[8] Basak, U., Sattari, S., Hossain, M., Horikawa, K., Komatsuzaki, T. An information-theoretic approach to infer the underlying interaction domain among elements from finite length trajectories in a noisy environment. J. Chem. Phys. 154, 034901 (2021). https://doi.org/10.1063/5.0034467

[9] Basak, U., Sattari, S., Hossain, M., Horikawa, K., Komatsuzaki, T. Transfer entropy dependent on distance among agents in quantifying leader-follower relationships. Biophys. Physicobiol. 18, 131-144 (2021). https://doi.org/10.2142/biophysico.bppb-v18.015

[10] Hayashi, S., Suzuki, H., Takemoto, T. The nephric mesenchyme lineage of intermediate mesoderm is derived from Tbx6-expressing derivatives of neuro-mesodermal progenitors via BMP-dependent Osr1 function. Dev. Biol. 478,

155-162 (2021). https://doi.org/10.1016/j.ydbio.2021.07.006
[11] Sugiura, D., Okazaki, I., Maeda, T., Maruhashi, T., Shimizu, K., Arakaki, R., et. al. PD-1 agonism by anti-CD80 inhibits T cell activation and alleviates autoimmunity. Nat. Immunol. 23, 399-410 (2022). https://doi.org/10.1038/s41590-021-01125-7