Control of cell elongation, division and fusion of minimal bacterium JCVI-syn3B

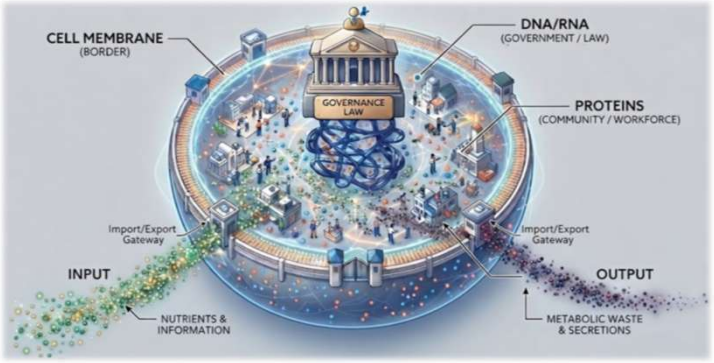
Muhammad Algiffari, Graduate School of Science

Abstract

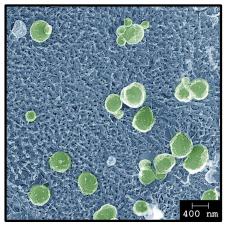
***Mycoplasma mycoides*-derived, chemically synthesized and reduced-genome bacterium JCVI-syn3B (syn3B) serves as a model for studying the minimal life system. While cellular behaviors are often regulated genetically, extrinsic control of the cell through physical and chemical conditions remains less explored. Here, we establish controllable experimental conditions to understand how minimal cells maintain their structure and organization through elongation induced by growth stage and pH medium, cell division via insertion of detergent to the membrane and cell fusion by polyethylene glycol. These results demonstrate that syn3B morphology and cytoplasmic organization can be robustly controlled, providing a platform to explore cellular behavior in a minimal cell.**

Introduction

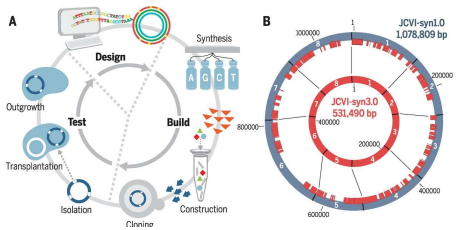
What is minimal requirements for live?



What is minimal genes requirements for live?



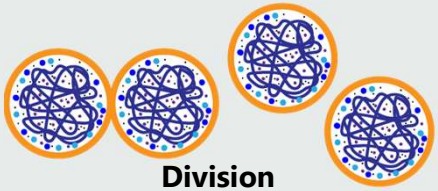
JCVI-syn3B



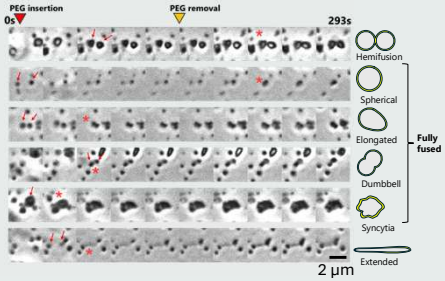
Minimal living organism defined by required genes

Research purpose : To establish the cell manipulation methods to better understand how minimal cells maintain their structure and organization

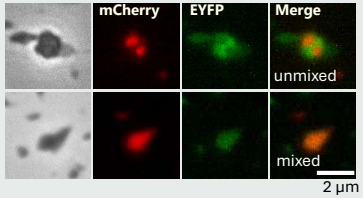
Results



Fusion changed the morphology of the cell

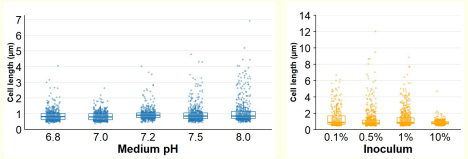


Cytoplasmic mixture behavior



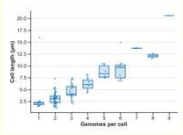
syn3B successfully fused by PEG and the cytoplasmic mixture behavior was observed

Strategy to generate elongated cells

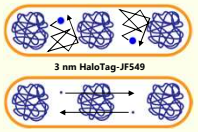


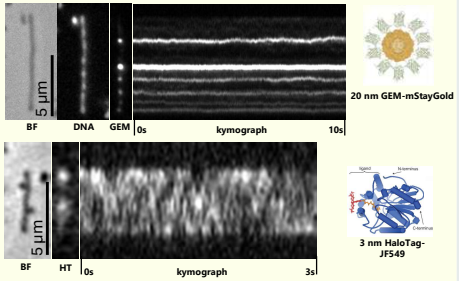
Elongated cell properties

Cell length vs. genome number



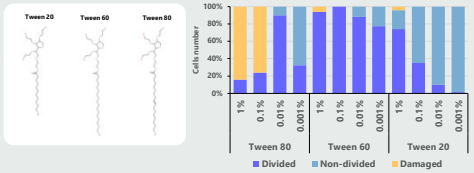
Size dependent particles fluctuation



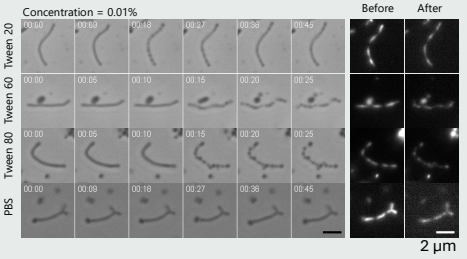


Elongated cell achieved by early-stage growth and alkaline medium, moreover the cytoplasmic organization and particle fluctuation was observed

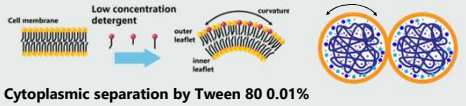
Cell division induced by low concentration of detergents



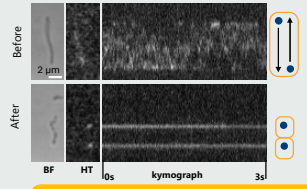
Detergents affect to the cells



Detergent-induced cell division mechanism

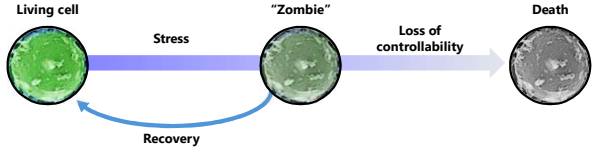


Cytoplasmic separation by Tween 80 0.01%



Detergents successfully induced the cell division, and cytoplasmic (DNA and molecules) partitioning were observed

Future study : Live-death boundary



References

[1] Hutchison, C. A. et al. Design and synthesis of a minimal bacterial genome. *Science* 351, aad6253 (2016).
[2] Kiyama, H., Kakizawa, S., Sasajima, Y., Tahara, Y. O. & Miyata, M. Reconstitution of a minimal motility system based on *Spiroplasma* swimming by two bacterial actins in a synthetic minimal bacterium. *Sci. Adv.* 8, eabo7490 (2023).
[3] Tarshis, M., Salman, M. & Rottem, S. Fusion of mycoplasmas: the formation of cell hybrids. *FEMS Microbiol. Lett.* 82, 67–71 (1991).
[4] Pelletier, J. F., Glass, J. I. & Strychalski, E. A. Cellular mechanics during division of a genomically minimal cell. *Trends Cell Biol.* 32, 900-907, (2022).