Bacterial Viruses Organize Subcellular Environments to Mediate Heterogeneous Development

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Spatial organization underpins biological processes in lifeforms. For complex organisms, body parts must develop properly in space, and within the cells making up these organisms, membranes separate organelles with different functions. Simpler organisms, like bacteria, lack intracellular membranes, but possess alternate strategies for organization, where certain proteins are confined to specific cellular locations to consolidate their functions. Viruses, among the simplest lifeforms, have also been reported to display organized development, further underscoring the essentiality of structure in biology.

Bacterial viruses, known as bacteriophages or phages, are utilized as model systems for studying advanced cellular functions. Phage lambda infects *Escherichia coli* and propagates via distinct pathways, either lysis or lysogeny, where this fate-selection is a paradigm for cellular decision-making. Recent efforts probing decision-making at the subcellular level have discovered that phages behave as individual interacting intracellular entities when processing decisions, generating considerable insights into how complex decision-making may occur [1-4]. However, little is known about this phage's spatial development within its host and how spatial organization might affect cellular decision-making.

Here, we characterize how phage lambda develops in subcellular space, from infection through lysis, using multiple fluorescence reporters targeting phage DNA replication, essential host resources, phage transcription, and phage assembly. We observe that these processes coalesce into distinct areas of the host cell during infection to coordinate phage development, reminiscent of virus factories, where multiple factories may be present in single cells. Remarkably, different intracellular factories quantitatively diverge at the viral DNA, mRNA and protein levels, suggesting the existence of bona-fide microenvironments within cells. We propose that individual viruses drive the formation of disparate territories, implying that phages may sense separate environments during decision-making and development. The conclusions from this work may shed significant light on the detailed molecular mechanisms of cell-fate bifurcation.

References

[1] Zeng L, Skinner SO, Sippy J., Feiss M, and Golding I. (2010) Decision Making at a Subcellular Level Determines the Outcome of Bacteriophage Infection, *Cell* **141(4)**, 682-691.

[2] Shao Q, Trinh JT, McIntosh CS, Christenson B, Balázsi G, and Zeng L (2016) Lysis-lysogeny Coexistence: Prophage Integration during Lytic Development, *Microbiologyopen* **6(1).**

[3] Trinh JT, Székely T, Shao Q, Balázsi G, and Zeng L (2017) Cell Fate Decisions Emerge as Phages Cooperate or Compete inside Their Host, *Nature Communications* **8**, 14341

[4] Cortes M, Trinh JT, Zeng L, and Balázsi G (2017) Late-arriving Signals Contribute Less to Cell Fate Decisions, *Biophysical Journal* **113(9)**, 2110-2120.

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