Decisions, decisions: How microbes choose lifestyles gives clue to origin of multicellular life

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The striking colors of Yellowstone's Grand Prismatic Springs arise from diverse communities of microbial biofilms. Credit: Jim Trodel/Flickr

Like many bacteria, the Bacillus subtilis lives a double life.

During adventurous phases, the microbes are free-swimming, independent explorers, searching for rare nutrient-rich niches amid an otherwise inhospitable environment. In more communal moments, B. subtilis cells trade their rotating flagellum for an extracellular matrix that causes them to stick together in chains that cling to the surfaces. These chains can eventually become the foundation for biofilms, dense communities of microbes that can grow on almost any moist or wet natural or industrial surface.

Though the two lifestyles are well known to microbiologists, the factors guiding the decision of when to choose each state have remained mysterious. Do the cells pick a lifestyle on a whim, or do they retain some memory of the past that influences what they do next?

Researchers led by Johan Paulsson, an associate professor of systems biology at Harvard Medical School, and Richard Losick, the Maria Moors Cabot Professor of Biology in the Harvard Faculty of Arts and Sciences, have developed new tools and techniques that allowed them to discover that random fluctuations in gene expression help drive the initiation of the sedentary communal state, and that the individual cells that make up these nascent colonies share an internal clock that commits them to communal life for an extended "trial period." The researchers said that these findings provide important insight about how individual cells can organize themselves into a cooperative community, an important first step in the development of multicellular life. The results of the study were published in *Nature*.

B. subtilis and other micro-organisms are known to form biofilms based on external signals from the environment, but until now it has not been understood whether a cell's past experience played a role in making the decision about whether to roam free or settle down.

Using a technique called microfluidics, the researchers constructed thousands of tiny habitats where individual bacteria and their descendants could be watched for very long periods of time.

The channels in the habitat were 1.6 micrometers wide, about 1/50th the width of a human hair. The bacteria were genetically modified so that mobile cells produced a green fluorescent protein and sedentary cells a red fluorescent protein. Images taken every few minutes tracked the color-coded state of the cells, for hundreds of consecutive generations per experiment.

The collected data on hundreds of thousands of generations of bacteria allowed the researchers to see patterns in the microbe's decision-making habits that would not be visible in a smaller data set.

Environmental conditions were identical in each of the habitats. A constant flow of nutrient-rich medium kept the bacteria well fed and also washed away any extracellular chemical signals that the cells may have been transmitting.

"With everything else in the environment static for an extended period of time, we were able to see what role the intrinsic dynamics within individual cells played in driving their decisions about which lifestyle to adapt," Paulsson said.

With these methods, the researchers could ask whether cells take their history into account when deciding which lifestyle to adopt. Surprisingly, motile cells decided to switch lifestyles in a completely random fashion—some lineages would remain free-swimming for only a handful of generations, and then become static, while others continued moving for hundreds. Though the choice was random, it was heavily biased toward staying in the motile state, the researchers said.

In contrast, once cells adopted the sedentary lifestyle, they were very particular about the length of time during which they adhered to form chains, persisting for almost exactly eight generations.

"When they're mobile, they don't bother to keep track of time, but in the sedentary, multicellular state, some sort of internal clock arises that coordinates activity across many different individuals," said Nathan

Lord, HMS research fellow in systems biology.

In the wild, these chains look for environmental feedback for insight into the value of maintaining the sedentary state. If conditions are right, the sticky chains continue to coalesce, eventually forming a biofilm. In the habitat constructed for the experiment, the rapid exchange of growing medium washed away extracellular information that would normally cue the chains to take the next step toward colonial life. Without a cue to stick together, the individual cells all went their separate ways.

"The degree of coordination is remarkable. When time runs out, all at once the cells in the chain revert to the independent, mobile state," said Thomas Norman, a graduate student researcher in the Paulsson and Losick labs.

The coordinated timing keeps the chain together long enough so that there's some benefit to being together, a "trial period" of multicellular growth whose continuation is periodically re-evaluated, the researchers said.

"Building a biofilm requires cooperation—the community can't grow successfully if cells casually jump ship," Losick said. As individual cells must relinquish their autonomy in order to benefit from living together, the internal clock provides a simple mechanism to force progeny to work together.

The study's simple approach—watching the cells for long periods under constant conditions—also allowed the researchers to use natural variation among cells to understand the genetic circuitry underlying the decision.

"Often in biology, there's an emphasis on the complexity of the molecular details," said Paulsson. "In this case, we found a hidden simplicity. The mechanisms behind founding the community and maintaining it operate independently of each other in a precise statistical manner. There's a lot of interest in understanding how individual cells make decisions, and this kind of modular behavior might make understanding these kinds of complex networks much easier. We're now working to reveal how the important parts we've identified work."

More information: Norman TM, Lord ND, Paulsson J, Losick R. Memory and modularity in cell-fate decision making. *Nature*. 2013;503(7477):481-6. dx.doi.org/10.1038/nature12804

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