A fluorescence microscopy image showing a network of bacterial cells. The cells are stained with two different fluorescent dyes: one in red and one in green. The red-stained cells form a dense, interconnected network, while the green-stained cells are more sparsely distributed and appear as individual rods or small clusters. The background is dark, making the fluorescent cells stand out.

Stochasticity and Cell Fate

Richard Losick

Harvard University

“I, at any rate, am convinced that He does not play dice.”

Albert Einstein

Consider life at the micron scale, the world inhabited by bacteria.

Bacteria must cope with an environment that undergoes changes in temperature, pH, availability of nutrients, and so forth.

One way bacteria cope with change is by ***sensing and responding to cues from the environment.***

In sensing systems, all cells in the population respond to environmental change in a more-or-less uniform manner.

Sometimes, however, the optimal strategy is to enter a specialized state ***prior*** to changed circumstances. That is, in anticipation of change.

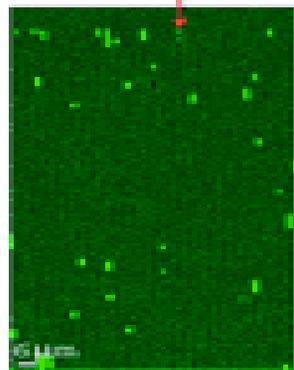
But bacteria cannot tell the future. Instead, they hedge their bets by ***stochastic switching.***

Consider the “persister” state. It has long been known that when challenged with ampicillin, certain cells in a population of bacteria mysteriously survive the antibiotic treatment without becoming resistant to it by mutation.

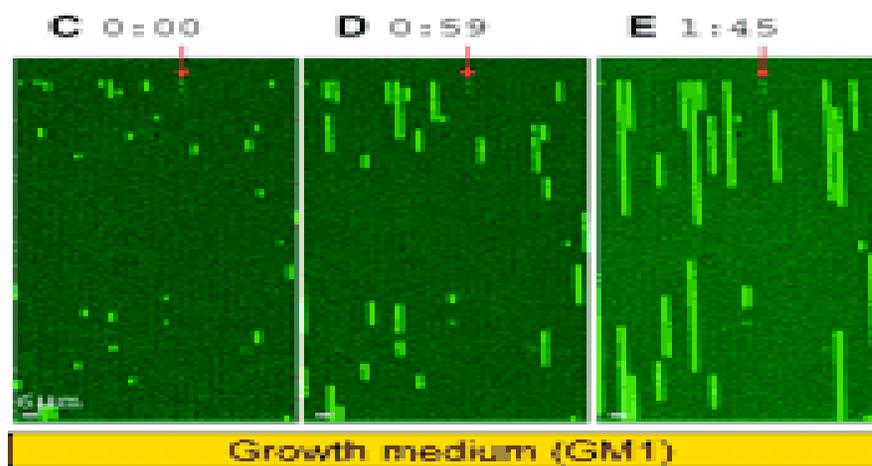
How is this possible? The answer is that a small number of cells spontaneously and temporarily enter a state in which they stop growing and thereby avoid being killed by ampicillin.

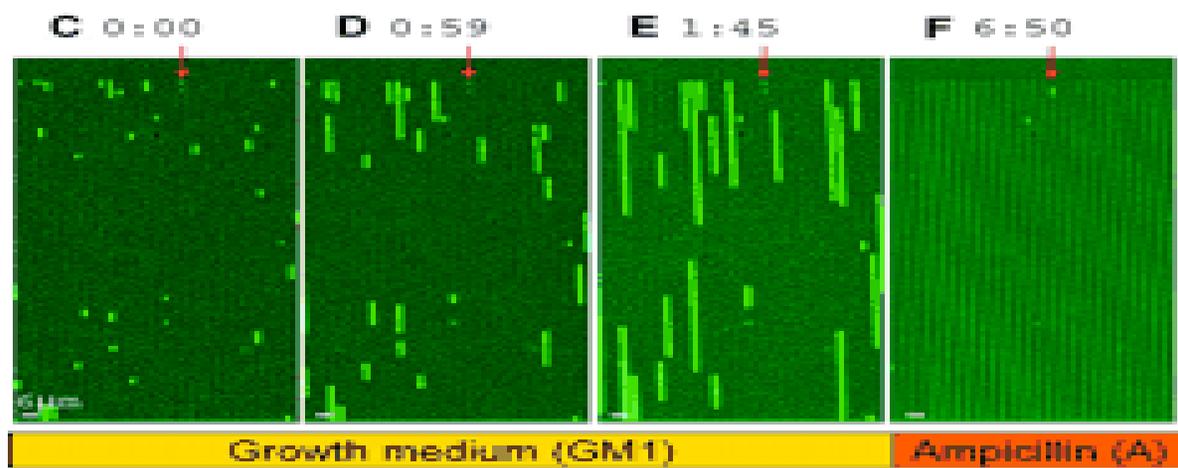
In other words, certain bacteria are bistable: they stochastically switch between a growing state and a non-growing state.

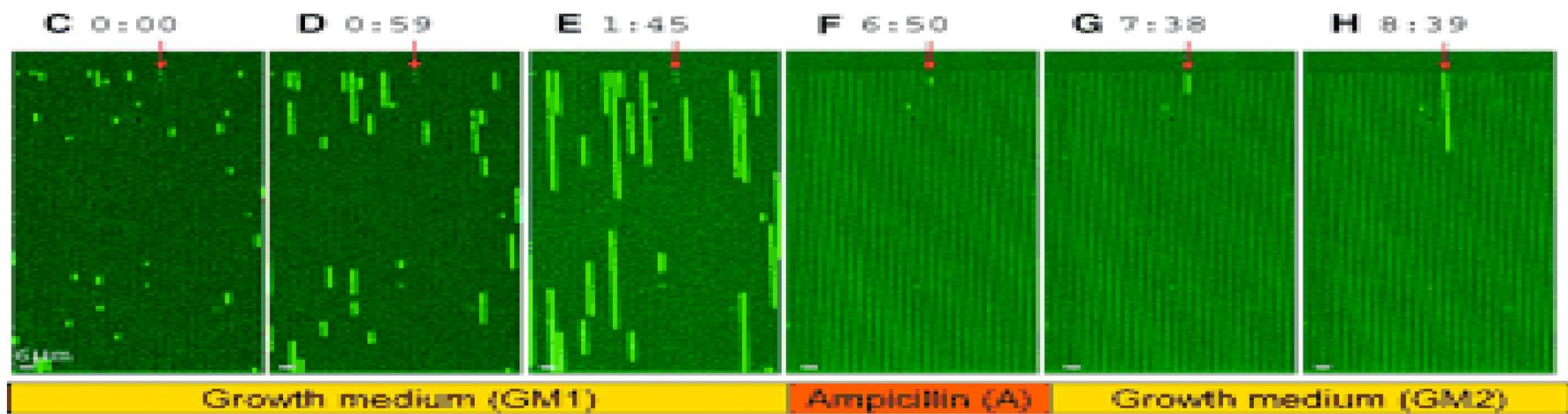
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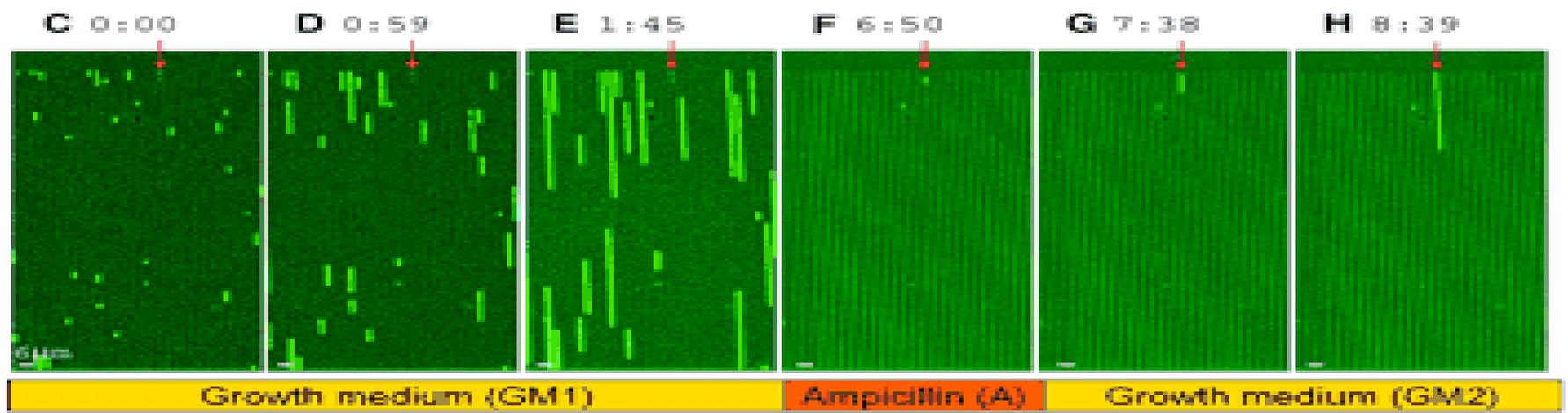


Gro









Beiträge
zur
Biologie der Pflanzen.

Herausgegeben

von

Dr. Ferdinand Cohn.

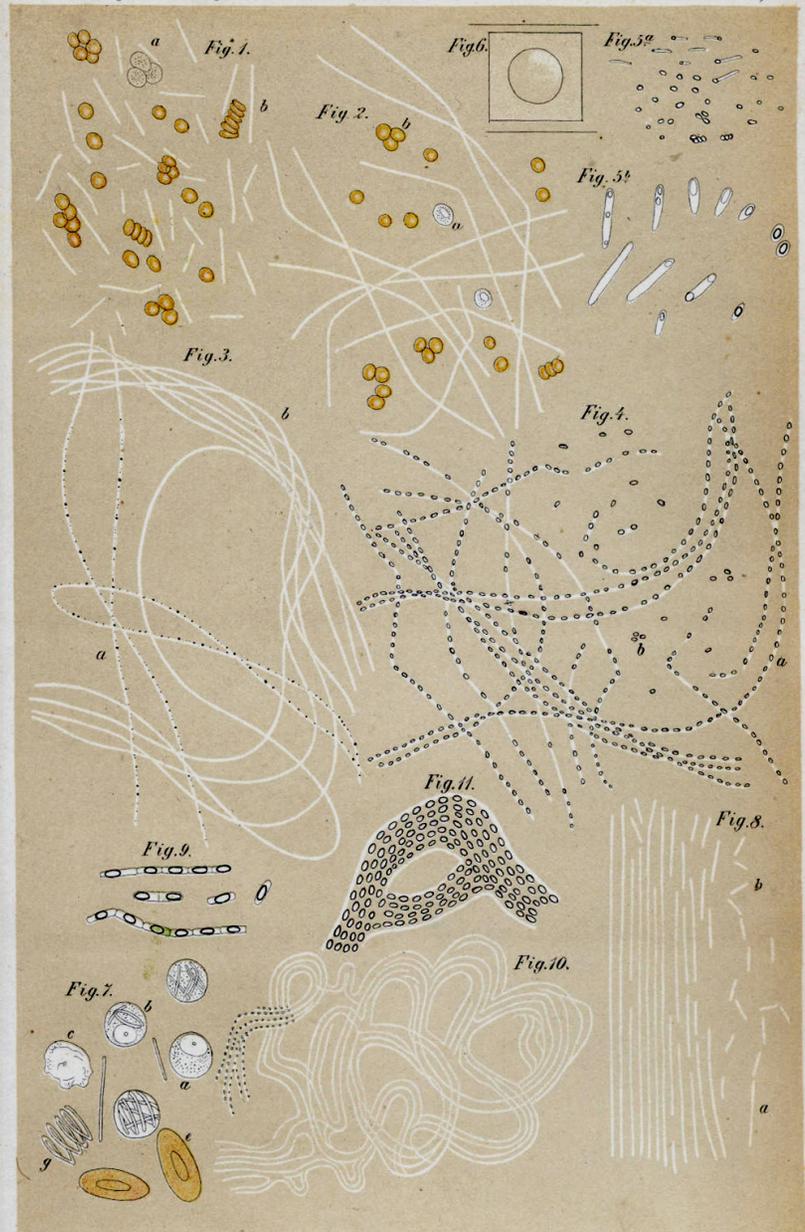
Zweiter Band.

Mit sechzehn Tafeln.

Breslau 1877.
J. U. Kern's Verlag
(Max Müller).

F. Cohn, Beiträge zur Biologie der Pflanzen.

Band II, Taf. XI.



Hoch u. Cohn ad nat. del.

Four examples from *B. subtilis*

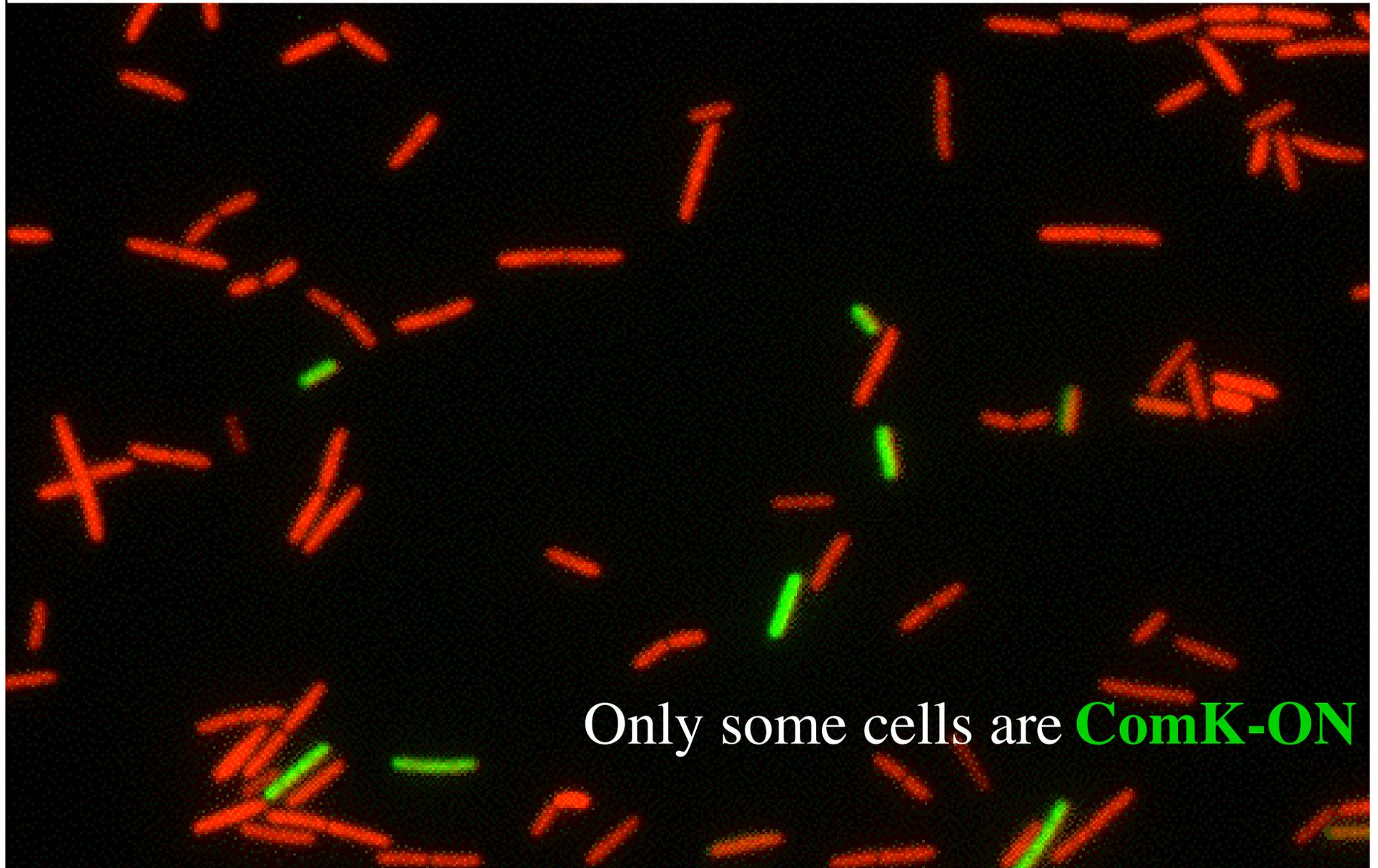
- Growth versus competence
- Swimming versus chaining
- Eating versus being eaten
- Community versus individuality

1. Growth versus Competence

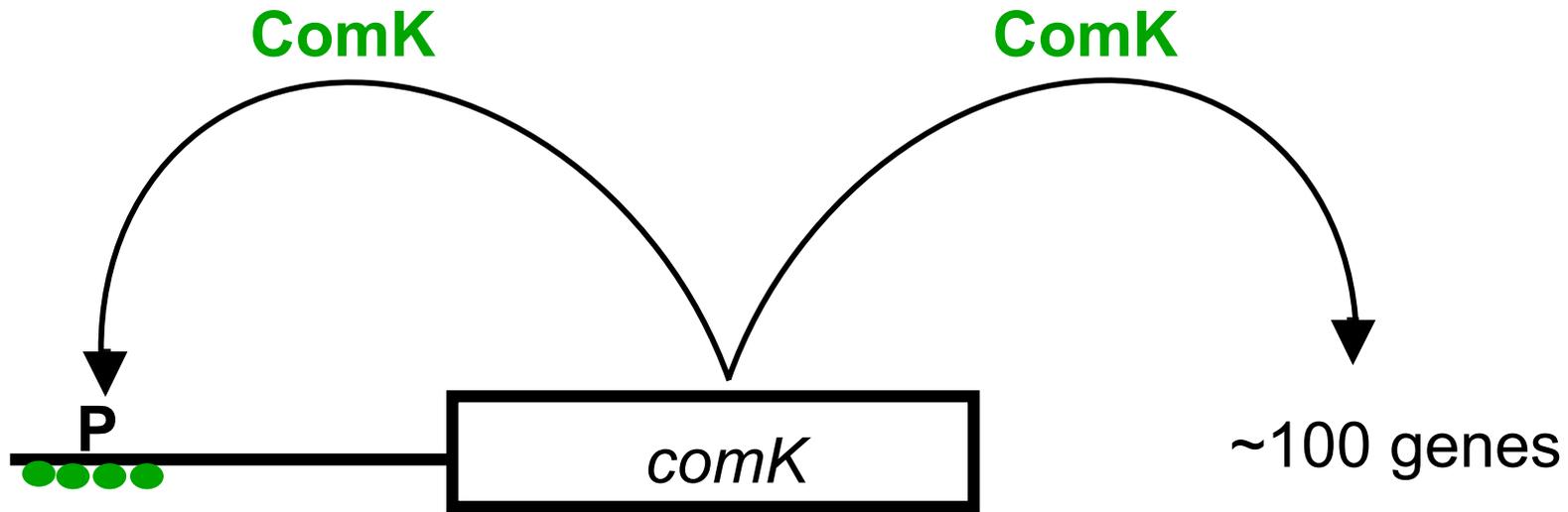
B. subtilis cells can enter a specialized state of “competence” in which they take up DNA from their environment.

Entry into competence is controlled by the regulatory protein **ComK**, whose synthesis is governed by a noise-driven stochastic switch.

Cells with **GFP** fused to a **ComK**-controlled gene.



Only some cells are **ComK-ON**

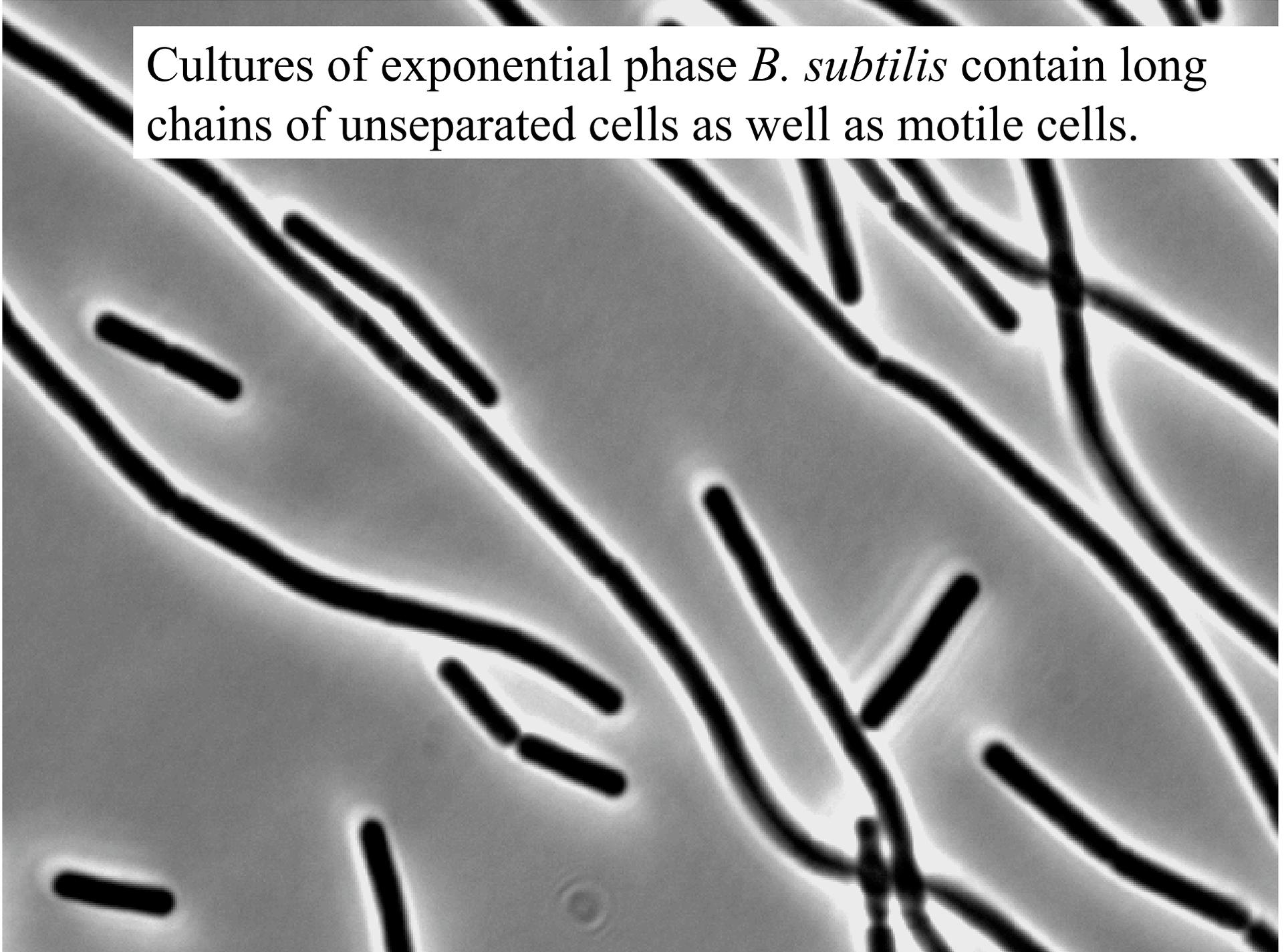


Expression of *comK* is governed by a positive feedback loop in which **ComK** stimulates transcription from the promoter for its own gene.

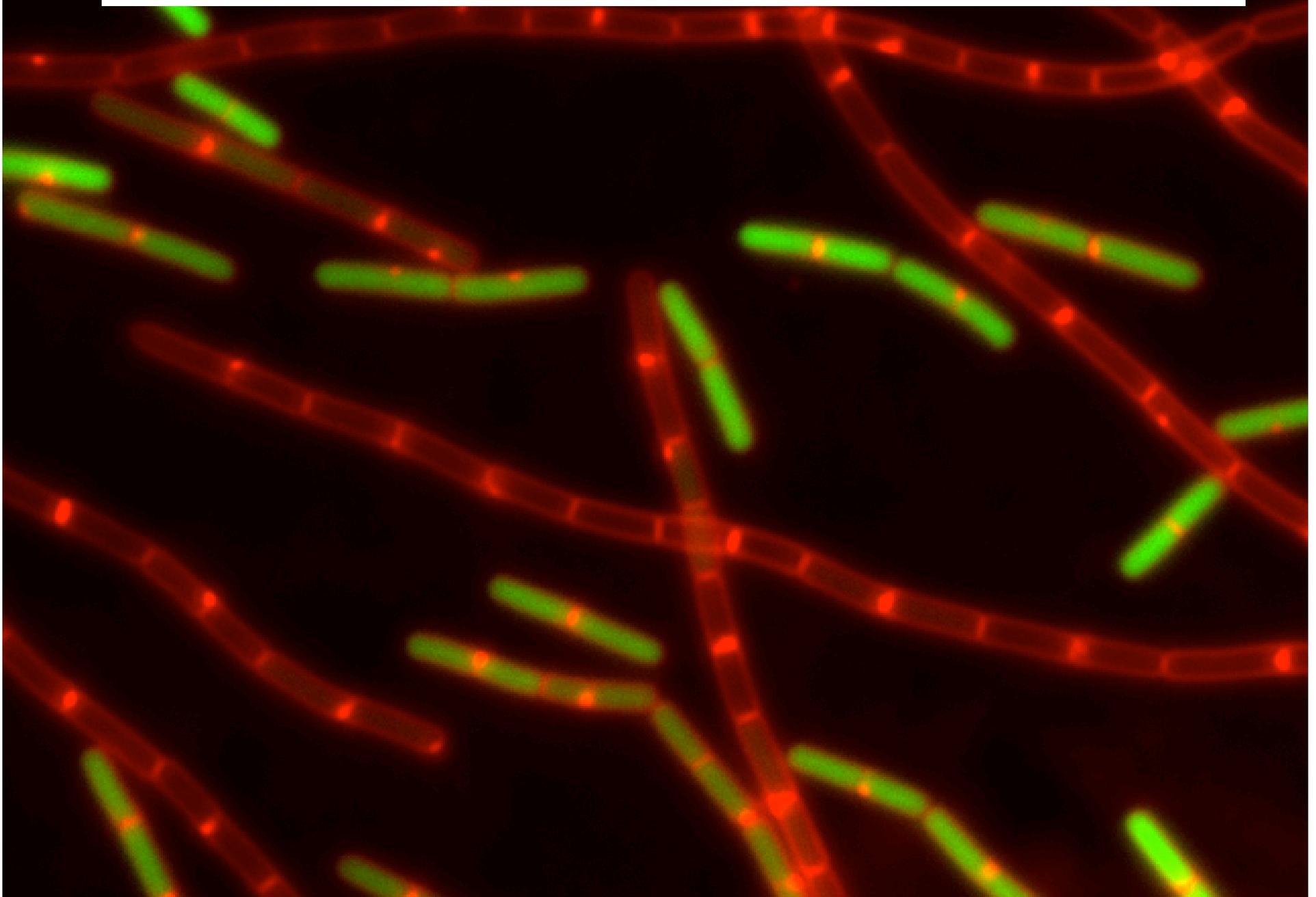
The loop is highly sensitive to stochastic fluctuations in **ComK** levels.

2. Motility versus chaining

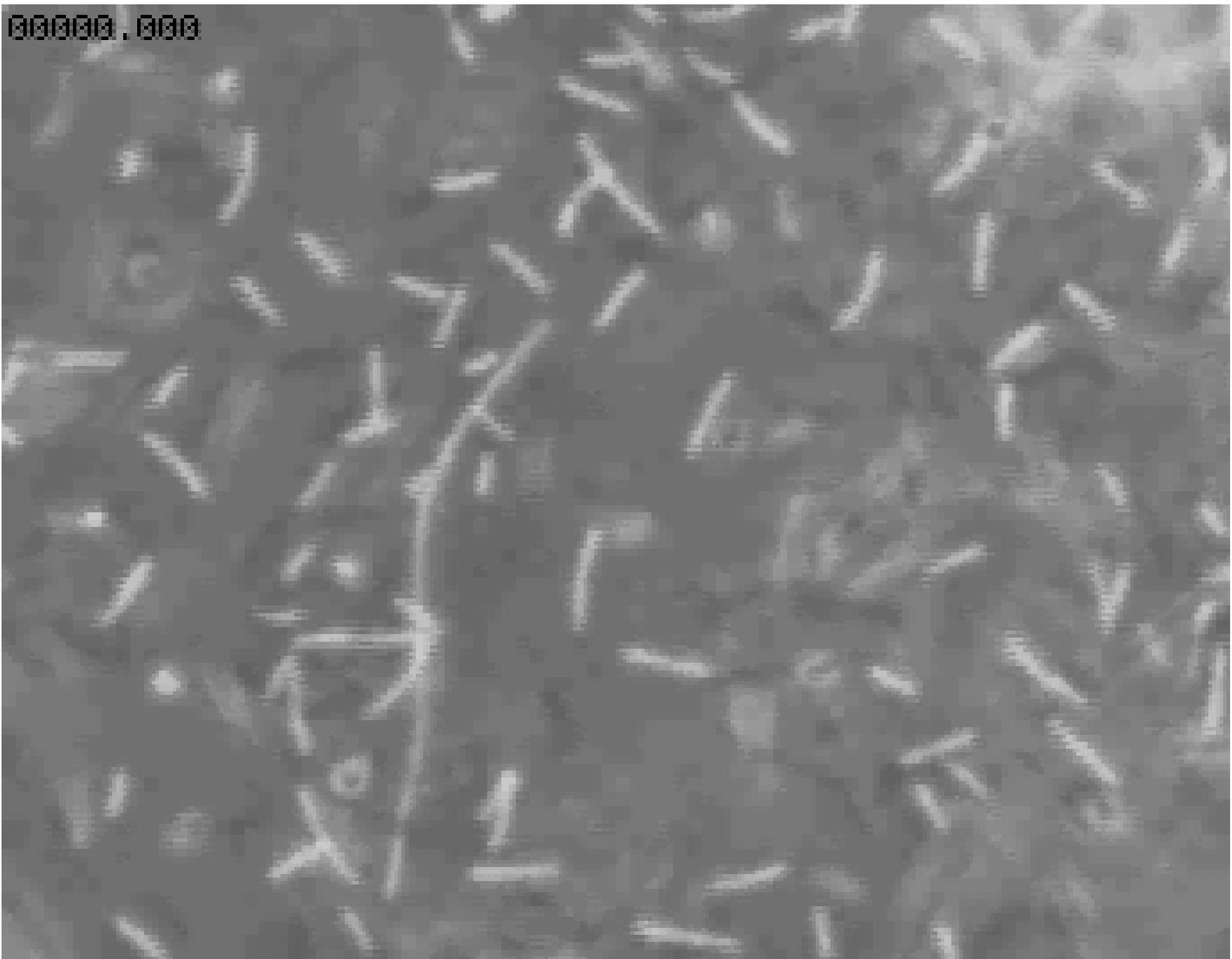
Cultures of exponential phase *B. subtilis* contain long chains of unseparated cells as well as motile cells.



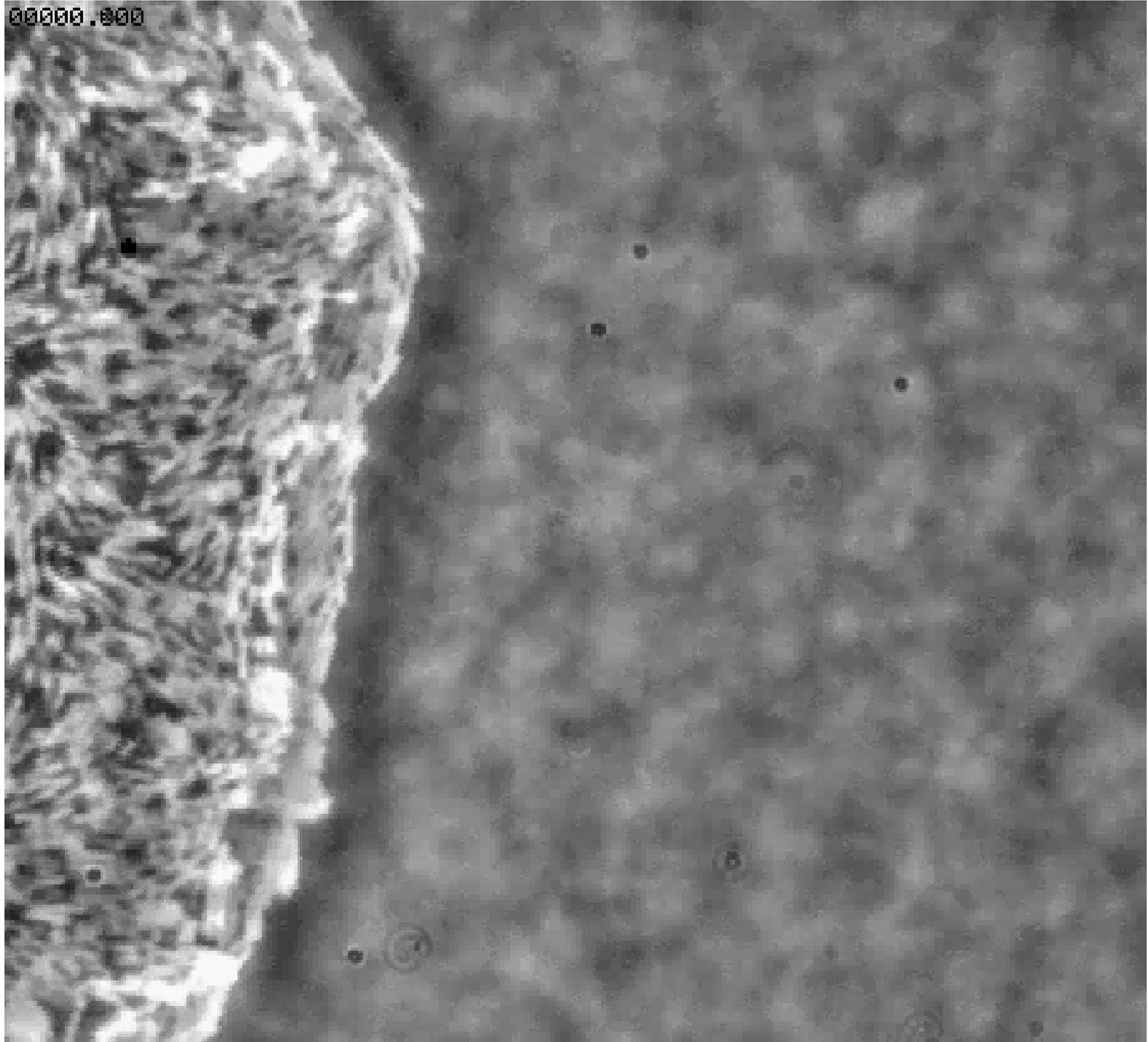
Chains with σ^D OFF and motile cells with σ^D ON.



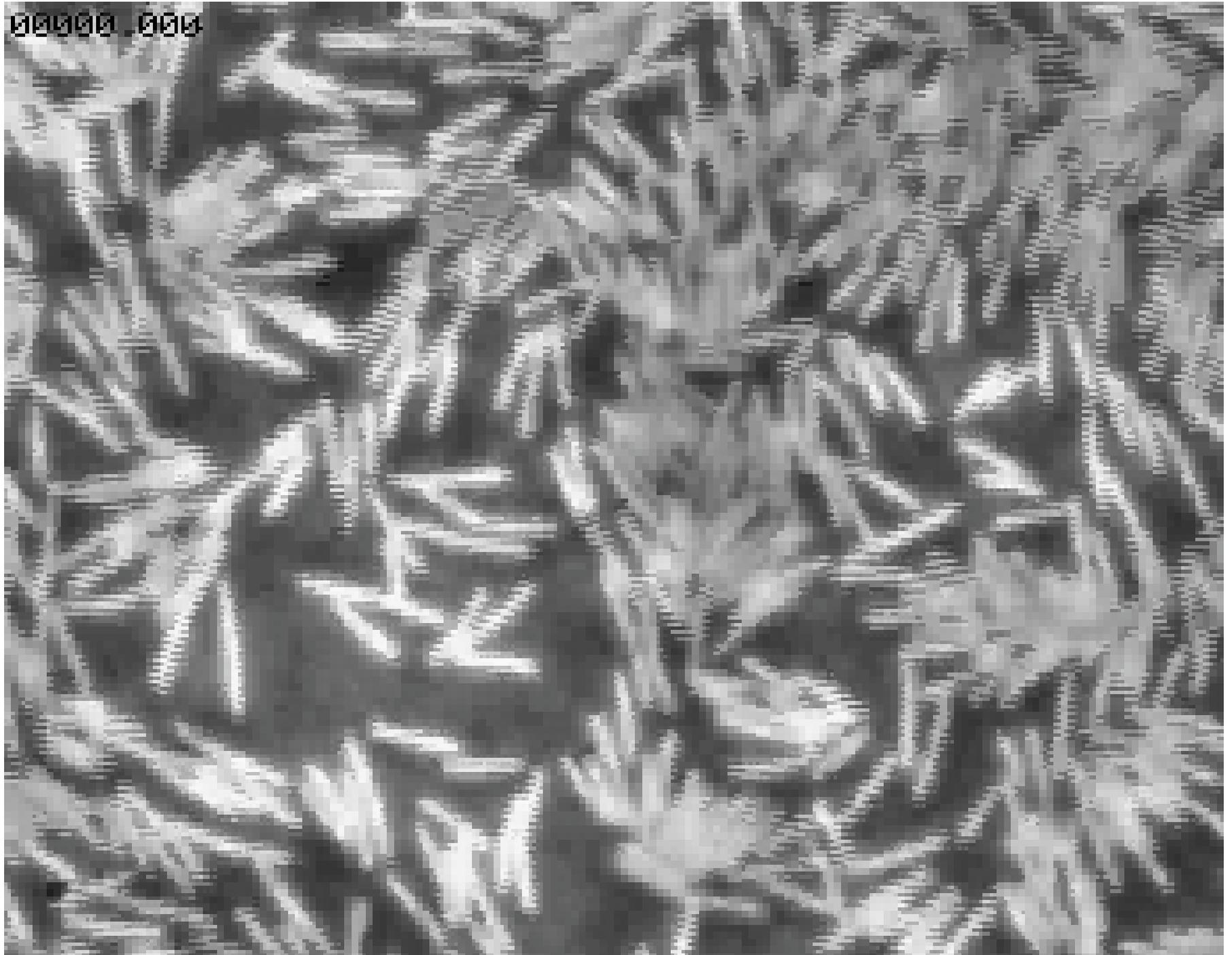
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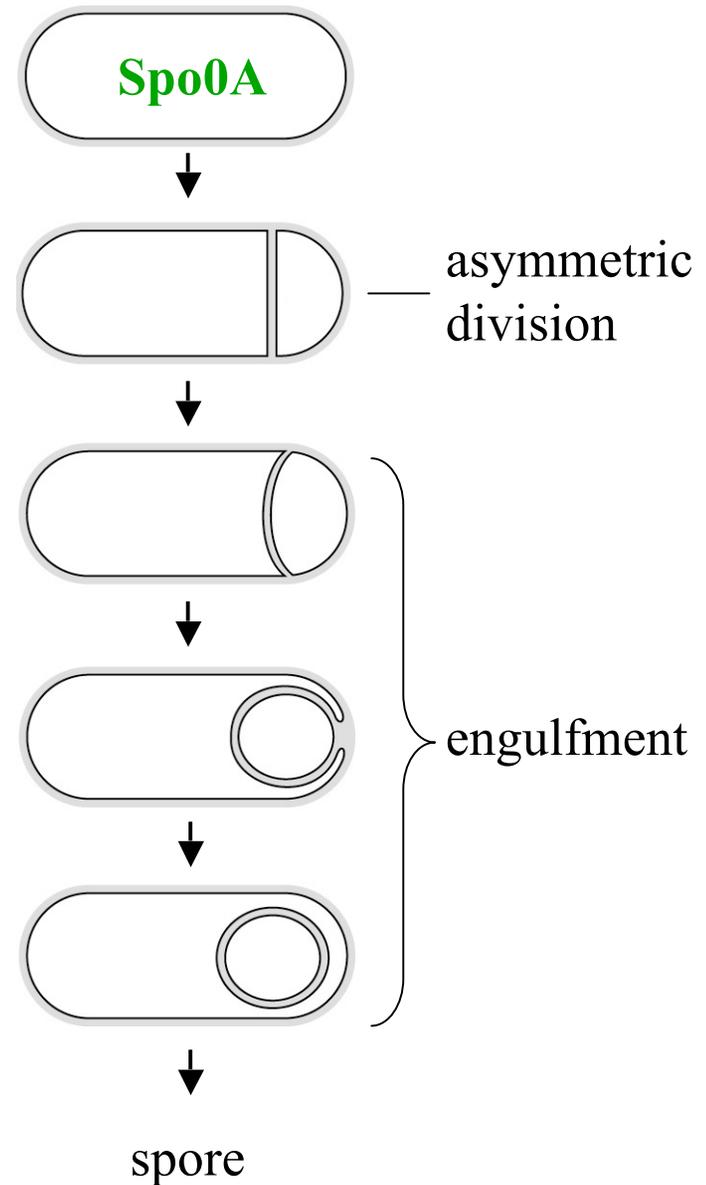


3. Eating versus being eaten

When starved for nutrients, *B. subtilis* enters a pathway that culminates in spore formation.

Entry in sporulation is governed by a master regulatory protein called **Spo0A**.

Sporulation takes time and energy

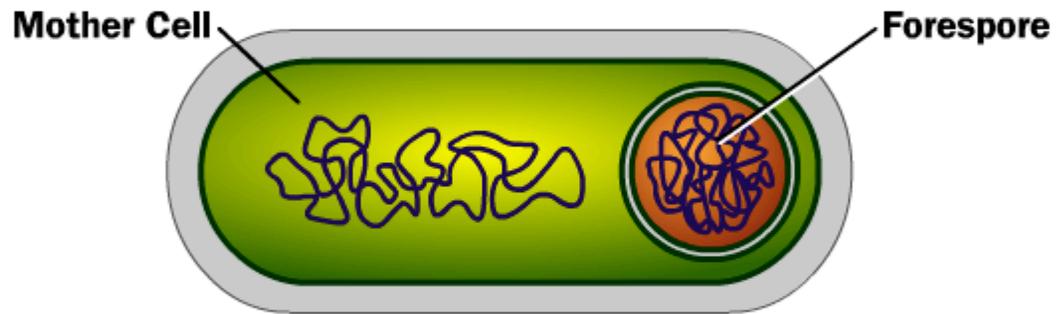


Sporulation

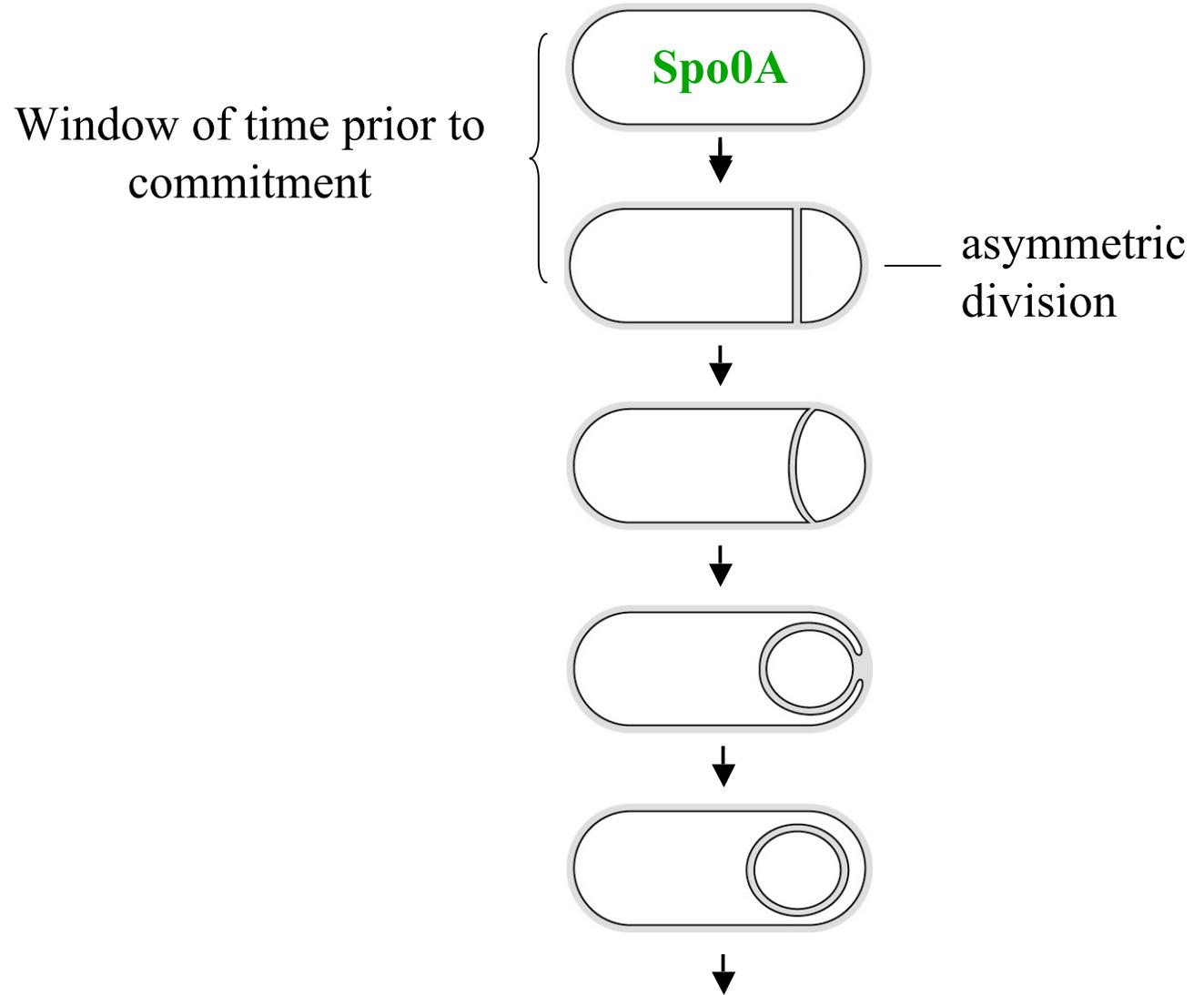
Overview

Regulatory Sequence

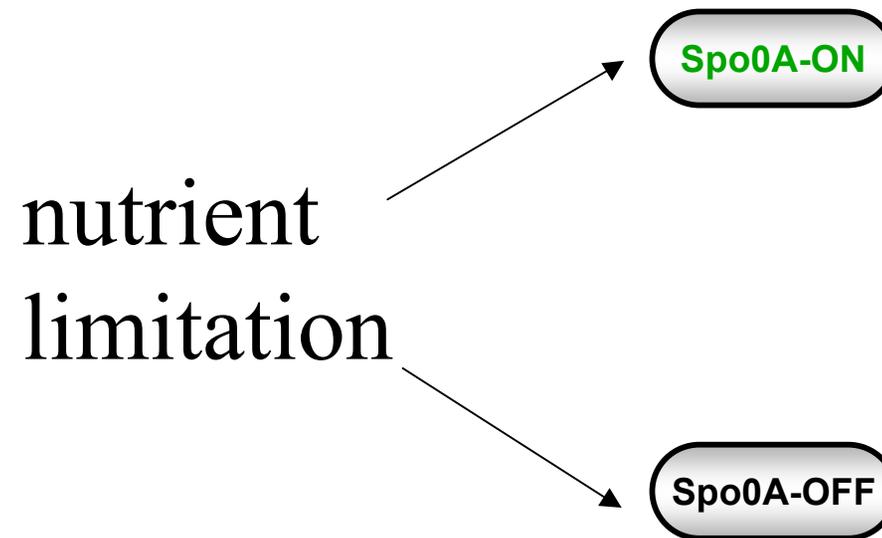
- Asymmetric Division*
- Chromosome Segregation*
- Engulfment (2D)*
- Engulfment (3D)*
- Coat and Cortex Formation*
- Germination*



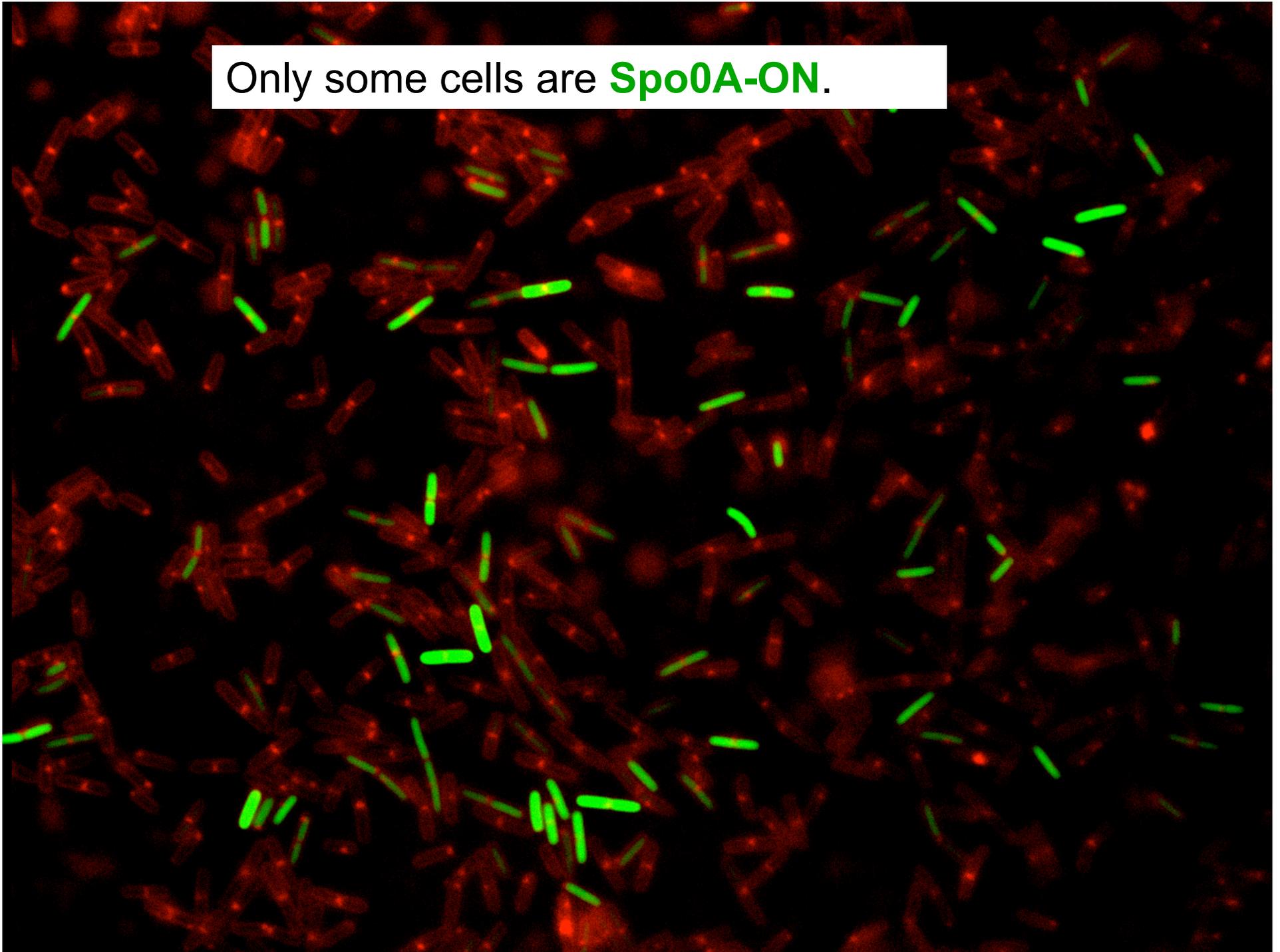
Sporulation is reversible until asymmetric division.



Activation of **Spo0A** is subject to a stochastic switch.

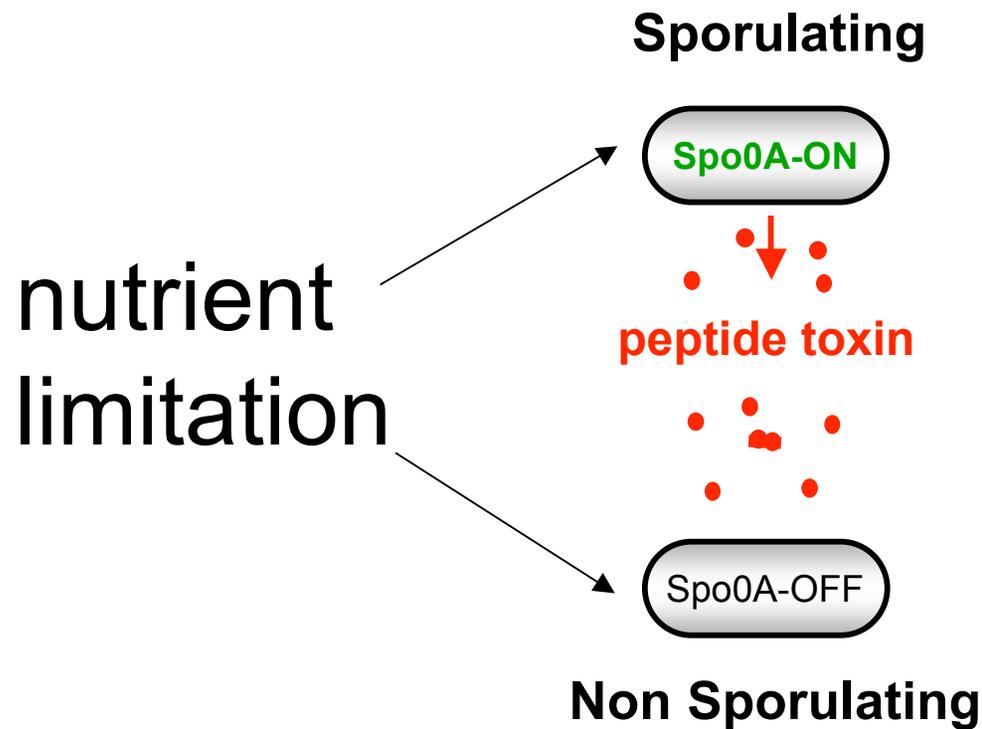


Only some cells are **Spo0A-ON**.

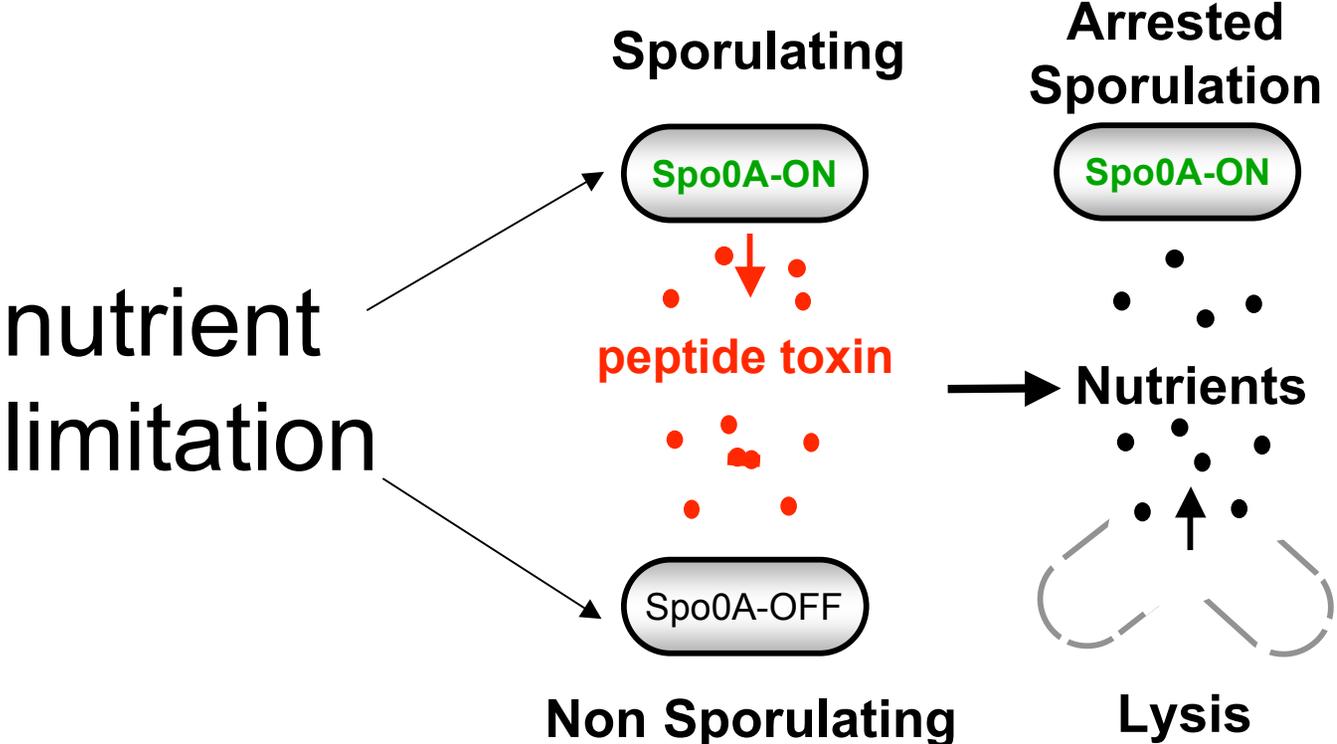


Why is Spo0A subject to a stochastic switch?

Bistability is the basis for cannibalism



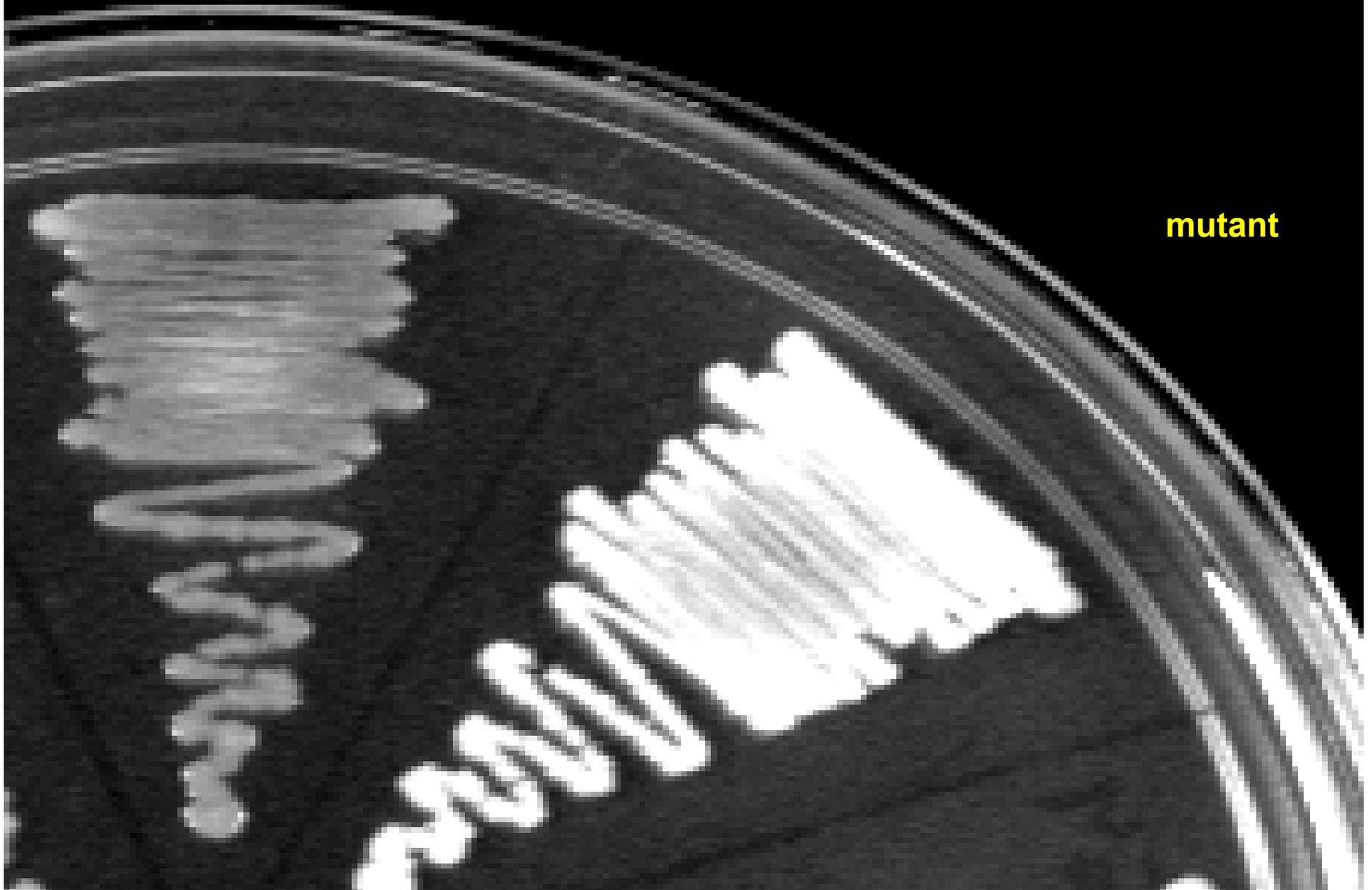
Bistability is the basis for cannibalism



Wild type

Cannibalism Mutants Sporulate Faster

mutant



Cannibalism allows *B. subtilis* to cope with uncertainty.

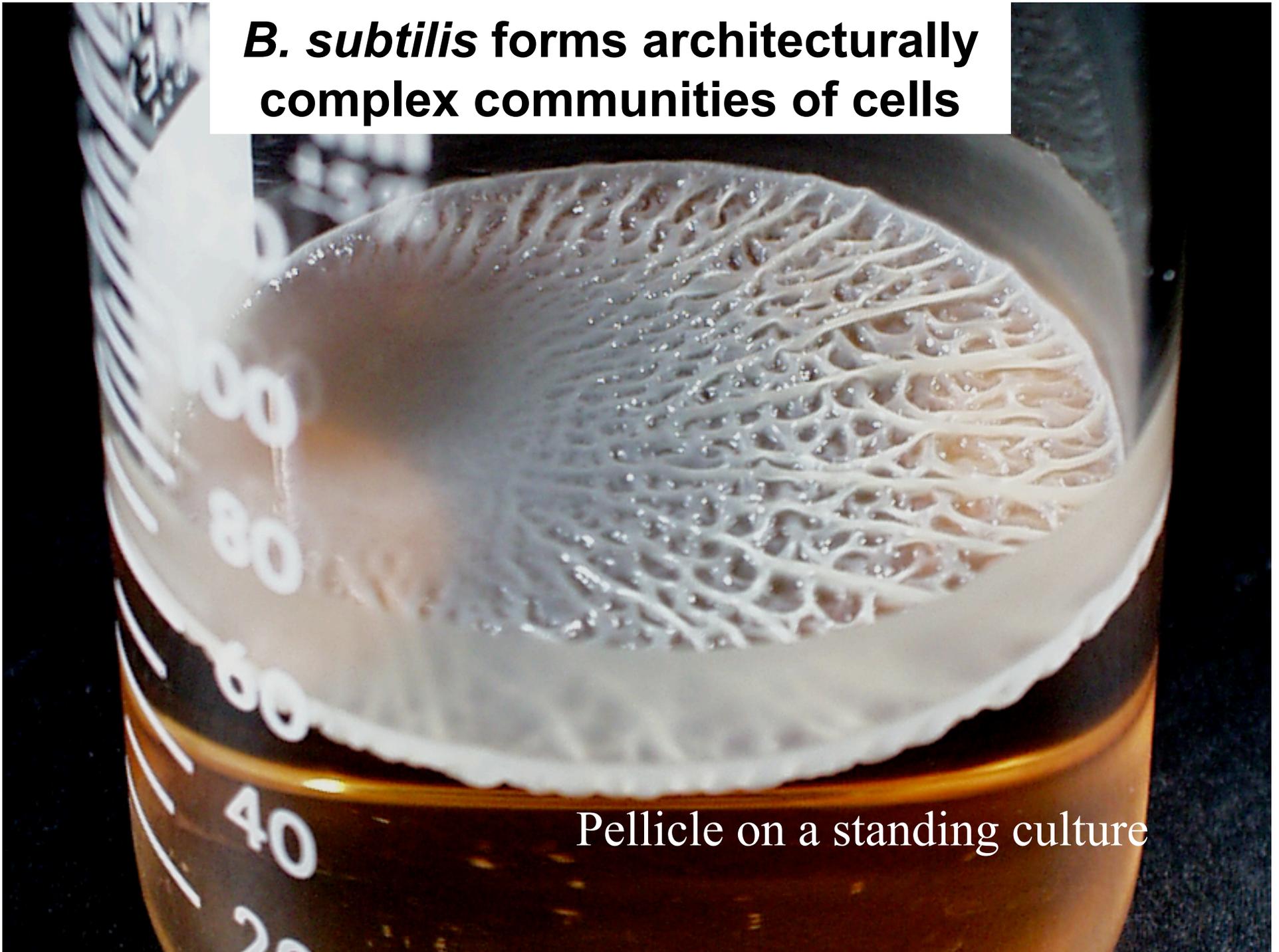
Making a spore is a commitment of time and energy. *B. subtilis* does not want to commit itself to making a spore if it is only experiencing a fluctuation in nutrient availability. This would put it at a disadvantage.

How does *B. subtilis* know if it is merely experiencing a temporary shortage of nutrients or is at the beginning of a famine?

B. subtilis cannot tell the future. So it has adopted a strategy in which it stalls for as long as possible by cannibalizing its siblings (fratricide).

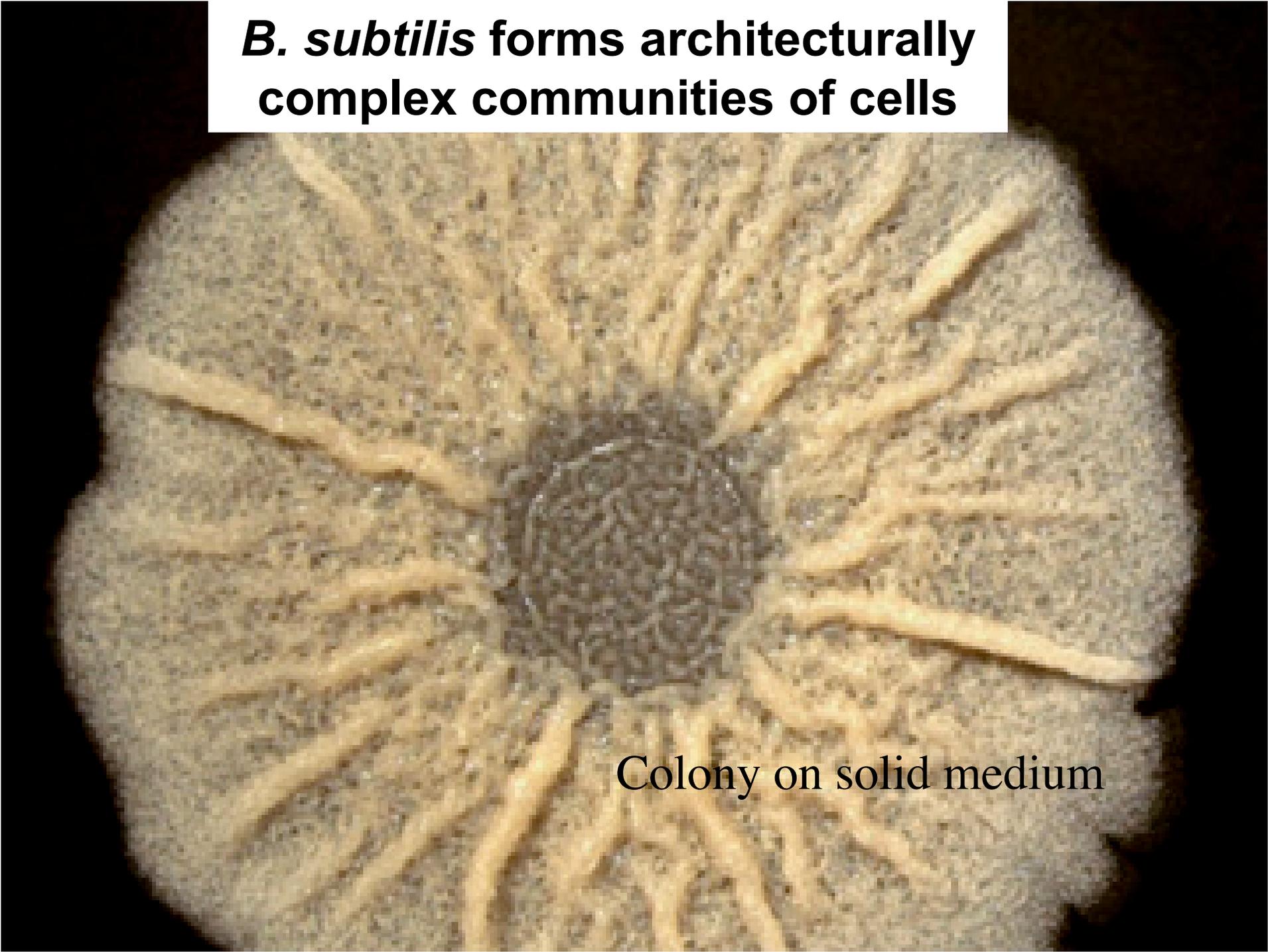
4. Individuality versus community

***B. subtilis* forms architecturally complex communities of cells**



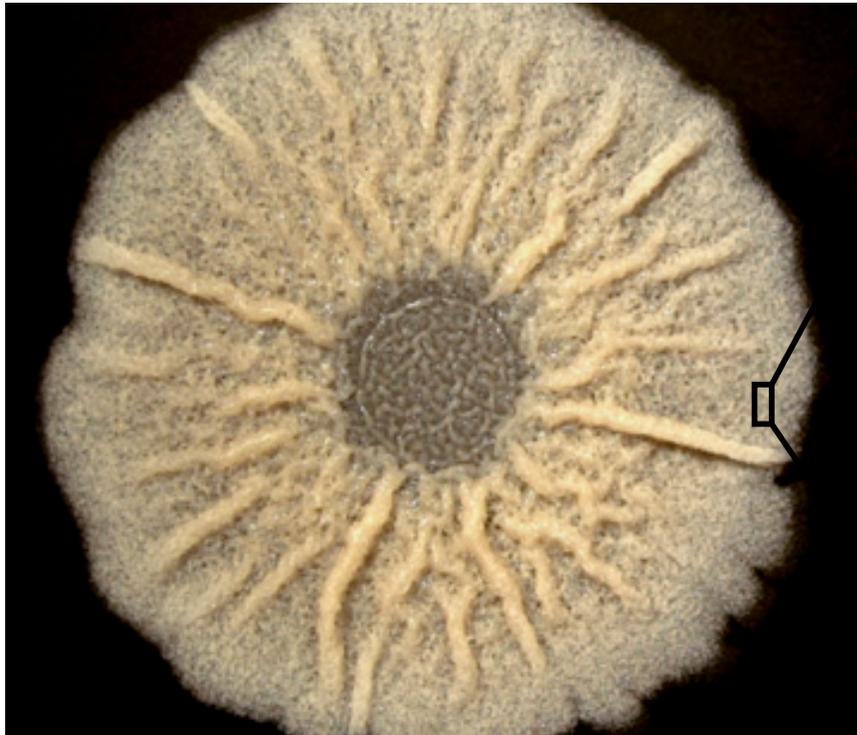
Pellicle on a standing culture

***B. subtilis* forms architecturally
complex communities of cells**

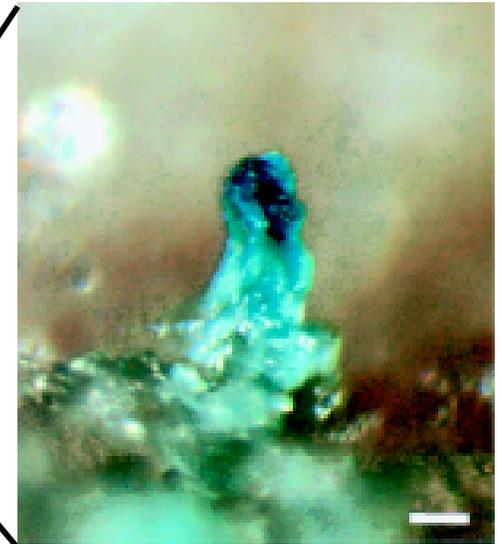
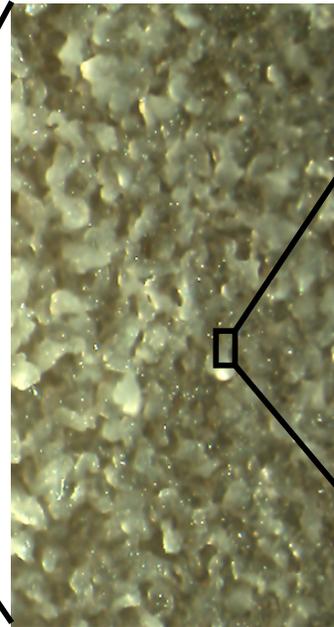


Colony on solid medium

Anatomy of a *B. subtilis* biofilm

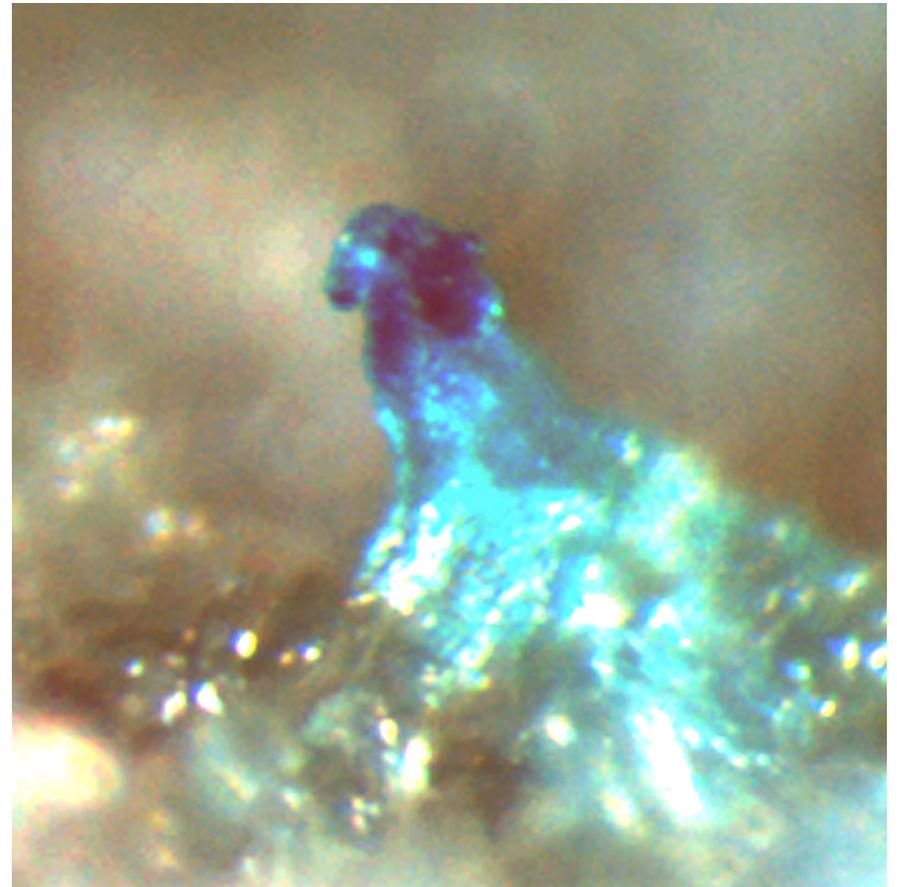
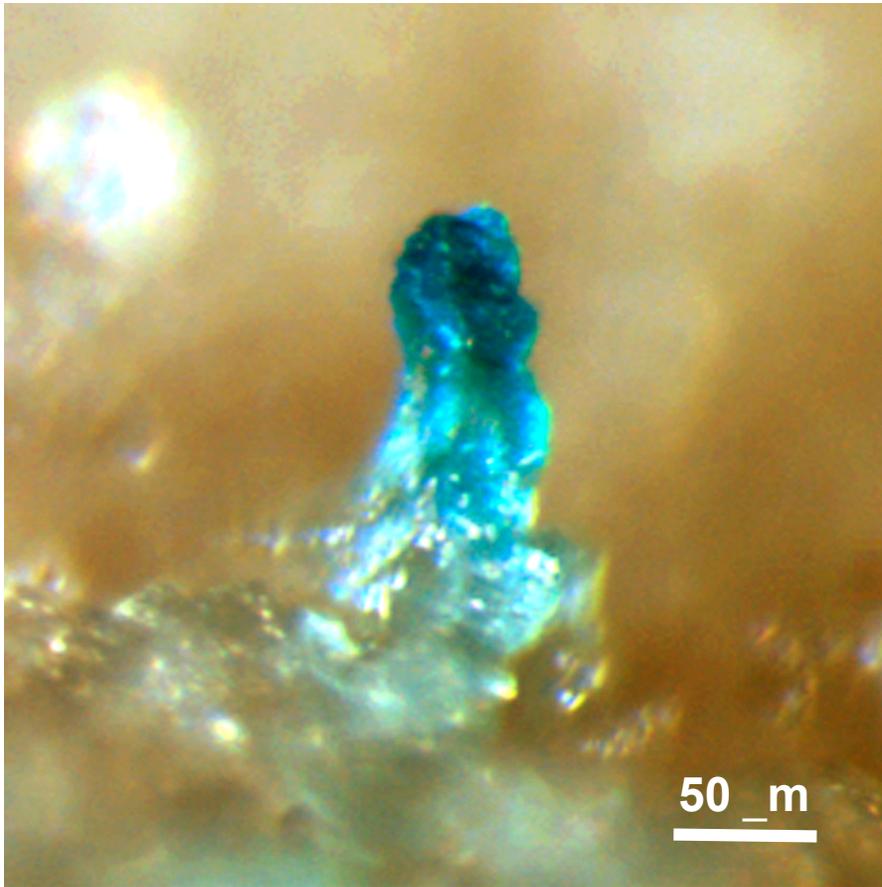


wild strain

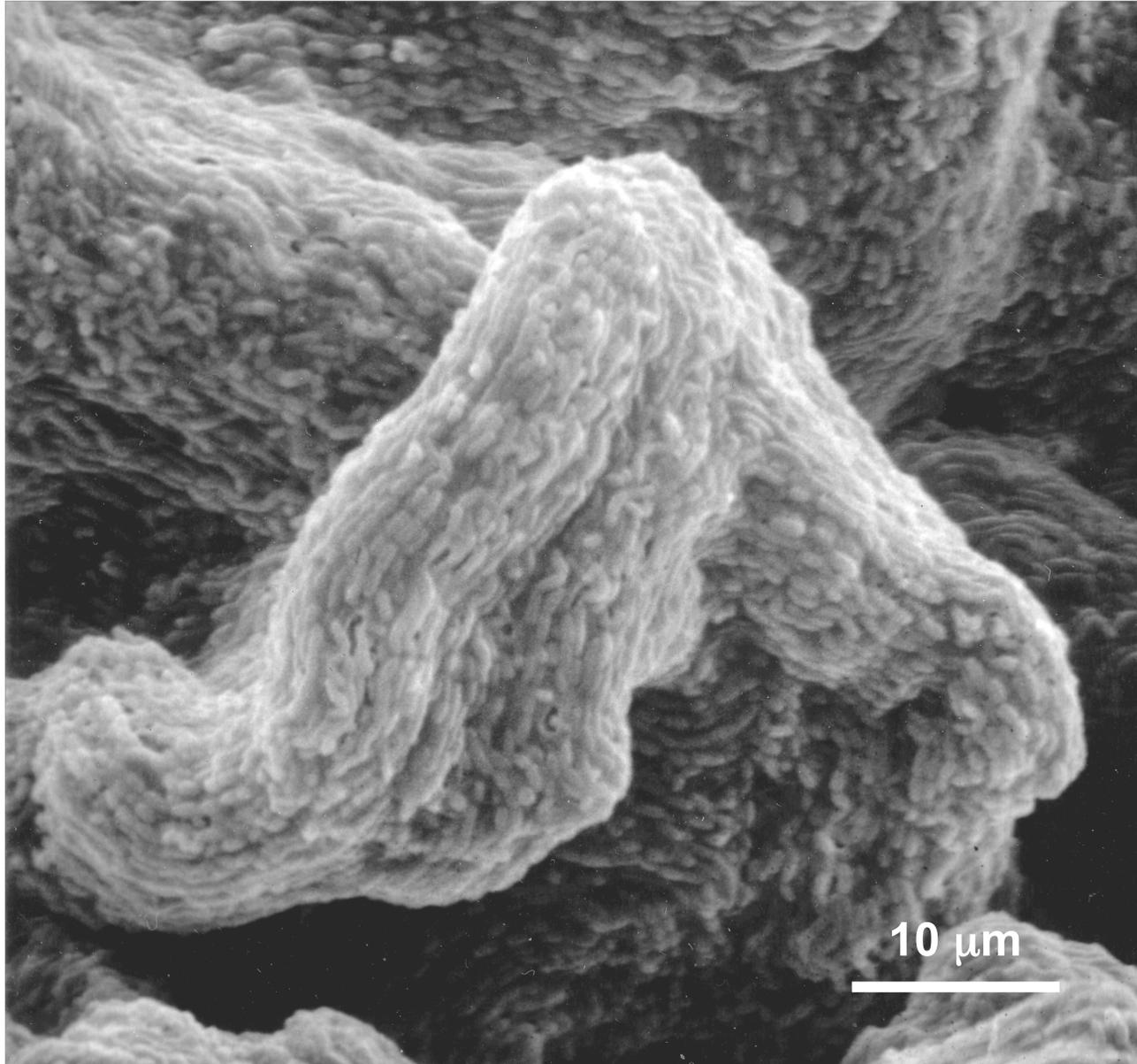


spores at tip

Spatial organization of sporulation



**Scanning electron micrograph of aerial structure
on the surface of a colony**



Cells in the biofilm are held together by an **extracellular matrix**.

The matrix consists of **polysaccharide** and **protein**.

Matrix production is by a **repressor**.

repressor → **matrix**

An **anti-repressor** inactivates the
repressor.

anti-repressor —| **repressor** —| **matrix**

Spo0A turns on the anti-repressor!

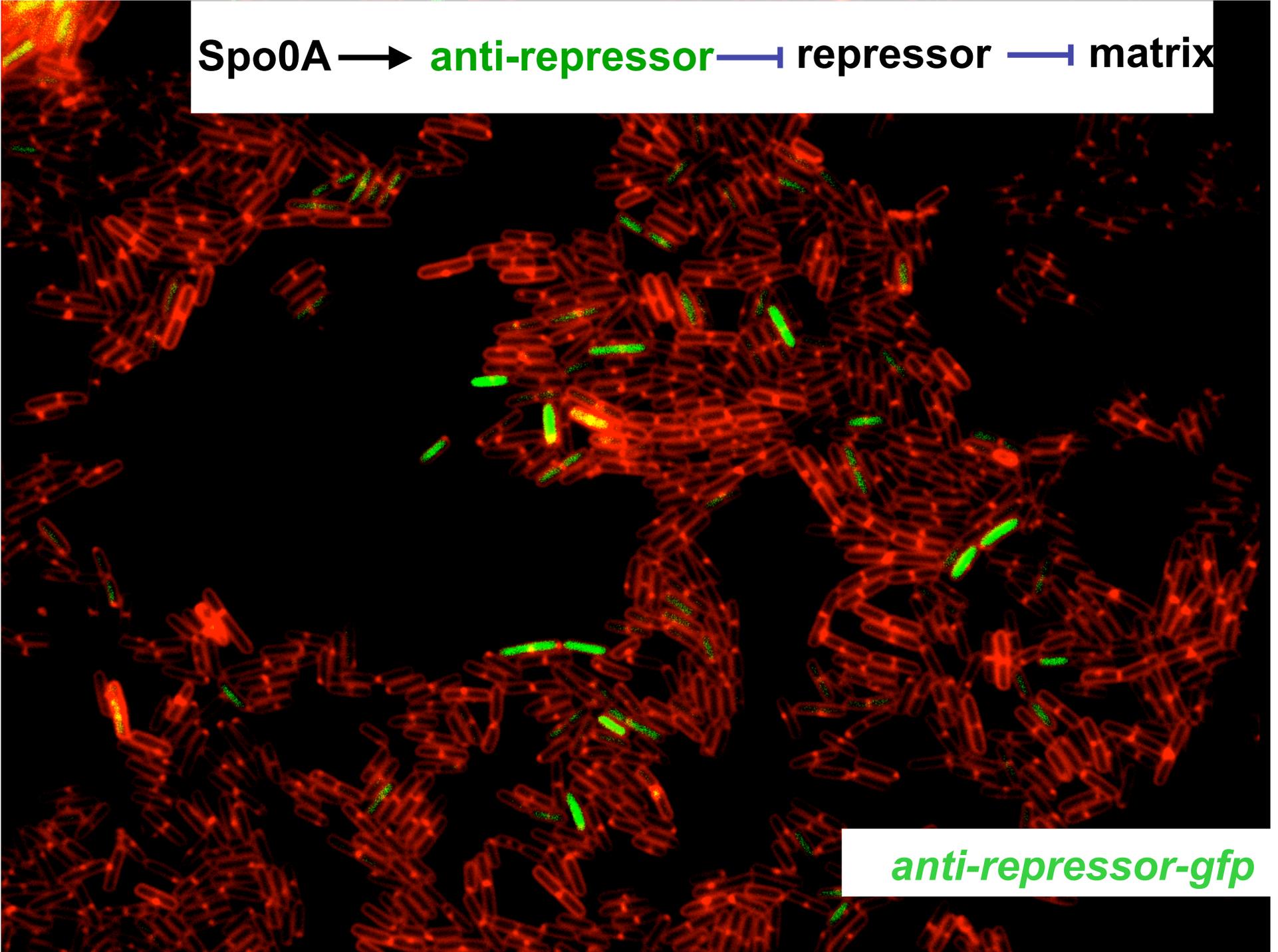
Spo0A → anti-repressor → repressor → matrix

Spo0A → anti-repressor —| repressor —| matrix



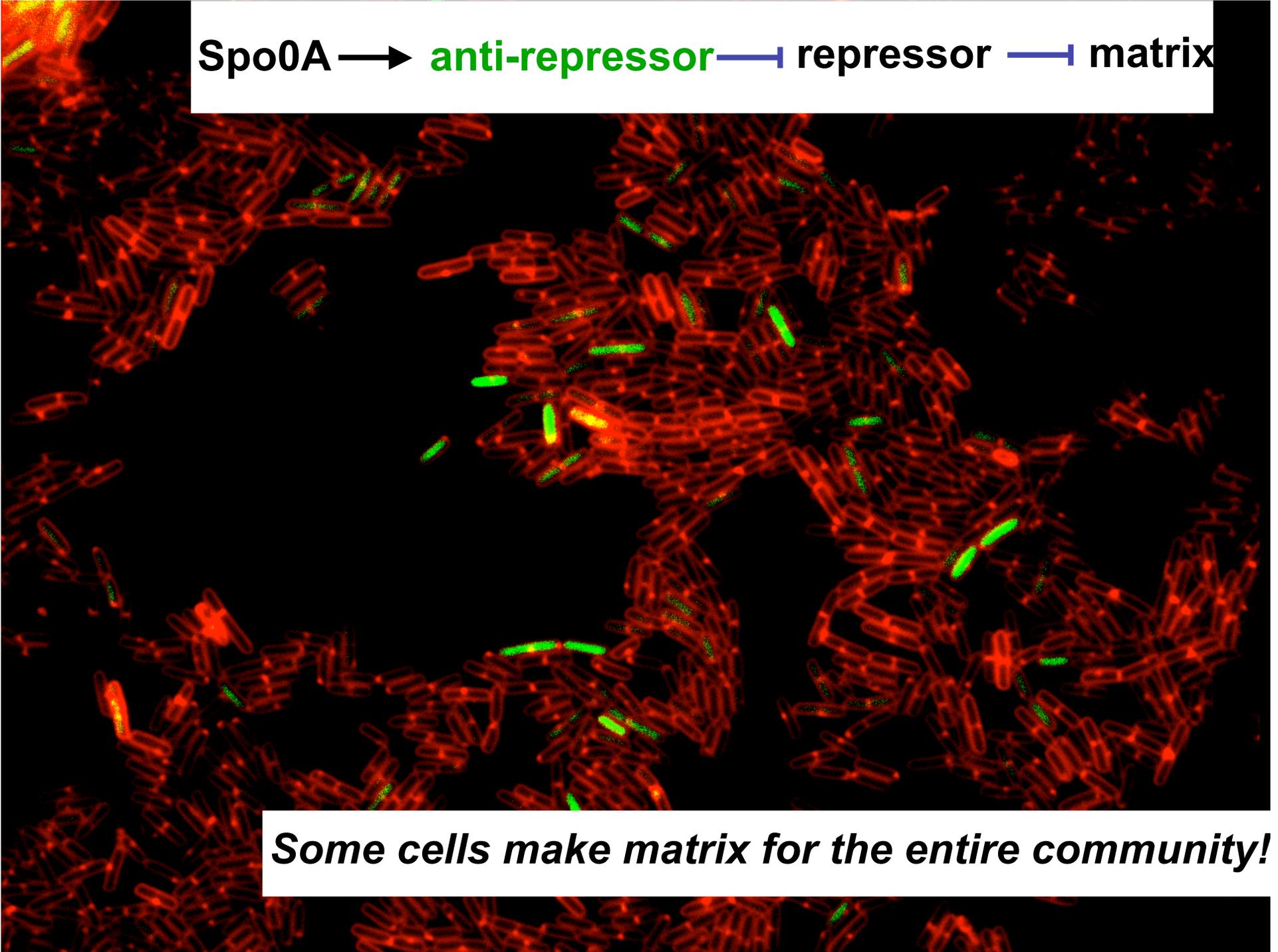
repressor-gfp

Spo0A → anti-repressor —| repressor —| matrix



anti-repressor-gfp

Spo0A → anti-repressor —| repressor —| matrix

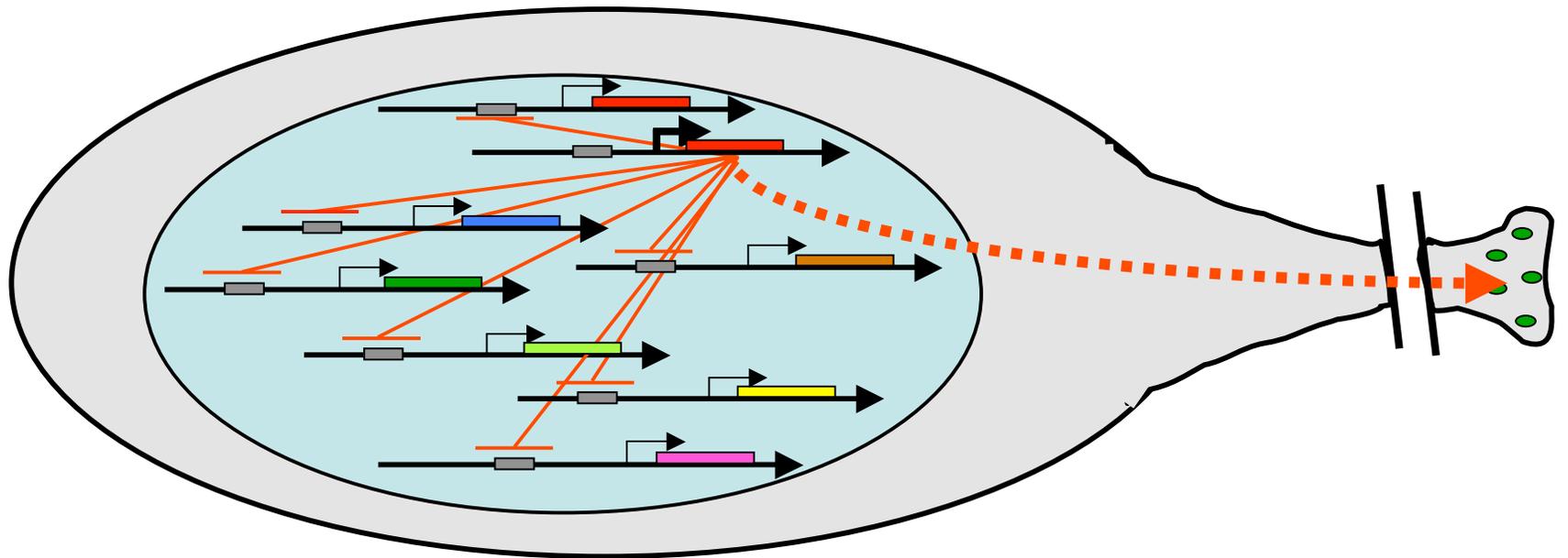


Some cells make matrix for the entire community!

Stochasticity is not unique to
bacteria.

Consider the mouse olfactory
neuron and the eye of the fly!

Mouse olfactory neuron



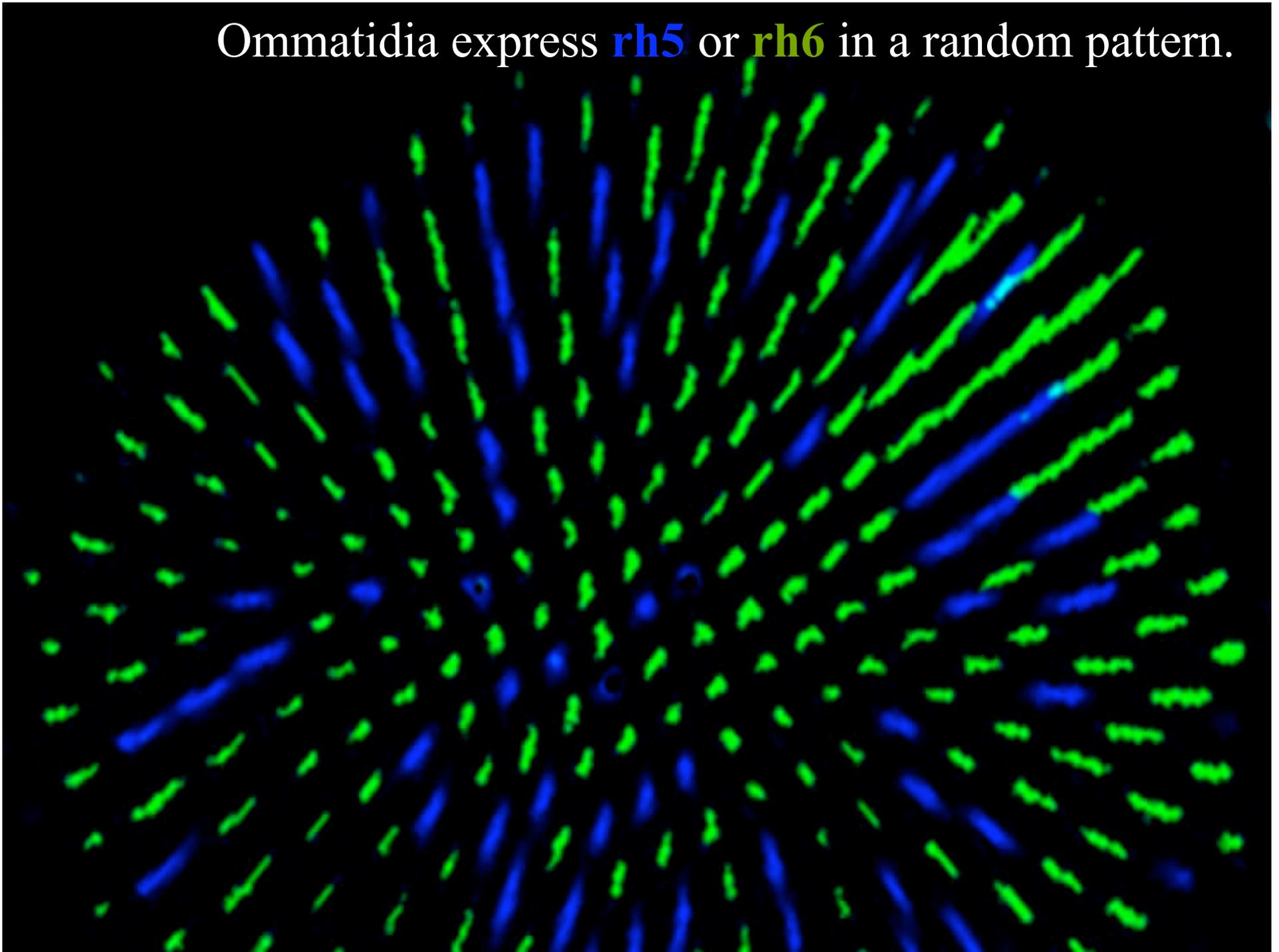
The eye of the fly consists of many clusters of light-sensitive cells called ommatidia.

The eye of the fly consists of many clusters of light-sensitive cells called ommatidia.

Ommatidia produce either of two color-sensitive rhodopsins, **rh5** or **rh6**.

The choice is stochastic!

Ommatidia express **rh5** or **rh6** in a random pattern.



Nature knows how to make deterministic decisions, but, in contrast to Einstein's view of the universe, she also knows how to leave certain decisions to a roll of the dice.