

Unicellular Whispers

Occasionally, they can be seen with the naked eye: small orange-yellow spherical structures. Closer examination reveals that they are accumulations of countless bacteria of the genus *Myxococcus*. The ability to form these fruiting bodies when food supplies are short requires a sophisticated system for intercellular communication that allows hundreds of thousands of single cells to interact in a coordinated fashion. **LOTTE**

SØGAARD-ANDERSEN and her team at the **MAX PLANCK INSTITUTE FOR TERRESTRIAL MICROBIOLOGY** in Marburg are on the hunt for the molecular basis of this complex social behavior.

Bacteria are unicellular organisms and tend to lead a relatively simple life. As independent individuals, they follow their own agenda, and intercellular communication is of no great importance to them – at least, that was previously the view. In recent years, however, it has been necessary to revise this perception. Bacteria do indeed communicate with each other, both within

their own species and between species. In this process, they exchange signal molecules, not words, in a form of chemical communication. Numerous processes typical of bacteria, such as infecting humans, animals and plants or stimulating plant growth, crucially depend on their ability to communicate.

My research group at the Max Planck Institute for Terrestrial Mi-

crobiology in Marburg uses the unique intercellular signaling system in *Myxococcus xanthus* to investigate how bacteria communicate with each other. Among the myxobacteria, *M. xanthus* is the ideal model organism to investigate in the laboratory. Its genome was completely sequenced recently, which allows us to carry out detailed functional genome analysis, and we can also in-



Myxococcus xanthus cells organize themselves into an orderly swarm.

organic matter and other microorganisms. They secrete enzymes that convert rotting wood, dead plants and the cell walls of bacteria and fungi into soluble metabolic products. However, what makes myxobacteria really fascinating is that they again and again cross the boundary between unicellular and multicellular organism: as long as there is sufficient food, the rod-shaped cells grow as single cells and form colonies that expand and feed together. These colonies consist of thousands of cells and are held together by intercellular signals. When the food supply falls short, the cells change their behavior and begin to create multicellular fruiting bodies.

Their ability to move and to regulate this movement allows the *M. xanthus* cells to make these two different structures. *M. xanthus* cells have two different mechanisms for locomotion: they can either push themselves forward by secreting slime from the rear pole or pull themselves forward with thread-like structures, called pili, at the front pole. This latter type of movement requires cell-to-cell contact and therefore works only in large groups of cells. Effective locomotion is possible only if the two propulsion systems are located at opposite cell poles. If a cell wants to change direction, the two engines have to switch poles.

In order to make a fruiting body, the cells begin to aggregate four to six hours after starvation has initiated, and first create small aggregation

centers. These grow as they are joined by other cells and pile up, ultimately creating symmetrical mounds. The aggregation process is complete after approximately 24 hours. The fruiting bodies thus created now consist of around 100,000 densely packed cells. All of the starving cells have the potential to develop into spores. However, only the cells inside the fruiting body differentiate into spores; cells on the outside remain rod-shaped or disintegrate. The spores are not damaged by lack of food or other chemical or physical stress factors, so the survival of the *Myxococcus* cells is assured even under adverse conditions.

CHEMICAL COMMANDS – THE BACTERIAL ABC

As soon as sufficient food is available again, the thousands of spores germinate into cells with an active metabolism, creating a new, communally expanding and feeding colony. The fruiting body thus ensures that the new vegetative cycle starts with a large community of cells and not just with one cell. In order to construct fruiting bodies, the cells need to be able to exchange signals to coordinate and synchronize their activities.

What types of intercellular signals are these? Thus far, the biochemistry and function of two of them, the A- and C-signals, have been investigated. The A-signal becomes important for fruiting body formation after two hours. It is part of a system that measures the density of starving cells and it helps guarantee that fruiting body formation is not initiated unless a sufficiently high number of cells are starving. The A-signal system, therefore, is intended to ensure that the cells adopt the course of forming a fruiting body only if the

investigate the social behavior of this bacterium at the molecular level. What kinds of signals do the bacteria use to communicate with each other? How do they produce, receive and then respond to the signals? In order to understand how the cells communicate with each other, we need to analyze all four stages of this communication process.

SURVIVAL STRATEGY IN TIMES OF FAMINE

Myxobacteria live in the upper layers of the soil, where they feed on

PHOTO: JÜRGEN BERGER, MPI FOR DEVELOPMENTAL BIOLOGY

starvation situation is genuinely serious for a large population of cells.

The C-signal takes action only if the cells have passed this checkpoint successfully. It coordinates three different processes: First, it triggers the aggregation of cells into the fruiting bodies; we will see later how that happens. In the late phase of fruiting body formation, the C-signal induces the sporulation process – that is, the conversion of the cells that have accumulated in the interior of the fruiting body into robust spores. At the same time, the C-signal switches on a set of developmental genes.

SIGNAL TRANSMISSION ONLY ON PHYSICAL CONTACT

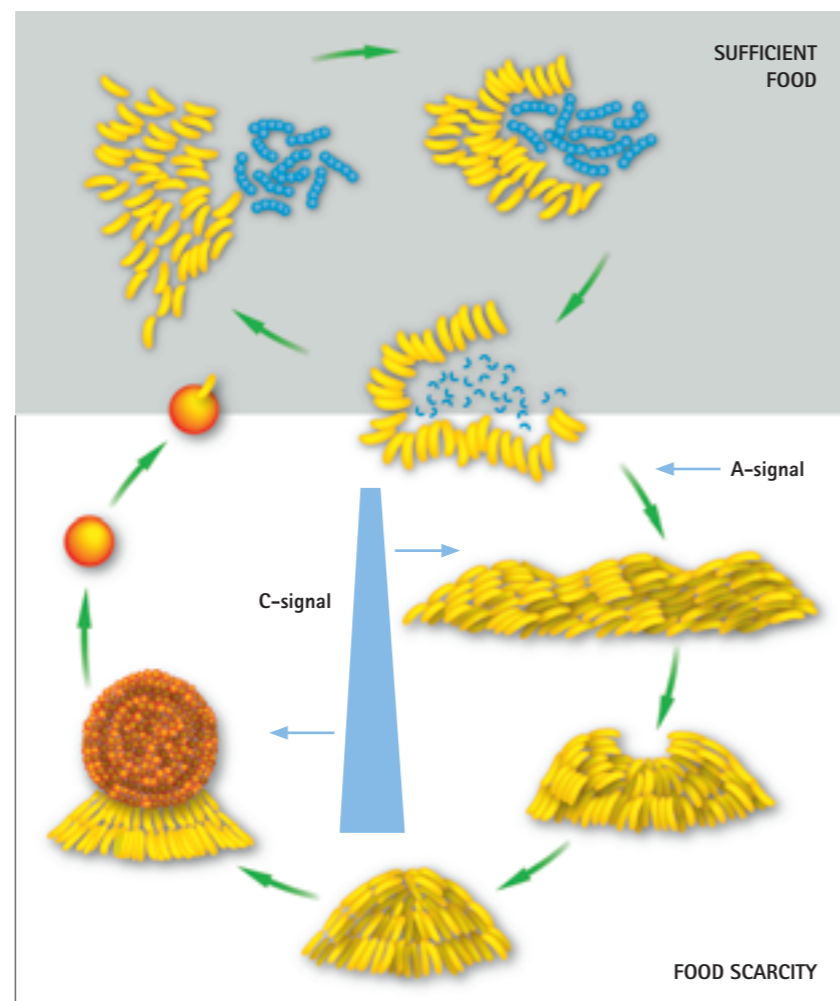
In order to understand how a signal coordinates three different processes that take place at different times and in different places, we have concentrated on explaining the pathway of the signal. What happens here exactly? First, the C-signal must be produced. As already noted, bacteria converse using molecules, not words. The synthesis of the C-signal molecule depends on the *csgA* gene. It is interesting that the protein exists in two forms: a long version, which has a mass of 25 kilodaltons and is therefore referred to as p25, and a shorter variant of only 17 kilodaltons, known as p17. We were able to show that p17 is the C-signal. It is made from p25 by proteolytic cleavage and then anchored in the outer membrane of the bacterial cell. The C-signal is thus a non-diffusibile protein located on the cell surface.

This is surprising, since most of the intercellular signals discovered in bacteria are small, diffusible molecules with a mass of less than 1,000 daltons. As a rule, they are part of a quorum sensing system that helps the bacteria estimate the

size of their population: when the density of cells in the bacteria colony increases, the signal molecules released by the bacteria accumulate. Once a certain threshold value has been exceeded, a signal cascade is triggered during which a wide variety of genes are switched on. In order to prevent, for example, the host organism from reacting prematurely to the bacterial attack, a quorum sensing system delays the produc-

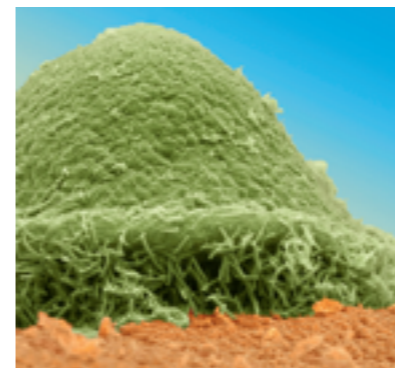
tion of virulence factors until the number of cells is high enough to trigger an efficient infection.

Why does *M. xanthus* use a cell-surface-associated signal molecule instead of a freely diffusible molecule? The answer is probably that the bacterium is simply too slow. The cells can only move at an average speed of two to five micrometers per minute (that would be the equivalent of a maximum of seven millimeters



When there is sufficient food, *Myxococcus xanthus* cells organize themselves into swarms that move and feed together (on other bacteria, for example). If food becomes scarce, the cells aggregate and eventually form a fruiting body packed full of spores. These germinate when food resources are again sufficient. The lifecycle is controlled by the A-signal and – depending on the threshold levels reached – by the C-signal.

FIG.: CHRISTOPH SCHNEIDER



In times of food scarcity, *Myxococcus xanthus* forms multicellular fruiting bodies.

PHOTO: JURGEN BERGER, MPI FOR DEVELOPMENTAL BIOLOGY

per day). This speed is so slow that the directional signals of a diffusible signal would have disappeared before the cells could orient themselves according to the concentration gradients – that is, the different concentrations of the signal in different locations. In order to circumvent this problem, *M. xanthus* has acquired a signal molecule that cannot help but move at the same speed as the cell because it is anchored in the cell membrane. It permits the cell to reorient itself without losing its sense of direction.

However, to allow the C-signal to be transmitted, two cells must come into direct contact with each other. It has not yet been possible to identify the C-signal receptor on the receiving cell. The first component on the receiving side that we know about is a DNA binding protein called FruA. The A-signal mentioned above induces the synthesis of this protein when the gene is transcribed, and the C-signal presumably triggers its phosphorylation (by attaching a phosphate group). At least, that is what we suspect, although we have not yet identified the relevant enzyme (a kinase). Phosphorylation is a widely used mechanism for activating proteins.

At this point, the signal system branches out. It can take two different routes: one route leads to aggregation, whereby a protein is modi-

fied that, combined with other proteins, regulates the behavior of the bacteria cell, or more precisely, controls the frequency with which it turns. Normally, *M. xanthus* cells change the direction in which they are moving. However, in cells in which the C-signal is switched on, the frequency with which the cells change direction decreases and these cells move directly to the aggregation center. The second branch leads to sporulation. The C-signal and FruA jointly regulate around 50 different genes.

EXCITING CONVERSATIONS WITH NEIGHBORING CELLS

During fruiting body formation, aggregation always precedes sporulation. The cells do not start sporulation until the aggregation process has been completed. Moreover, in order for cells to sporulate, they must be located inside a fruiting body. As the C-signal controls the aggregation and the sporulation, the question arises how it can control these two processes in time and space. The mechanism functions comparatively easily. We were able to show that aggregation and sporulation are triggered at different threshold values. The C-signal is amplified via two control circuits. As the density of cells increases, more and more neighboring cells come into contact with each other and transmit their C-signal. This results in increased transcription of *csgA* in the cells, and thus in an increase in the C-signal. The cells can now transmit the C-signal at a higher level to its neighbors. All the cells involved in the transmission of the C-signal are thus exposed to ever-increasing C-signal activity, which, at a moderate level, triggers first the aggregation, and at a higher level, sporulation.

This mechanism ensures that the C-signal reflects the cell density and thus also the position of the individual cells. Only the cells in the interior of the fruiting body have sufficient contact with neighboring cells to reach the high signal activity that finally causes conversion to spores. Using the C-signal, a cell can decode its position relative to the other cells and adjust the gene expression and sporulation according to their position.

In order to understand the whole of the C-signal pathway, we are now trying to identify the other components in the pathway. These include the proteases, which cut the p25 protein down to the actual C-signal molecule, p17, the C-signal receptor, and the kinase that phosphorylates the FruA protein.



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PROF. LOTTE SØGAARD-ANDERSEN, 48, has made a pet out of the bacterium *Myxococcus xanthus*. She conducts research into the communication between bacteria, how the signals are transmitted in the cell, the morphogenetic movement of cells, and the ability of bacteria to form patterns. She was born in Denmark and is now a Director at the Max Planck Institute for Terrestrial Microbiology in Marburg. She previously held a professorship at the University of Southern Denmark.



PHOTO: PRIVATE

DR. CHRISTINA BECK, 42, studied biology in Hamburg and wrote her doctoral thesis in the mid-1990s at the Max Planck Institute for Biochemistry in Martinsried. Since then, she has become very familiar with unicellular organisms. She has worked as a Scientific Editor in the Press Office at the Max Planck Society since 1998 and regularly contributes to MAXPLANCKRESEARCH.