Cells on **the Catwalk**

Life is motion and interaction with the environment. This is equally true of cells within an organism, but for cells to get from one place to another, they not only have to be able to move, they also have to interact with their environment. **Joachim Spatz** and his team at the **Max Planck Institute for Medical Research** in Heidelberg are studying how cells manage this. In his search for answers, the winner of the 2017 Leibniz Prize puts cells through their paces on catwalks and obstacle courses to test their adhesive properties.

TEXT **CATARINA PIETSCHMANN**

The adult human body comprises 100 trillion cells – an almost unimaginably large number, a one followed by 14 zeroes. The cells of our bodies form organs such as the heart and kidneys, and tissues such as the skin and nerves. Some drift through the highly branched vascular system in the form of blood cells, while others patrol the body for the immune system. But regardless of the task they perform for the body as a whole, every cell is an individual. "Each must be able to perceive its environment and respond to it," says Joachim Spatz. The biophysicist headed the New Material and Biosystems Department of the Max Planck Institute for Intelligent Systems until the end of 2015. In 2016, he and his team moved to the Max Planck Institute for Medical Research in Heidelberg. Together, they are devising biophysical experiments, measuring techniques and model systems to investigate the motility and adhesion of individual cells and cell collectives.

Many cells have to cover distances of various lengths within the body: during embryonic development, for example, but also during continuous remodeling processes in the adult body, cells must migrate from their place of origin to the sites where they are needed. To do this, they have to know where they are and where they need to go.

RECEPTORS SENSE THE ENVIRONMENT

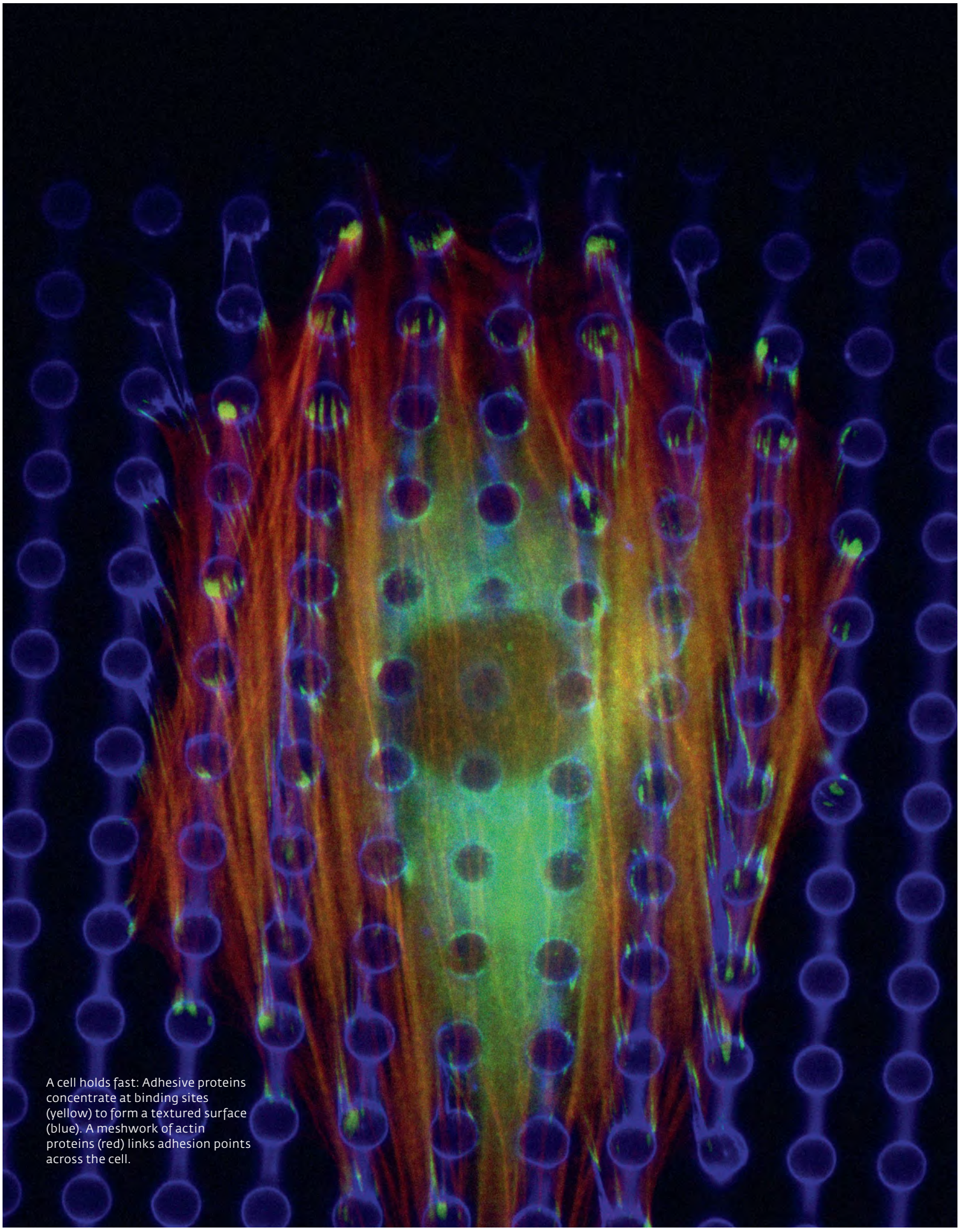
But how does a cell interact with its environment? "For one thing, it detects chemical signals via receptors on the cell membrane," Spatz explains. Small molecules attach themselves to membrane-spanning proteins and activate signaling pathways in the cell's interior. Depending on the information transmitted, genes are revved up, throttled or switched on or off.

In addition, the cell also perceives its underlying surface tactically. It can distinguish whether the surface is hard or soft by essentially tugging on it. "It's like us probing the ground," Spatz says. "If it's soft enough, I can safely fall to the ground. If it's made of stone, I'd better not. "Cells adapt their behavior in a similar manner. "If a stem cell perceives a hard environment, it will mature into bone or tissue cells. If, in contrast, the substrate is soft, it matures into a nerve cell."

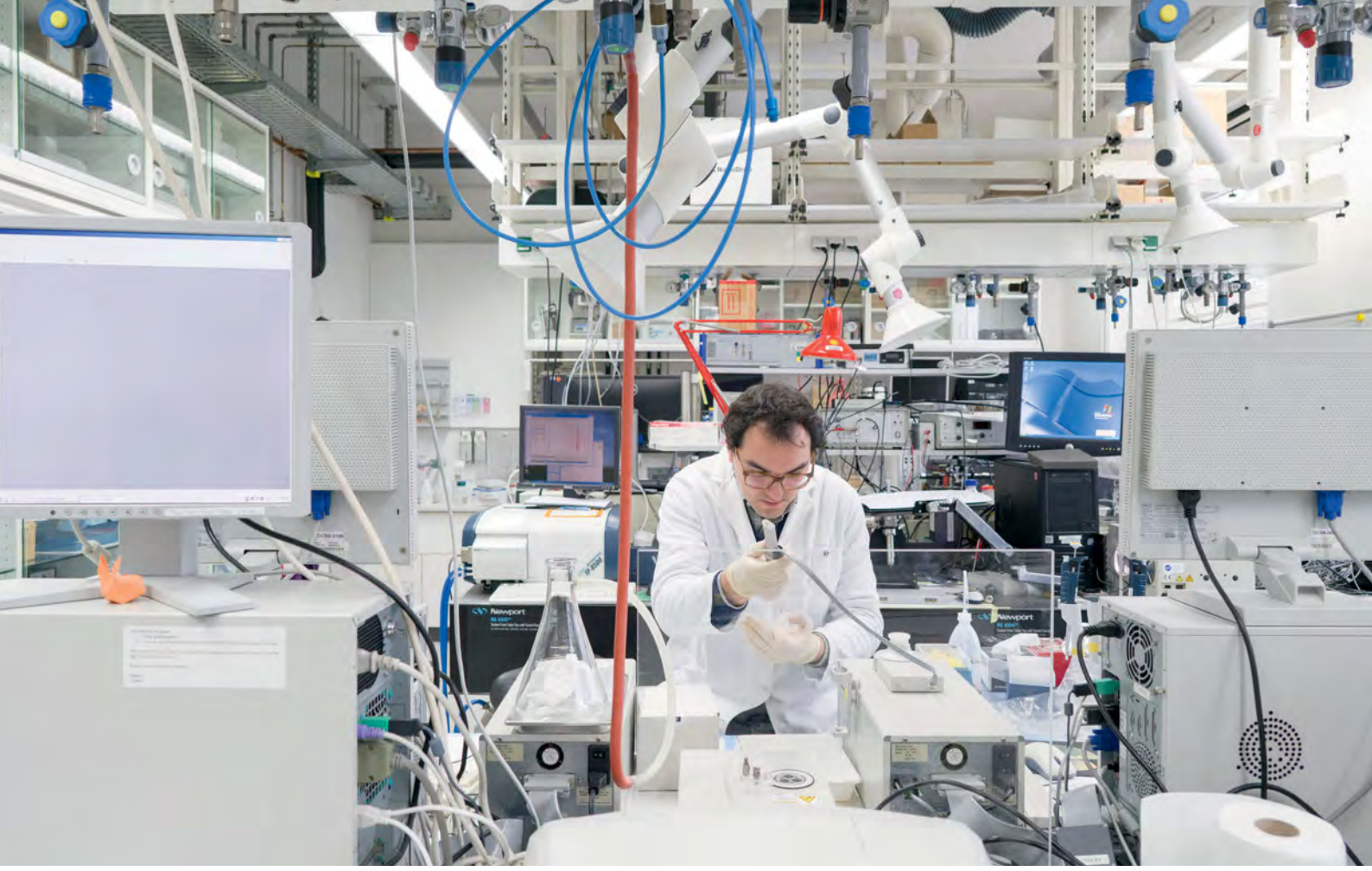
Cells are even able to estimate the number of small molecules near them. This gives them a sense of how important the latter are as chemical messengers and whether a response is appropriate. "It is also important for migrating cells to know how densely a surface is covered by molecules," Spatz says. "If the distance between the molecules is 60 nanometers or more, the cells are unable to read the information."

In order to observe the behavior of individual cells, Spatz's coworkers have developed a sort of cellular catwalk, which they call cellwalks. Each cellwalk

Photo: MPI for Intelligent Systems



A cell holds fast: Adhesive proteins concentrate at binding sites (yellow) to form a textured surface (blue). A meshwork of actin proteins (red) links adhesion points across the cell.



Jacopo Di Russo studies the migration behavior of human skin cells and measures the force the cells exert to cling to a surface. To do this, he binds various proteins to gels to alter the properties of the surface.

consists of a polymer surface coated with ultrafine gold particles. The gold acts as anchoring points for biomolecules – peptides, for example, or antibodies, to which, in turn, cell receptors can bind. This provides support for the cell, because without these "nubs," the surface would be far too slippery. By varying the base polymer, the spacing between the anchoring points can be adjusted to 30, 50, 70, 100 or 150 nanometers. For the cellwalk, a small piece of the synthetic surface is placed in a culture dish with nutrient solution. Individual cells are then placed on top of it, the microscope is focused and the camera is activated.

MIGRATION AT A SNAIL'S PACE

How fast do cells actually move? "On average, 30 micrometers an hour, but they can reach a brisk 50 micrometers an hour," says Spatz with a chuckle. They can travel about one millimeter a day, assuming they are fit enough. However, the speed of travel depends on the cell type. Even within a single cell line there are "tortoises" and "hares" because the speed is also determined by the cell's stage of development.

Cells, of course, have no feet, but they do have something similar. They crawl forward on sheet-like extensions, called lamellipodia. The cell membrane bulges out and spreads forward. "It's comparable to us taking a step forward," Spatz explains. The lamellipodium adheres to the substrate by rearranging and aligning protein molecules in the cell's interior – integrins as well as actin and myosin filaments. The rest of the cell body is then pulled forward, and the anchoring site is released again. This process is repeated for each "step" – a form of locomotion also used by amoebas.

In recent years some members of Spatz's team have conducted research not only in Stuttgart but also on the campus of the University of Heidelberg, where word about their work on cell migration soon got around. One day parasitologist Friedrich Frischknecht from Heidelberg University Hospital approached Spatz: couldn't they put a "real" protozoon, namely the malaria organism *Plasmodium*, on the cellwalk for a change?

The tropical disease, which is caused by the bite of an infected *Anopheles* mosquito, still claims half a million lives every year. The mobility of the pathogens, which are injected into the human skin from the mosquito's salivary glands, is crucial for the "success" of the infection. "The sickle-shaped sporozoites move at a top speed of 10 micrometers per second, nearly 100 times faster than human cells," Frischknecht explains. "We're interested in finding out how they do it."

Sporozoites drill through the skin until they reach a blood capillary. They are then carried by the bloodstream to the liver, where they multiply for the first time. Until now it was a puzzle exactly how sporozoites move forward. The only thing that was clear was that they neither crawl nor paddle like bacteria and single-cell algae, because they

Photo: Wolfram Scheible

» Malaria pathogens move nearly 100 times faster than human cells.

form neither lamellipodia nor flagella. "Sporozoites glide gracefully without changing their shape thanks to special proteins on their surface," Kai Matuschewski explains. The scientist at the Max Planck Institute for Infection Biology in Berlin and professor at Humboldt University is the third member of the project.

The *Plasmodium* experiments didn't require a non-slip cellwalk, but an obstacle course to imitate the parasite's relentless progress through the skin. Spatz constructed a miniature bed of nails, of sorts, from polymers through which the parasites can glide as if through a forest of ultrafine needles.

A comparison of various mutants, each of which lacks a different protein, yielded fresh insights. "We can now directly observe what actually happens. If protein X is missing, the parasite is unable to hold on; if Y is missing, it sticks fast to the surface," Matuschewski explains.

TINY PROTEIN FEET

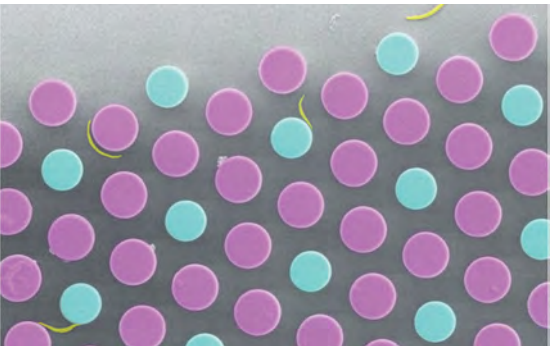
In its cell membrane, a sporozoite has a huge range of proteins that are required for locomotion, some of which perform identical functions. Hundreds of them are situated at the anterior end of the tiny cell body, to which they are connected by a meshwork of actin molecules. "The parasite causes the membrane proteins to move toward the posterior end and then repels them," Matuschuweski explains. The motion is similar to that of a millipede – except that these so-called feet consist only of single molecules and are so small that they can't even be seen under an electron microscope. The force the parasite has to exert to hold and then release itself can be measured with the help of optical tweezers. This provided further insights into how the single-cell organism moves.

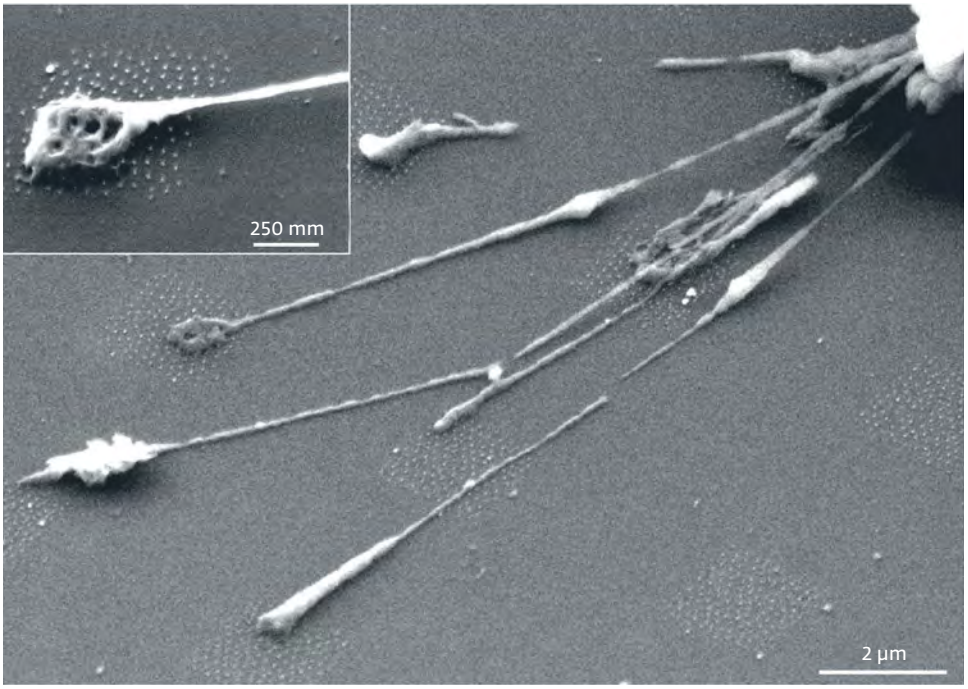
The malaria organism has a complex life cycle, in the course of which

Photos and graphic: MPI for Intelligent Systems (2)

Below Malaria parasites (yellow) crawl over a surface studded with tiny columns. If the single-cell organisms have the same curvature as the columns, the pathogens, which measure just one hundredth of a millimeter in length, begin to circle around the obstacles.

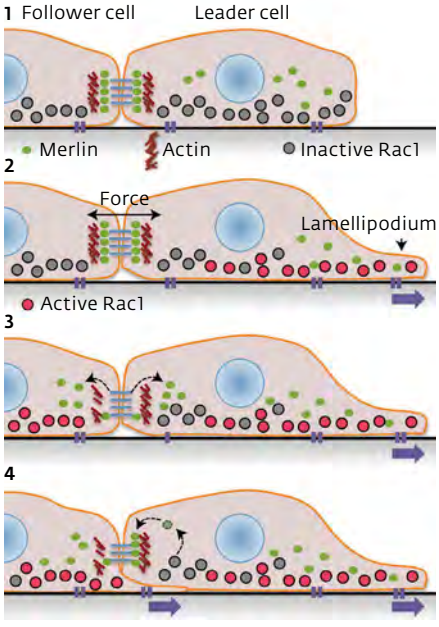
Right A migrating cell. It forms contact points with the surface at specially structured sites located at the ends of thread-like processes (small image). At these points, it senses the properties of the surface and uses the anchoring points to pull itself forward.





Above Jacopo Di Russo, Katharina Quadt and Medhavi Vishwakarma (from left) preparing a new experiment in which they will modify and measure the movement of cell collectives.

Below The protein merlin (green dots) controls the direction of travel of follower cells: **1** Without physical or chemical stimulation, merlin blocks the formation of lamellipodia. Without these protuberances, the cell is unable to move. **2 + 3** If, in contrast, the leader cell pulls on the cell behind it, merlin in both cells leaves its site on the cell membrane, allowing a lamellipodium to form. The follower cells are then able to follow the leader cells. The signal molecule Rac1 (red dots) must also be activated for a lamellipodium to form. The cells always migrate toward areas of higher concentrations of activated Rac1. **4** If Rac1 is inactivated (gray dots), merlin again blocks the formation of lamellipodia at the cell membrane.



it takes on various forms. However, experts agree that the sporozoite is a promising – if not *the most* promising – target for a vaccine, for the sole reason that only around 100 pathogens are present immediately after an infection. In the later stages of infection, there are billions. "Ideal vaccine candidates are antibodies that block two or three of the pathogen's proteins involved in movement," Matuschewski says. If the sporozoite is unable to glide, it literally gets stuck in the skin, and the infection is stopped in its tracks.

Having studied the movements of individual cells in detail, Spatz's team turned its attention to the migration behavior of entire groups of cells. They posed the question: how exactly does a wound heal?

Whether it's a small cut to a finger, a scraped knee or deep cuts following an operation, epithelial cells have to "rush" into the wound to close it and begin rebuilding the tissue. This sounds simple, but it is a highly complex process. It has been compared to marching in lockstep. Joachim Spatz calls it collective cell migration. It is one of the processes that no longer function properly in chronic wounds.

MIGRATION UNDER A MICROSCOPE

The scientists needed a suitable wound model for their experiments. They covered the bottom of a petri dish with a nutrient medium and grew epithelial cells on it. They blocked a region of the dish to prevent the cells from migrat-

Photo: Wolfram Scheible, graphic: MPI for Intelligent Systems

» Mechanical tension at tissue edges promotes wound healing.

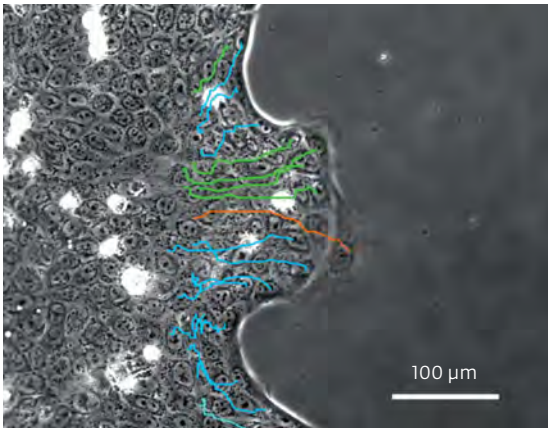
ing into it. As soon as they removed the blockade, the cells started to colonize the free space. Their movements were recorded by a microscope camera, which took snapshots of the process every ten minutes.

After just a few hours, bulges appeared in the originally straight wound edges. "That's because some cells move faster than others," Spatz says. In other words, the repair crews don't advance in ordered lines. Rather, there are leaders, "pioneering" cells that the others trail along behind. "They form the vanguard of the collective and can easily be identified because they're bigger and move ahead of the others."

What makes a cell a leader? And how do they communicate with the other cells in their tow? To answer these questions, the researchers varied their experiments. They covered the bottom of the petri dishes with a hydrogel, which cells find slippery, and sowed the epithelial cells within only a small geometrical shape in the center. As soon as the colonies filled these circular, triangular or square areas, the researchers briefly exposed the gel to UV light. This transformed the slippery surface into a non-slip surface, and the epithelial cells quickly set off. "We found that leader cells tend to form at places with a pronounced curvature, for example in the corners," Spatz says. This is easily explained: the contacts between the cells are mechanically stabilized by what are known as tight junctions – short struts of membrane proteins such as cadherins. If the cadherins of cell A match those of cell B, the extracellular domains of those proteins click together like snaps. At the same time, the actin skeleton in the interior of the cell reaches out to neighboring cells for stability. "The edge of a cell collective is sort of like a herd of sheep that has been penned behind a fence," Spatz says. The actin fence is under mechanical tension. If the curvature is the same everywhere, as in the case of a circle or a straight line, it's difficult to break away from the herd. "Neighboring cells take off together and jockey for the lead. Eventually, one prevails and the others fall behind." However, a cell that marches out of the corner of a square, for example, has no neighbors and automatically becomes a leader. Surgeons unknowingly exploit this effect when they make zigzag scalpel cuts rather than straight ones, thus stimulating subsequent cell migration into the wound.

ONE LEADS, THE COLLECTIVE FOLLOWS

The researchers suspect that, at least in principle, every cell has leadership potential. Whether they can exploit that potential depends largely on their position at the edge of the wound. If the position is not crucial, for example if the edge of the wound is straight, then the cell rows in the rear determine which cell becomes the leader. Ultimately, the decision regarding the leadership of a collective is not made independently by the leader cell, but by the collective as a whole. This decision is physically regulated in the collective by purely mechanical means. The question that remains is how the leader communicates with its followers.



Wound healing in a petri dish: Epithelial skin cells migrate together into a region with no cells. The colored paths show the cells' movements over a period of more than five hours.

The researchers first measured the speed and direction of motion of each cell in a collective. "That showed us that domains of 20 to 30 cells form that then quickly march together in one direction," Spatz explains. These cells form what is termed the persistence length, that is, the distance over which cells march in a coordinated manner in one direction.

Then the real work began. "In molecular cell biology, we know hundreds of proteins that serve as signal molecules for cell migration," Spatz says. "We switched off the gene for each migration-associated protein so that the cell was unable to synthesize that protein. Then we measured the persistence length of the mutants by biophysical means." The scientists were amazed to discover that very few of the proteins

Photo: MPI for Intelligent Systems

Medhavi Vishwakarma, Freddy Frischknecht, Joachim Spatz, Jacopo di Russo and Katharina Quadt (from left) analyzing data. The scientists have established that the protein merlin plays a key role in the collective migration of cells.

have anything to do with collective movement. Only if the membrane protein merlin is missing does the collective break apart.

It is astonishing that the cell relies on just a single type of molecule and doesn't have at least one backup protein at the ready. Merlin is not a new discovery – it was already known from cancer biology. Unlike in the case of wound healing, a metastatic growth wants to prevent the cellular "herd instinct" because single cancer cells are more efficient and can progress into tissue more quickly than a group. They let the bloodstream carry them to distant regions of the body, where they establish themselves at suitable locations and begin to divide uncontrollably. "Merlin is a metastasis inhibitor," Spatz explains. "It's a good sign when the protein is very active in cancer cells. The cells then tend to stay together and are thus less likely to form metastases."

It was already known that merlin is positioned between the cell membrane and the cellular skeleton. The researchers observed collective cell migration after labeling merlin with a dye, and found that the protein leaves its original location to reappear in the interior of the cell. But why? They hypothesize that the leader cell rushing ahead creates tension between the cells. "It's like a runner pulling another runner behind him by the hand," Spatz explains. Merlin is like a force sensor that responds to this tension. It disappears into the interior of the cell, leaving room on the membrane for the follower cell to extend its lamellipodium and follow the leader. "If merlin didn't do that, the cell would be unable to follow, because membrane-bound merlin acts essentially as a lamellipodium brake." And because the protein makes room only at places where the leader cell pulls, the direction is automatically established. This process continues down the rows of the cell collective – literally pull by pull. So mechanical tension promotes the formation of cell collectives and thus wound healing.

And how does the lead cell know which direction it must go in? "In our model, it can move in only one direction, namely into the free space. In the case of a real wound, however, signaling substances are released that convey directional information to the cells along the wound's edge." Spatz's team, together with the University of Heidelberg, has since repeated and confirmed the experiments on human skin models.

UNCOORDINATED MOTION WITHOUT MERLIN

The researchers also discovered that, without merlin, more leader cells form, but the wound healing proceeds more slowly, because the mechanism by which the cell collective moves is no longer coordinated.

Spatz is currently also looking more closely at the movement of cancer cells. "Unlike healthy cells, they have a tendency to ignore their environment. That's bad for patients, but good for the cancer!" Metastatic cancer cells reduce not only their merlin production, but also that of cadherin contact proteins. They are therefore able to make themselves extremely long and narrow and slip between tissue cells to invade new regions of the body without being detected or stopped by other cells.

Photo: Wolfram Scheible

However, cell migration is not specific to wounds and cancer cells. It occurs continuously everywhere in the body. "If cells did not cluster together, communicate with each other and migrate together, we wouldn't exist. The migration and interaction behavior of cells was the prerequisite for the evolution of multicellular organisms – and thus for life as we know it."

Communication with the environment is the lifeblood of cells, something they have in common with us, their one-hundred-trillion-cell collectives. What happens to people when communication is cut off for extended periods? They become lonely. Some may become despondent, while others might find that they are sufficient unto themselves. Yet others would seek out old acquaintances or establish new ones. Cells, in contrast, have no choice: they activate a built-in suicide program and die. ◂

TO THE POINT

- **Membrane-bound proteins convey information about a cell's environment, such as the nature of the substrate. By remodeling its internal skeleton, the cell can form small protuberances in the membrane, which it uses to move about.**
- **When epithelial cells migrate into an uncolonized area, some of them become leader cells that the others follow. Such leader cells tend to develop at sites where the tissue edge has a pronounced curvature, for example at corners and edges.**
- **Merlin, a membrane protein, coordinates groups of migrating cells. It senses the physical tension that arises when a leader cell pulls on the follower cells, enabling the formation of small protuberances at the pulling points. The protuberances are then used for locomotion.**

GLOSSARY

Lamellipodia: Broad protuberances of the membrane at the anterior end of motile cells. They are also known as pseudopodia. A two-dimensional meshwork of thread-like actin proteins provides stability and tensile force. Lamellipodia are used for locomotion and navigation, and for engulfing nutrient particles (phagocytosis).

Optical tweezers: A method that allows scientists to measure the forces that individual molecules exert on each other. Each of the molecules to be measured is placed on a plastic bead that is held in position by two laser beams. As soon as the molecules start to interact and attract each other, sensors measure the force that the lasers must exert to hold the beads apart.