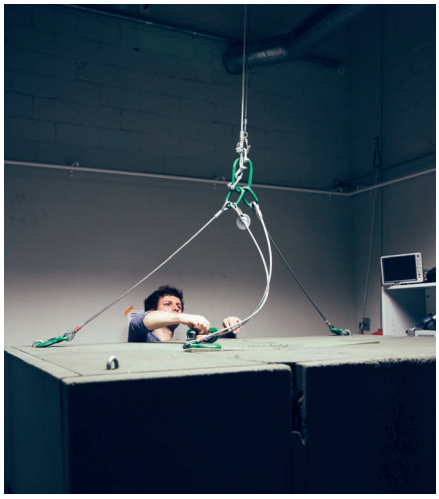
Tweezers Made of Light

Many biomolecules move through cells like microscopic machines. Often, however, it isn't known what forces these molecules generate or how fast the molecules act or move. That's why **Stephan Grill** from the **Max Planck Institute of Molecular Cell Biology and Genetics** in Dresden decided to specialize in measuring molecular forces. He uses optical tweezers to pull gently on DNA strands. His method is shedding light on the proteins that read genetic information.

TEXT **TIM SCHRÖDER**

Springs are ubiquitous in the modern world, whether in pens or on the grips of garden clippers. A spring is compressed or stretched, depending on its function. This requires force, as the spring resists any attempt to change its shape. Springs can be very strong: the leaf springs of trucks, for example, easily support 20 to 30 tons.

To express the strength of a spring, physicists perform a measurement that determines the spring constant. The spring constant is measured by determining how much force is needed to stretch or compress a spring through a given distance. The force required depends on the thickness and material of the spring. For example, stiff shock absorbers for sports cars shorten by just one millimeter in response to a force of 70 newtons acting on them – corresponding to the force generated as a car drives over cobblestones.



TINY POWER PACKS

Stephan Grill has nothing to do with shock absorbers, sports cars or leaf springs. He deals with forces on a completely different scale. The physicist from the Max Planck Institute of Molecular Cell Biology and Genetics measures forces that are a billion times weaker than these.

Grill isn't interested in newtons and kilonewtons, the units of force in everyday life, but in piconewtons, less than a billionth of a newton, to express the forces acting between biomolecules. "Biomolecules aren't rigid and static," says Grill. "Some proteins resemble microscopic machines powered by tiny forces. This is tremendously fascinating." Our muscles contract, for example, because millions of elongated proteins slide along each other over miniscule distances. By combining all these tiny shifts, muscles are able to carry out large movements.

The movements that interest Grill, however, are smaller than those associated with muscle proteins. He wants to understand how RNA polymerase travels along DNA molecules while reading the genetic information the DNA encodes – a process known as transcription.

After decades of intensive research, biologists, biophysicists and geneticists now understand which molecules are involved in transcription, what intermediate products are formed, and where the energy for the finely tuned genetic machinery comes from – but not the forces that are involved. "If we really want to understand the transcription engine, we need to find out what drives it," says Grill. "It's like any other machine. If you don't know how much force it generates, you don't really understand how it works." >

Photos: Sven Doering

Left: A foam box protects the optical tweezers from environmental factors such as temperature fluctuations and noise vibrations. A hoist can be used to lift the entire box or to open the lid.

Right: Stephan Grill can load the sample to be investigated, or make changes to the setup through the open lid. The researcher wears special goggles to protect his eyes against the laser light.

Photo: Sven Doering

Measuring forces – at the molecular level, this is no easy feat. For this, Grill constructed a sophisticated experimental setup when he was leader of a joint group at the Max Planck Institute of Molecular Cell Biology and Genetics and the Max Planck Institute for the Physics of Complex Systems before 2013. Together with his colleagues, he combines mirrors, lenses and high-precision lasers to form an optoelectronic maze. And his tool for measuring biomolecular forces is light.

Grill, who is now also a professor at the TU Dresden, is in the process of moving and shuttles back and forth between the Max Planck Institute and the TU laboratories – four minutes by bike. The laboratory at the Max Planck Institute is currently housed in a small room that is almost entirely filled by a large gray styrofoam box. Next to it are a chair and a table on which three computer monitors sit. Grill's colleague Christoph Ehrlich opens the lid of the styrofoam box. Inside is a perforated plate the size of a tabletop. Mounted on the plate are lenses, mirrors and thin tubes that end in fist-sized chunks of stainless steel. "When the system is running and we're taking measurements, there can be no movements," says Ehrlich. "After all, we're dealing with a high level of precision to within a millionth of a millimeter."

What occupies a large box and a whole laboratory room is known by experts simply as optical tweezers. The sole purpose of the entire apparatus is to capture a protein, pull on it as if it were a spring, and measure its resistance. It's difficult to imagine how laser light can capture molecules, but the method, developed by US physicist Arthur Ashkin in the 1980s, is well established.

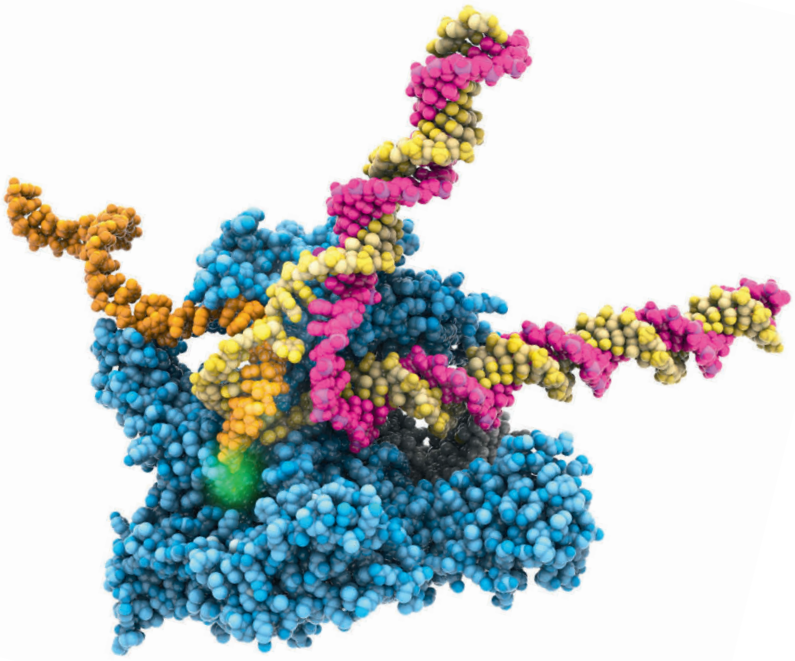
BILLIARDS WITH PHOTONS

The dual nature of light as a wave and a particle has been known for a century. Light particles, called photons, possess momentum that can be imparted to an object. This happens, for example, when a photon flies through a tiny transparent bead and is deflected from its path by light refraction. The photon propels itself away, pushing against the bead. If the light is strong enough, the bead can actually be nudged to the side.

But Arthur Ashkin wanted to achieve the opposite effect, namely to capture the beads with laser light. To do so, he used a trick based on a finely focused laser beam. Like billiard balls, the photons impinge on the bead from all directions and hold it in place. It's possible to capture and balance a ping pong bead on the stream of air from a hair dryer. Ashkin achieved essentially the same thing with molecules and light.

Many research groups have since refined this prototype of optical tweezers. In recent years, researchers have used optical tweezers to study various biomolecules, including myosin and kinesin. Myosin, a motor protein that is present in all our muscle cells, moves in 14-nanometer increments. As it does so, it causes muscle fibers to contract. Kinesin moves through the cell in 8-nanometer steps as it transports cell components. "We've perfected our optical tweezers to the extent that we can even measure the movements of RNA polymerase, which are ten times smaller," says Grill.

DNA is the blueprint according to which the cell produces proteins. For polymerase to read the genetic information contained in genes, it must first open up the DNA molecule, which consists of two intertwined strands, like a zipper. It travels, like the slide of a zip-



Left: While Veronika Fitz and Stephan Grill look at data on the screen, Marcus Jahnel (left) prepares the optical tweezers for a new experiment.

Right: RNA polymerase II reading a gene. The enzyme (blue) moves along the coiled DNA strands (yellow, pink), untwists them and transcribes the genetic information into an RNA molecule (orange). If the polymerase makes an error, the faulty RNA section is snipped off. The molecule then reverses and reads the gene segment again. The RNA molecule eventually serves as a template for protein synthesis.

per, from one DNA building block to the next, always in 0.34-nanometer steps. These building blocks, called dinucleotides, represent the letters of the genetic alphabet.

As it reads the DNA molecule, polymerase strings together the RNA counterparts of the dinucleotides, thus creating an RNA copy of the DNA strand. "We want to know the strength of the forces involved as polymerase moves along the strand of DNA, and how quickly the enzyme progresses," says Grill. "This is extremely difficult in view of the short increments involved."

The trick Grill and his colleagues use to measure the forces is impressive. Because the RNA-polymerase complex can't be captured easily in an optical trap, Grill uses a kind of support structure: two small plastic beads measuring just a few micrometers across. He attaches a polymerase molecule to one of the plastic beads, and a DNA strand to the other. This is where the optical tweezers – or more precisely, two optical tweezers – come into play. Grill and his colleagues use one to hold the bead with the attached polymerase-DNA construct, and the other to hold the second bead. They then carefully move the two beads closer together until the DNA from the first bead binds to the second bead, thus forming a tether between the two beads. The pair of beads connected by the long DNA strand resembles a tiny dumbbell.

At first, nothing happens, because the polymerase requires energy to travel along the DNA. Only when the researchers add energy-rich RNA nucleotides as fuel do things get moving. The polymerase moves in increments from one nucleotide to the next. But because the two beads are held firmly in the traps, a tensile force is created between the beads, like a rope on which two opponents are pulling in a tug-of-war match.

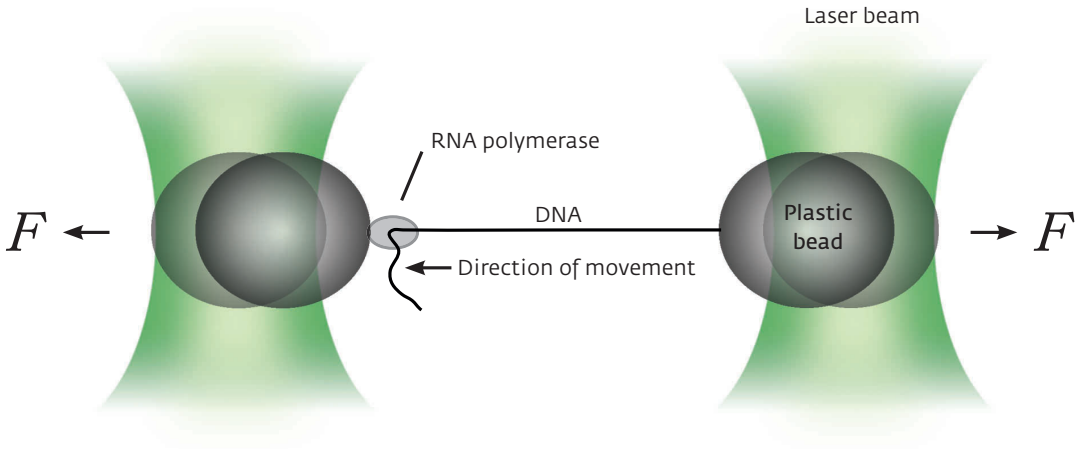
The polymerase must therefore exert a force to move from nucleotide to nucleotide. After each step, it remains stationary for a few seconds, during which the force on the other bead remains constant. When the polymerase takes the next step, it has to apply even more force against the growing resistance. While the polymerase progresses and the tension on the DNA increases, the beads are gradually pulled out of the focus of the laser beams by just a few nanometers. High-resolution light detectors sense this deviation. A computer program then converts the deviation of the beads away from the center of the traps into the force that the polymerase generates.

What's more, the researchers are also able to move the two beads back and forth, pulling on the DNA like a miniscule spring. The spring constant of DNA is infinitesimally small: stretching a DNA molecule by one micron requires a spring force of just one tenth of a piconewton. And because the polymerase is bound to and moves along the DNA, it has to act against these forces.

BEADS IN THE TRAP

Although the laser technique is extremely complex, and the mirror laser beams must be precisely adjusted, the trap is amazingly simple to operate. Grill's doctoral student Veronika Fitz holds a joystick while gazing at a computer screen. Somewhere in the depths of the styrofoam box, the beads move through a small container, and their magnified images appear as tiny dots on the screen. The light beams of the two traps appear on the screen as crosshairs. >

Photo: SPL-Agentur Focus



The optical tweezers work like a tug-of-war: When one person pulls on the end of a rope, the other person pulls the other way. The opponents in this case are two small beads to which a polymerase molecule (left bead) or a DNA molecule (right bead) are attached. Two laser beams (green) hold the beads in position. The polymerase travels along the DNA, pulling the bead to which it is attached from its original position. Sensors measure this movement and calculate the forces involved.

Fitz skillfully places the crosshairs on a bead as it floats past. The bead stops. Trapped! Fitz switches to the other crosshairs, captures a second bead, and moves it toward the first. It takes only a few seconds for the DNA to bind to the bead, thus linking the two beads. Fitz tugs a little. "Yes, they're firmly coupled now."

Despite the joystick and crosshairs, the experiments are tricky to perform. Often, the DNA thread snaps. Sometimes the polymerase simply refuses to move. Fitz manages only three to four successful runs a day. "We're dealing with biology, which is sometimes unpredictable," she says. Grill is proud that they are able to perform measurements on living objects. "While other techniques exist for measuring molecular forces, they are completely unsuitable for this type of biological experiment."

With the help of the optical tweezers, the researchers can get right down to business. Grill uses the tweezers to investigate the three different types of polymerase that occur in the cells of higher organisms: polymerases I, II and III. They differ from one another in how they read the DNA. "Polymerase I is quick and dirty," says Grill. "It transcribes only those building blocks that are required to make ribosomes," the cell's protein factories, he says. Quick and dirty means that polymerase I frequently makes reading errors and inserts the wrong nucleotides in the RNA. That's not so bad as far as the synthesis of ribosomes is concerned. If a ribosome doesn't work, a new one is synthesized.

CORRECTING ERRORS IN REVERSE GEAR

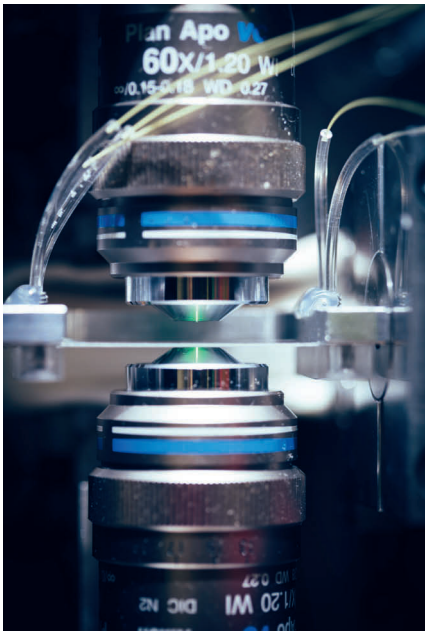
The situation is different with polymerase II. It reads those sections of DNA that contain information for synthesizing vital metabolic and structural proteins, so that transcription errors must be few and far between. Polymerase II therefore works much more slowly.

If the wrong nucleotide is nevertheless inserted into the RNA chain, the transcription process halts. The polymerase travels backward and cuts off the faulty piece of RNA. "We can also detect this reset action by the forces involved, and we can even measure the time required. The polymerase takes around ten seconds to carry out the repair," says Grill. Such data is still unique.

Grill is seeking to improve our knowledge about how DNA is read and to shed light on the phenomenon of gene regulation. Because genes can be switched on or off, the synthesis of many proteins can be upregulated or downregulated as required. In cancer cells, for example, which grow rapidly and divide in record time, gene regulation has gone haywire.

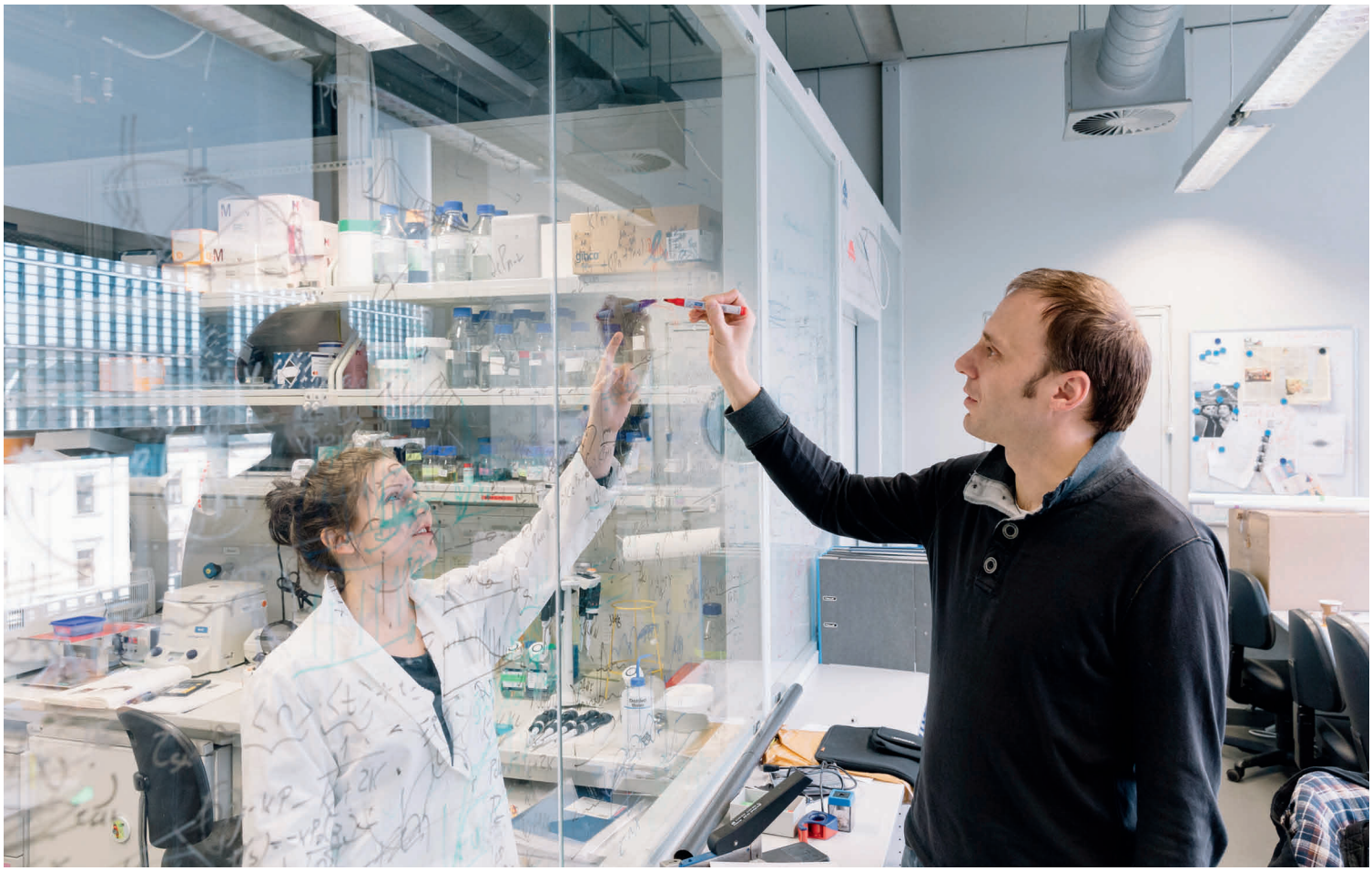
The activity of polymerases is key to such processes. It is known that some genes are switched on very rapidly, such as genes that code for heat shock proteins, which protect the body against excessive heat.

Grill and other researchers suspect that polymerase remains permanently attached to some of these rapidly acti-



Right: A microscope lens (top) focuses the laser beams on a point. A condenser recaptures the scattered light (bottom). Between them is the flow cell in which the experiment takes place. The researchers load the optical tweezers through the tubes.

Graphic: designergold, based on original material from the MPI for Molecular Cell Biology and Genetics; photo: Sven Doering



It is relatively easy to operate the optical tweezers, but the underlying physics is complicated. Veronika Fitz and Stephan Grill therefore have to perform a whole series of calculations before every experiment.

vated genes. "Presumably, the polymerase is parked right in front of the gene segment in question and is just waiting for a starting signal," says Grill. Such signals may be small proteins, known as transcription factors, such as TFIIS, that liberate polymerase II from its parking bay. Grill, in collaboration with Patrick Cramer from the Max Planck Institute for Biophysical Chemistry in Göttingen, is currently using optical tweezers to investigate exactly how this works. Initial results show that this liberation process plays an important role, especially after long pauses.

The experiments also show that polymerase is even able to unravel DNA from the structures that DNA is normally wound around. A DNA strand is wound on a framework of proteins known as histones – like yarn on a spindle. Evidently, as it progresses along a strand of DNA, polymerase pushes the histones ahead of it so that the DNA slowly unwinds. In the next few months, Veronika Fitz's measurements are expected to show how hard polymerase has to work to achieve this. ◂

TO THE POINT

- **Optical tweezers enable researchers to measure the tiny forces that act between biomolecules. A focused laser beam holds the proteins in place.**
- **As it reads the DNA strand, RNA polymerase moves in 0.34-nanometer steps from one DNA building block to the next, creating an RNA copy of the DNA as it does so. If it inserts an incorrect RNA building block, it backs up and repairs the faulty section.**
- **Genes that can be activated very rapidly probably have their own dedicated RNA-polymerase molecule. Once activated by a transcription factor, it can then begin its work immediately.**

GLOSSARY

RNA polymerase: An enzyme that transcribes DNA into an mRNA copy. Polymerase docks to a specific sequence of the DNA strand, known as the promoter. Once a signal has been received, the polymerase begins to read the DNA. The transcription process is halted when the polymerase reaches a stop sequence. Besides bacterial RNA, three different forms occur in nucleated cells. In addition to polymerase itself, many other proteins are involved in the transcription process. Scientists therefore often speak of an RNA-polymerase complex.

Transcription: Transcription is the process by which DNA is read. During transcription, an RNA copy, called messenger RNA, is constructed from a DNA template. Instead of the four letters of the DNA alphabet – A, C, G and T – it contains the letters A, C, G and U. The messenger RNA leaves the cell nucleus and then serves as a blueprint for the synthesis of proteins.

Transcription factors are proteins that switch genes on and off. They bind either directly to one of the proteins of the RNA-polymerase complex or to specific sequences on the DNA molecule (promoters, enhancers or silencers). Transcription factors are controlled by various factors. For example, they can be activated or inhibited by hormones and enzymes.

Photos: Sven Doering