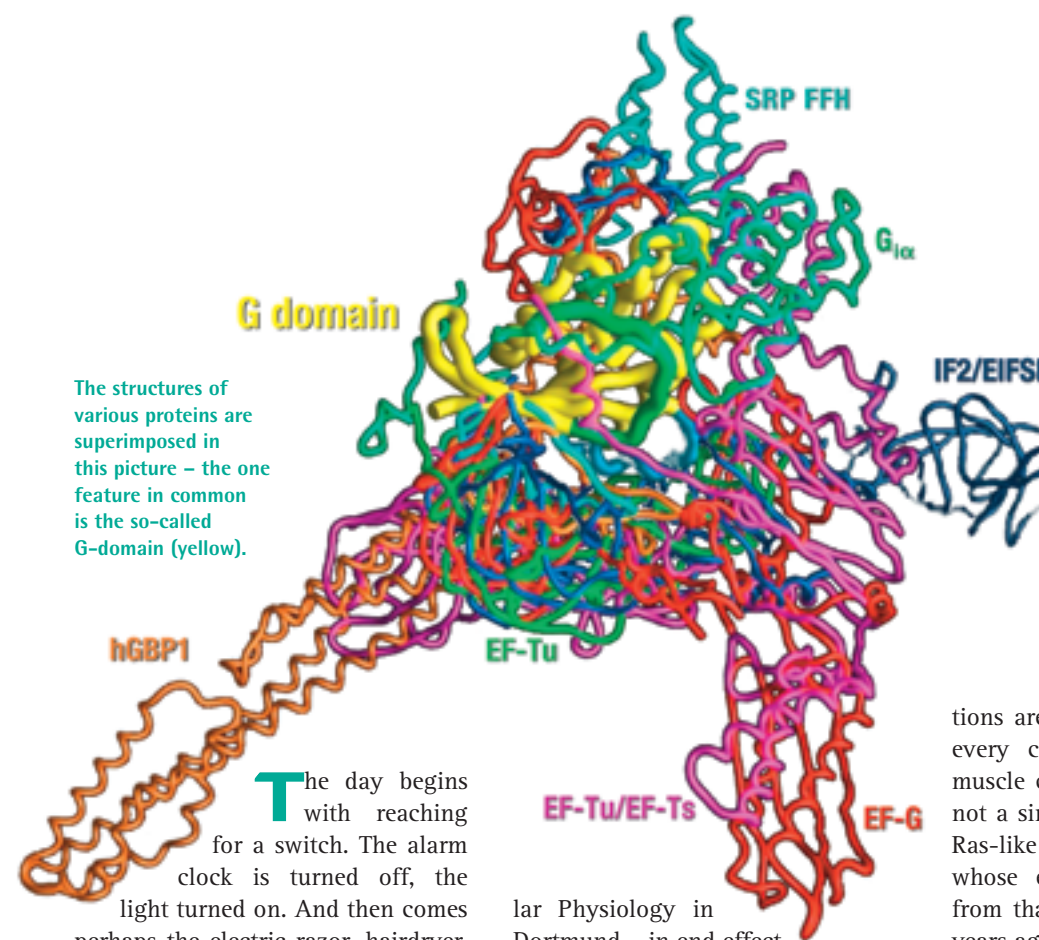
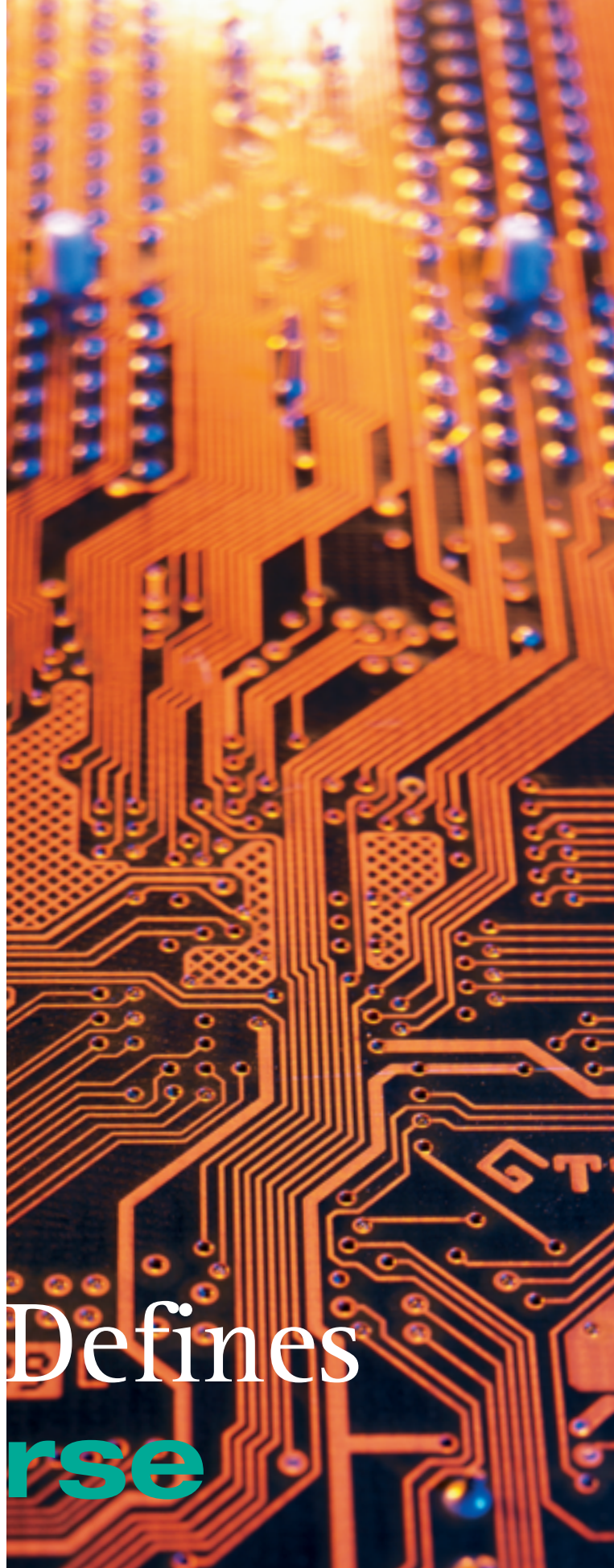


There's a lot to control in cells. Processes must constantly be started or stopped at the right time. These vital life functions are taken on by protein switches. One such switch, called "Ras", is the focus of a group led by

PROF. ALFRED WITTINGHOFFER,
Director at the **MAX PLANCK INSTITUTE FOR MOLECULAR PHYSIOLOGY** *in Dortmund.*

Interest surrounding this protein extends beyond basic research – given that the Ras switch plays a vital role in causing cancer.

A Switch Defines the Course



The structures of various proteins are superimposed in this picture – the one feature in common is the so-called G-domain (yellow).

The day begins with reaching for a switch. The alarm clock is turned off, the light turned on. And then comes perhaps the electric razor, hairdryer, radio, coffee machine or toaster. By the time we've left the house in the morning we've already pressed a button, turned a lever or touched a sensor key two or three dozen times. By evening it might be more than a hundred clicks of a switch, mostly without really being aware of it.

But this omnipresence of switches is nothing compared to the constant on and off taking place simultaneously in the body. Living cells are crammed full of cascades of biological levers, sensors and buttons that continually switch on – and when necessary switch off – very varied metabolic processes, cell growth and movement. And just like in everyday life, the purpose of these switches is to start processes at the correct time – and to switch them off again when they are no longer needed. "This saves on resources and allows the cell to move back and forth between alternative metabolic demands," says Prof. Alfred Wittinghofer, Director at the Max Planck Institute for Molecu-

lar Physiology in Dortmund – in end effect to react to the changing demands made by the environment. Wittinghofer knows what he's talking about. Within sight of the Westphalian stadium, the 60-year-old Borussia Dortmund soccer season-ticket holder leads the department for structural biology, where currently a group of 16 are working towards a better understanding of roughly a dozen different cell switches.

Solely the switch type that Wittinghofer's lab has been concentrating on has thus far been identified by researchers in more than 100 different variations. When nature reuses a blueprint so often, this is bound to be a fundamental success story. "Biological switches belong to the oldest of life's inventions," says Wittinghofer. His group is interested in the different variants of one switch that resembles a protein discovered in the eighties: "Ras", as this protein is called for short nowadays, is a member of a switching molecule family of which several dozen varia-

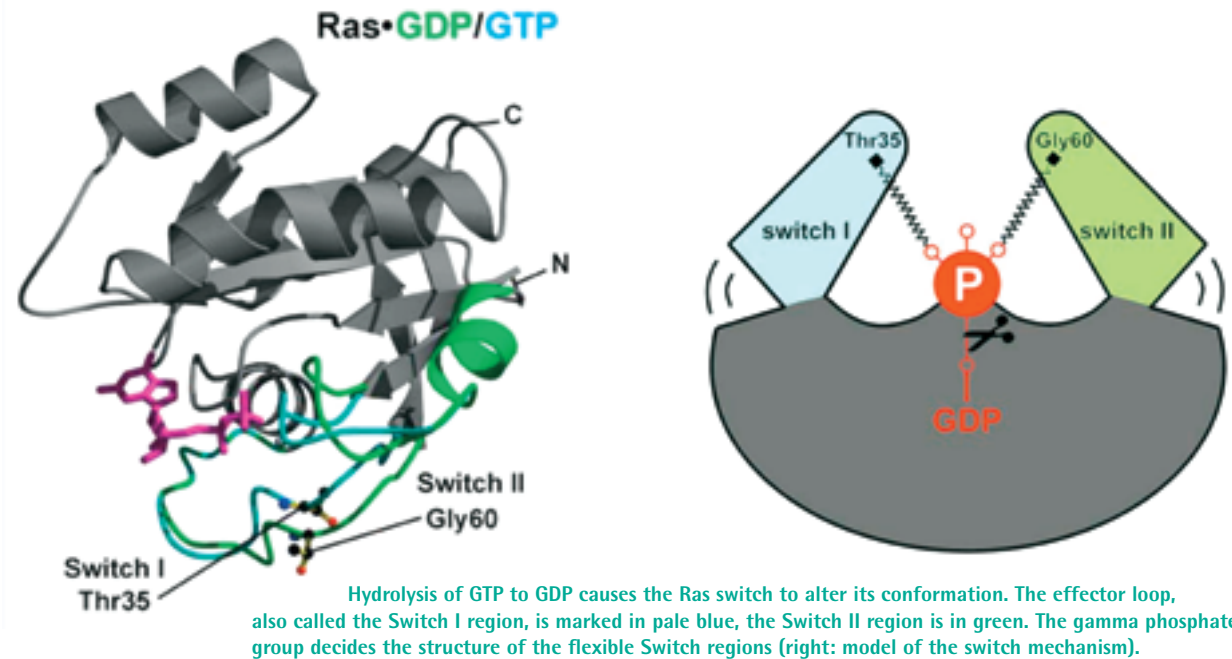
tions are active at the same time in every cell. Skin cells, nerve cell, muscle cells, immune cells – there's not a single cell which does not use Ras-like switches. Even bacteria, whose evolutionary path diverged from that of humans several billion years ago, use related proteins.

THE STRUCTURE DETERMINES THE SWITCH FUNCTION

Among other things, Ras-switches regulate growth and cell movement. And if specific switches have been seriously damaged, they can also lend a hand by diseases such as cancer. "Ras is an example of how basic research can lead to important applications," says Wittinghofer. No wonder, then, that researchers want to know exactly how a switch functions within the overall cell infrastructure.

Alfred Wittinghofer belongs to a worldwide research community who, for more than a decade, have been working on the questions of how Ras family members switch metabolic processes on and off in the cell, in addition to who makes use of these switches. In the last ten years the scientists have come very close to answering these questions. "We now know that the shape of the switch molecule plays a vital role in its

PHOTO: OKAFIA, MUNICH / GRAPHICS: MPI FOR MOLECULAR PHYSIOLOGY, ALFRED WITTINGHOFFER



function,” says Wittinghofer. This is his special area: to measure the outer and inner structure of such cell switches in three dimensions, atom for atom. Like all proteins, Ras is made up of chain-like, sequentially linked building blocks, the so-called amino acids. Twenty different amino acids, each distinguished by the chemical properties of their side-chains, serve in nature to make up proteins. The exact order of the amino acids in the protein is encoded in the corresponding gene. The gene contains code words, for example, for amino acids with “large” or “small”, or positively or negatively charged side-chains. Protein amino acids can be positioned far from each other along the chain; depending however on their chemical characteristics certain protein amino acids that fit together can combine in pairs or groups. In this way the amino acid chain folds up, for the most part independently, into the intact protein – with a three-dimensional structure that determines its function.

Generally, however, the rules by which proteins fold up inside a cell are not yet well enough understood to be able to predict the three-dimensional protein shape solely from its amino acid sequence. Scientists depend much more on generating kinds of protein “pictures” for working out the inner structure and outer shape. With a protein molecule only a few billionth part of a millimeter, however, this can only be achieved in an indirect way: Proteins are too small to be observed directly under a microscope.

X-RAYS ILLUMINATE MOLECULAR ARCHITECTURE

Nevertheless, there is an alternative method. As a prerequisite for the structural determination, the researchers must produce a regularly formed crystal of the protein. “Often this is the most tedious part of our work – the search for appropriate conditions under which our relevant protein forms suitable crystals,” says Wittinghofer. Once this has been achieved these crystals, less than a

millimeter in dimension, are then illuminated using X-rays. The atoms of the protein molecule bend or diffract the beam and create a pattern of spots that are electronically recorded by a detector. Using such diffraction patterns and computer analysis, the researchers can then decipher the arrangement of particular atoms in the protein interior. Using a combination of complex mathematics and simple trial and error they devise models of how the amino acid chain might be folded. Over the last decade, the Dortmund group has used these methods to solve the structure of around ten proteins under various conditions and in combinations with other proteins.

By similar analyses the scientists have also found out what happens inside a Ras molecule when it flips between on and off. Since the chains only fold up loosely into a bundle, proteins always have a certain inner flexibility, meaning that a loop in the chain is sometimes able to move with relative freedom. In fact, Ras has two regions in the protein chain

with this ability; in other words to synchronously change their position. This shape change signifies the two switch positions: one protein conformation denotes on, the other off. The driving power for flipping the switch is chemical energy that Ras draws from the cleavage of a “fuel” molecule: The protein has a pocket in which a molecule called “GTP” (guanosine triphosphate) fits exactly. As soon as Ras has picked up a GTP molecule, it becomes fixed in a state where the flexible regions resemble a loaded spring. They remain in this position until the fuel molecule is used up. This happens when the GTP molecule is cleaved into two parts. The larger of the two cleaved parts, known as GDP (*guanosine di-phosphate*) remains bound to Ras. With this cleavage, the two loops fall back into their off position again – the spring is unwound. Only when GDP is replaced by a new GTP molecule does Ras flip back to its on state. “This cycle does not run randomly, but is regulated by a system of pro-

tein partners,” explains Wittinghofer. There is an additional protein that helps Ras expel the remaining GDP cleavage product out of its pocket so that a molecule of the cellular abundant GTP can occupy the free site. Ultimately, it is this partner protein that switches Ras on, and there is a second partner that considerably accelerates the cleavage of docked GTP – switching Ras off again. “Normally, Ras remains activated for only a few minutes,” says Wittinghofer.

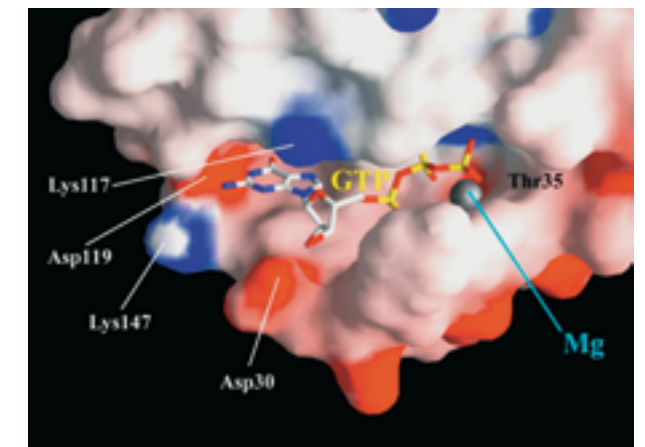
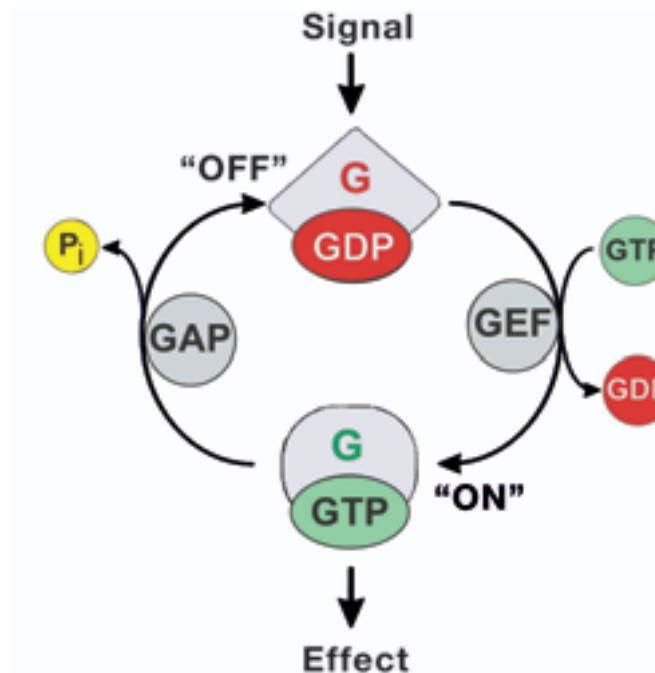
THE EFFECT OF HORMONE SIGNALS

This protein trio, in turn, is merely a part of a much larger protein machine. How this functions is best investigated through hormone signal transmission – for example, after a skin injury, when new cells must be generated by cell division for wound repair. Directly following the injury, cells surrounding the wound start to release growth hormone. This hormone docks with the matching receptors – reception antennae located

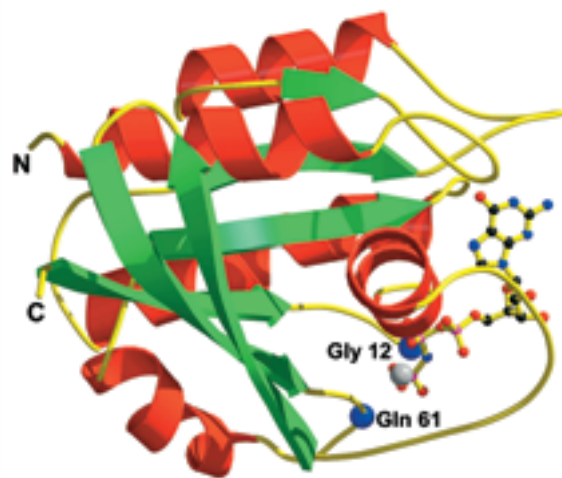
on the surface of neighboring cells. This docking starts a flow of protein-protein interactions. Initially, hormone binding causes the two halves of the receptor to join into one functional unit.

The receptor is embedded in the thin envelope (plasma membrane) of the cell in such a way that one of its ends pokes inside the cell. As soon as the receptor halves are more or less joined together by the hormone, the ends inside the cell reciprocally change each other through a chemical reaction. The hormone therefore triggers the first chemical reaction inside the cell – signifying the arrival of the signal in the cell.

In turn, it is these receptor molecule ends that contact and activate Ras and its partner molecules that are waiting on the cell membrane’s inner side. Through this signal cascade Ras converts to the on state within a few seconds following hormonal contact. Now the Ras protein has become the signal distributor, since further proteins are gathered



GTP binds on the surface of Ras (right). The switch is “on” when GTP is bound, and “off” when GDP is bound. Replacement of bound GDT is carried out by exchange factors (Guanine Nucleotide Exchange Factors, GEFs), which accelerate the dissociation rate of the nucleotide by several orders of magnitude. The hydrolysis of bound GTP can similarly be accelerated by GTPase-activated proteins, or GAPs.



Molecular details of the Ras-GTP structure: In the activated, GTP-bound state, Ras interacts with various target proteins that represent the next members in the Ras signal transduction pathway. Ras-GTP is switched off by various Ras-GAPs. The best known are p120GAP and Neurofibromin, a gene product of a tumor suppressor gene.

around Ras on the inner side of the cell envelope, immediately sensing its change in shape. This contact then leads to enzyme activation that amplifies the signal, passing it on in a branched reaction network: “Ras is located at the beginning a domino effect that ends with the cell duplicating its genetic material and initiating cell division,” describes Wittinghofer. “It is quite possible that only a few activated Ras molecules are sufficient to alter the behavior of the entire cell.”

RELATIVES HELP WITH THE TRANSPORT

However, cell division is by no means the only process in whose regulation such Ras-like switches are involved. Ras belongs to a modular construction system with hundreds of components. There is not only a long list of various switch proteins, but also an equally specific spectrum of different on and off factors. And so are diverse Ras-like switches able to be involved in various processes. For example, molecule relatives help to shift “loads” into, or out of the cell

nucleus through special pores. Other Ras relatives control movements within the cell, as well as cellular component transport. Small bubbles are continually pinched off from the cell membrane – so-called vesicles – that are passed on within the cell, reloaded and then transported back again to finally fuse with the cell membrane. The cell uses this cycle to incorporate particles from the outside or to throw out surplus ballast.

Since Ras-like switches control this transport, a range of pathogens have learned in the course of evolution how to target such regulation. Several bacteria, for example, possess poisons that switch off the transport switch of defense cells, preventing the pathogens from being “eaten”. Other bacteria, including the diarrhea-causing *Salmonella typhi*, inject targeted proteins into cells of

the intestinal wall that keep the Ras-like switches there active and promote bacteria uptake into the intestinal cells. In fact, a normal cell is stuffed so full of different switches that the scientists seriously question how the cell can decide at all which switch to use to control which metabolic process. According to what the scientists know so far, the switch proteins are not randomly mixed up in the cell but are pre-assembled into units and switch circuits – like components in a complex machine. “This ensures that signals are rapidly passed on and that they reach the correct address,” says Wittinghofer.

Meanwhile, interest in Ras and its relatives reaches way beyond basic

research. In the search for new drugs against cancer a group of pharmaceutical companies are interested in Ras. Ras was first discovered in the eighties as a virus protein that can cause cancer in rats. Later, it became apparent that an almost identical Ras gene was present in every healthy cell. But only almost identical: the virus variant of the protein is altered in specific positions.”

These mutations result in Ras losing its ability to cleave GTP,” explains Wittinghofer. The result: in virus infected cells a switch for cell division is constantly in the on position, like the replay button of a CD player. Medics have discovered that these mutations can also arise spontaneously in normal cells in the body, initiating a development that turns a normal cell into a cancer cell. Altogether, almost every third cancer patient possesses a defect in the Ras switch in their tumor cells. In particular, mutations are repeatedly found in intestinal, breast and bladder carcinomas. Numerous research groups have therefore been searching for some years for ways to switch off the function of the altered Ras protein in such cancer cells.

There are various options for such anti-Ras drugs. The most advanced are investigations with compounds that prevent Ras from finding its predetermined place at the cell membrane. The protein is anchored in the cell membrane by a fatty-like tail. Currently, a number of pharmaceutical companies are trying out inhibitors that might prevent the Ras enzyme from attaching to just such a fatty tail. “These so-called farnesyl-transferase inhibitors have functioned well in the test tube and in animal experiments,” says Wittinghofer. In clinical trials with humans, however, results so far have been

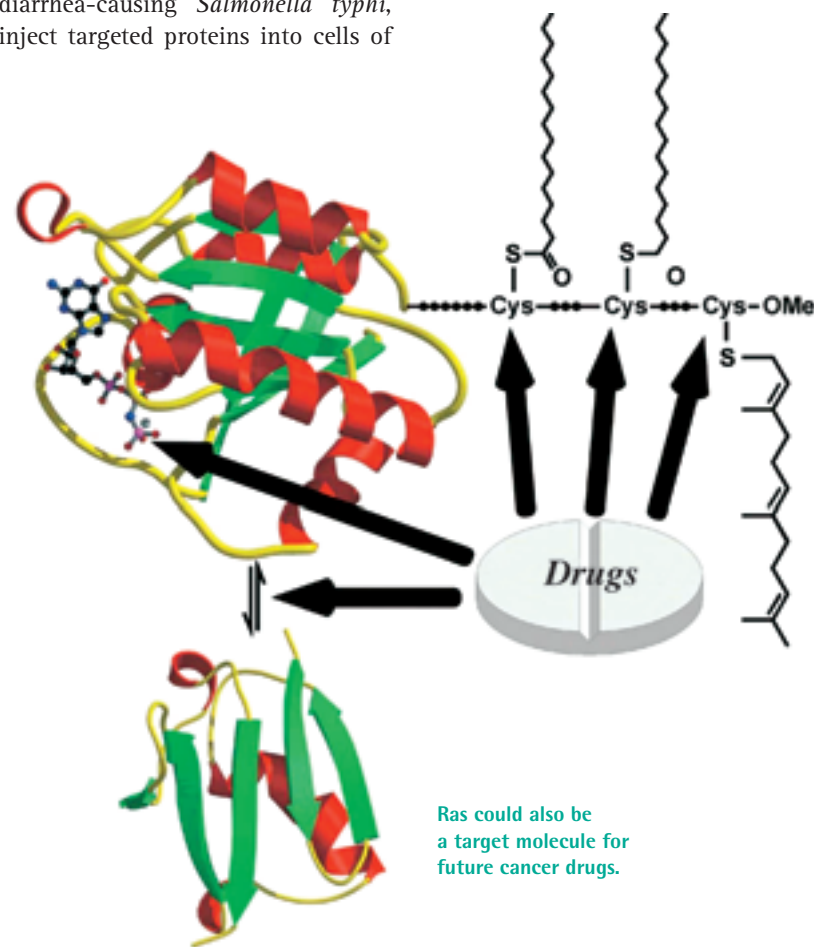
contradictory. Apparently, these substances have indeed led to tumor regression in some patients, but with others they have failed completely. “I’m rather skeptical of this approach,” says Alfred Wittinghofer. All the same, his department has also started the search for possible cancer drugs: “We hope that with relatively non-toxic substances it will be possible to persuade the mutated Ras to cleave GTP after all.”

RAS AS TARGET MOLECULE FOR NEW DRUGS

At this time the Dortmund team is working together with the Hamburg Biotech company Evotec. In the first phase of the cooperation candidates have to first be identified that can bind to Ras. Among the 90,000 or so substances tested so far, the group has discovered several interesting compounds that are currently under investigation. Wittinghofer’s group is following up a second idea together with Prof. Herbert Waldmann, who leads the Department for Chemical Biology at the Dortmund Max Planck Institute. Both groups are currently trying to find small protein fragments – so-called peptides – which work like enzymes that accelerate GTP cleavage, and can similarly inactivate Ras. The search is prolonged and will not necessarily lead to any rapid solutions.

For the time being, basic research will remain the focus in Dortmund, says Wittinghofer. Nevertheless, Ras would not be the first example of apparently “clinically remote” work years later becoming the starting point for a successful medical or technological application. “That is, after all, precisely the principle behind basic research,” says Wittinghofer: “One can never predict where it will lead.”

KLAUS KOCH



Ras could also be a target molecule for future cancer drugs.