Histones are important proteins for epigenetics. Consequently, delegates at the Epigenetics Conference are greeted with the amino acid code of the histone protein H3.

Photo: Alice Jessica Hath

Operating the **Genome Switches**

Research into epigenetics is a rapidly growing field. A recent conference at the **Max Planck Institute of Immunobiology** in Freiburg shed light on the reasons. The new science investigates permanent biochemical switches that control genetic activity. These switches give a cell its identity and memory. They are what enable organisms to develop and adapt to their environment.

TEXT **PETER SPORK**

The different types presented by Shelley Berger display an amazing variety. "One is a typical command receiver," explains the cell biologist, "while the next one tells the first what to do." And the third is not only the boss – on top of that, it lives a lot longer than the others and is the only one that is fertile. Although it is not too surprising that such differences occur, what is astonishing is that the three types have identical genomes. Yet the lively speaker from the University of Pennsylvania, USA, is not presenting a study on triplets or cloned breeding animals. She is describing natural processes exhibited by the Florida carpenter ant *Camponotus floridanus*. The behavior and the physical form of the insects in the three boxes display marked differences. And these are not even remotely genetically fixed.

But what else could have such a strong modifying effect on the mind and body? There are structures in the genome that, while having no effect on the genes themselves, still determine which of them can use a somatic cell and which can't. These structures define the way a cell is built and how it functions by means of a gene activation program. Berger shows what this does to the ants. She states that, in the genome of brain cells, there are systematic, non-genetic differences that ensure that the subordinate female worker ants are more sensitive than their dominant sisters to their inhibitory messenger substances.

A SENSE OF EXCITEMENT IN THE FIELD OF EPIGENETICS

Epigenetics as a research field focuses on the ingenious mechanisms that underlie gene regulation. There is scarcely a scientific discipline that is developing as rapidly as this new branch of genetics. And with good reason: epigenetic structures affect practically every life form – they are the memory and

identity marker of every cell. Their influence penetrates into the very essence of life, even in us humans.

EVEN ACQUIRED FEATURES CAN BE INHERITED

Just five years ago, quite a number of biologists had no idea what epigenetics meant. Today, even doctors, educationalists, psychologists and politicians are familiar with the term. The Greek prefix 'epi-' means 'subsidiary', 'additional' or 'over'. In fact, epigenetics is a sort of "additional genetics," associated by definition with everything that a cell passes on to its daughter cells by way of residual information over and above the DNA base code. Once a so-called epigenetic switch has been set in response to an external stimulus – such as a developmental signal – it determines, for example, whether a cell will form part of nerve, skin or liver tissue.

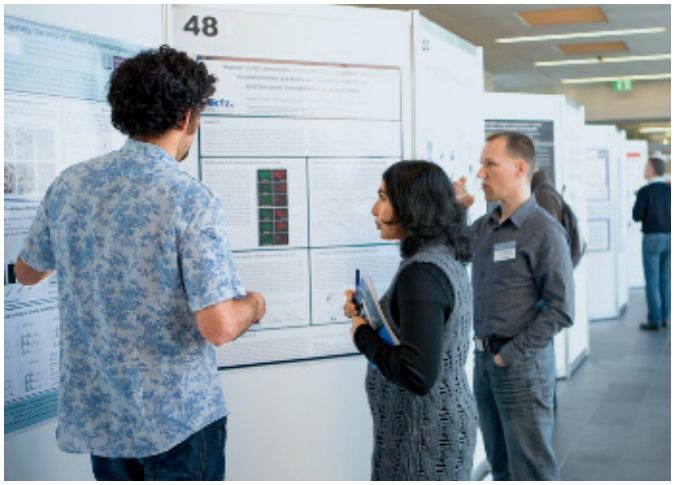
The sum of all of the switches forms the epigenome of the cell, and each cell has its own specific epigenome, which it passes on when the cell divides. However, cells also respond to their environment by modifying their epigenome. They can, as it were, recall former states induced by external stimuli and store them. For example, an early childhood trauma in humans can trigger the permanent reprogramming of brain cells and make an individual prone to depression decades later. Or over-nutrition in the womb can alter metabolic cells to the extent that an individual will be more likely than other people to develop type 2 diabetes in old age.

Shelley Berger does not have to explain these things to her listeners. They are experts in the field. Early in December 2010, she spoke to an audience of more than 100 from around the world at the first Max Planck Freiburg Epigenetics Meeting. In three days alone, 40 lectures were given at the Max Planck Institute of Immunobiology and Epigenetics. A significant number of them were presented by world-renowned specialists such as Phil Avner, Geneviève Almouzni, Amanda Fisher, Edith Heard, Barbara Meyer, Steve Henikoff, Gunter Reuter, Wolf Reik, Yang Shi, Brad Bernstein, Martin Vingron, Danny Reinberg, Roland Schüle, Susan Gasser and Meinrad Busslinger.

Each of the lectures was subsequently discussed in unusual depth by the experts, who generally focused on biochemical details. But the burning issues surrounding epigenetics really came to a head in conversations held away from the formal setting. Researchers pondered over lunch whether the tofu in the vegetarian dishes provided protection against cancer via an epigenetic mechanism. And they wondered over dinner whether a substance called resveratrol in red wine could have a life-prolonging effect given that it can, at least in a test tube, epigenetically halt cell aging.

EPIGENETICS INFLUENCES MANY ILLNESSES

This difference – hard-core molecular biology on the one hand and possible solutions to humanity's great issues on the other – illustrates the fascination that this new discipline inspires. The science is unbelievably complex, but it is also well on its way to making life both easier and better for humans. "There is a direct link between the body's metabolic processes and the



Science thrives on an exchange of ideas, especially in such a rapidly growing research field as epigenetics. Delegates at the congress in Freiburg discuss the latest findings – sometimes even over a glass of red wine.

epigenetics of metabolic cells," claims Paolo Sassone-Corsi from the University of California in Irvine, USA. He is investigating the inner clock, a vivid example of this link.

There is a clock ticking in every cell, he says, and it has the genome firmly in its grip. "The activity of at least 15 percent of the genes in a cell oscillates in a 24-hour rhythm." He and his team have just discovered an epigenetically active enzyme associated with this clock. It translates the time sense of the cells into a gene activation program. Called MLL1, it binds to proteins that act rhythmically and determines the time of day at which some genes can be read and others can't. It is now known, says Sassone-Corsi, that when the inner clock is disrupted – which can occur, for example, when a person does regular shift work – conditions are more favorable for diabetes and many other metabolic disorders.

Tests show that genetically identical mice fed with the same food either become ill or remain healthy depending on whether they get the food at the right time or not. There is much to indicate that such disruption alters the "epigenetic memory" of the cells and thus upsets the equilibrium of the whole body, rendering the individual susceptible to illness. "Epigenetics touches many different fields. It starts with nutrition and ends with trauma," believes Herbert Jäckle from the MPI for Biophysical Chemistry in Göttingen.

NEW TREATMENTS FOR STRESS DISORDER

The Ministry of Defense is now interested in the epigenetic explanation for post-traumatic stress disorder as well. "Increasing numbers of soldiers are returning from foreign deployments with this disorder." There is felt to be an urgent need for new treatments, and epigenetics might be able to deliver them. The time is now ripe, he says, not least because of the recent findings, to take a proactive approach to advance the discipline. That is why the Max Planck Society supports the Freiburg Meeting, and also why it resolved to expand the work of the city's Max Planck Institute for Immunobiology and change its name to refer to both epigenetics and immunobiology.

The new name was announced the day before the conference, an occasion that could not have been better and one that had been long awaited by co-organizer Thomas Jenuwein. Back in 2008, when the epigenetics pioneer was appointed Director at the Freiburg Max Planck Institute, he said, "We are standing on the threshold of a new way of thinking in biology, on the threshold of the post-genome society." The glorious age of genetics lasted five decades, he pointed out: "It began in 1953 with the announcement of the DNA structure and ended in 2003 with the publication of the almost complete human DNA sequence." In saying this, Jenuwein in no way wishes to diminish the extraordinary achievements of the geneticists. Rather, he is keen to build on their findings to drive biology forward and reveal what is passed on over and above the genes.

There are different epigenetic switch systems. For example, if methyl groups are bound directly to DNA (DNA methylation), that generally inactivates the affected gene. Changes to the histones are a great deal more variable. Histones are proteins around

Photos: Alice Jessica Hath (3)

which the DNA coils – rather like a cable reel. How firmly the DNA binds and which enzymes still have access to the gene being read depends on the biochemical structure of the histones. As the cell fixes various different chemical attachments – acetyl, methyl, ubiquitin or phosphate groups – to various parts of the histones or detaches them again, one of the decisions it makes is whether the base sequence of individual genes is transcribed into proteins or not. Proteins with regulatory roles also dock onto the "cable reels" (known as nucleosomes), depending on their nature. And the whole DNA and protein mixture, called chromatin, can dock onto the membrane of the cell nucleus – provided a particular histone modification has taken place – and this also affects whether or not individual genes can be activated.

CHANGES TO HISTONES REGULATE GENE ACTIVITY

"More than 50 histone modifications are now known," says Robert Schneider, though "we can hardly be anywhere near the full total yet." Schneider, who became head of an epigenetics group at the Freiburg Max Planck Institute as early as 2004, introduced conference delegates to a previously unknown chemical modification in the nucleosome center. Until now, it was especially the so-called termini of the histones, which project out of the nucleosome like tails, that had been regarded as the main access point for modifications. Apparently the experts need to think again: "The histone modification that we discovered probably opens a window that gives enzymes access to a DNA binding site," explains Schneider. Discoveries such as these might even help fight diseases that are very difficult to treat, stresses the epigeneticist.

It has long been clear that cancer and many other illnesses are linked to wrongly regulated epigenomes, he says, so every newly discovered epigenetic switch provides a potential point of attack for future medications. This is because, unlike gene mutations, wrongly configured epigenetic switches can, in theory, be returned to their previous configuration. Cancer researchers in

"We are more than the sum of our genes"

Thomas Jenuwein is regarded as a pioneer of epigenetics. In 2000, he and his team discovered the first enzyme that, in humans and mice, deposits methyl groups on histones, effectively switching off genes permanently. The molecular biologist has been a Director at the Max Planck Institute of Immunobiology and Epigenetics in Freiburg since 2008. His research field is having a profound impact on contemporary biology, as Jenuwein reveals in an interview. The staggering discoveries in epigenetics and their enormous implications are already transforming society today.

Mr. Jenuwein, you say that we are living in a post-genomic society. Why?
Thomas Jenuwein: Because we have decoded the human genome and are forced to acknowledge that we are more than the sum of our genes. The DNA sequence taken in isolation is not enough to provide a full explanation for either normal or aberrant development. We are now in the age of epigenetics and chromatin – the unit composed of DNA and the attached proteins. It's all about understanding cell identity from a functional perspective.

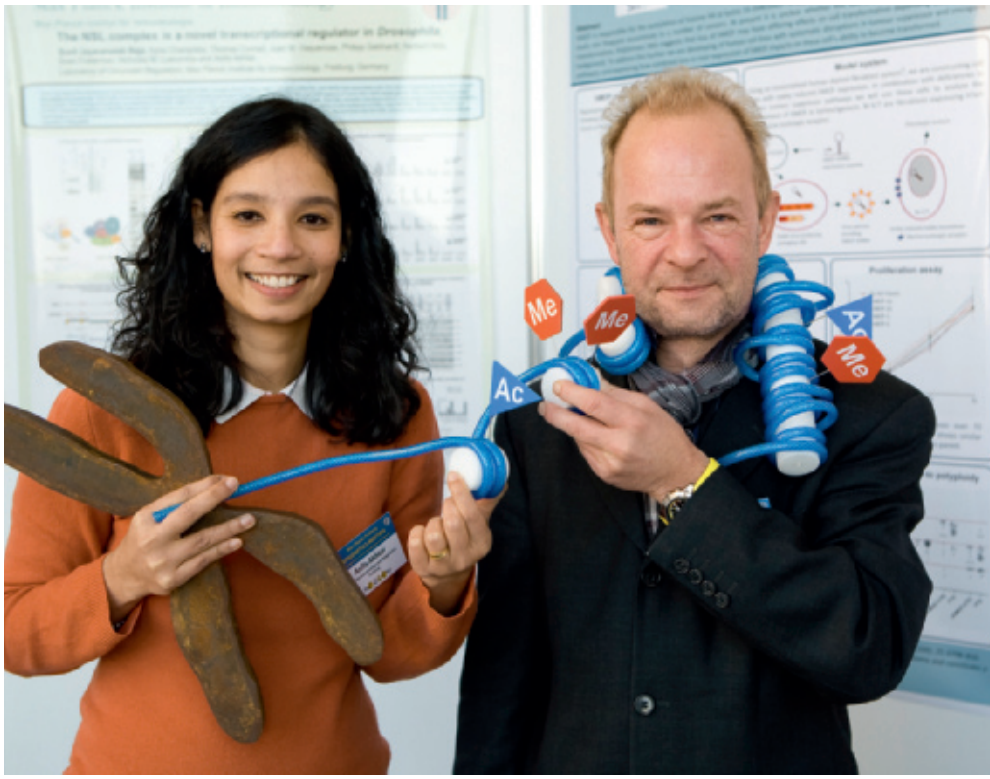
So your science is now acknowledged as such?
Absolutely. You need only count how many studies are published on epigenetics. Whereas there used to be no more than 400 per year, there are now around 8,000.

How did this development come about?
The key breakthrough was the discovery of the enzymes that chemically mark chromatin. That led to an explosion of new avenues of research. This was because the discovery created a pathway to controlling genetic activity, to cell identity and to different chromatin states. The main thing, however, was that we finally understood how changes in metabolism and energy consumption, as well as changes caused by environmental factors, could have a lasting effect on cells – it was because they could be transferred to chromatin via enzymes and their cofactors.

So epigenomes really do give cells a memory? Will we be able to manipulate this memory one day?
Our recognition of the way enzymes modify chromatin has opened up potential ways of doing this, because enzymes can be inhibited. And this takes us to the heart of treatment approaches. Take cancer, for

particular have high hopes for this new science – provided the epigeneticists can shed light on other fundamental principles. There is a new experimental model that may help: Herbert Jäckle is using it in a bid to systematize the world of histone modification. "The term histone code signifies nothing more than the fact that, in certain combinations, certain markings on the histones cause certain phenomena. This is the very thing that we want to test now."

In his sensational main lecture, Jäckle presented the latest findings on the fruit fly *Drosophila*. The genome is



The conference organizers: Asifa Akhtar holds a chromosome model, while Thomas Jenuwein wears a representation of chromatin with epigenetic modifications around his neck.

example. Clinics already have such substances as HDAC inhibitors and DNMT inhibitors. Treatment based on epigenetics is already a fact of life in cancer research.

Are there other examples?
Epigenetics also helps in reprogramming somatic cells to become stem cells. I am convinced that, in five years' time, we will have a success rate of 40 to 50 percent in this field – and it will be thanks to the specific influence of epigenetic enzymes. At present, the success rate is only 1 to 2 percent. The third point is the continued expansion of our understanding of how dietary habits and stress signals affect the epigenomes of cells. This opens up quite clear avenues toward a new kind of prevention.

This is somewhat reminiscent of the promises – as-yet unrealized – held out for the human genome project, that is, new and effective treatments for the major widespread diseases. Why do you think we are now closer to that goal?
Because we know the interrelationships. We know what makes the chromatin in a stem cell different from that in a mature cell. Looking at the epigenetic markings on chromatin, I can now see how old a cell is, what type of cell it is and whether it is healthy or not. So we have our fingers on the right switches. This ultimately means that any conceivable thing in this field is possible. In theory at least, diabetes cells can be made fully functional again, malignant cancer cells can at least be made more benign, and brain cells can be made less susceptible to stress.

Is DNA unimportant, then?
The driving force in the cell continues to be the DNA sequence, without question. But the crucial factor is that we can modify epigenetics. Adrian Bird once said that even if epigenetics affected only 0.1 percent of development, it would be absolutely sufficient – given the huge number of human cells and cell types – to reconstruct all sorts of adaptations, both good and bad.

The Max Planck Society has itself recognized the significance of the new science and changed the name of the Freiburg institute to incorporate both immunobiology and epigenetics. Are you proud of this?
Naturally. But it was also a logical development that began long before my time in Freiburg. Immunobiology and epigenetics are inextricably linked. The first Epigenetics Research Group has been at work here for five years. The key thing is that researchers at Freiburg understood very early on how innovative epigenetics was. Next came the call for applications, the new building and the inauguration of a new department, which I was asked to head. In addition, arrangements were made for Davor Solter – co-discoverer of imprinting and a pioneer of modern epigenetics – to be succeeded by Asifa Akhtar, an outstanding epigeneticist. And then the institute was renamed – more or less as a beacon to the world.

Interview: Peter Spork

Photo: Alice Jessica Hath

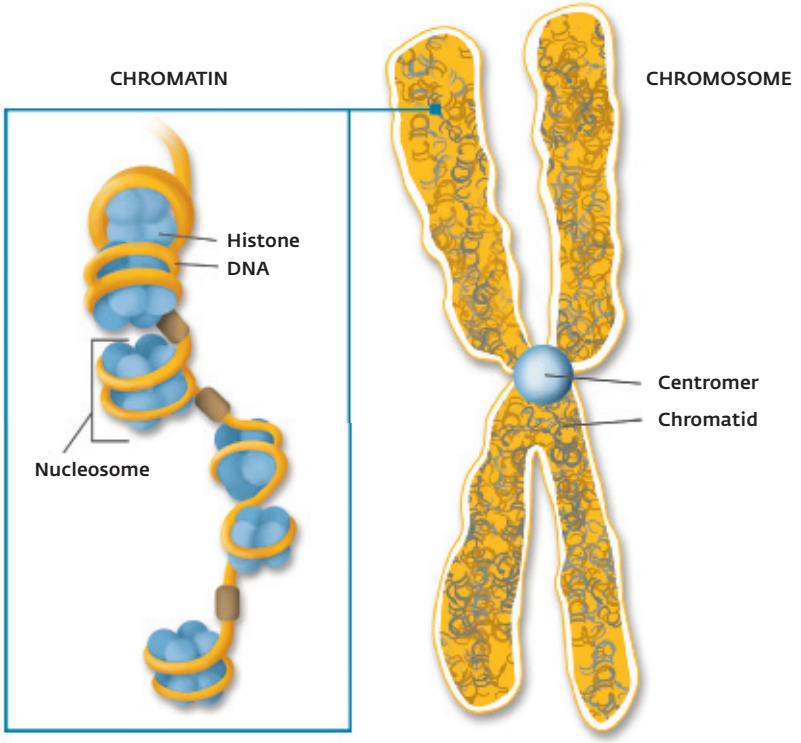
a storehouse for quite a number of gene copies, several hundred in the higher organisms, which are needed for the production of histones. This explains why it was previously thought impossible to switch off these gene copies and replace them with genetically modified histone variants. Jäckle's team removed all the histone genes from the flies, which caused the insects to die off after the fourteenth cell division. However, following the introduction of a critical number of gene copies, the flies regained their ability to survive and reproduce. In the next stage, the Göttingen-based researchers want to return particular histone genes to the insects. These genes have been modified to the extent that they lack docking sites, for example, for certain chemical groups. This would prevent certain histone modifications. "That would enable us to work through the histone code section by section to identify which biological effect is produced by which histone markings," explains Jäckle.

RNA FRAGMENTS SILENCE GENES

There is another important epigenetic switch system, the non-coding RNAs, or ncRNAs for short. The very sections of DNA that geneticists used to regard as functionless and refer to disparagingly as "junk DNA" actually code for these RNA fragments. They are, as it were, siblings of the messenger RNAs and, though a little underdeveloped, are definitely not junk. But unlike messenger RNAs, they do not contain instructions for producing proteins. On the contrary, one of their tasks is to remove from circulation any messenger

Epigenetics is a sure talking point: Herbert Jäckle from the Max Planck Institute of Biophysical Chemistry in Göttingen (top), Danny Reinberg (New York University, middle) and Peter Becker (Ludwig Maximilian University of Munich, bottom).

Photos: MPI of Immunobiology (top), Alice Jessica Hath (2)



The two-meter-long strand of DNA has to be packed very tightly to fit into the cell nucleus. To achieve this, the DNA (yellow) is coiled around complexes composed of eight histones (blue) each. These are then linked together like beads on a string (left). The diameter of a chromosome is just one thousandth of a millimeter (right).

RNAs whose base code matches their own. Accordingly, the transcript can no longer be translated into a protein and the effect of a gene is thus weakened or even completely silenced. Known as RNA interference, this principle, discovered in 2006, was honored with the Nobel Prize for Medicine. These days, almost every week sees the publication of new studies that demonstrate the importance of these processes in, for example, the prevention or the development of cancer.

Ingrid Grummt from the German Cancer Research Center in Heidelberg reports in Freiburg on another of the tasks of ncRNAs. She discovered ncRNAs that attach themselves near a gene to a DNA strand that matches their base sequence and, in doing so, insinuate themselves solidly into the DNA double helix. This produces a triple helix. Enzymes, in turn, bind to this structure and attach a methyl group directly to the DNA, thus silencing genes. Since more than half of the human genome can be transcribed into ncRNAs, Grummt suspects she has discovered a very general mechanism. "It is certainly conceivable that all genes that are silenced for any length of time have precisely matching ncRNAs," she says. There's no doubt whatsoever that epigeneticists are in the grip of gold fever.

"Just now, our field of research is getting increasingly complex and difficult to take in," explains Renato Paro from the Department of Biosystems at the Technical University of Zurich. His colleague Peter Becker from Munich's Ludwig Maximilian University concurs, saying, "The more research I do, the less I understand." He is delivering a paper on a problem that faces all living creatures that determine their sex via sex chromosomes. They need to ensure that genetic activity is organized on a balanced basis. In humans, for example, women, unlike men, have two X chromosomes. Without compensation, the genes lodged there would be twice as active in women. That is why one X chromosome is switched to be epigenetically silent in all female cells.

"We used to think that all organisms did this in the same way," says Asifa Akhtar, head of the Laboratory of Chromatin Regulation at the Freiburg Max Planck Institute. But the epigenetic toolkit is way too variable for nature not to have found other solutions as well. "*Drosophila*, for example, does the opposite. In these creatures, the X chromosome activity in males is nearly twice as strong," explains Akhtar. She is investigating proteins that are involved in this regulation, but that also play a role in human epigenetics.

BETWEEN IMMUNOBIOLOGY AND EPIGENETICS

One of her objectives is to compare the epigenetics of humans and flies. Ultimately, she is as concerned as most of her colleagues to get to the bottom of this complex new field of research, as far as this is possible. In this sense, the Freiburg Meeting, organized jointly by Asifa Akhtar and Thomas Jenuwein, Monika Lachner and other colleagues, might well have helped already. "It all went off so well, it was a wonderful start. We managed to get Freiburg on the epigenetic research map," she says, summing up the outcome. "And now we will try to repeat the meeting every two years in December."

Immunobiologist Rudolf Grosschedl, current Managing Director of the Freiburg institute, is also keen on the idea of repeating the conference. "We would very much welcome this, preferably alternating it with a conference on immunobiology." On the whole, everyone was "thoroughly satisfied" that they managed to bring epigenetics to Freiburg. This field, he says, enriches key research areas of the future and is also indispensable for immunobiology.

Most of the epigeneticists would like to return, that much is clear. Shelley Berger would then probably be able to provide fresh reports from the ant kingdom. One of the questions might be why some people – similar to ant queens – live very much longer than others. For, as Berger knows, "ants are a wonderful model for studying aging processes." ◂

Graphic: Haak & Nakat, based on original material from the MPS