Human Biology

The marks that mold us

by Martin Thurau

Source: Jan Greune

The genetic code is not everything: Heinrich Leonhardt, Professor of Human Biology at LMU, is currently dissecting the epigenetic mechanisms that determine which genes are activated or repressed in each of the diverse cell types that make up multicellular organisms.

Self-doubt was not on the agenda on 26 June 2000. At press conferences held in cities around the globe, molecular biologists celebrated a collective achievement, acclaimed by many as one of mankind's greatest accomplishments, comparable to splitting the atom or landing on the Moon. The leaders of the publicly funded Human Genome Project announced that they had deciphered the sequence of the human genome. The data were incomplete and riddled with errors, but 85% of the genome had been successfully assembled. But the decision to reveal this preliminary result was precipitated by the protagonists of the rival project, led by Craig Venter and carried out by his company Celera, who gave a similar press conference on their sequence data at the same time. Three years later a much more accurate and virtually complete version was published, revealing that the 3 billion base-pairs in the human genome encoded some 25,000 genes. In the succeeding decade, however, it has become clear that even this sequence does not provide the answers to many crucial questions.

Why for example do nerve cells differ from skin cells or muscle cells, given that essentially all cells in the body harbor the same genomic DNA sequence? How do their divergent forms and functions arise? Why do identical (monozygotic) twins often differ from one another more than one might expect, although both carry the same genome? Why are they not perfect *Doppelgänger*? Heinrich Leonhardt has what sounds like a simple answer: Not all genes in the genome are active in all cells, and cell differentiation relies on a network of interactions, a distinctive epigenetic level of control that determines which genes are activated and which are repressed in each specific cell type. As Professor of Human Biology and BioImaging at LMU, Leonhardt studies the exceedingly complex mechanisms that underlie this epigenetic program, which plays a central role in development.

"Nature vs. Nurture"

The epigenetic code is itself a highly dynamic system, Leonhardt explains. It is not a rigid blueprint like the genetic code, which specifies how the nucleotide sequences that make up the genes are translated into the amino-acid sequences of proteins. For instance, it can mediate the transient repression of a gene or set of genes for periods of hours or days not – as was once naively thought – permanently. This of course raises the next question: How are such modifications regulated? The organism basically reacts to every stimulus from its internal or external environment that alters its physiological state, and thus activates signaling networks that orchestrate an appropriate response. Researchers now know that nutritional factors have long-term effects on this control system, as does physical or cognitive activity, air pollution or drug consumption. Epigenetic changes are involved in the pathogenesis of many disorders, in diabetes and depression, cancer and cardiovascular disease. Biologist Rudolf Jaenisch (Massachusetts Institute of Technology) calls them "the language the genome uses to communicate with the environment".

Genes and Environment have generally been understood as opposing forces, as in "Nature vs. Nurture". They were the banners raised in ideological battles over the issue of which factor exercised the greater influence on the individual's personal development, one's genetic heritage or one's environment. Leonhardt may have mild reservations about the wording of Jaenisch's remark regarding epigenetic communication with the environment, but he is in no doubt about the significance of the dynamically responsive interface between genome and environment for the organism, its development and its metabolism: "All roads lead onto the epigenetic plane," he says. If one wants to understand how humans function, how the individual organism develops and operates, how it reacts at the biological level to its environment,

what makes it vulnerable to particular diseases and why, it is essential to understand how the logic elements that make up epigenetic circuits work. This is where Version 2.0 of the human genetic system begins.

From the chemical point of view, the system appears quite banal. The basic mechanisms involve the attachment and removal of very simple functional groups, such as methyl groups (CH3-), to the DNA. Like little flags, these tags mark some of the nucleotide bases that constitute the building blocks of the double-stranded DNA molecules that make up the genome. And they tell the cellular machinery responsible for gene expression which genes are unavailable for transcription – for the moment at least. However, combinations of different tags give rise to complex patterns, providing a supplementary code that is superimposed on the triplet combinations of the four bases A, C, G and T that form the basis of the classical genetic code.

Hanging drops: Stem cells growing in culture dishes can be induced to differentiate into certain cell types, such as nerve cells, or can be reprogrammed to produce others. This allows one to study the epigenetic mechanisms that underlie their (re-)programming. *Source: Jan Greune*

The crucial importance of methylation for the survival of higher organisms was demonstrated by a simple experiment done by Heinrich Leonhardt's group. Using a mouse model system, they genetically disabled the protein that attaches methyl groups to DNA. The enzyme retained only 10% of its normal activity – and the consequences were devastating. The mutant mice were underweight at birth, and developed highly aggressive tumors within a few months – "the first direct evidence that DNA modifications can lead to cancer," Leonhardt notes. The paper appeared in 2003 in the leading journal "Science" (the work was done in collaboration with Professor Rudolf Jaenisch of MIT in Cambridge, Mass.). Until then, physicians and biologists had believed that mutations – errors in the replication of the nucleotide sequence itself – were the root cause of cancer. Massive alterations in the epigenetic code, which according to Leonhardt can be found in all tumor cells, were regarded as consequences – not as a potential cause – of tumorigenesis.

How then might epigenetic modifications cause a tumor to develop? Leonhardt favors the following model. Every cell must find the appropriate balance "between proliferation-promoting and -inhibiting factors". If growth factors begin to dominate, the cell may start to divide uncontrollably. "To get through traffic, your car needs an accelerator and brakes. If your brakes are defective, you will end up hitting a wall," he says. In the cell, the primary defect may be a mutation in a tumor suppressor gene that normally keeps cell proliferation in check. But an epigenetic modification that represses expression of that same gene will have the same effect.

Nature has also come up with a second "game with marked cards", as Leonhardt likes to call it. This system methylates the histone proteins that package the DNA into chromosomes by forming disks around which the DNA is wound, like thread on a spool. Like all proteins, histones consist of defined sequences of amino acids, and specific positions in these chains can also be marked with methyl groups. These modifications then serve as binding sites for so-called repressor proteins that prevent the transcriptional machinery from gaining access to the packaged genes, effectively switching them off. Molecular biologist Professor Peter Becker at LMU's Adolf Butenandt Institute and many other researchers have been studying this class of epigenetic control mechanism for several years.

Only a few years ago, chemists like Professor Thomas Carell at LMU discovered

three new epigenetic marks attached to nucleotide bases in DNA. Structurally, these differ only a little from methyl groups, and they were at first thought to be products of rare side-reactions during normal metabolism. It is now known that they represent intermediates in a specific breakdown pathway, which the cell uses to erase methyl marks from the DNA, thus derepressing previously repressed genes. Moreover, it turns out that this pathway exploits mechanisms that are otherwise used for the repair of damaged DNA.

All these findings underline the complexity and flexibility of epigenetic control systems, and they have focused attention on the network of enzymes that attach and erase epigenetic marks. The resulting research has revealed unexpected connections between the genetic apparatus and other areas of metabolism. For example, together with a team at the University Medical Center in Grosshadern, Leonhardt's group discovered that an enzyme called TET2 was defective in a subset of leukemia patients. Because TET2 converts those methyl flags into an alternative tag, loss of its function results in drastic changes in the pattern of epigenetic marks in developing white blood cells, leading the researchers to believe that the defect was linked to the disease. Strikingly, in some patients, a second enzyme defect was found. This enzyme is involved in a central metabolic circuit and appeared to have nothing to do with marking of DNA. But it emerged that it normally produces a cofactor that is essential for TET2 activity. This case exemplifies the intimate connections between cell metabolism and epigenetic regulation. "And this is just the tip of the iceberg," says Leonhardt

So how far does the long arm of epigenetics reach? What sorts of signals cause the organism to adjust the modifications in its genome? And what effects do such changes have? Here are three random examples from the current literature. Researchers now suspect that susceptibility to stress may be largely determined by the patterns of epigenetic marks set up in the brain and the endocrine system during the months just before and after birth. The evidence comes from experiments in rats. Thus rats that were neglected by their mothers in early postnatal life were found to have life-long problems in adapting to stress. They showed abnormal epigenetic patterns in the hippocampus, and the cells in this brain region produced very few receptors for the stress hormone cortisol. The animals reacted sluggishly to increasing levels of the hormone, displayed signs of anxiety and were less able to cope with stressful situations.

Are epigenetic patterns heritable?

This work prompts one to ask how early trauma might impinge on epigenetic systems? Munich psychiatrists have studied a human gene that is a major regulator of stress-hormone secretion and is present in several genetic variants in the population. One of these is correlated with enhanced risk for depression and anxiety disorders, specifically if the carrier has experienced trauma during childhood. Experiments on nerve-cell cultures revealed that this gene variant undergoes aberrant epigenetic modification.

What about physical exercise? Does it leave its marks on the genome, and if so, how soon do they appear? To find out, Swedish scientists put a group of sedentary men through a 6-month fitness program, and compared the epigenetic patterns in fat cells taken from their subjects before and after the training phase. The differences were enormous, and genes linked to increased risk for obesity and diabetes were among those affected. Similar experiments showed just how flexible the system is: The first changes in DNA methylation in muscle cells appeared after only a few minutes on an exercise bike.

While Leonhardt finds results like these plausible, he points out that definitive proof is difficult to come by in such cases. That can only be done in animal models, he says. One can extrapolate from such models to human subjects, but this does not constitute secure scientific evidence – quite apart from the issues raised by all attempts to reduce highly complex phenomena to simple correlations, he adds. Many experiments carried out on humans do not meet scientific standards because the conditions cannot be precisely controlled and the sample sizes are too small.

To detect epigenetic changes, one must be able to access the relevant tissues, isolate the cells and analyze the patterns directly, Leonhardt asserts, and normally that is not possible with humans. "There are articles in the literature that verge on the macabre." On the basis of post-mortem material taken from suicide victims, researchers report epigenetic changes in the gene for a stress-hormone receptor – and go on to suggest that these are related to the suicidal state, he remarks dismissively. "Things like that make waves, but don't meet the criteria for good science." And they certainly don't afford insight into "the biology of suicide", as the headlines claimed.

A substantial body of evidence now suggests that environmental factors begin to influence the epigenome during prenatal development. But are epigenetic patterns heritable? This notion

has galvanized the imaginations of a number of researchers, because it recalls theories of heredity that had been consigned to the dustbin of history. The 18th-century biologist Jean-Baptiste de Lamarck proposed a model in which the heritability of acquired traits was viewed as the driving force of biological evolution. This idea was later comprehensively displaced by Charles Darwin's postulate that new species originate from the effects of selection on existing genetic variation.

Studies of the Dutch famine winter of 1944/45 have reignited the debate, says Leonhardt. For the German occupation during the war years not only persists in the nation's memory, it had long-term effects on the health of the population. Thus children who were born during or soon after the famine were underweight at birth. Not only that, as adults, they were more likely to be overweight, and to develop diabetes and cardiovascular disease, than their siblings who were born in less trying times. They were undernourished in the womb, because their mothers didn't get enough to eat. Epigenetic changes enabled fetal metabolism to adapt to the lack, but was apparently maladaptive when conditions improved in later years. Generally speaking, everything that reaches the embryo via the placenta will have an effect on the developing organism, Leonhardt says, including all forms of stress, which of course impinges on all aspects of the mother's physiology.

But can epigenetic patterns be directly transmitted to the genotypes of later generations? Findings which indicate that the grandchildren of those famished Dutch women also tend to be underweight at birth, even though *their* mothers had never undergone privation, hint that the answer is yes. But Heinrich Leonhardt is skeptical. After conception, when the fertilized egg cell has developed into the blastocyst that implants in the wall of the uterus, he says, virtually all of the epigenetic information in the embryo's genome is erased. This enables the embryo's stem cells to begin from scratch, as it were, and establish new lineages that give rise to their own tissues. The only genes that do not undergo such a reset are members of the small group of imprinted genes, which retain marks that distinguish the paternal from the maternal copies (alleles) at these loci. The age-old battles over the dominance of genes or environment were always dominated by ideological absolutes, and the dispute continues to smolder in places.

Irrespective of the question of heritability, the epigenetic system certainly has direct effects on genes. Can it perhaps be seen as reconciling the opposing philosophical positions? Let's give Heinrich Leonhardt the last word: "The genes set limits, but they leave a significant amount of leeway, an it is up to us to make the most of it. The more we learn about epigenetics, the clearer this becomes."

Prof. Dr. Heinrich Leonhardt
Chair of Human Biology and BioImaging at LMU since 2012. Born in 1961, Leonhardt earned his doctorate in Biochemistry at the Free University in Berlin. After a stint as a Postdoctoral Fellow at Harvard Medical School in Boston, he went on to lead research groups at the Franz Volhard Clinic and the Max Delbrück Center in Berlin-Buch, before being appointed to a professorship at LMU in 2002.

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Translation: Paul Hardy,