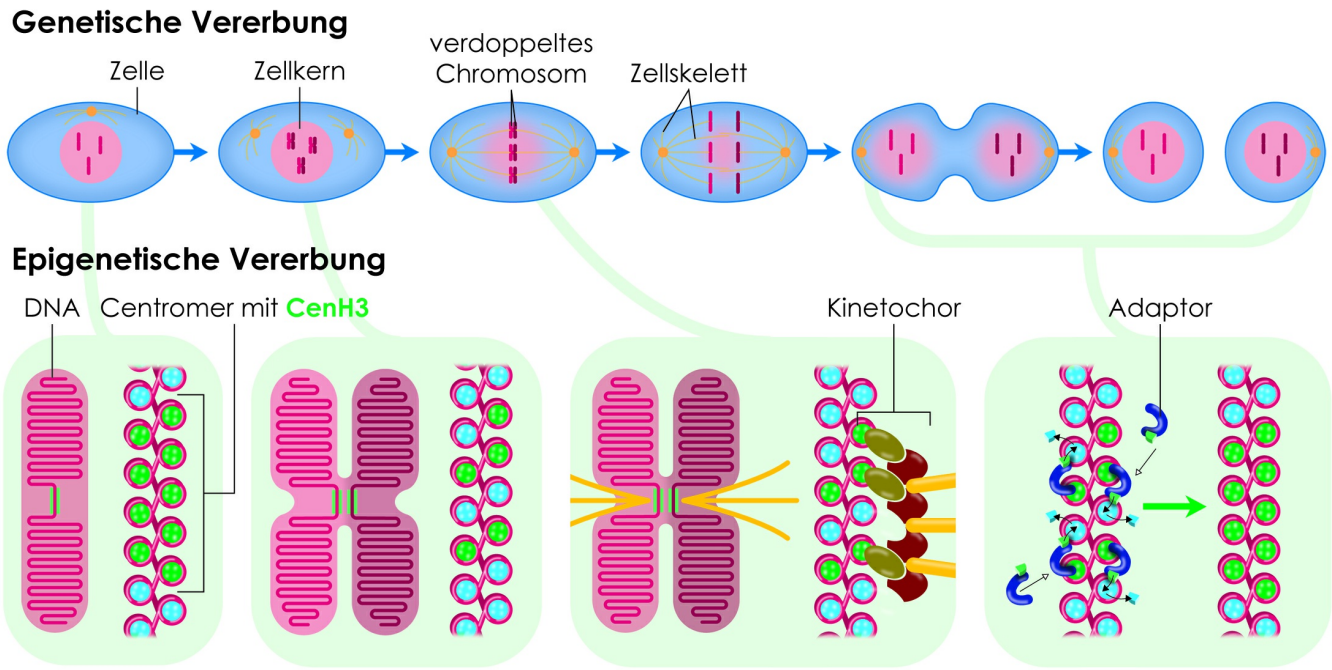
Patrick Heun: The DNA tangle and how it is organised

Cell nuclei should not be taken for a bowl of spaghetti that cannot be untangled. Dr. Patrick Heun and his group of researchers at the Max Planck Institute (MPI) of Immunobiology and Epigenetics in Freiburg are working on elucidating the spatial organisation of cell nuclei and how the structure of chromosomes is passed on to daughter cells during the division of cells. The researchers have come up with clear proof that the formation of centromeres – located in the centre of chromosomes – depends on a single molecule, the histone CenH3. This molecular warden is part of what is known as the epigenetic memory of cells and also appears to play a key role in controlling the spatial structures in cell nuclei.

The DNA (deoxyribonucleic acid) inside the nucleus of a human cell resembles a ball of wool; it exists in the form of coiled threads that would be about 2 metres long (in diploid cells) if laid out lengthwise. However, this apparent chaos is more organised than it first appears when looked at through an electron microscope. The cell division process clearly shows the thin DNA thread to be more than just a ball of wool and it also shows the organisation of 23 chromosomes (46 in diploid cells). Before the cell divides, the chromosomes duplicate and condense into short X-shaped structures; they line up at a specific plane in the nucleus and the sister chromatids are pulled apart by clusters of microtubules. They are pulled towards the opposite sides of the cell and a new nuclear envelope forms around each set, giving rise to two daughter cells. Chromosomes have a characteristic X-shaped form, with the centromere located near the centre, the site where two identical sister chromatids come into closest contact. The human centromere DNA region is packed with CenH3 histone molecules which form a docking site for other proteins that in turn form a kind of super complex to which the microtubules attach during cell division. This protein structure is known as kinetochore. “The 23 chromosome pairs can only be distributed equally to the two daughter cells when each chromosome possesses a centromere,” said Dr. Patrick Heun from the Max Planck Institute (MPI) of Immunobiology and Epigenetics in Freiburg. “It is therefore of crucial importance to thoroughly understand how this chromosomal structure is established.”



Cell division (top) and epigenetic inheritance of the centromeres (bottom).

An additional kind of memory

Heun has been interested in the mechanisms underlying the organisation of human DNA in the cell nucleus for a long time. The complex of DNA and proteins within the nucleus is called chromatin. Its functions include packaging DNA into a smaller volume so that it fits into the cell and controlling gene expression and DNA replication. Chromatin affects DNA packaging; less tightly folded DNA becomes accessible to the enzymes that transcribe the DNA; more tightly compacted DNA cannot be accessed by transcription or replication enzymes and leads to the silencing of genes, even across many generations. This inheritance of traits is known as epigenetic memory, i.e. epigenetic alterations that occur due to chemical changes rather than in the coding sequence of DNA. For example, epigenetic memory causes female mice to pass on different fur colours (and other traits) to their progeny in relation to the type of food (e.g., DNA-methylating foods) the female mice eat. The environment can also affect the traits of offspring due to different interactions with the chromatin. The DNA sequence (i.e. genetic code) plays no role in epigenetic inheritance.

Chromatin is the combination of DNA and proteins, mainly histones that compact the DNA. A histone molecule also plays a key role in the formation of centromeres, a DNA region that is typically found near the middle of a chromosome. Centromeres are the most condensed regions of chromosomes to which the spindle fibre is attached during cell division. "Many years ago, it was shown that Drosophila chromosomes do not form centromeres in the absence of the CID histone," said Heun. CID is the fruit fly homologue of the human CenH3 histone. During his postdoctoral period in Berkeley, Heun was able to generate artificial kinetochores (i.e. the protein structure on chromatids where the microtubules attach during cell division, thereby enabling the chromosomes to move to the opposite poles of the cell) in other regions of fly chromosomes by way of genetic manipulation that led to elevated concentrations of CID in the cell nucleus. When Heun returned from the USA, he established a method he learned from using baker's yeast that enabled him to direct the CID histone to specific regions in fruit fly chromosomes. He introduced the sequence for a known DNA binding site (lac operon) into the DNA of fruit fly cells and coupled the CID histone with a protein that specifically recognised and bound to this DNA sequence.

Dr. Patrick Heun from the Max Planck Institute of Immunobiology and Epigenetics in Freiburg
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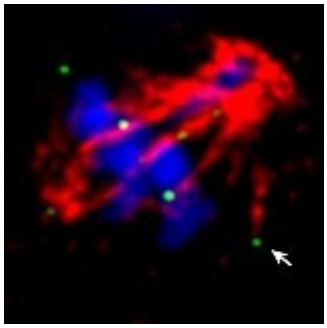
Artificial microchromosomes

This synthetic method enabled Heun and his team from the MPI in Freiburg to show that it was possible to generate kinetochores at desired chromosome regions. However, kinetochores are different from centromeres. Kinetochores break up after cell division; centromeres are maintained across many cell generations. As the cells used by the Freiburg researchers never lived long enough to pass on their DNA to their "grandchildren" and "great-grandchildren", the researchers were unable to experimentally differentiate between the two structures. "Despite promising results, we were nevertheless unable to prove without any doubt that the CID protein alone was sufficient to give rise to a true centromere in a specific region of a fruit fly chromosome," said Heun explaining that this was because chromosomes with more than one centromere tended to be torn apart during the cell division process. However, the researchers were finally able to achieve a breakthrough a few months ago: they were able to demonstrate that the CID histone determines the position, function and inheritance of the centromere.

Working in cooperation with researchers from the Helmholtz Research Centre in Munich, the



The photo shows a cell during mitosis. The chromosomes are lined up in the metaphase plate. The green spot (arrow) shown on the bottom right-hand side is a plasmid that was used to induce the generation of a centromere with the help of CenH3. This plasmid has been shown to be able to assemble kinetochore proteins and bind the microtubules of the mitotic spindle. Green = CenH3 = centromeres, red = microtubules, blue = DNA.

Freiburg researchers engineered artificial DNA rings (known as plasmids) that contained a CID binding domain (lac operon) at a specific site. They introduced the plasmids into the cells, ensuring that the artificially introduced CID bound to the target structures in the plasmids. They found that the CID protein bound to a DNA region where a centromere does not normally form. The plasmids behaved like small chromosomes and were passed on from one cell generation to another. They remained stable across a large number of cell divisions. The artificially introduced CID histone appeared to recruit natural CID from the cell, thereby ensuring that sufficient CID was available at the centromere after each cell division. If this did not occur, the CID proteins available would be halved in number after each cell division. "The self-recruitment of CID to the centromere structure is a prerequisite for epigenetic inheritance, i.e. the passing on of the centromere position from generation to generation," said Heun.

Light at the end of the tunnel

The Max Planck and Helmholtz researchers were thus able to show that CID alone is sufficient to trigger the formation of centromeres. Nevertheless, natural centromeres in living cells are far more stable than the artificial ones. Future research will help Heun and his colleagues to discover further mechanisms that ensure this stability across hundreds of cell generations. From a practical perspective, Heun's research could also prove important for medicine, as scientists have been trying for many years to introduce into cells therapeutic genes (to treat cancer for example) that remain active across many cell divisions. The artificial microchromosomes produced by Heun and his colleagues might act as alternatives to gene therapy using viruses in that they have the potential to be used as vehicles that are able to retain such genes in the cell for a prolonged period. "We still have a long way to go before our artificial microchromosomes are likely to be used in clinical application," said Heun explaining that his group will now go on to transfer the construct to human cell cultures and find out how it behaves.

In addition to the aforementioned research, Heun's group of researchers is also focused on issues related to spatial organisation in the cell nucleus. The researchers discovered, for example, that CenH3/CID has something to do with the predominant positioning of centromeres close to the nucleoli, structures composed of proteins and nucleic acids in the nucleus where the protein biosynthesis machinery (ribosomes) is assembled. It appears that everything in the cell nucleus is well organised and Heun and his colleagues are working hard to decipher how this organisation is maintained.

In 2012, Patrick Heun was awarded a ERC Starting Grant worth 1.75 million euros for his research project entitled "Dissection of centromeric chromatin and components: A biosynthetic approach".

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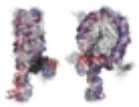

Epigenetics – heritable traits without changing the DNA sequence