Epigenetics

Reading domains detect and identify histone modifications

Genes can be switched on and off, and their expression thus regulated, by the chemical modification of DNA or histone proteins. Such modifications are therefore essential in the development and function of healthy cells, and hence organisms. It comes as no surprise that the study of these modifications is paramount in basic and clinical research. Up until now, specific biochemical tests and antibodies have been used to detect the modifications. However, this method has a number of drawbacks. Prof. Dr. Albert Jeltsch from the Institute of Biochemistry at the University of Stuttgart and his team of researchers have now presented an alternative method to detect and identify changes in the post-translational modifications of histones. They envisage that their new method, which uses parts of natural proteins, so-called reading domains, will make such tests cheaper and simpler, and experimental data more reliable.

DNA in the nuclei of eukaryotic cells is wrapped around histones. Chemical modifications such as the attachment of methyl or acetyl groups to histones or the methylation of DNA greatly influence gene expression. In other words, genes are turned on or off by such modifications, something that is essential for the proper function of all living organisms. Abnormal modification patterns can lead to disease. For example, tumour cells often have DNA methylation patterns that differ significantly from those of healthy tissue. Such modifications of nucleic acids and proteins are referred to as epigenetic modifications and may be inheritable.

Prof. Dr. Albert Jeltsch, director of the Institute of Biochemistry at the University of Stuttgart, and his team have been studying epigenetic issues like these for quite some time: "All our research revolves around how genes are regulated," says Jeltsch. The researchers are investigating a broad range of different epigenetic modification processes. They are studying how methyl transferases work, i.e. enzymes that attach methyl groups to DNA and histones, how these enzymes are regulated and how the methylation pattern is generated.

Modifications determine functional chromatin state

Prof. Dr. Albert Jeltsch is director of the Institute of Biochemistry at the University of Stuttgart. He has presented a new method for reliably detecting and identifying incorrect histone modifications that are implicated in disease.
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They are also carrying out another major research project on how histone modifications are read. Jeltsch comments: "We are interested in finding out how cells realise that a histone has been modified. We already know that so-called reading domains play an important role. Reading domains are protein domains that bind to nucleosomes with a specific modification, thus inducing a biological response like switching on a gene." This means that the functional state of chromatin is largely determined by its chemical modifications. Studying these modifications is therefore crucially important for a variety of issues, including tumour characterisation.

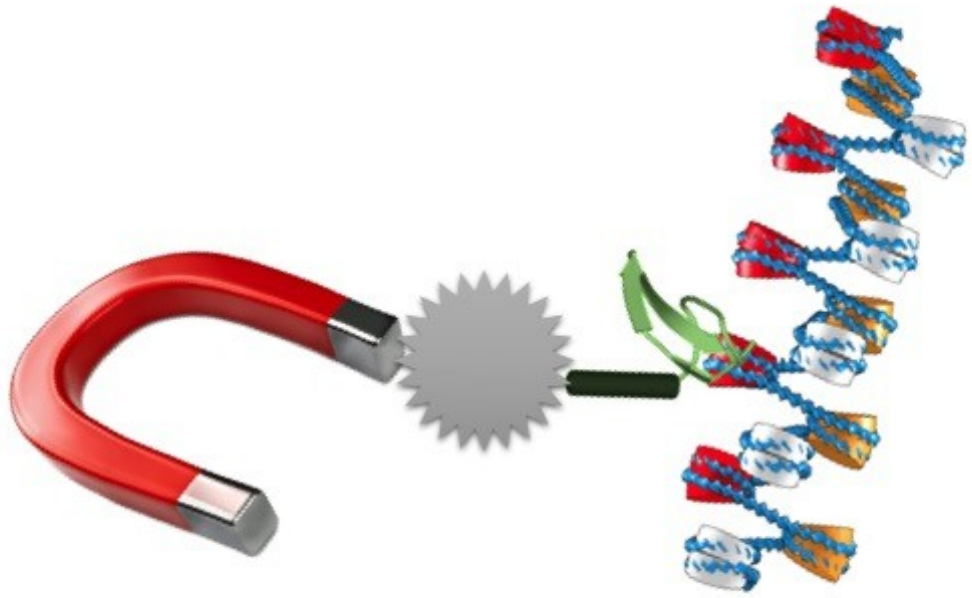

Diagram illustrating how chromatin immunoprecipitation (ChIP) works: incubating mononucleosomes with specific antibodies enables the detection of histone modifications.
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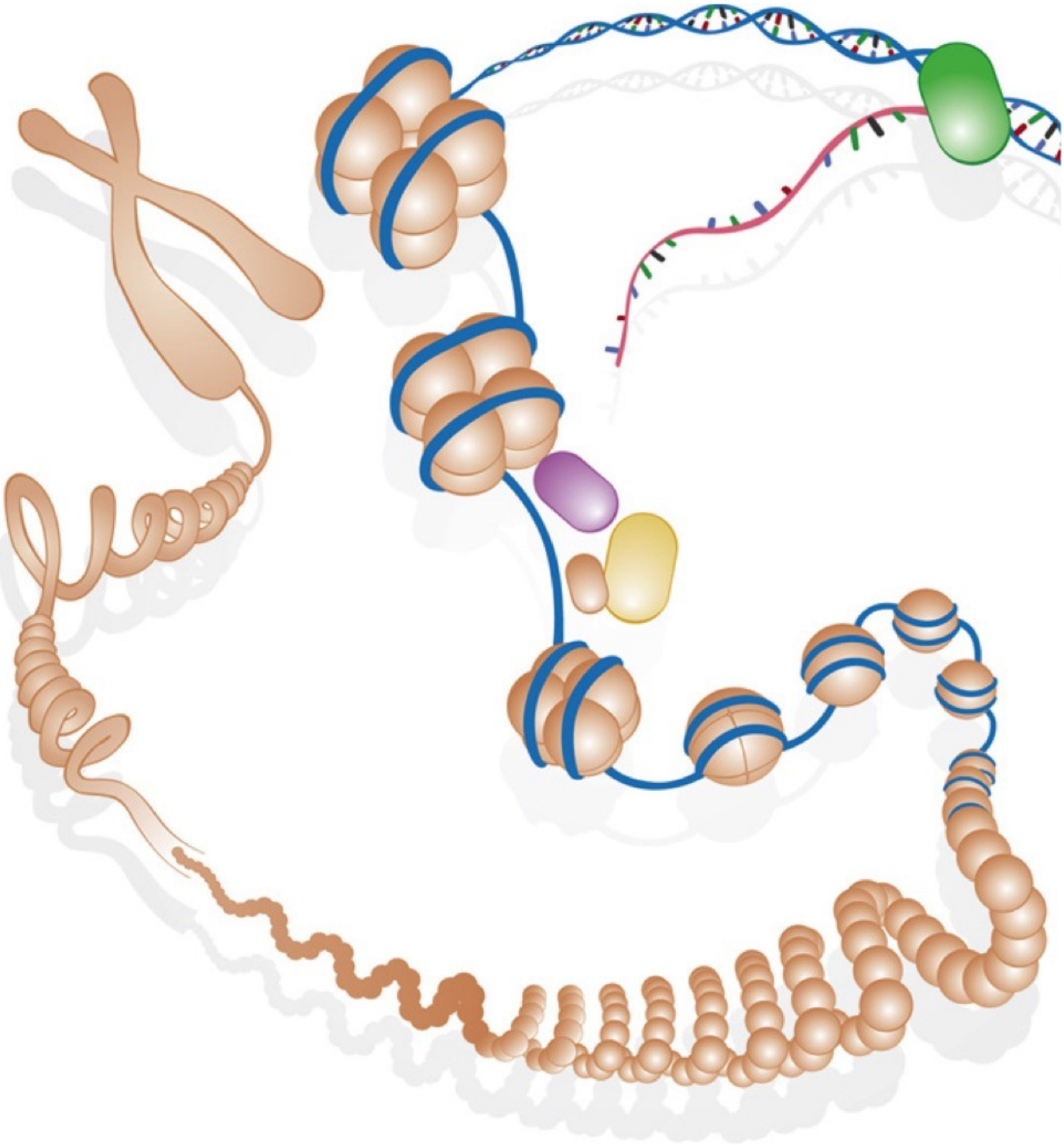
While powerful techniques for the analysis of DNA modifications have long been available, the range of methods suitable for studying histone modifications is still relatively limited. A method that has been used up until now is chromatin immunoprecipitation (ChIP), in which chromatin is broken down into mononucleosomes and incubated with antibodies that bind to histones with specific modifications. "The work of an entire research field still relies on an antibody that is specific for one single modification," says Jeltsch. The method is also associated with a number of drawbacks, such as the cross-reactivity of antibodies that recognise different modifications from those they are expected to recognise, or false-negative results when an antibody is unable to correctly bind due to additional modifications in close vicinity to the target modification."

Antibody tests are often not reproducible

Another serious problem is the availability of antibodies with invariable properties. "When antibodies in a certain batch are used up, the researcher makes new ones. These always differ from the old

batch, especially with regard to the features that are critical for a certain experiment, with the result that experiments cannot be reproduced," says Jeltsch, going on to add, "there is a high batch-to-batch variability and the antibodies have insufficient specificity. Moreover, companies cannot produce antibodies with invariable properties due to complex production methods. A major scientific issue that needs to be taken into consideration is the long-term reproducibility of results." In addition, producing antibodies is rather costly. This is why the researchers from Stuttgart eventually came up with the idea of using biological reading domains in place of histone modification antibodies.



Chromatin with DNA and histone modifications as well as associated reading domains.

Such reading domains (also called histone modification interacting domains) are part of natural proteins that bind to and detect the modified histone under investigation. This technology enables

recombinant reading domains with high yields in E. coli to be used relatively easily and cost-effectively using specific vectors. "Once you have the vector, you can produce a specific protein again and again, and it will still have the same properties in 20 years' time," explains Jeltsch. "Our approach therefore guarantees consistent reagent quality and hence reproducibility of results as properties will be invariable across different batches." The only drawback of reading domains is that their binding affinity is lower than that of antibodies. However, this can be compensated for by using larger substrate quantities. At the moment, Jeltsch does not anticipate any technical hurdles: "They are as good as antibodies. Whether this can be generalised, only time will tell. In any case, we are using protein design techniques to further optimise the reading domains."

Reading domains as a business idea

Since reading domains can be produced in test tubes with the same high quality over a period of many years, the scientists are now considering commercially exploiting their technology. With the financial support of the University of Stuttgart, they have just filed a patent application. Jeltsch and his colleagues are also looking for cooperation partners. "We are currently trying to find a distribution partner for our reading domains. We will then produce and test the proteins before handing them over to our cooperation partner. In the meantime, we will continue to work on the development of further reading domains. But all this is still a pipe dream." The laboratory production of the reading domains in bacteria is well established, and Professor Jeltsch is confident that upscaling the technology will not be a problem.

Article

10-Aug-2015
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BioRegio STERN

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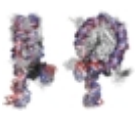

Epigenetics – heritable traits without changing the DNA sequence

epigenetics histone