The epigenetic switchboard

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Epigenetic signals help determine which genes are activated at which time in a given cell. A novel analytical method enables systematic characterization of the relevant epigenetic tags, and reveals that the system adapts to the loss of single epigenetic writer and eraser enzymes.

With a few exceptions, every cell type in a multicellular organism carries the same endowment of genetic instructions encoded in the nucleotide sequence of its DNA genome. Nevertheless, each cell type expresses only those genes required for its specific function. Muscle cells and nerve cells, for instance, implement quite different genetic programs directed by subsets of the genes in the whole genome. Which genes are activated and at which time is largely determined by cell-type-specific chemical modification of the proteins that package the genes in the cell nucleus, the histones. The pattern of modification is determined by the

interplay between enzymes that attach or remove the various tags (writers and erasers, respectively), which is in turn controlled by so-called epigenetic signal pathways that respond to changes in the cell's local environment. "Perturbations of these signaling pathways can precipitate the development of diseases, such as cancer or Alzheimer's," says LMU's Professor Peter Becker. Together with his doctoral student Christian Feller, Becker has now been able to characterize the enzymes involved in one of these signal pathways, which is based on the attachment of so-called acetyl groups to histones – the proteins that package the DNA molecules into the highly compact form (called "chromatin") found in the cell nucleus. The results of the new study appear in the leading cell biology journal "*Molecular Cell*".

The four major types of histone proteins interact to form spools around which the nuclear DNA is tightly wrapped. This packaging interaction effectively makes the DNA inaccessible to the enzymes that read out the genetic information. However, the attachment of acetyl groups to certain exposed sites on the histones weakens their hold on the DNA. Hence localized acetylation "unmasks" the corresponding genes and renders them susceptible to activation. "Although histone acetylation as such has been known for a long time, we know relatively little about the target sites in the histones, how the presence of groups at neighboring sites gives rise to so-called motifs, and how frequently such motifs appear in the cell nucleus," Becker explains. Different acetylation motifs are presumably targeted through different epigenetic signaling pathways.

Mediators of histone acetylation

Histone acetylation is carried out by a large set of specialized enzymes called acetyltransferases. The human genome codes for more than 60 putative histone acetyltransferases, while the fruitfly Drosophila melanogaster possesses more than 40, most of them very similar to those found in humans. "However, up to now it has not been technically

possible to identify the contributions of single acetyltransferase enzymes to the generation of acetylation motifs" says Becker.

But by adapting an existing analytical method, based on enzymatic fragmentation of histones and mass spectrometry, the LMU researchers were able to reliably quantify both single histone modifications and their various combinations. "Our close cooperation with two experts in proteomics, Axel Imhof and Ignasi Fornè, was crucial to our success. It enabled us to develop an optimized procedure for mass spectrometric analysis of histone fragments that can identify many of the acetylation motifs in the cell," says Christian Feller, first author of the new study. By genetically inhibiting the function of each individual acetyltransferase (and deacetylase) in fly cells, the researchers were able to define the contributions of most enzymes expressed in Drosophila to placing each different acetylation motif. The experiments also revealed that the presence of adjacent acetyl groups and other modifications have a potent impact on the target sites recognized by many of the enzymes.

Compensatory mechanisms

"Our most surprising finding was that the depletion of single acetyltransferases often leads to the attachment of novel acetylation tags at nearby sites, so that the overall level of acetylation is often very similar to the normal one," says Feller. The ability of biological systems to compensate, at least in the short term, for the loss of individual functional components is well known. "However, the degree to which the histone acetylation system is able to accomplish this feat was a big surprise," Becker adds, "and it illustrates the complexity of the circuitry that links the various epigenetic signaling pathways."

The new findings lay the basis for future work by Becker and his colleagues. They would now like to know, for instance, how closely the target sites of the acetyltransferases in the fruitfly resemble those of

their counterparts in human cells. Is compensatory acetylation a widespread phenomenon and, if so, what is its function? And finally, how can the results of the new study be exploited for the development of more effective inhibitors of acetyltransferases for use in cancer therapy? The results of the current study will help many in the field to explore these interesting questions in the future.

More information: "Global and Specific Responses of the Histone Acetylome to Systematic Perturbation." DOI: dx.doi.org/10.1016/j.molcel.2014.12.008

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