The importance of mixed motifs

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Local modifications in histone proteins alter DNA packing density in the cell nucleus to regulate gene activity. They also form the basis of a code in which the significance of a given pattern or motif depends on its broader context.

Virtually all the cells in any given multicellular organism contain the same complement of genes. Nevertheless, the genetic program implemented in a muscle cell, for instance, differs from that of a liver or a nerve cell. Which subset of genes is expressed in a given cell type at any given time is determined by, among other factors, targeted chemical

modifications of the proteins that interact with the genomic DNA in the cell nucleus. A team of researchers led by Professor Peter Becker at Ludwig-Maximilians-Universitaet (LMU) in Munich's new Biomedical Center (BMC) and Dr. Carsten Marr at the Helmholtz Center Munich has now shown that whether and how each individual target site is modified depends on the nature and pattern of the neighboring marks. Their findings, which appear in the journal *Cell Systems*, provide unexpected insights into the complexity of the epigenetic mechanisms that mediate gene regulation in higher organisms.

Acetylation is one of the most important classes of protein modifications involved in regulating gene expression. In the cell nucleus, histone proteins interact with the DNA molecules that carry the genetic information, causing them to fold up into a tightly packed and compact form.The attachment of acetyl groups (CH3COO) to histones alters the accessibility of the DNA to the enzymes required for gene expression. In this way, these chemical tags serve as molecular switches to regulate gene activity- with acetyl groups in particular generally functioning as activators of gene expression. Both acetylations and deacetylations are carried out by a special set of dedicated enzymes. Moreover, each of the four types of histone possesses several target sites for acetylation. As a result, histones can exhibit specific combinations of modifications, which are referred to as motifs."We therefore assume that not only the individual acetylated site but also the pattern of acetylations is of functional significance," says Peter Becker. In an earlier study, in which his group systematically deleted the genes for each acetylase enzyme, the researchers were surprised to find that novel patterns of acetylation were often observed in the vicinity of a known target site for the deleted enzyme, so that the overall level of acetylation remained virtually unchanged.

Intricate patterns of interaction

"Contrary to what has generally been thought, the likelihood that a given acetylation site will be modified seems to be influenced by the state of neighboring sites," says Christian Feller, who made a significant contribution to the newly published paper while working on his doctoral thesis. "Certain motifs turn up more often than others, which also argues that acetylation motifs are not randomly distributed. However, because the effects of existing acetylations on the various enzymes involved are so complex, one cannot hope to understand the mechanisms that determine the distribution of the different motifs with the help of biochemical methods alone," he explains.

For this reason, Becker and Feller teamed up with the mathematicians around Marr, who reanalyzed the experimental data using a novel theoretical approach. At one end of the Drosophila histone H4 molecule, one finds four potential targets for acetylation immediately adjacent to one another. If acetylation occurs at random, one would expect to find up to 16 different motifs in this region - depending on whether each site is acetylated or not.

Since Becker had experimentally determined the actual distribution of motifs in Drosophila H4 in the previous study, Carsten Marr and his colleagues used various mathematical approaches to simulate the patterns expected when various boundary conditions - particularly in relation to rates of acetylation - were imposed. They then compared the resulting distribution of motifs with the experimental results in order to identify the theoretical model that most closely reproduced the measured values.

Over a billion models tested

The best models were then successively refined by systematically altering the parameters and new distributions were calculated. "We actually had to check more than a billion models before we identified the

optimal fit to the actual data," says Marr. And this model is based on two assumptions: That specific enzymes modify their targets at different rates, and that pre-existing acetylations influence the rates at which neighboring acceptor sites are acetylated."

In other words, the mathematical modeling confirms that acetylation occurs in a motif-specific fashion. In addition, the results elucidate the reaction pathways involved, and provide a more precise picture of the acetylation networks used in cells. Moreover, by applying the optimal model to an experimental dataset that had not been used in the original modeling studies, the theoreticians confirmed that the corresponding simulation is able to predict the effects of the deletion of particular acetylases on the overall acetylation pattern.

Hence Becker and his colleagues believe that the new model provides a useful tool for investigating the impact of modifying enzymes for which experimental data is still lacking. "We can use it to obtain a deeper insight into acetylation mechanisms, which will perhaps make it possible to modulate histone acetylation in a targeted manner," Becker says."Since errors in histone acetylation are known to contribute to the development of many medical disorders, this work also has potential therapeutic relevance."

More information: Thomas Blasi et al. Combinatorial Histone Acetylation Patterns Are Generated by Motif-Specific Reactions, *Cell Systems* (2016). DOI: 10.1016/j.cels.2016.01.002

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