Scientists develop tool to study a deadly parasite's histone code

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(PhysOrg.com) -- In the Japanese art of paper folding, a series of folds can make the same sheet of paper into a ballerina or baby elephant. But try unfolding the baby elephant and making it into a ballerina. It's like trying to make a neuron from a kidney cell. Epigenetics, it turns out, isn't much different from this old Japanese art: Each fold, or epigenetic crease, both limits and permits further potential folds in a way that mirrors how epigenetic changes seal a cell's fate.

The changes occur on the tails of histones, the globular proteins around which DNA winds itself to make chromatin, the stuff of chromosomes. When the strings of amino acids that make up the tails undergo epigenetic modifications — chemical alterations such as methylation or acetylation — chromatin's structure changes in order to either seal off DNA or make it available for transcription. Like each fold of the paper, each modification ultimately shapes chromatin's structure.

In a genome-wide study led by George A.M. Cross, head of the Laboratory of Molecular Parasitology, and T. Nicolai Siegel, a graduate student in the lab, scientists at Rockefeller University have mapped epigenetic changes that are likely to play a role in initiating the transcription of genes in Trypanosoma brucei, the deadly single-celled parasite responsible for African sleeping sickness. The advance marks the first time scientists have been able to develop the tools to map these changes across the entire genome of the evolutionarily ancient parasite.

"Histones in trypanosomes are extraordinarily divergent from histones in

other organisms, so we couldn't use the same commercially available antibodies we use for mammals and yeast to isolate them and study their modifications," says Cross. "If we were interested in histone modifications, we couldn't reliably predict which amino acids in the histone tails would be modified and had a role in transcription. We now have the means to do that."

Two years ago, Cross and his colleagues were the first to identify histone modifications that exist in T. brucei, focusing on modifications that occur on H4, one of the four pairs of core histones. Building off that research, Siegel was able to create an antibody specific to the modified histone, whose 10th amino acid was acetylated. When the antibody was exposed to the trypanosome genome, it attached to the modified histones, allowing Siegel to extract them — along with the DNA coiled around them — from the parasite's nucleus.

"What I had was all these DNA fragments, which I could then map back to the genome and see every location where this modification occurred," says Siegel.

The results were striking. This modification of H4 occurred along every probable transcription start site across the trypanosome genome, suggesting that this modification serves as a loading dock for transcription factors. The team proposes that at these transcription start sites, H4's tail is acetylated, which helps open up chromatin to make room for factors that initiate transcription.

Siegel then decided to repeat the procedure for every histone variant (in trypanosomes, each core histone has one variant), revealing that two of them occur at transcription termination sites and two at probable transcription start sites, with the two at the start sites always occurring together.

They further found that the two variants at the start sites make the histone unstable. When histones become unstable, they are ejected from the chromatin structure and the chromatin collapses. So all that DNA that is wound tightly around the histones loosens up, becoming more accessible to factors that initiate transcription.

“The research gives us important clues about how transcription is initiated in this deadly parasite,” says Cross. “If we can block transcription, we may be able to gain the upper hand in the cat-and-mouse game this parasite plays with our immune system.”

More information: *Genes and Development* 23(9) 1063-1076 (May 1, 2009) Four histone variants mark the boundaries of polycistronic transcription units in Trypanosoma brucei; T. Nicolai Siegel, Doeke R. Hekstra, Louise E. Kemp, Luisa M. Figueiredo, Joanna E. Lowell, David Fenyo, Xuning Wang, Scott Dewell and George A.M. Cross.

Provided by Rockefeller University (news : web)