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Seeing the Shape of Gene Silencing

Researchers have devised clever new techniques to mimic the chemical marks on tightly wound DNA-protein complexes that silence gene expression. The scientists have used these complexes to visualize gene silencing in its natural context for the first time.

Howard Hughes Medical Institute investigators Karolin Luger, Kevan Shokat, and their colleagues published their findings on September 14, 2008, in an advance online publication in the journal *Nature Structural & Molecular Biology*. Luger is at Colorado State University and Shokat is at the University of California, San Francisco.

During growth and development, genes that should not be expressed are physically tagged with chemical groups such as methyl groups. Genes can also be silenced by modification of the histone proteins that make up the “smart stuffing” in chromosomes.

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- Karolin Luger

Histones make up the spool of proteins around which DNA winds so that it is packaged compactly in the nucleus. The protein-DNA nucleosome complexes, in turn, are packed into repeating units called chromatin, which is the building block of chromosomes. When methyl groups are added to a histone, they modify its properties and alter the frequency at which a particular gene is expressed. Histone proteins both protect DNA and regulate genes as they combine with DNA to form chromatin.

Despite its importance, histone methylation has remained enigmatic. “Although methylation has been appreciated as an important epigenetic marker, very little was known mechanistically about what methylation does to chromatin,” said Luger. “Experimentally, it has been an intractable area.”

The new experiments involved creating artificial nucleosomes bearing chemically synthesized methylation marks that mimic natural gene-silencing tags. With this new set of tools, Luger and her colleagues did x-ray crystallographic and biophysical studies to see how methylation affects the structure of chromatin and nucleosomes.

The researchers began their work with the goal of learning how two different methylation marks affect the overall structure of nucleosomes and chromatin. They chose to study one mark that activates genes and one that represses genes. The activating mark consisted of two methyl groups attached to the amino acid lysine (also called a lysine residue) in a specific portion of the histone H3. The repressive mark was composed of three methyl groups attached to a lysine on the H4 histone. Histones H3 and H4 are two of the five main histones that regulate the structure of chromatin. Knowing the specific location of the lysines is important because histones contain many lysine residues and activation or repression of genes only occurs when the methylation marks are attached to the correct lysine.

Past structural studies of methylation were hindered by the extreme difficulty of constructing homogeneous samples of histones bearing methyl groups attached to specific lysine residues. However, Shokat and his colleagues had synthesized a chemical structure in the lab that mimics lysine with the methyl groups attached. With this methyl-group mimic in hand, his team could then synthesize purified histones with methyl groups inserted at any desired point on the histone structure. Matthew Simon, a postdoctoral fellow in Shokat's laboratory, produced pure samples of H3 and H4 histones with the desired methylation marks.

Luger and her colleagues next packaged the modified histones they received from Shokat's group into recombinant nucleosomes and chromatin. After they had successfully created their nucleosomes, Xu Lu, a postdoctoral fellow in Luger's lab set about crystallizing the nucleosomes so that they could analyze their structure using x-ray crystallography. In this widely used technique, x-rays are directed through crystals of a protein, and its structure deduced from the pattern of diffraction of the beam. "These structures revealed conclusively that the structure of the nucleosome itself is not significantly altered by the two types of methylation," said Luger.

"The ability of our laboratory to make homogeneous histones with the appropriate methylation was essential for the crystallography," said Shokat. "If there had been any significant contamination, it would have been almost impossible to solve the structure. And the nice thing about the synthesis technique we developed is that it is incredibly scalable. We can make kilograms of the material if we need it, which is a real advantage for crystallography."

In a second set of analyses, co-author Jeffrey Hansen and Xu Lu measured how methylation affected the condensation of recombinant chromatin formed from the methylated histones. Hansen, who is at Colorado State, used

analytical ultracentrifugation to assess compaction. This analysis revealed that activating methyl marks on the H3 histone caused little alteration in the structure of chromatin when compared to nonmethylated counterparts. However, the repressive methyl groups on the H4 histone caused the arrays of nucleosomes to become significantly more compacted. “They were much more able to form chromosomal fibers,” said Luger.

The researchers' experimental approach offers new tools and methods to explore the structural effects of methylation, said Luger. “We believe that the nucleosomes will always retain their basic shape,” she said. “But the interesting question will be whether their dynamics change with methylation—whether individual nucleosomes may have a higher propensity to unfold, to unravel slightly or just to be remodeled.”

Luger and her colleagues are also setting their sights on studying how combinations of histone modifications--a more common gene control mechanism--affect the structure of nucleosomes and higher order chromatin. “In this initial study, we used a very simplified system in which every nucleosome carried the same modifications” said Luger. “But clearly, one can imagine biological systems using complex combinatorics of markers for epigenetic control.”