

Meeting Report

K4/K9: A single switch for chromatin?

This will be remembered as the K4/K9 meeting," remarked one participant at a recent Colorado gathering. The meeting covered all of chromatin biology, but talks kept returning to methyl modifications at lysine 4 (K4) and lysine 9 (K9) of histone H3. Methylation of K4 is associated with gene activation; methylation of K9 with gene repression.

The K4/K9 story is representative of a field that was, until recently, a backwater. Its rejuvenation can be traced to two innovations—a switch from purified to messy assay systems, and the isolation of antibodies that recognize modified histones. These innovations have allowed the identification of critical enzymes that modify histones by adding and removing moieties such as methyl and acetyl groups. Gene expression, in turn, is regulated by proteins that recognize the presence or absence of these modifications.

K4/K9 modifications have the highest profile at present, but they are not the whole story. "It would be premature to

give it billing as the central switch," said David Allis (University of Virginia, Charlottesville, VA). "More for historic reasons a huge amount of activity has centered around histone H3, but we have probably got at least eight more identified methylation sites on histones. Some of these look like they are very much players."

All those modifications may add up to a combinatorial histone code, as suggested by Allis and others. Whether any of the modifications act as developmental switches is not known, and Allis feels that our understanding of the code is far from complete. "If it [histone modification] is a really important mechanism, there will be back-up and redundancy," he said. "We're going to have to inch our way residue by residue through these histone tails. I think we're going to encounter hundreds of surprises." ■

References: Strahl, B.D., and C.D. Allis. 2000. *Nature*. 403:41–45. Jenuwein, T., and C.D. Allis. 2001. *Science*. 293:1074–1080.

Dynamic repression

Elements used to shut down large blocks of heterochromatin are also used for dynamic regulation of genes that turn on and off every cell cycle, according to Tony Kouzarides (Wellcome/CRC Institute, Cambridge, UK).

Kouzarides and colleagues began with a search for enzymes that put methyl groups on histone H3. The first such enzyme was discovered by Thomas Jenuwein (Research Institute of Molecular Pathology (IMP), Vienna, Austria) and colleagues, who characterized SUV39H1 as a methylase for lysine 9 (K9) of H3.

The Cambridge group had a hunch that some histone methylases would be associated with transcription factors. Indeed, an immunoprecipitation of the tumor suppressor and transcriptional repressor Rb brought down SUV39H1.

The K9 modification has been associated with repressed chromatin, and Kouzarides found that both Rb and SUV39H1 are needed for in vivo repression of certain regulated genes. At one promoter, that of cyclin E, the repression only occurs in G1, raising a question of reversibility. "The chemistry of how you would demethylate is not known," said Gary Felsenfeld (NIH, Bethesda, MD).

"No such enzyme has been identified."

The repressive mechanism appears to involve HP1, which is known to bind K9-methylated H3. HP1 is better known as a mediator of the long-lived shut-down seen in heterochromatin. But Kouzarides found that Rb triggered both K9 methylation and HP1 association at a nucleosome positioned over the transcription start site of the cyclin E promoter.

"Because the nucleosome overlaps the initiation site there may be targeting factors that allow the methylase to

distribution of these modifications; these regions apparently include the active, pseudoautosomal regions of the inactive X.

Jenuwein also reported that K9-methylated H3 covers much of the inactive X, but in addition he found the same variant in the heterochromatin around centromeres. Loss of this methylation causes chromosomes to pair incorrectly during spermatogenesis and stick together near their kinetochores, perhaps secondary to an opening up of the centric heterochromatin. Geneviève Almouzni

(Institut Curie, Paris, France) found that the K9 staining at centric heterochromatin is lost following RNase treatment, raising the intriguing possibility that RNAs are critical to heterochromatin formation.

Now that K9 modifications are known to be common to both impenetrably repressed heterochromatin and a dynamically repressed cell cycle promoter, it is not clear what makes the two types of repression distinct. At the very least there appears to be more than one way of translating K9 methylation into repression, as early evidence suggests that HP1 is not bound to the K9-methylated inactive X. ■

References: Rea, S., et al. 2001. *Nature*. 406:593–599. Nielsen, S.J., et al. 2001. *Nature*. 412:561–565.



Activating acetylation (left) makes way for repressive methylation.

associate with a particular nucleosome," said Kouzarides. The second nucleosome over from the modified nucleosome did not show the same modifications. "What stops it from spreading is uncharacterized," said Kouzarides.

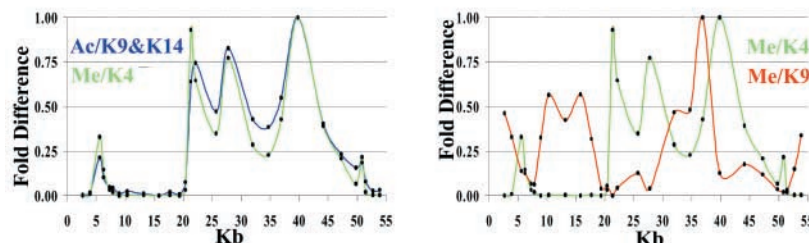
Spreading of repressive environments does occur in heterochromatin, such as on the inactive X chromosome. David Allis (University of Virginia, Charlottesville, VA) reported that most of the inactive X is covered with K9-methylated H3 but lacks K4-methylated H3. The exceptions to this rule are small regions with an opposite

Stopping the spread

Dramatic correlations and anticorrelations between different histone H3 modifications, as reported by Gary Felsenfeld (National Institutes of Health, Bethesda, MD), are yielding clues about how chromatin is divided into active and inactive regions.

Felsenfeld used chromatin immunoprecipitation to track H3 methylation at lysines 4 (K4) and 9 (K9) and a double acetylation of H3 at lysines 9 and 14. He found that acetylation and K4 methylation, both modifications associated with gene activation, closely mirrored each other in different regions of the chicken β -globin locus. In contrast, these modifications were anticorrelated with the repressive K9 methylation.

K9 methylation is particularly prominent in a large region of condensed chromatin. Surrounding this region are peaks of acetylation, one of which is associated with a known insulator element that shelters the



Correlation (left) and anticorrelation (right) across the globin locus.

globin locus from repressive effects.

"The model we are developing is that acetylation at K9 of H3 is actually preventing the methylation on that residue and therefore the propagation of silencing," said Felsenfeld. "That's the terminator of the chain."

A different view of chromatin domains came from Peter Fraser (Babraham Institute, Cambridge, UK). He is studying patients with a particular form of β -thalassemia in which a globin locus boundary element has been deleted. This results in down-regulation of adult β -globin expression and a failure in silencing of fetal

globin genes, suggesting that the boundary elements delimit a fetal domain. Fraser suspects that derepression is related to chromatin remodeling factors that are associated with elongating RNA polymerases during intergenic transcription.

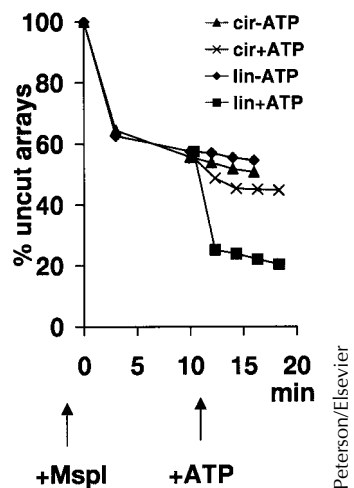
How many of the regions are set up in the first place remains completely mysterious. "Which of these is the first step," asks Felsenfeld, "and how does the cell know how to start off?" ■

Reference: Litt, M.D., et al. 2001. *Science*. 10.1126/science.1064413
<http://www.sciencemag.org/cgi/content/abstract/1064413>

Felsenfeld/AAAS

Time to remodel

Adding core histones to in vitro transcription reactions gave a more realistic picture of what transcription factors were up against, and how they might work in the context of chromatin.



Small DNA circles do not remodel.

Constrain the DNA in a small circle and remodeling fails, but add topoisomerase to the reaction and remodeling activity can once again be seen. This experiment led to the suggestion

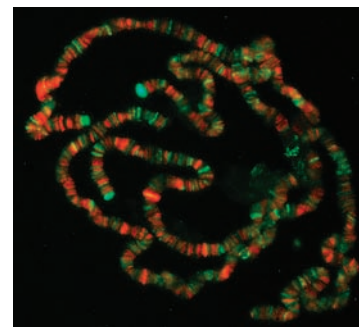
that remodeling complexes affect DNA twist (rotation), writhe (the path of the DNA) or both.

But adding H1 to in vitro reactions shuts down remodeling. Perhaps, said Peterson, this can be explained by H1's ability to prevent changes in DNA topology. H1 binds to DNA as it enters and exits the nucleosome, thus locking the DNA in place. But now Peterson has found that phosphorylation by cyclin-dependent kinases may alleviate this locking, allowing SWI/SNF to do its job.

The topology model is also consistent with data from Robert Kingston (Massachusetts General Hospital, Boston, MA), who contends that remodeling complexes act like chaperones, cycling the DNA through many different topologies until one of those topologies is "trapped" by the binding of a transcription factor.

"For the first time in six years we're pretty much in agreement," said Peterson. "We're all thinking about these topological models." ■

Reference: Gavin, I., et al. 2001. *Mol. Cell*. 7:97–104.



A remodeling complex (green) in regions lacking RNA polymerase (red).

Tamkun/Elsevier