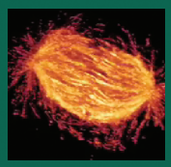

Views from Lake Placid

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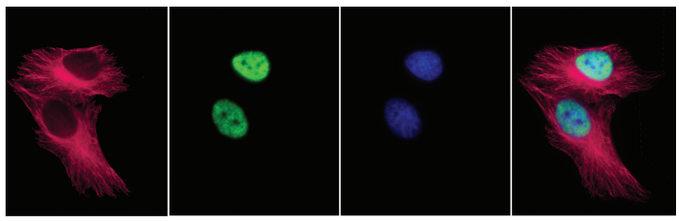
A Brief Survey of the Chromatin Landscape

The process of organizing the millions of base pairs of genetic material in the eukaryotic nucleus has profound effects on DNA-dependant events, such as transcription, recombination, replication and repair. DNA is organized by its incorporation into chromatin, the basic subunit of chromatin being the nucleosome. A nucleosome is composed of 147 base pairs of DNA coiled around an octamer of histone proteins, two molecules each of histone H2A, H2B, H3 and H4. Histone H1 associates with chromatin outside the core octamer unit and regulates higher order chromatin structure. Chromatin is packaged into chromosomes by proteins such as the condensins and the chromatin assembly proteins, but chromatin and chromosomes undergo dramatic and dynamic changes in organization in response to a myriad of cellular signals. Chromosomes condense and relax during the cell division process. Damaged DNA adopts a unique structure that facilitates repair. And critical to cell function, most of the genome must remain in a transcriptionally silent state, save for specific combinations of genes which vary significantly between cell types. These processes must be tightly regulated to maintain the integrity of the genome and the proper function of the cell.

Multiple mechanisms exist within the nucleus that allow the function and organization of the genome to be dynamically regulated, responding rapidly to different signals or insults. During the past fifteen years, it has become evident that ATP-dependant enzymes that reorganize chromatin at the level of the nucleosome (Swi/Snf, Rsc, Iswi…) play an important part in the regulation of genomic organization. In mammals, CpG methylation of DNA is also involved in DNA-dependant processes, tied inextricably to other chromatin-based mechanisms of modulating genome function. Most notably though, a large body of evidence dating back forty years or more has accumulated to indicate that post-translational modification of the histone proteins that form the core of the nucleosome is crucial to all genome-based activity (Table 1; see Kouzarides, 2007 for a recent and comprehensive review of the subject). The recruitment of transcriptional regulators and chromosomal proteins brings along enzymes that modify (by either addition or removal) specific functional groups on histones, and these dynamic addition and subtraction events have profound affects on the structure and function of chromatin. Many of the proteins involved in the deposition or removal of modifications are involved in important cellular process such as cell division, DNA damage repair, cell fate determination, and the maintenance of stem cell pluripotency (see Ringrose and Paro, 2007; Spivakov and Fisher, 2007). So important are these proteins that the alteration in function of a histone modifying enzyme is frequently associated with specific cancers, making histone modifiers attractive targets for therapeutic intervention (see Swamanathan et al., 2007).



Immunodetection of Histone Acetylation in HeLa Cells: *Red: Anti-alpha-Tubulin (AM-0191), Green: Anti-acetyl-Lys23 Histone H3 (AR-0104), Blue: DAPI (CK-0156. Far right: Merge.*

Generally, acetylation of histones is involved with loosening chromatin structure and the activation of gene expression. Deacetylation is correlated with the tightening of chromatin structure and the repression of expression. The only acetylation mark that appears to have an identified role in specific transcriptional processes is Ac-Lys 16 of histone H4 (see Shia et al, 2006). Histone methylation has a more complex role in controlling access to specific regions of the genome than acetylation. Both lysine and arginine residues in histones can be methylated, and both are subject to multiple forms of methylation (mono-, di-, trimethylation of lysines; mono-, symmetric or asymmetric dimethylation of arginines). Also, many more examples exist where the methylation of a specific amino acid is associated with a unique chromosomal phenomenon (e.g., lysine 36 methylation of H3 and transcriptional elongation). With regard to histone phosphorylation, many such events are involved in chromosome condensation and segregation. Other marks, such as sumoylation and ubiquitylation have functional roles just now being made clear.

continued...

As complex as the histone modification landscape is, it is even more so since the identification of a co-repressor protein complex member, BHC110, as the first histone demethylase. This groundbreaking discovery helped make possible the identification of dozens more demethylating enzymes and has opened up a new field of study for researchers studying chromatin biology and its influence on the regulation of genomic activity (see Shi, 2007). Many of these novel demethylases have been previously identified and studied for their roles in development, or have been found to exist as part of complexes containing widely studied transcription factors. A large amount of work is going regarding demethylases, not the least of which involves identifying the catalytic mechanisms of the different families of demethylases, the substrate specificity and the importance of demethylation in the grand scheme of genome regulation and organization.

As the role of a few of the vast number of histone modifications was uncovered, the existence of a "histone code" was put forth, helping to make sense of the panoply of histone modifications and to try to predict and define cellular events associated with specific modification patterns. The histone code hypothesis suggests that post-translational modifications (or combinations of modifications) confer specific biological functions and that modified histones recruit specialized chromatin-interacting proteins that facilitate the defined function. It is believed that these histone-effector protein interactions give rise to downstream protein recruitment and enzyme and substrate interactions (Table 1). Proteins such as HP1 and Pc bind to repression-associated methylation events on histone H3 to maintain regions of the genome in a repressed state. The Bromo domain binds to acetylated histones and appears to be involved in recruitment of histone modifying complexes. It is likely that other such domains remain to be discovered, which will help identify the molecular mechanisms by which histone modifications exert their influence on the genome.

Table 1:

Histone Variant	Identity vs. Core Histone	Function
H2A.X	96%	DNA damage repair; ser in SQEY motif in mammalian H2AX phosphorylated by ATM and ATR in response to double strand DNA breaks
H2A.Z	60-65%	Transcriptional regulation, DNA damage, chromosome stability
macroH2A	64%	Enriched on inactive mammalian X chromosome; unique N-terminal addition
CENP-A	highly diverged	Centromere-specific; specialized protein for attachment of kinetochore
H3.3	97%	Replaces H3.1 at transcriptionally active regions; varies at 4 amino acids

In addition to the core histones, several unique versions of the histone proteins exist that are not identical to the canonical core proteins. These "variant" histones have unique functions, unique expression patterns and in some cases are restricted to specific sub-nuclear domains (see Table 2 and Henikoff and Ahmad, 2005).

Currently, the chromatin immunoprecipitation (ChIP) technique is the most precise method to identify specific proteins associated with a region of the genome, or conversely, identification of regions of the genome associated with specific proteins. However, much of the recent usage of ChIP has been for identifying and analyzing transcription factor binding sites.

Table 2:

Modification	Associated Pathway	Modification Binding Domain
Acetylation	Transcriptional Regulation; DNA Repair; Chromosome Condensation	Bromo
Methylation-Lysine: mono, di-, tri-	Transcriptional Regulation; DNA Repair; Heterochromatin Formation	Chromo, MBT, Tudor, PHD
Methylation-Arginine: mono-, asym. di-, sym. di-	Transcription	
Phosphorylation: Ser, Thr	Chromosome Condensation; Transcriptional Regulation	14-3-3
Ubiquitylation: Lys	Transcriptional Regulation	
Sumoylation: Lys	Transcriptional Regulation	
ADP-ribosylation: Glu	Transcriptional Regulation	

New techniques that combine the ChIP technique with microarray analysis facilitate the determination of genome-wide distribution patterns for chromosomal proteins and histone modifications. With this combined technique, researchers can ask sweeping questions regarding cellular functions, and place the protein or modification in the larger context of regulatory network interactions. Recent advances in high-throughput DNA sequencing threaten to make ChIP on chip obsolete, as vastly larger and more accurate data sets can be generated (see Barski et al., 2007). As powerful as the ChIP technique is, the chromatin field is in need of other such versatile techniques that can help researchers understand the nature of histone modifications, chromatin organization and their role in biology and disease.

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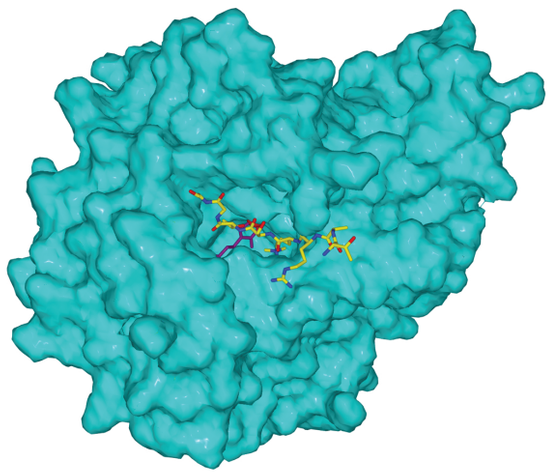

Resetting Histone Methylation: *Crystal structure of the catalytic domain of the histone demethylase JMJD2A bound to a peptide derived from the amino terminus of histone H3, trimethylated at lysine 9 (yellow carbon atoms). Also shown is the substrate alpha-ketoglutarate (purple) and a Ni(II) cation (red) which is substituted for the Fe(II) normally present. Image courtesy Dr. Ray Trievel, Department of Biological Chemistry, University of Michigan.*

Antibodies and Purified Proteins

		Description	Applications	Reactivity	Cat. No.	Vial Size	USD
ASH2		Anti-ASH2	WB IF IP	Hu WR*	AR-0175-200	200 µl	$275
ATM		Anti-p-ATM (Ser1981), Clone 10H11.E12	IF	Hu Ms	AM-0173-200	200 µl	$275
Copine I		Anti-Copine I	WB	Hu WR*	AR-0167-200	200 µl	$275
EZH2		Anti-EZH2, polyclonal	WB IF ChIP IHC	Hu Ms	AR-0163-200	200 µl	$275
Hbo1	***NEW***	Anti-Hbo1, polyclonal	WB	Hu Ve*	AR-0197-200	200 µl	$275
HDAC		Anti-HDAC1, clone 10E2	WB IF IHC IP ChIP	Hu Ms Rt	AM-0131-200	200 µl	$275
		Anti-HDAC2, clone 3F3	WB IF IHC IP ChIP	Hu Ms Rt	AM-0132-200	200 µl	$275
Histone H2A		Anti-Histone H2A, acidic patch	WB	Hu WR*	AR-0189-200	200 µl	$275
	NEW	Anti-phospho-Ser1 Histone H2A / Histone H4	WB	Hu WR*	AR-0200-200	200 µl	$275
	NEW	Anti-acetyl-Lys5 Histone H2A	WB	Hu WR*	AR-0201-200	200 µl	$275
	NEW	Anti-acetyl-Lys9 Histone H2A	WB	Hu WR*	AR-0256-200	200 µl	$275
Histone H2AX		Anti-phospho-Ser139 Histone H2AX	WB IF	Hu Ma*	AR-0149-200	200 µl	$275
Histone H2A.Z	***NEW***	Anti-Histone H2A.Z	WB IF	Hu Ma*	AR-0255-200	200 µl	$275
Histone H2B		Anti-Histone H2B	WB IF	Hu Ma*	AR-0139-200	200 µl	$275
	NEW	Anti-acetyl-Lys5 Histone H2B	WB IF	Hu WR*	AR-0228-200	200 µl	$275
	NEW	Anti-acetyl-Lys16 Histone H2B	WB IF	Hu WR*	AR-0227-200	200 µl	$275
Histone H3		Anti-Histone H3, CT	WB IF ChIP	Hu Sc WR*	AR-0144-200	200 µl	$275
Histone H3 acetyl		Anti-acetyl Histone H3	WB	Hu WR*	AR-0143-200	200 µl	$275
		Anti-acetyl-Lys9 Histone H3	WB	Hu WR*	AR-0102-200	200 µl	$275
		Anti-acetyl-Lys14 Histone H3	WB	Hu WR*	AR-0142-200	200 µl	$275
		Anti-acetyl-Lys18 Histone H3	WB IF	Hu WR*	AR-0103-200	200 µl	$275
		Anti-acetyl-Lys23 Histone H3	WB IF	Hu WR*	AR-0104-200	200 µl	$275
		Anti-acetyl-Lys27 Histone H3	WB ChIP	Hu Sc WR*	AR-0105-200	200 µl	$275
		Anti-acetyl-Lys27 Histone H3	WB IF ChIP	Hu Sc WR*	AR-0129-50	50 µl	$150
Histone H3 phospho		Anti-phospho-Ser10,28 Histone H3	WB	Hu WR*	AR-0140-200	200 µl	$275
	NEW	Anti-phospho-Thr11 Histone H3	WB	Hu WR*	AR-0257-200	200 µl	$275
	NEW	Anti-phospho-Ser28 Histone H3	WB	Hu WR*	AR-0198-200	200 µl	$275
Histone H3 methyl	***NEW***	Anti-dimethyl-Lys4 Histone H3	WB IF	Hu WR*	AR-0168-200	200 µl	$275
	NEW	Anti-trimethyl-Lys4 Histone H3	WB IF ChIP	Hu WR*	AR-0169-200	200 µl	$275
		Anti-dimethyl-Lys9 Histone H3	WB IF ChIP	Hu WR*	AR-0108-50	50 µl	$150
	NEW	Anti-trimethyl-Lys9 Histone H3	WB IF	Hu WR*	AR-0170-200	200 µl	$275
		Anti-trimethyl-Lys27 Histone H3	WB IF	Hu WR*	AM-0150-200	200 µl	$275
	NEW	Anti-trimethyl-Lys27 Histone H3	WB ChIP	Hu WR*	AM-0174-200	200 µl	$275
	NEW	Anti-trimethyl-Lys27 Histone H3	WB IF	Hu WR*	AR-0171-200	200 µl	$275
	NEW	Anti-trimethyl-Lys27 Histone H3	WB IF	Hu WR*	AR-0199-200	200 µl	$275
	NEW	Anti-monomethyl-Lys79 Histone H3	WB IF ChIP	Hu WR*	AR-0172-200	200 µl	$275
	NEW	Anti-dimethyl-Lys79 Histone H3	WB	Hu WR*	AR-0177-200	200 µl	$275
Histone H4 acetyl		Anti-tetra-acetyl Histone H4	WB ChIP	Hu Sc WR*	AR-0109-50	50 µl	$150
		Anti-tetra-acetyl Histone H4	WB IF	Hu Sc WR*	AR-0120-50	50 µl	$150
		Anti-acetyl-Lys5 Histone H4	WB IF ChIP	Hu Sc WR*	AR-0119-200	200 µl	$275
		Anti-acetyl-Lys8 Histone H4	WB	Hu WR*	AR-0138-200	200 µl	$275
		Anti-acetyl-Lys12 Histone H4	WB IF ChIP	Hu Sc WR*	AR-0106-200	200 µl	$275
		Anti-acetyl-Lys16 Histone H4	WB ChIP	Hu Sc WR*	AR-0107-200	200 µl	$275
Histone H4 methyl		Anti-monomethyl-Lys20 Histone H4	WB IF	Hu WR*	AR-0134-200	200 µl	$275
		Anti-dimethyl-Lys20 Histone H4	WB IF	Hu WR*	AR-0135-200	200 µl	$275
		Anti-dimethyl-Lys20 Histone H4	WB	Hu WR*	AM-0165-200	200 µl	$275
		Anti-trimethyl-Lys20 Histone H4	WB IF ChIP	Hu WR*	AR-0136-200	200 µl	$275
L3MBT		Anti-L3MBTL1	WB IP ChIP	Hu Ve*	AR-0160-200	200 µl	$275
LexA		Anti-LexA DNA Binding Domain	WB IP	tagged fusions	AR-0166-200	200 µl	$275
LSD1		Anti-LSD1	WB IP	Hu Ve*	AR-0162-200	200 µl	$275

continued...

Antibodies (AR-#### are rabbit polylconal; AM-#### are mouse monoclonal)

Applications Key: **WB**-Western **IP**-Immunoprecipitation **IHC**-Immunohistochemistry **IF**-Immunofluorescence **ChIP**-Chromatin IP **FC**-Flow cytometry

Species Key: **Hu**-Human **Ms**-Mouse **Rt**-Rat **Sc**-Budding yeast **Sp**-Fission yeast **Dr**-Drosophila **Ve**-Vertebrate **Ma**-Mammal **Eu**-Eukaryote ***-predicted

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		Description	Applications	Reactivity	Cat. No.	Vial Size	USD
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PP2A	*NEW*	Anti-PP2A	WB	Hu WR*	AR-0190-200	200 µl	$275
Rad51		Anti-Rad51	WB IF IHC IP	Hu	AR-0100-200	200 µl	$275
Rad52		Anti-Rad52	IF IHC	Hu	AR-0101-200	200 µl	$275
RbAp46/48	*NEW*	Anti-RbAp46/48	WB ChIP	Hu WR*	AR-0178-200	200 µl	$275
SNF2		Anti-SNF2h	WB IP	Hu Ma	AM-0152-200	200 µl	$275
Tubulin	*NEW*	Anti-alpha-Tubulin, clone 5-B-1-2	WB IF IP	Hu Ms WR*	AM-0191-200	200 µl	$275

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		Chromatin Immunopreciptiation Kit			KA-013-100	100 assays	$500

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