

A Guide to Epigenetics



Introduction

Epigenetics refers to heritable changes in gene expression that occur without alteration in DNA sequence. These changes may be induced spontaneously, induced by environmental factors or as a consequence of specific mutations.

There are two primary and interconnected epigenetic mechanisms: DNA methylation and covalent modification of histones. In addition, it has become apparent in the last years that non-coding RNA is also intimately involved in this process.

The different mechanisms that control epigenetic changes do not stand alone, and there is a clear interconnection and interdependency between:

- DNA methylation
- Histone modification and incorporation of histone variants
- Non-coding RNA-mediated epigenetic regulation

These mechanisms, together with action of transcription factors and ATP-dependent chromatin remodeling, will establish unique epigenetic states resulting in alterations of gene expression that for example can determine cellular diversity with virtually no differences in DNA sequences.

Epigenetic modifications are central to many cellular processes and essential to many organism functions, such as imprinting, X chromosome inactivation, cellular reprogramming and senescence. But if these modifications occur improperly, they can lead to major adverse health effects such as cancer or congenital diseases. Because of their role in cancer development, epigenetic modifications offer promise as potential novel biomarkers for early cancer detection and prognosis. Furthermore, the possibility of reversing epigenetic modifications represents a potential target of novel therapeutic strategies and medication design.

DNA methylation

DNA methylation, the addition of a methyl- group to one of the bases in the deoxyribonucleic acid chain, does not change the primary DNA sequence and it is therefore considered to be an epigenetic modification. DNA methylation is generally repressive to transcription, therefore constituting an important mechanism for gene silencing in embryonic development and inactivation of defined tumor suppressor genes in human cancers.

Although cytosine methylation is the most studied modification, adenine has been found to be methylated in prokaryotes and plants. In prokaryotes, DNA methylation is involved in processes such as determination of DNA-host specificity, virulence, cell cycle regulation and gene expression. In higher eukaryotes, DNA methylation is involved in the regulation of several cellular processes such as chromatin stability, imprinting, X chromosome inactivation and carcinogenesis.

In mammals, DNA methylation occurs mainly on the fifth carbon of the cytosine base, forming what is known as 5-methylcytosine or **5-methylcytidine (5-mC)**, and it is almost exclusively found at CpG dinucleotides. 5-mC is a potent epigenetic marker and regulator of gene expression. Methylated CpG clusters – named **CpG islands** – at gene promoters have been associated with gene inactivation. DNA methylation is catalyzed by a family of enzymes called DNA methyltransferase and includes DNMT1, DNMT3a and DNMT3b. **DNMT3a** and **DNMT3b** are known as **de novo methyltransferases** and they are able to methylate previously unmethylated CpG dinucleotides. In contrast, **DNMT1**, is known as a **maintenance methyltransferase**, and it methylates hemi-methylated DNA during replication.

The reverse reaction - DNA demethylation – is only beginning to be elucidated. It is believed to involve the successive oxidation of 5-mC to 5-hydroxymethyl- (5-hmC), 5-formyl- (5-fC), and 5-carboxy- (5-caC) cytosine in a process that involves the Tet family of enzymes. A glycosylase of the BER (Base Excision Repair) pathway, TDG, was also recently involved in an alternative demethylation pathway, where it can repair the 5-hydroxymethyluracil which can result from deamination of 5-hmC (see figure 1).

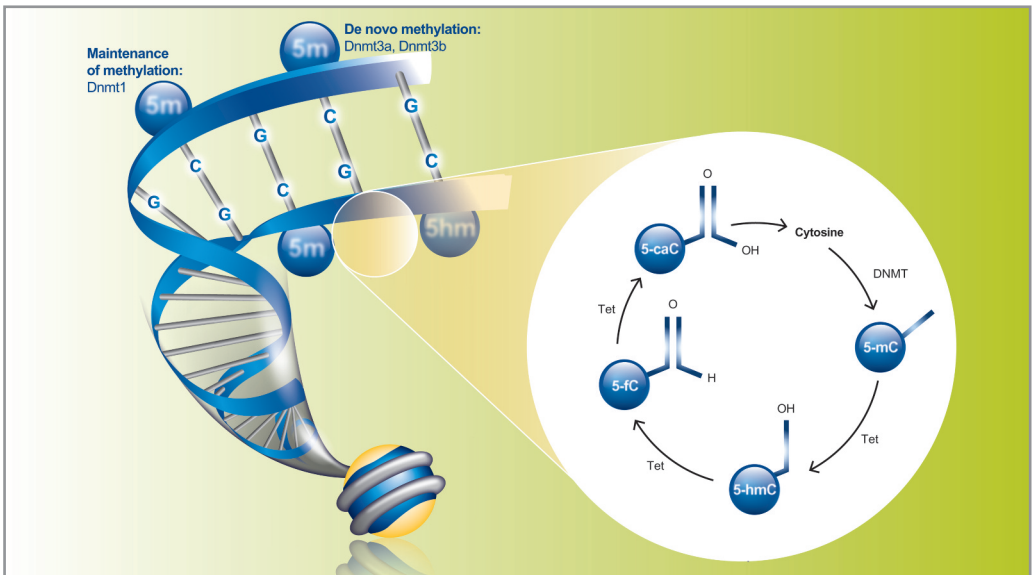


Figure 1. DNA methylation and demethylation overview

Currently, the main approaches to study DNA methylation are:

- a) Sodium bisulfite modification
- b) Sequence-specific enzyme digestion
- c) Capture/quantification of methylated DNA

a) Sodium bisulfite modification

Sodium bisulfite modification, also known as **bisulfite conversion**, is one of the most useful tools for analyzing cytosine methylation. This method is based on treating DNA with sodium bisulfite in order to determine its methylation pattern.

Treatment of DNA with sodium bisulfite leads to the deamination of cytosine residues and converts them to uracil, while 5-mC residues remain the same.

The treatment will generate specific changes in the DNA sequence that will depend on the methylation status of individual cytosine residues, potentially providing single-nucleotide resolution information about the methylation status of a DNA region: cytosine residues that have been converted to uracil will be detected as thymidine residues whereas methylated cytosine residues will be detected as cytosines.

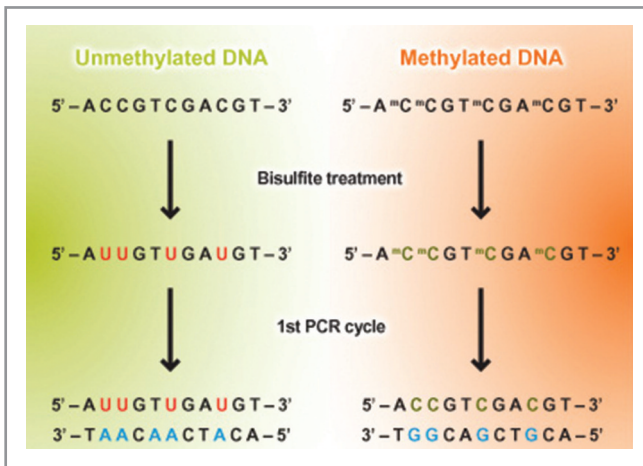


Figure 2. Overview scheme of bisulfite modification

Bisulfite-modified DNA can be analyzed by PCR methods that can discriminate the methylation state of cytosines in specific genomic regions. Alternatively, bisulfite treatment can be coupled to next generation sequencing to achieve single nucleotide resolution mapping of cytosine methylation across the whole genome.

b) Sequence-specific enzyme digestion

This method involves the use of methylation-sensitive restriction endonucleases to produce DNA fragments based on the methylation status of the sequence. In general, a methylation-sensitive enzyme (i.e., an enzyme that cannot cut methylated DNA) and a methylation-insensitive isoschizomer (i.e., an enzyme which cuts the same DNA sequence regardless of whether this is methylated or not) are used to digest the same DNA sample. Comparison of the sensitive versus insensitive digestion leads to a differentiated band digestion pattern on an agarose gel which indicates the methylation status of the cytosines in the analyzed DNA sequence.

This technique can also be used in combination with other methods such DNA sequencing, end point and RT-PCR or Southern blotting to provide methylation status information.

c) Capture/quantification of methylated DNA

The use of methylated DNA-binding proteins or antibodies that specifically recognize methylated DNA is another common method for studying methylated DNA.

Antibodies can be used in an ELISA-type assay to specifically detect the percentage of methylated or hydroxymethylated cytosine residues in the sample: the higher the signal, the higher percentage of methylated DNA.

Antibodies can be also used to immunoprecipitate methylated DNA, thereby enriching a DNA sample in methylated DNA. This technique is also known as **MeDIP (Methylated DNA Immunoprecipitation)** and it can be coupled to locus specific PCR or to whole genome approaches such as microarrays or next generation sequencing. Similar techniques have been developed using methylated DNA-binding proteins instead of antibodies.

Highlighted Products

Product highlights

Product name	Application	Product code
EpiSeeker Bisulfite Modification Kit – Whole Cell	Bisulfite modification of methylated DNA directly in cells	ab117126
EpiSeeker methylated DNA Quantification Kit (Colorimetric)/(Fluorometric)	Quantification of methylated DNA using an ELISA-based method	ab117128 ab117129
EpiSeeker hydroxymethylated DNA Quantification Kit (Colorimetric)/(Fluorometric)	Quantification of hydroxymethylated DNA using an ELISA-based method	ab117130 ab117131
EpiSeeker MeDIP Kit – DNA/cells/tissue	Immunoprecipitation of methylated DNA using DNA/cells/tissue as starting material	ab117133 ab117135 ab117136
EpiSeeker hMeDIP Kit	Immunoprecipitation of hydroxymethylated DNA using DNA as starting material	ab117134
Rat monoclonal [AB3/63.3] to 5-hydroxymehtyl Cytidine	To be used in ICC/IF, IP or MeDIP	ab106918
Mouse monoclonal [33D3] to 5-methyl Cytidine – CHIP grade	To be used in WB, MeDIP or CHIP	ab10805
Rabbit monoclonal [EPR3521(2)] to Dnmt1	To be used in WB, IHC-P or Flow Cyt	ab134148
Rabbit monoclonal [EPR2408] to Dnmt3a	To be used in WB or IP	ab134142
5-Aza-2'-deoxycytidine	Potent DNA methyltransferase inhibitor; epigenetic modifier (99% purity)	ab120842
Chlorogenic acid hemihydrate	DNMT1 inhibitor (99% purity)	ab120973

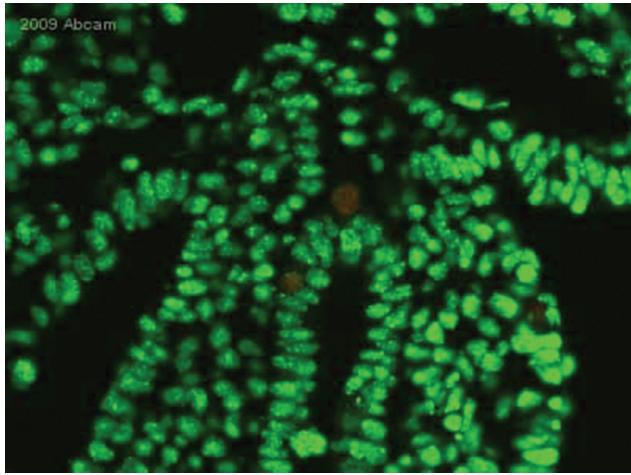


Figure 3. 5-methyl cytidine staining with mouse monoclonal [33D3] anti-5-methyl cytidine antibody (ab10805).

Image shows a paraformaldehyde-fixed section of pig embryo (15 – 17 days).

Image courtesy of an Abreview submitted by Ms Sara Maj Wätjen Hyldig.

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Chromatin

Chromatin is comprised of histones and DNA: a 147bp of DNA chain wrapped around the 8 core histone forms the basic chromatin unit, the nucleosome. The primary functions of chromatin are to package DNA into a smaller volume to fit in the cell, to strengthen the DNA to allow mitosis and meiosis and prevent chromosome breakage, and to control gene expression and DNA replication. In mammals, chromatin is mainly found as a condensed transcriptionally silent form called **heterochromatin**, which constitutes telomeres, pericentric regions and areas rich in repetitive sequences. **Euchromatin** is instead less condensed, and it contains most actively transcribed genes.

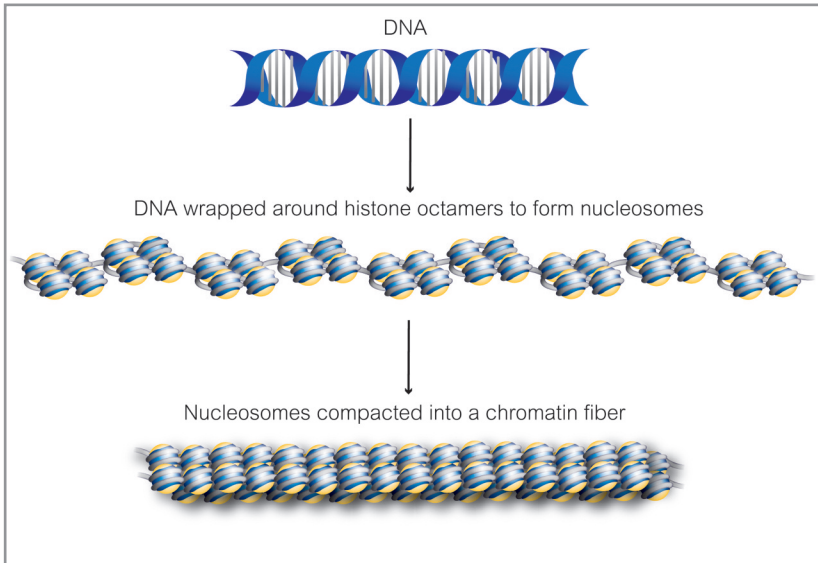


Figure 4. DNA wraps around the histone proteins to form nucleosomes; these in turn couple to become the chromatin fiber.

A vast amount of proteins participate in shaping chromatin structure, including histones and other chromatin interacting proteins such as transcription factors and DNA repair proteins. Chromatin remodeling complexes have the ability of changing the chromatin architecture by modulating the interaction between nucleosomes and DNA, which is often achieved by adding post-translational modifications to histones.

Analysis of chromatin associated factors

In general, heterochromatin is difficult to analyze, not the least because of its condensed state and generally repetitive DNA sequence, whereas euchromatin poses less challenges as it contains active genes and maintains an open and extended structure.

Chromatin Immunoprecipitation (ChIP) has become the optimal technique of choice to study chromatin modifications, as it can be used to dissect the spatial and temporal dynamics of the interactions of chromatin and its associated factors, including transcription factors and histone modifications. This technique allows to map minute-by-minute changes of chromatin associated proteins at a single promoter, or to determine their binding sites over the entire human genome.

The principle of ChIP is simple: the selective enrichment of a chromatin fraction containing a specific antigen. Antibodies that recognize a protein or protein modification of interest can be used to determine the relative abundance of that antigen at one or more locations (loci) in the genome.

Traditionally, end point and/or quantitative PCR are performed after ChIP to verify whether a particular DNA sequence is associated with the protein of interest. Using this classical approach, researchers can evaluate the interaction of a protein of interest with a limited number of targets. However, genome-wide studies are now possible through the use of 'ChIP on chip' and 'ChIP-seq', which couple ChIP with either microarray platforms or next generation sequencing.

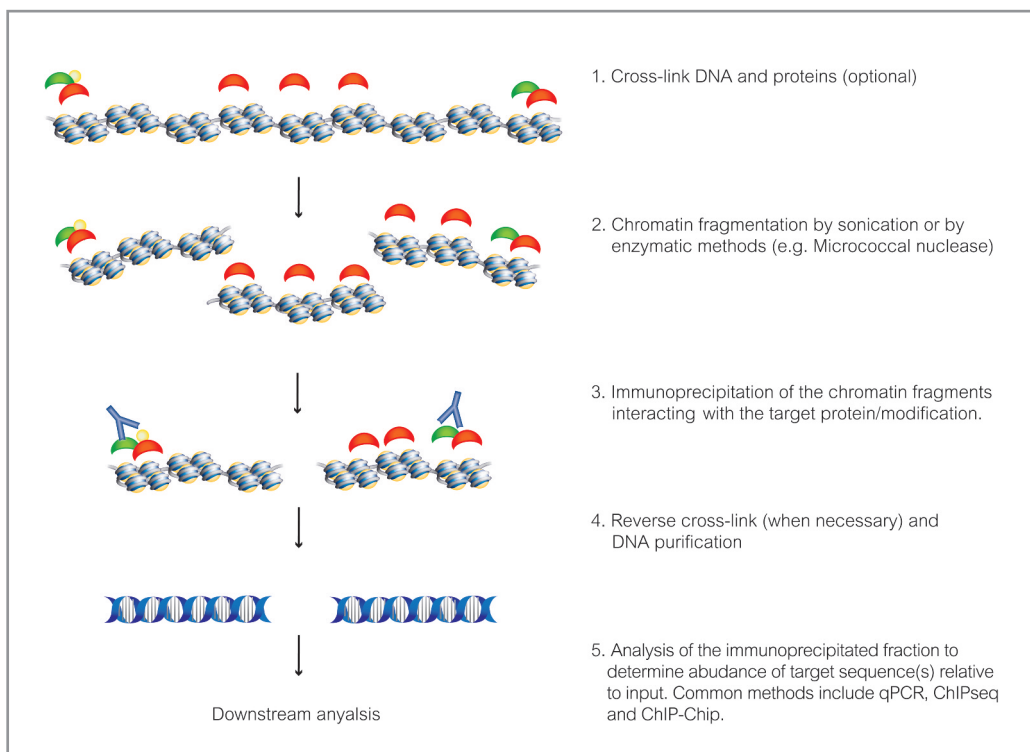


Figure 5. Schematic overview of ChIP protocol

Product name	Application	Product code
Anti-Histone H3 (tri methyl K4) antibody – ChIP grade	Can be used as positive control for ChIP	ab8580
Anti-GFP antibody – ChIP grade	Can be used as negative control for ChIP	ab290
EpiSeeker ChIP Kit – One Step	Perform ChIP analysis in only 5 hours; use with your ChIP-grade antibody of choice	ab117138
EpiSeeker ChIP Kit - Plants	Specifically design to be used with plant cells	ab117137

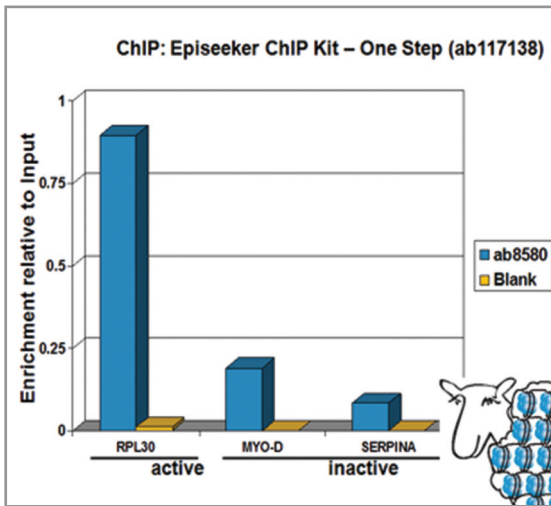


Figure 6. ChIP performed on HeLa cells using anti-histone H3 tri methyl K4 antibody (ab8580) together with EpiSeeker ChIP Kit – One Step (ab117138). No antibody was added to the blank (yellow). The immunoprecipitated DNA was quantified by RT-PCR. Primers and probes are located in the first Kb of the transcribed region.

Tips for ChIP

Cross-link: necessary or not?

The aim of cross-linking is to fix the antigen of interest to its chromatin binding site, so the further away the interaction of interest lies the less effective ChIP will be without cross-linking. ChIP for histone modifications is unlikely to require cross-linking whereas non-histone proteins such as transcription factors and proteins contained within DNA binding complexes will probably need cross-linking.

If cross-linking is used, ChIP reaction will be known as X-ChIP, whereas a native ChIP reaction will be known as N-ChIP.

Chromatin fragmentation

Fragmentation of the chromatin is required to make interactions accessible to antibody reagents. The method chosen for fragmentation will depend on the type of ChIP experiment performed.

N-ChIP: enzymatic digestion with micrococcal nuclease should be able to generate single monosomes (~175bp). This enzymatic cleavage will not produce “random” sections of chromatin, as micrococcal nuclease favors certain areas of genome sequence over others and will not digest DNA evenly. Be sure to run a new time course every time you set up an experiment.

X-ChIP: sonication is necessary as formaldehyde cross-linking restricts the access of enzymes to their targets. Sonication creates randomly sized DNA fragments (average of 500 – 700bp), with no preferential cleavage for a section of the genome.

Antibodies for ChIP

If available, use an antibody that has been fully characterized and labeled as ChIP-grade. If there is none available, then an antibody that works in normal immunoprecipitation is a good candidate.

A polyclonal antibody is preferable to a monoclonal: monoclonal antibodies recognize only a single epitope, which could be masked during the cross-linking process.

As a positive antibody control for the technique, histone H3 (tri methyl K4) is a popular positive control to use when chipping active genes. As a negative control, use an antibody that recognizes a non-chromatin epitope such as anti-GFP antibody. Remember though that these antibodies are not positive and negative controls *per se*, as this will depend on the locus you are studying: if there is no histone H3 (tri methyl K4) at the particular locus of interest, the best ChIP antibody in the world will not immunoprecipitate anything from this region and therefore will not be an appropriate positive control.

For more information scan here



Histones

Histones pack and order the DNA into nucleosomes, the building blocks of chromatin. Each nucleosome contains two subunits each of histones H2A, H2B, H3 and H4, known as the **core histones**. The **linker histone** H1 does not form part of the nucleosome itself but seems to act as stabilizer of the internucleosomal DNA.

Histones are characterized by a large number of post-translational modifications, which serve to allocate the genome into “active” regions or euchromatin where DNA is accessible for transcription, and “inactive” regions or heterochromatin where DNA is more compact and therefore less accessible for transcription.

At least nine different types of histone modifications have been described, each catalyzed by a specific set of enzymes. The best understood modifications are lysine acetylation, lysine and arginine methylation, serine/threonine/tyrosine phosphorylation, and serine/threonine ubiquitylation. Other modifications include GlcNAcylation, citrullination, crotonylation and proline isomerization.

It is now well established that histone modifications do not stand alone and that there is an intense cross-talk between them, which can occur on the same histone (cross-talk in cis), between different histones within the same nucleosome (cross-talk in trans), or across different nucleosomes (nucleosome cross-talk).

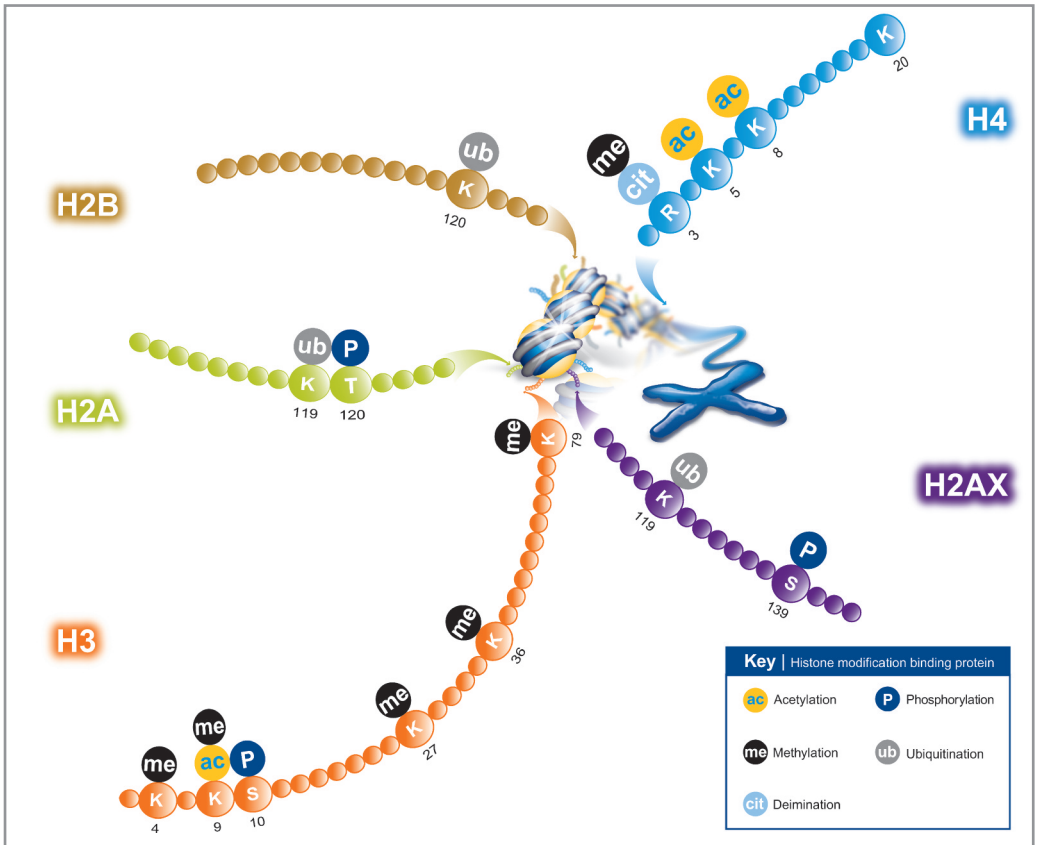


Figure 7. Schematic of the most common epigenetic modifications.

Histone Acetylation

Acetylation is one of the most widely studied histone modifications, as it was one of the first described and linked to transcriptional regulation.

Acetylation on lysine residues leads to relaxation of the chromatin structure and allows the binding of transcription factors and significantly increases gene expression. The enzymes responsible for regulating the acetylation of histone tails are histone acetyltransferases (HAT) and deacetylases (HDAC).

While all histones can be acetylated, lysine residues within H3 and H4 are a preferential target for HAT complexes. Histone acetylation is largely targeted to promoter regions, known as promoter-localized acetylation; however, low levels of global acetylation are also found throughout transcribed genes although the function of this type of acetylation is still unclear.

Histone Methylation

Unlike acetylation, histone methylation does not alter the charge of the modified residues and it is therefore less likely to directly alter nucleosomal interactions required for chromatin folding. This probably explains why histone methylation can either repress or activate transcription depending on location.

Arginine methylation of histone H3 and H4 promotes transcriptional activation, whereas lysine methylation of histone H3 and H4 is implicated in both transcriptional activation and repression, depending on the methylation site. In addition, lysine residues can be methylated in the form of mono-, di-, or tri-methylation, providing further functional diversity to each site of lysine methylation. For example, tri-methylation on K4 of Histone H3 (H3K4me3) is generally associated with transcriptional activation, whereas tri-methylation on K9 and K27 of histone H3 (H3K9me3 & H3K27me3) are generally associated with transcriptional repression.

For many years, histone methylation was thought to be irreversible as it is a stable mark propagated through multiple cell divisions. However, it was recently shown that, similarly to histone acetylation, methylation is an actively regulated and reversible process.

There are two types of histone methyltransferases: lysine-specific histone methyltransferases, which can be SET-domain containing or non-SET-domain containing, and arginine-specific histone methyltransferases belonging to the PRMT (protein arginine methyltransferases) family.

Histone demethylases can also be classified based on the residue they modify: KDM1/LSD1 (lysine specific demethylase 1) is involved in demethylation of mono- and di-methylated lysines, while JmjC (Jumonji domain-containing) histone demethylases are able to demethylate mono-, di-, or tri-methylated lysines.

Removal of arginine methylation is mediated through conversion of the methylated arginine to citrulline by PAD4/PADI4, and citrullinated histones are then either replaced or converted back to unmodified histone by an unknown mechanism.

Histone Phosphorylation

It has recently been shown that all nucleosome core histones are phosphorylated, and that this modification is critical as intermediate step in chromosome condensation during cell division, transcriptional regulation and DNA damage repair. Contrary to acetylation and methylation, histone phosphorylation seems to function by establishing interactions between other histone modifications and serving as platform for effector proteins, leading to a downstream cascade of events.

Known markers for mitosis are phosphorylation of histone H3 at S10, involved in chromatin compaction and phospho-T120 in histone H2A, linked to regulation of chromatin structure and function during mitosis. On the other hand, phosphorylation of H2AX at S139 (resulting in γ H2AX), has been identified as one of the earliest event occurring after DNA double-strand break and serves as recruiting point for DNA damage repair proteins. Histone H2B phosphorylation hasn't been studied as well as H3 and H2A phosphorylation, but recent findings suggest that this modification facilitates apoptosis-related chromatin condensation, DNA fragmentation and cell death.

Histone Ubiquitylation

Histone H2A and H2B are two of the most abundant ubiquitylated proteins found in the nucleus, although ubiquitylated histone H3 and H4 have also been described. The most abundant forms of ubiquitylated histones are monoubiquitylated H2A on K119 and monoubiquitylated H2B on K123 (yeast)/K120 (vertebrates). However, polyubiquitylated histones have also been described, such as K63-linked polyubiquitylation of H2A and H2AX. Monoubiquitylation of H2A is catalyzed by Polycomb group proteins, and it is mostly associated with gene silencing. The main enzyme responsible for monoubiquitylated H2B is Bre1 in yeast and its homologues RNF20/RNF40 in mammals. Contrary to H2A, monoubiquitylated H2B is mainly associated with transcription activation. Like other histone modifications, monoubiquitylation of H2A and H2B is reversible, and it is tightly regulated by histone ubiquitin ligases and deubiquitylating enzymes.

Histone ubiquitylation plays a central role in the DNA damage response: RNF8/RNF168 catalyzed K63-linked polyubiquitylation of histone H2A/H2AX provides a recognition site for RAP80 and other DNA repair proteins, and monoubiquitylation of histones H2A, H2B, and H2AX is also found at the sites of DNA DSBs (double strand-breaks).

Biological Functions of Histone Modifications

Here are few examples of some of the processes in which histone modifications play an important role and how to study them:

Process	Which histone is involved?	How to study?	What product to use (abID)?
DNA damage	Phosphorylated S139 on H2A.X (γH2AX)	IF staining of γH2AX foci	Specific antibody that works in IF/ICC (ab2893)
Mitosis	Phosphorylated S10 on H3	IF staining of phosphoS10-H3 foci	Specific antibody that works in IF/ICC (ab5176)
		FACS analysis of cells expressing phosphoS10-H3	Specific antibody that works in Flow Cytometry (ab5176)
		ELISA-based <i>in situ</i> quantification of phosphoS10-H3 in cells	Microplate ELISA-based assay for <i>in situ</i> quantification (ab115126)
Development/differentiation	Histone H2A, histone H2B, histone H3 or histone H4	IF/ICC staining or IHC of modification on cells in different development stages	Specific antibody that works in IF/ICC (ab5823)
		WB comparing modification expression on cells in different development stages	Specific antibody that works in WB (ab8580)
		ELISA-based quantification using cell/ tissue lysates in different development stages	Microplate ELISA-based assay for specific quantification (ab115108)
		ChIP during differentiation experiments	Specific ChIP-grade antibodies for histone modifications & transcription factors (ab9050, ab1) ChIP Kits (ab117142)
Chromatin structure and dynamics	Acetylated and methylated histone H3 and histone H4	WB comparing modification levels	Specific antibody that works in WB (ab8580)
		IF/ICC staining or IHC of modification on cells in different development stages	Specific antibody that works in IF/ICC (ab5823)
		ELISA-based quantification using cell/ tissue lysates in different development stages	Microplate ELISA-based assay for specific quantification (ab115108)
Gene Activation	Histone H3 (acetyl K9), H3 (methyl K4), H3 (methyl K36) and H3 (methyl K79)	ChIP	ChIP-grade antibodies for the specific histone modifications (ab10812, ab8580, ab9050, ab2621)
			ChIP kits for specific histone modifications (ab117143)
Gene Repression	Histone H3 (methyl K9) and H3 (methyl K27)	ChIP	ChIP-grade antibodies for the specific histone modifications (ab9045, ab2886)
			ChIP kits for specific histone modifications (ab117141, ab117146)

NON-CODING RNAs

Recent studies have revealed that about 90% of the eukaryotic genome is transcribed. Interestingly, only 1 – 2% of these transcripts encode for proteins; the majority are transcribed as **non-coding RNAs (ncRNAs)**. It is increasingly becoming clear that ncRNAs play a big part in epigenetic regulation of gene expression in addition to their roles at the transcriptional and post-transcriptional level.

Non-coding RNAs can be divided into two main types: infrastructural and regulatory ncRNAs. Infrastructural ncRNAs seem to have a housekeeping role in translation and splicing and include species such as ribosomal, transfer, small nuclear RNAs. Regulatory ncRNAs are more interesting from an epigenetic point of view as they are involved in the modification of other RNAs. They can be further classified into:

MicroRNAs (miRNAs)

MiRNAs are small single-stranded molecules (20 – 24 nt) that derive from transcripts forming distinctive hairpin structures called pre-miRNA. The hairpin is processed into mature miRNA and forms the RNA-induced silencing complex (RISC), which contains miRNA-interacting proteins such as Dicer. The miRNAs will pair with complementary sequences on target mRNAs transcripts through the 3'UTR, leading to gene silencing of the target.

Piwi-interacting RNAs (piRNAs)

PiRNAs are small ncRNA (24 – 31 nt) that are able to form complexes with Piwi proteins of the Argonaute family. piRNAs are characterized by a uridine at the 5'end and a 2'-O-methyl modification at the 3' end. Their main role is the silencing of transposable elements during germ line development.

Small interfering RNAs (siRNAs)

SiRNAs are long linear dsRNA processed by Dicer into mature 20 – 24 nt siRNAs that direct silencing when loaded onto RISC. They mediate post-transcriptional silencing similarly to miRNA by a process called RNA interference (RNAi), where siRNA interferes with the expression of a complementary nucleotide sequence.

Long non-coding RNAs (lncRNAs)

The majority of non-coding RNAs belong to this group, as **lncRNAs** are considered as non-protein coding transcripts > 200 nt in length. Many of the lncRNAs are subject to splicing, polyadenylation, and other post-transcriptional modifications, and can be classified according to their proximity to protein coding genes.

A subgroup of lncRNAs, named large intergenic non-coding RNAs (lincRNAs) are marked by trimethylation of K4 on histone H3 (H3K4me3) at their promoter and trimethylation of K36 on histone H3 (H3K36me3) along the transcribed region. lincRNAs are involved in epigenetic gene silencing, such as the role of Xist (X-inactive specific transcript), and in tumor development by promoting expression of genes involved in metastasis and angiogenesis.

Enhancer RNAs (eRNAs) and Promoter-associated RNAs (PARs)

These two types of ncRNAs have been described recently and their roles are still unclear. **eRNAs** are non-coding transcripts with an average of 800 nt (ranging between 0.1 – 9 kB), and they are produced from regions enriched with monomethylated lysine 4 on H3, RNA Pol II and coactivators such as P300, which differentiates them from lncRNAs. It has been postulated that eRNAs function as transcriptional activators.

PARs are non-coding transcripts that range from 16 – 36 nt to 200 nt, and they are generally expressed near the TSS (Transcription start site) or in upstream elements of the promoter. Most of the PARs are associated with highly expressed genes, but they are weakly expressed and with short half-lives. It has been postulated that PARs are connected with transcriptional activation and repression.

Analysis of RNAs-protein interactions

Interest in RNA-protein interactions is booming as we begin to appreciate the role of RNA, not just in well-established processes such as transcription, splicing, and translation, but also in newer fields such as RNA interference and epigenetic regulation of gene expression by non-coding RNAs as previously described.

The two most common techniques to study RNA-protein interactions are called RIP (**RNA Immunoprecipitation**) and **CLIP (UV Cross-Linking and Immunoprecipitation)**. Both are variations of the more popular ChIP, as they both function with a similar principle.

RIP is used to map RNA-protein interactions *in vivo* by immunoprecipitating the RNA binding protein of interest together with its associated RNA. The purified RNA-protein complexes can be then separated by performing an RNA extraction and the identity of the RNA can be determined by RT-PCR. As research into RNA in the last decade has intensified, so has the interest in RIP, leading to development of better RIP protocols.

CLIP is a related technique that differs from RIP in the use of UV radiation to cross-link RNA binding proteins to the RNA that they are bound to. This covalent bond is irreversible, allowing stringent purification conditions to remove non-specific contaminating RNA. Unlike RIP, CLIP provides information about the actual protein binding site on the RNA. CLIP is increasingly used to map transcriptome-wide binding sites of RNA-binding proteins.

RIP

Product name	Product code
Rabbit polyclonal to KMT6 / EZH	ab3748
Rabbit polyclonal to SUZ12 – ChIP grade	ab12073
Mouse monoclonal [7H1G1] to SRA1	ab36625
Rabbit monoclonal [EPR3472] to RPA70	ab79398
Rabbit polyclonal to CPEB1	ab73287
Rabbit polyclonal to Survivin	ab469

CLIP

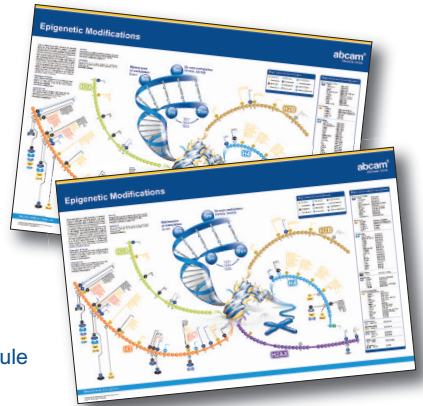
Product name	Product code
Rabbit polyclonal to TRA2B	ab31353
Mouse monoclonal [4C8] to HuR / ELAVL1 – ChIP grade	ab136542
Rabbit polyclonal to KDM6B / JMJD3 – ChIP grade	ab85392



Resources

Posters and pathway cards

- Chromatin remodeling and the transcription cycle
- Epigenetic Modifications
- Histone glossary
- Production and actions of small RNAs
- Replication Chromatin Assembly
- RNAi pathway
- Readout of Chromatin marks by histone-binding module



Find all these pathways and posters at www.abcam.com/posters

Protocols & Technical Information

- A Beginner's Guide to ChIP
- Cross-linking chromatin immunoprecipitation (X-ChIP) protocol
- Native chromatin immunoprecipitation protocol
- ChIP tips
- ChIP FAQ
- ChIP troubleshooting tips
- ChIP using plant samples – Arabidopsis
- Chromatin preparation from tissues for ChIP
- RNA Immunoprecipitation (RIP)
- UV Cross-Linking and Immunoprecipitation (CLIP)



Find all these protocols and more troubleshooting tips at www.abcam.com/protocols

Other resources

CpG Island Detection - http://pbil.univ-lyon1.fr/software/cpgprod_query.html

MethylCancer Database - <http://methycancer.psych.ac.cn/>

Histone Systematic Mutation Database - <http://histonehits.org>

Discover more at abcam.com/epigenetics

