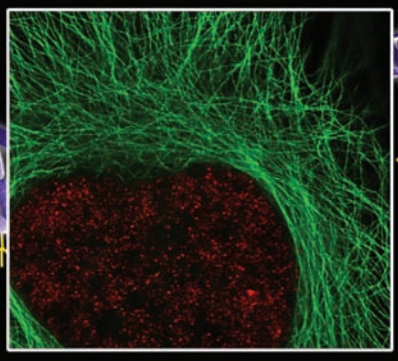
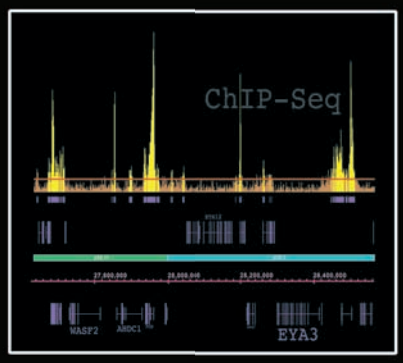
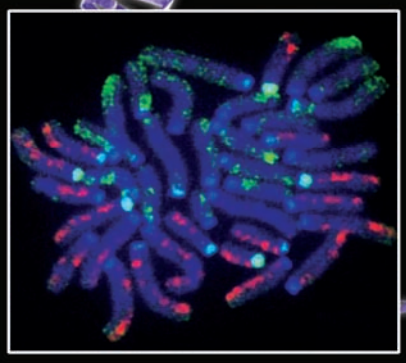
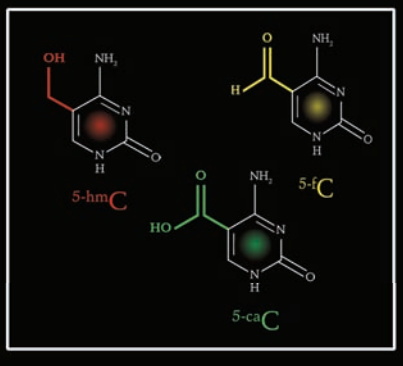
ANTIBODIES

APPLICATIONS IN EPIGENETICS RESEARCH

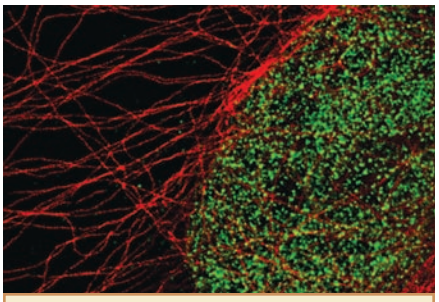








ACTIVE MOTIF®



INSIDE:

Antibodies & Advances in Epigenetics

The emerging role of epigenetics in development and disease

Epigenetics, or the heritable changes in gene function that occur independent of DNA sequence, has claimed a prominent position in research of developmental and disease processes. Countless studies link epigenetic alterations to developmental processes, including X-chromosome inactivation, genomic imprinting, axis patterning and differentiation, as well as to childhood and adult diseases such as cancer, chromosomal instabilities and mental retardation. Because genetics provides an insufficient explanation for the complexity of these processes, a deeper understanding of the role of epigenetics will be one of the keys to unraveling the underlying mechanisms responsible for regulating development and disease.

Using epigenetics as a tool to understand development and disease

Epigenetic modifications are heritable and traceable. These characteristics serve as a powerful tool for the analysis of DNA and histone modifications. The "epigenetic code" consists of modifications including the acetyl, methyl and phosphoryl groups that modify the histone tails, as well as the methyl groups and their derivatives that modify DNA. This epigenetic information is deposited, maintained and processed by "Writers", "Readers" and "Erasers" and serves to signal the activation and silencing of genes. New epigenetic techniques are now available that enable researchers to look directly at the patterns of epigenetic modifications and link those patterns to phenotype. Comparison of epigenetic patterns can be used to demonstrate epigenetic variance across populations, in exposed vs. unexposed groups, or between normal and disease states. This has proven especially valuable in the field of public health, where short- and long-term effects caused by environmental or socioeconomic factors can be evaluated.

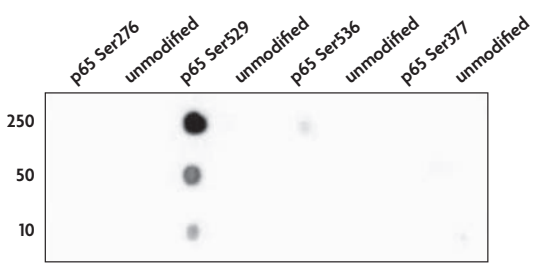
Antibodies to study epigenetics

At Active Motif, we are committed to providing the highest quality antibodies for studying epigenetics in the context of histone and DNA modifications. Our antibodies are manufactured in-house and undergo rigorous development and validation procedures to ensure their quality and performance. We are the only company to test our histone modification antibodies for specificity using our ground-breaking MODified™ Histone Peptide Array. Our extensive line of antibodies to study epigenetics ranges from antibodies against histone and DNA modifications to transcription factors, chromatin modifiers and stem cell regulators. Our staff scientists validate these antibodies for use in the applications you need them to work in, such as chromatin immunoprecipitation (ChIP), ChIP-chip, ChIP-Seq, Western blot and immunofluorescence. For a complete list of our antibodies and their validated applications, please visit www.activemotif.com/abs.

Antibody Development and Validation

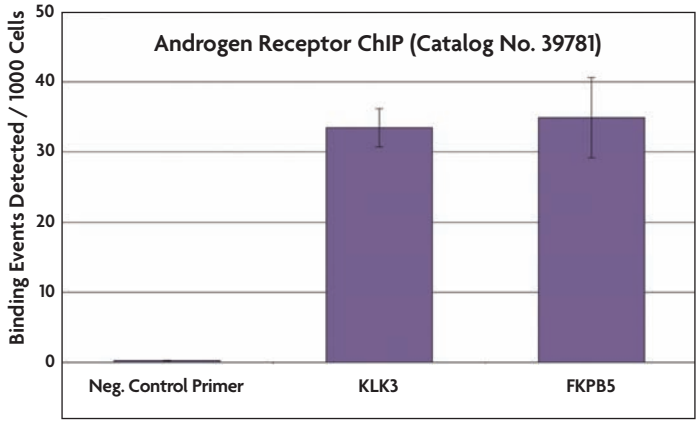
Our antibodies are tested in multiple applications to ensure quality. At Active Motif, antibody development and testing is an important, tightly controlled, multi-step process. From the design of the immunogen to specificity screening and technique validation, our years of expertise in antibody development and performance testing ensure that only the highest quality antibodies are offered for use in your research.

Dot Blot Analysis

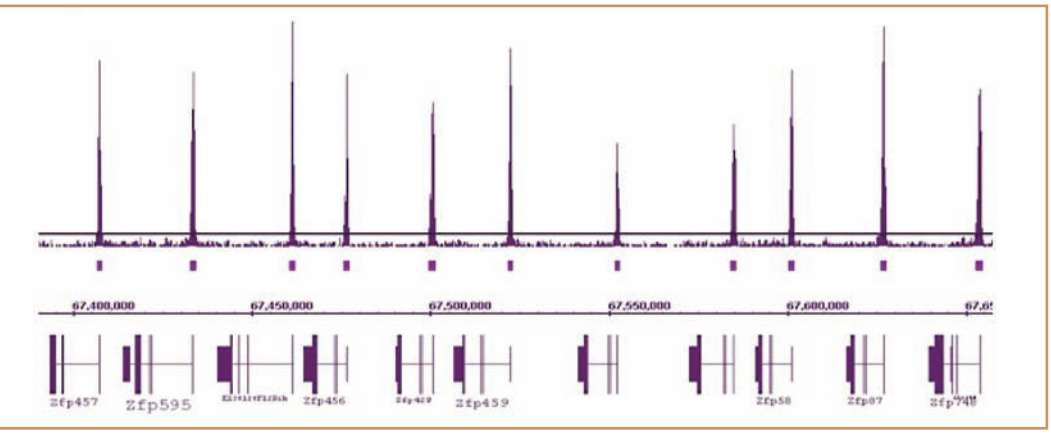


NFκB p65 phospho Ser529 pAb (Catalog No. 39691

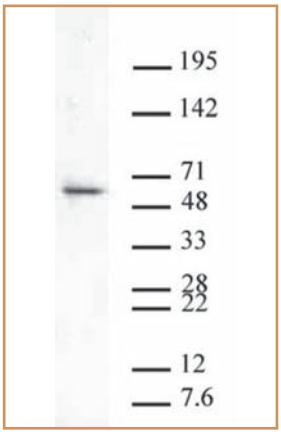
Chromatin Immunoprecipitation (ChIP)



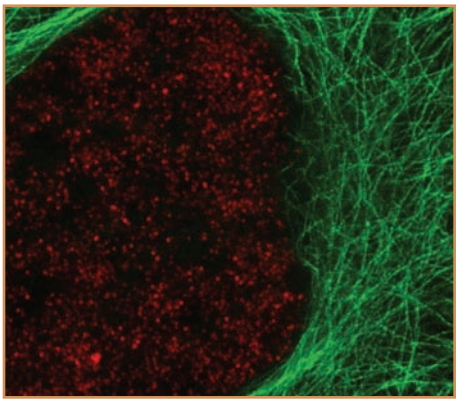
ChIP-Sequencing / ChIP-chip



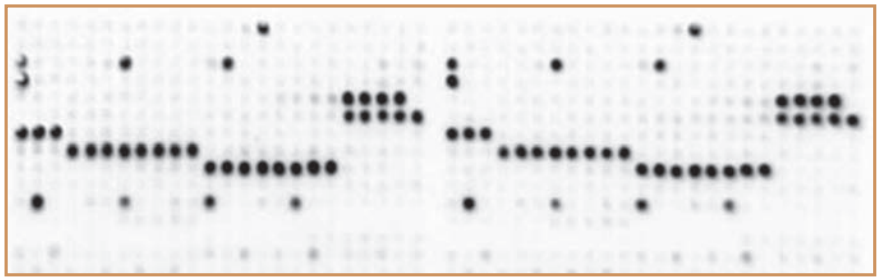
Western Blot



Immunofluorescence



Histone Peptide Array



Active Motif's MODified™ Histone Peptide Array (Catalog No. 13001) can be used to screen histone antibodies for cross-reactivity to over 384 unique histone modifications, so you can be sure your antibody binds specifically to its intended target.

DNA Methylation and Gene Regulation

DNA methylation is an important regulator of gene expression and genomic organization. It occurs at the cytosine bases of eukaryotic DNA, which are converted to 5-methylcytosine by DNA methyltransferase enzymes. DNA methylation appears almost exclusively in the context of CpG dinucleotides. These dinucleotides are relatively rare in the mammalian genome, and tend to be clustered in what are called CpG islands. Approximately 60% of gene promoters are associated with CpG islands, and are normally unmethylated. CpG methylation of gene promoters is usually associated with transcriptional silencing, which can occur through a number of mechanisms including the recruitment of methyl binding domain (MBD) proteins. DNA methylation is involved in a number of cellular functions such as embryonic development, genetic imprinting, X chromosome inactivation and control of gene expression. Alterations in normal DNA methylation patterns, either local increases that result in silencing of specific cell cycle regulatory genes or a global reduction in DNA methylation, are involved in many different types of cancer.

DNA methylation variants

A role for 5-hydroxymethylation

In 2009, 5-hydroxymethylcytosine (5-hmC) was first identified in mammalian cells[1,2]. This novel variant of DNA methylation results from the enzymatic conversion of 5-methylcytosine (5-mC) into 5-hmC by the TET family of cytosine oxygenases. This form of methylation is prevalent in neurons and embryonic stem cells. In 2011, researchers studying cerebellar and hippocampal cells showed that 5-hmC patterns change significantly during development and aging in mice[3], raising speculation that 5-hmC may represent another layer of gene regulation.

Although a role for 5-hmC as a true epigenetic mark remains possible, no specific 5-hmC binding proteins have been described to date. However, several studies have shown its potential as a demethylation intermediate, suggesting that this may be its main cellular role.

5-caC & 5-fC

In addition to 5-hydroxymethylcytosine, other DNA variants have been identified and characterized in mouse embryonic stem (ES) cells. Researchers have shown that the TET family of cytosine oxygenase enzymes, which convert 5-methylcytosine into 5-hydroxymethylcytosine (5-hmC), can further oxidize 5-hmC into 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). Both of these DNA modifications have been shown to exist in mouse ES cells, and 5-fC has also been identified in major mouse organs. Both 5-fC and 5-caC exist in the paternal pronucleus, concomitant with the disappearance of 5-methylcytosine, suggesting that these DNA variants may be involved in DNA demethylation. One hypothesis for the mechanism of DNA methylation suggests that 5-caC is excised from genomic DNA by thymine DNA glycosylase, thereby returning DNA to an unmethylated state. Other research suggests that replication-dependent dilution accounts for paternal DNA demethylation during preimplantation development.

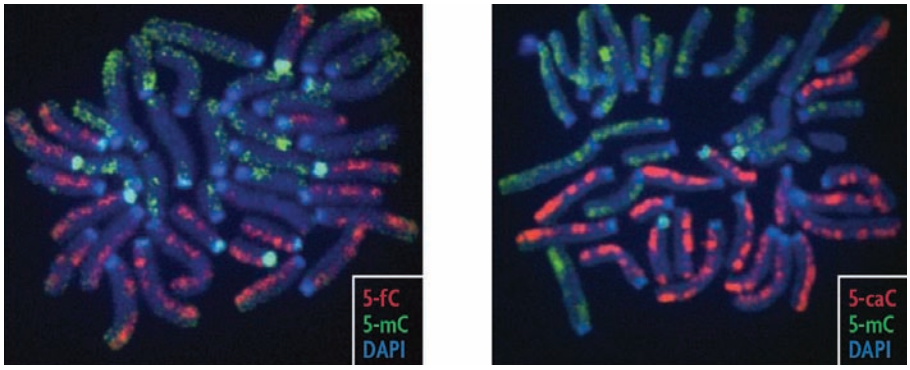


Figure 1: Differential staining of maternally and paternally derived chromosomes with 5-methylcytosine and 5-carboxymethylcytosine or 5-formylcytosine antibodies.
Shown are representative immunofluorescent images of mitotic chromosome spreads that have been co-stained with Active Motif's 5-formylcytosine (5-fC) or 5-carboxylcytosine (5-caC) antibodies (red, Catalog Nos. 61223 and 61225, respectively), a 5-methylcytosine (5-mC) antibody (green) and DAPI (blue) from two-cell stage embryos. The 5-fC and 5-caC antibodies were used at a 1:2000 dilution. The images reveal that at the two-cell stage, half the chromosomes are enriched for 5-fC (left) or 5-caC (right), and half are stained with 5-mC (Inoue *et al.* (2011)). This is consistent with similar findings that show maternal DNA is methylated and paternal DNA is hydroxymethylated.

References

1. Kriaucionis S, Heintz N (2009) *Science* 324(5929):929-930.
2. Tahiliani M *et al.* (2009) *Science* 324(5929):930-935.
3. Szulwach KE *et al.* (2011) *Nature Neurosci* 14(12):1607-1616.

Enzymes involved in DNA methylation

CpG methylation involves the transfer of a methyl group to position 5 of cytosine (5-methylcytosine, or 5-mC) by the action of the DNA methyltransferase enzymes (DNMTs).

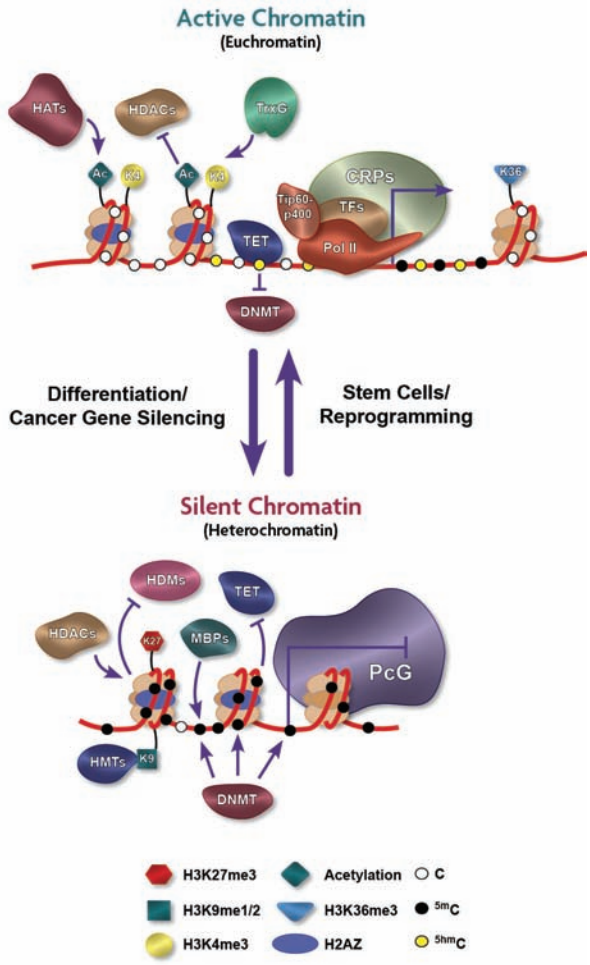


Figure 2: DNA methylation and gene silencing.
DNA methylation-mediated gene silencing is a critical regulatory event in both differentiation and tumorigenesis. Active genes have an open chromatin (euchromatin) structure and display DNA hypomethylation at gene promoter regions. They are also characterized by H3K4 & H3K36 methylation and H3 & H4 acetylation. These modifications function to neutralize histone charges and recruit chromatin remodeling proteins (CRPs) that lead to unraveling of the chromatin structure, allowing access to the basal transcriptional machinery. In contrast, gene silencing results in the recruitment of DNA methylation machinery (DNMTs, MBPs), chromatin modifiers (HDACs, HMTs) and repressive complexes, such as Polycomb (PcG) proteins. This leads to chromatin condensation and DNA hypermethylation. Condensed chromatin is characterized by H3K27 & H3K9 methylation. Together, the cofactors and regulatory proteins effecting these epigenetic modifications define the chromatin landscape that dictates the expression profile of the cell.
TrxG, Trithorax group; PcG, Polycomb group; HATs, Histone acetyltransferases; HDACs, Histone deacetylases; TFs, Transcription factors; HDMs, Histone demethylases; DNMT, DNA methyltransferase; TET, Ten-eleven translocation enzymes; HMTs, Histone methyltransferases; MBPs, Methyl binding proteins.

Three families of DNMTs have been identified: DNMT1, DNMT2 and DNMT3. The DNMT3 family contains two active methyltransferases, DNMT3A, DNMT3B and one DNMT3-Like protein (DNMT3L) which has no DNA methyltransferase activity but does function as a co-factor of DNMT3A. The defined role of DNMT1 is to ensure the maintenance of cytosine methylation during DNA replication, while DNMT2 does not methylate DNA, but has been identified as the first RNA cytosine methyltransferase.

The complex series of events leading to a repressive chromatin state involves the coordinated regulation of DNA methyltransferases, Methyl-CpG binding proteins (MBD proteins) and the Kaiso family of proteins. The family of MBD proteins include MeCP2, MBD1, MBD2, MBD3 and MBD4. (Figure 2).

DNA METHYLATION ANTIBODIES

Due to the large role that it plays in development and disease, much research depends on the ability to accurately detect and quantify DNA methylation. Active Motif offers a number of products specific for this area of research, including kits and antibodies that enrich for DNA fragments that contain 5-mC and 5-hmC. Many of our DNA methylation antibodies have been validated for use in ChIP, MeDIP and/or immunofluorescence. For complete details on our DNA methylation products, please visit us at www.activemotif.com/dnamt.

Product	Format	Catalog No.
3-Methylcytosine (3-mC) antibody (pAb)	100 µl	61179
5-Carboxylcytosine (5-caC) antibody (pAb)	100 µl	61225
5-Formylcytosine (5-fC) antibody (pAb)	100 µl	61223
5-Hydroxymethylcytosine (5-hmC) antibody (pAb)	100 µl	39769
5-Methylcytosine (5-mC) antibody (mAb)	100 µg	39649
5-Methylcytosine (5-mC) antibody (pAb)	100 µg	61255
DNMT1 antibody (mAb)	100 µg	39204
DNMT3A antibody (mAb)	100 µg	39206
DNMT3B antibody (mAb)	100 µg	39207
DNMT3B antibody (pAb)	100 µl	39899
DNMT3L antibody (pAb)	100 µl	39907
MBD1 antibody (mAb)	100 µg	39215
MBD2 antibody (pAb)	200 µl	39547
MBD3 antibody (mAb)	100 µg	39216
MBD4 antibody (pAb)	100 µg	39217
MeCP2 antibody (mAb)	100 µg	61285
MeCP2 antibody (mAb)	100 µg	61291
MeCP2 antibody (pAb)	100 µg	39218
MeCP2 antibody (pAb)	200 µl	39188

Epigenetics in Cancer

The epigenetic state of the cell is controlled by the activity of proteins that add and remove small chemical modifications to histones and directly to DNA. Aberrant epigenetic regulation can lead to changes in gene expression and the development of cancer. The link between epigenetics and cancer has been substantiated through the identification of mutations in or altered expression of epigenetic regulator proteins in many different types of cancer. These mutations have been identified in all three major classes of epigenetic proteins: the Writers (enzymes that deposit modifications), the Erasers (enzymes that remove modifications) and the Readers (proteins that recognize and bind epigenetic modifications). Recent drug development strategies that target these enzymes have resulted in four FDA approved cancer drugs, with many more on the horizon.

DNA methylation and hydroxymethylation

Changes in DNA methylation have been well characterized in cancer. In general, the cancer epigenome is characterized by global DNA hypomethylation and promoter-specific DNA hypermethylation, which often leads to silencing of tumor suppressor genes. This link to cancer is supported by the finding that mutations in DNMT3A are found in acute myeloid leukemia, and there are currently 2 FDA approved cancer drugs that target DNMTs. The recently characterized DNA modification 5-hydroxymethylcytosine (5-hmC) has also been linked to cancer. Both a reduction in 5-hmC and a reduction in expression of the TET enzymes that convert 5-mC to 5-hmC have been reported in breast, liver, lung, prostate and pancreatic cancers.

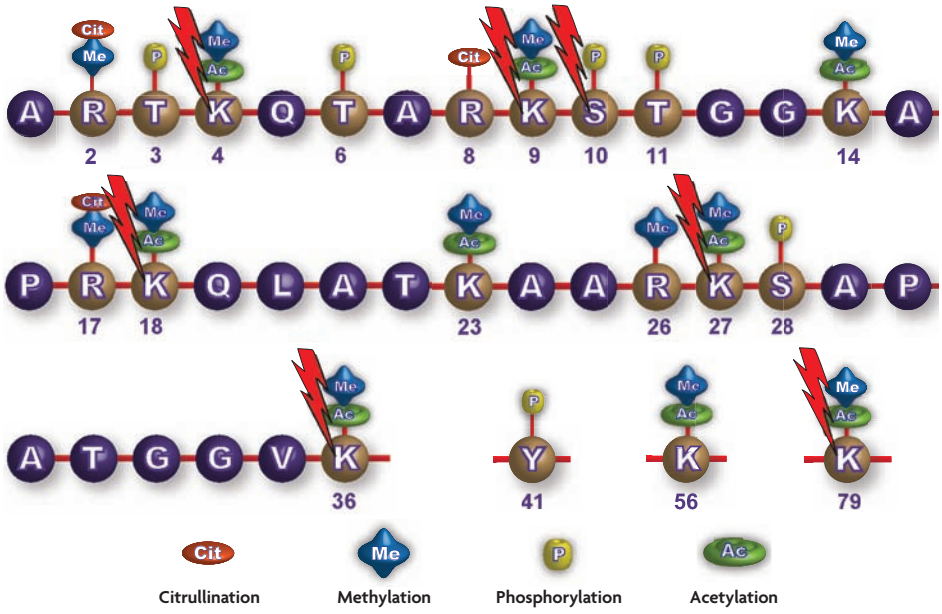


Figure 1: Alterations in histone modifications are associated with cancer.
Normal modifications that occur on Histone H3 are depicted. Arrows indicate known disruptions of the normal patterns that have been associated with cancer.

Writers add modifications

The "Writers" are a group of enzymes that act on histones and covalently add small modifications such as methyl and acetyl groups. Enzymes that add acetyl groups to histones are called HATs, while enzymes that add methyl groups to histones are called HMTs. Mutations and/or misregulation of these genes are found in many different human tumors. For example, EZH2 (mAb 39875), an H3K27 methyltransferase, is overexpressed in many tumors and EZH2 inactivating-mutations are found in large B cell lymphomas. In another example, the methyltransferase MLL (pAb 61295) is mutated via rearrangements and translocations in mixed-lineage leukemia, and more than 60 known MLL fusion partners have been described. MLL associates with the H3K79 methyltransferase DOT1L (pAb 39953), and this association has led to the discovery that enhanced H3K79 methylation (pAb 39145, 39143) is associated with overexpressed genes in MLL-fusion leukemias.

EPIGENETICS NEWS

Visit www.activemotif.com and sign up to receive "Epigenetics News" monthly and keep up with the latest findings in the field.

Erasers remove modifications

The "Erasers" are a group of enzymes that act on histones to remove small covalent modifications. Enzymes that remove acetyl groups from histones are called HDACs, while those that remove methyl groups from histones are called HDMs. Mutation and misregulation of many different HDAC (mAb 39533, pAb 40971) and Sirtuin (mAb 39353, pAb 39903) genes have been associated with cancer. There are currently two FDA approved drugs (and four more that have progressed to phase II clinical trials) that function by inhibiting HDAC activity. Additionally, the histone demethylase LSD1 (pAb 39186) has been implicated in cancer. LSD1 is an H3K4/K9 demethylase that is over-expressed in multiple cancer types, and small molecule inhibitors have been identified that have potential as therapeutics.

Readers help interpret modifications

The "Readers" are a group of proteins that do not have enzymatic activity, but that function by recognizing and binding to specific epigenetic modifications. These proteins are thought to influence chromatin structure and function either by recruiting other proteins or by blocking Writers and Erasers from accessing specific modifications. The recent discovery of compounds that specifically inhibit BRD4 (pAb 39909) binding to acetylated lysines in H3 and H4 has created much excitement around this emergent class of new drug targets.

Recombinant Proteins Available

In addition to our wide range of antibodies against cancer-related proteins, Active Motif also offers the proteins themselves as full-length recombinant proteins or as protein domains. Many of our kits and technologies are directly applicable to cancer research.

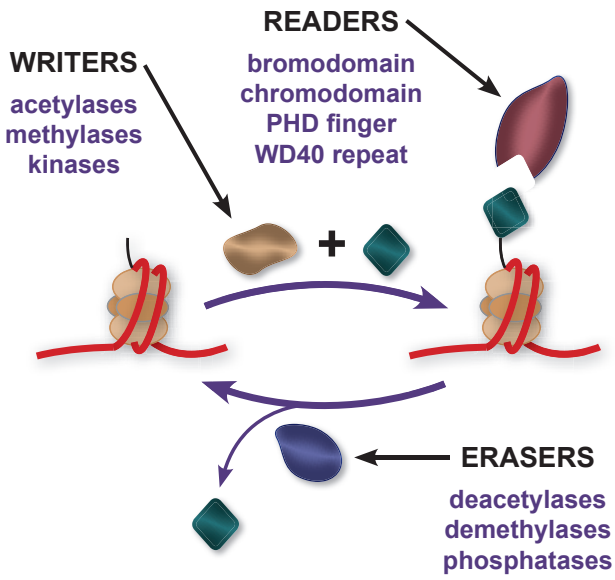


Figure 2: Regulating the histone code.
The "histone code" is comprised of post-translational modifications that occur on the histone tails. These modifications are generated, interpreted and edited by proteins coined "Writers", "Readers" and "Erasers".

HISTONE MODIFICATION ANTIBODIES

Active Motif has a large, ever-growing collection of antibodies that target proteins and histone modifications implicated in cancer, some of which are shown below. Please visit us at www.activemotif.com/chromabs for a complete, up-to-date list of antibodies.

Product	Format	Catalog No.
BRG-1 antibody (mAb)	100 µg	39807
BRM antibody (mAb)	100 µg	39805
DNMT1 antibody (mAb)	100 µg	39204
DNMT3A antibody (mAb)	100 µg	39206
DNMT3B antibody (mAb)	100 µg	39207
EZH2 antibody (mAb)	100 µg	39875
HDAC2 antibody (mAb)	200 µl	39533
HDAC6 antibody (pAb)	100 µg	40971
JARID1C antibody (pAb)	200 µl	39229
LSD1 antibody (pAb)	200 µl	39186
MBD1 antibody (mAb)	100 µg	39215
MBD2 antibody (pAb)	200 µl	39547
MBD3 antibody (mAb)	100 µg	39216
MeCP2 antibody (mAb)	100 µg	61285
MLL antibody (pAb)	100 µg	61295
MMSET / WHSC1 antibody (mAb)	100 µg	39879
MTA2 antibody (pAb)	200 µl	39359
SIRT2 antibody (pAb)	100 µl	39903
SNF5 antibody (pAb)	100 µl	39775

Polycomb and Stem Cells

Embryonic stem cells (ESCs) are cells derived from the inner cell mass of the developing pre-implantation embryo that gives rise to endoderm, ectoderm and mesoderm. ESCs are unique from all other cells types in that they can differentiate into essentially all cell types in the developing and adult organism, a phenomenon termed pluripotency. They also have the ability to divide into cells that have the same developmental state as the parent (self-renewal). The ability of a cell to remain pluripotent and to self-renew requires the establishment of very complex gene regulation programs. ESCs must facilitate the expression of genes required for self-renewal and pluripotency, but at the same time maintain all genes involved in lineage commitment and differentiation in a repressed state.

Unlimited developmental potential

Unlike the cells that are derived from them, embryonic stem cells by their very nature have unlimited developmental potential. In contrast, differentiated cells like neuron or muscle cells have lost the ability (without intervention by the researcher) to be anything other than what they have become. Because ESCs are genetically identical to all of the cells that are derived from them, the primary difference between them is not explained by genetics but rather by epigenetics.

Stem cell identity

The Polycomb proteins are important epigenetic determinants of stem cell identity. They play an important role, serving to help determine whether stem cells remain pluripotent or commit to a more specific cell fate. Originally identified in *Drosophila* as repressors of genes involved in body plan, Polycomb proteins are segregated into one of two protein complexes, Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2; see Table 1). As some Polycomb complex subunits have multiple potential component proteins (*e.g.* there exist four isoforms of EED), a number of different versions of PRC1 and PRC2 have been identified.

PRC1

Protein	Function
BMI-1, MEL18, MBLR, NSPC1	Unknown, required for self-renewal
CBX2/PC1, CBX4/PC2, CBX6, CBX7 & CBX8/PC3	Chromodomain proteins, bind H3K27me3
PHC1, PHC2, PHC3	Unknown
RING1, RING1B/RNF2	Monoubiquitylation of H2AK119

PRC2

Protein	Function
EED (four isoforms)	Stimulation of Lys27 methylation
EZH2, EZH1	H3K27 HMTase
SUZ12	Unknown, required for ESC differentiation
RbAp48	Histone chaperone

Table 1: The subunits and functions of Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2).

Polycomb Response Elements (PREs)

In flies, Polycomb complexes are recruited to specific DNA sequences (termed Polycomb Response Elements) through the DNA-binding protein Polyhomeotic. Although no mammalian PRE sequences have been identified, there are several potential DNA-binding recruitment proteins, including OCT4, YY1, JARID2, PCL2/MTF2 and AEBP2. Non-coding RNAs, such as HOTAIR, have also been implicated in the recruitment of PRC2.

Once it has associated with chromatin, the PRC2 subunit EZH2 catalyzes trimethylation of histone H3 at Lys27 (H3K27me3; see Figure 1). H3K27me3 has long been known as a hallmark of regions of repressed chromatin. Trimethylation of Lys27 leads to the recruitment of PRC1 through the binding of H3K27me3 by chromodomain-containing proteins in PRC1. Interaction of PRC1 with non-adjacent regions of chromatin marked by H3 Lys27 trimethylation may

contribute to silencing through the establishment of a domain of specialized chromatin structure that is refractory to gene expression. PRC1 also includes a histone modifying enzyme, RING1B, that catalyzes monoubiquitylation of histone H2A at Lys119 (H2AK119ub1). H2AK119ub1 has been found to repress transcript elongation by RNA polymerase II.

The above recruitment and repression model is more complex than described, as many key regulatory genes in stem cells are found in "bivalent" chromatin domains. These bivalent domains, which exhibit hallmarks of both active (H3K4me3) and repressed (H3K27me3) chromatin, are thought to represent a gene poised for either expression or repression. Upon differentiation, bivalent domains are resolved, depending upon the resulting expression state. Genes that are expressed in the target cell type maintain histone H3 Lys4 methylation. Markers of other cell types lose Lys4 methylation but retain Lys27 methylation, and thus go from poised to silent.

In addition to cell type-specific genes and those involved in lineage commitment and differentiation, targets of Polycomb repression in stem cells include tumor suppressors, which prevent cell senescence and potentially promote proliferation. Genes involved in apoptosis are also repressed in a Polycomb-dependant fashion. In terminally differentiated cells, Polycomb proteins participate in the repression of pluripotency and ESC-specific genes, as well as genes expressed in other cell types.

Summary

It is essential to regulate stem cell self-renewal and orchestrate the differentiation process, and it is clear that Polycomb proteins are a crucial part of this regulation. Cells lacking EED or SUZ12 show aberrant expression of lineage-specific genes, which interferes with their ability to differentiate properly. Differentiation is also hampered by the inability to repress pluripotency genes. Mutations in Polycomb proteins are also linked to the establishment of cancer, as lack of Polycomb function can produce cancer stem cells that are unable to differentiate and, as a result, perpetually self-renew.

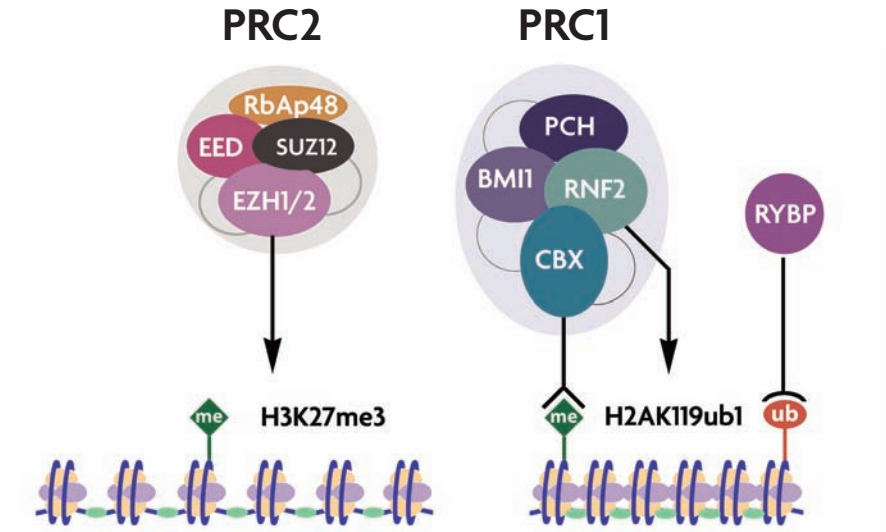


Figure 1: Simplified model of Polycomb Group (PcG)-mediated repression.
PRC2 is recruited to chromatin which allows trimethylation of histone H3 Lys27 by EZH2. PRC1 is then recruited to chromatin through the recognition of H3K27me3 by chromobox (CBX) proteins via the chromodomain. RNF2/RING1 homologs are E3 ubiquitin ligases for H2A, which is monoubiquitylated at Lys119. The RYBP repressor protein recognizes H2A monoubiquitylation, contributing to transcriptional repression. Combined, these activities induce and maintain transcriptional repression. The gray circular outlines in the image above depict other PRC subunits and associated proteins.

STEM CELL ANTIBODIES

Active Motif has a large, growing collection of antibodies to proteins important in stem cell biology. Visit www.activemotif.com/stemcellabs for the complete, up-to-date list.

Product	Format	Catalog No.
BMI-1 antibody (mAb)	100 µg	39993
CBX8 antibody (pAb)	100 µl	61237
EED antibody (mAb)	100 µg	61203
EZH2 antibody (mAb)	100 µg	39875
EZH2 phospho Thr345 antibody (pAb)	100 µl	61241
Histone H3K27me3 antibody (mAb)	100 µg	61017
PCL2 antibody (mAb)	100 µg	61153
Phc1 antibody (mAb)	100 µg	39723
Phc2 antibody (mAb)	100 µg	39661
Ring1B antibody (mAb)	100 µg	39663
Suz12 antibody (mAb)	100 µg	39877
YY1 antibody (pAb)	100 µl	39071

Transcription and Stem Cells

The fate of embryonic stem cells (ESCs) is governed by a multi-layered transcriptional control mechanism that includes the Oct-4, Sox2 and Nanog transcriptional regulatory network, the integration of external signaling cues via signal transduction pathways, and changes in chromatin structure regulating gene accessibility. The complexity of the gene expression program endows stem cells with the flexibility and adaptability to differentiate into essentially any cell type.

Stem cell pluripotency and self-renewal require a complex transcriptional regulatory network that allows for stem cells to propagate yet remain poised to differentiate into essentially all cell types in response to developmental cues. The transcriptional network involves a regulatory hierarchy with the core transcription factors Oct-4, Nanog and Sox2 at the top. Oct-4 and Sox2 form a heterodimer that is recruited with Nanog to a large number of promoters, including their own. These proteins create a positive feedback, auto-regulatory loop that maintains proper levels of the three core regulators in ESCs. Oct-4, Nanog and Sox2 serve to activate a network of genes required for stem cell pluripotency and self-renewal (Figure 1). If any of the core regulators is absent in embryonic stem cells, they lose pluripotency and begin to differentiate. So important are these genes that expression of a subset of them in differentiated cells is sufficient to induce pluripotency.

A large number of transcription factors and co-factors cooperate with Oct-4, Nanog and Sox2 to regulate gene expression in stem cells (Table 1 and Figure 2, Activation). Many of these proteins serve to enhance expression from genes whose promoters are occupied by the three core regulators. The c-Myc protein assists in the release of RNA polymerase II from a paused to an elongating state. While Oct-4, Nanog and Sox2 specify which genes to activate, c-Myc and the enhancer-binding proteins play a role in the efficiency of activation. c-Myc also serves to activate the expression of genes involved in cell proliferation (Figure 2, Activation). Much of the genome in ESCs remains in an open chromatin state, permissive to transcription. Indeed, a large proportion of genes in ESCs are expressed, albeit at low levels. Keeping ESC chromatin open involves many transcriptional co-activators, such as p300, BRG-1, Mediator complex and the PAF complex. Other transcription factors, like the SMAD, Notch and STAT proteins, serve to integrate cues from important cell signaling pathways into the Oct-4/Sox2/Nanog regulatory cascade. The Wnt and TGF-β signaling pathways are crucial for stem cells to maintain their identity and function. The role of small, non-coding RNAs in stem cell function is emerging, but it seems that they assist in regulating the levels of transcript produced from pluripotency and self-renewal genes.

Gene	Function
DAX-1	Activates Oct-4; SC pluripotency
ERR-β	Activation of Oct-4
FOXD3	Pluripotency
KLF4	LIF signaling; self-renewal
LRH-1	Activation of Oct-4
c-Myc	Proliferation
NAC-1	Stem cell pluripotency
Nanog	Core regulator
Oct-4	Core regulator
PRDM14	ESC identity
REST	Repression of neuronal genes
Ronin	Metabolism
SALL4	Embryonic regulator
SMAD1	BMP signaling to core regulators
SMAD2/3	TGF-β/Activin/Nodal signaling
Sox2	Core regulator
STAT3	LIF signaling to core regulators
TBX3	Mediates LIF signaling
TCF3	Wnt signaling to core regulators
ZFX	Self-renewal

Table 1: Transcription factors crucial to stem cell function and identity.
Adapted from Young RA (2011) *Cell* 144(6):940-954.

Oct-4, Sox2 and Nanog also function to repress genes specific for differentiation and lineage commitment. It is crucial that genes encoding master regulator transcription factors (*e.g.* MyoD, which specifies muscle cell fate) be kept silent in stem cells in order that they retain their pluripotent state (Figure 2, Repression). As with transcriptional activation, many other proteins play a role in this process. Polycomb proteins are responsible at some level for repression of many of these lineage-specific regulators and may be directly recruited by Oct-4. The REST protein keeps neuron-specific genes repressed in ESCs prior to differentiation. The H3K9 methyltransferase ESET/SETDB1 and members of the NuRD complex are also implicated in the silencing of this class of genes.

Stem cells possess a fascinating system of gene regulation. Using genome-wide methods to examine transcription factor localization, complex gene interaction maps have been created. While these provide tremendous insight into stem cell biology and how ESCs maintain their identity, much remains to be discovered.

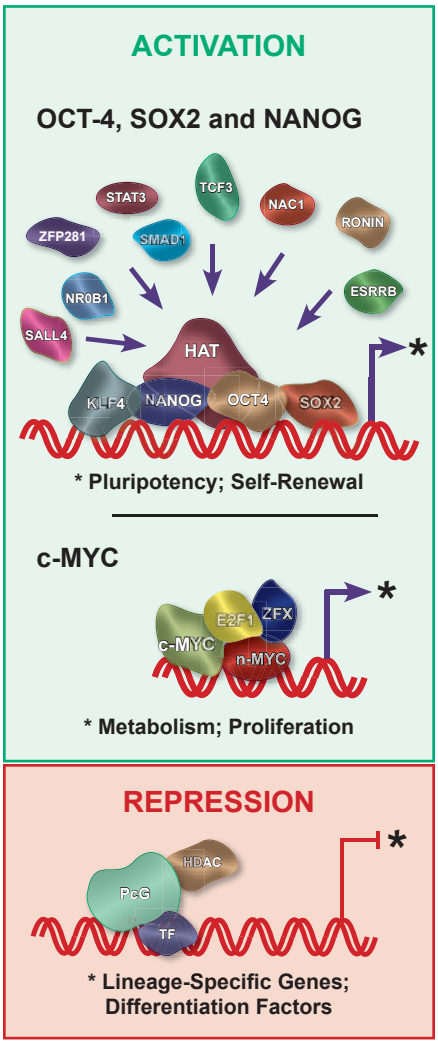


Figure 2: Transcriptional networks and stem cell identity.
Transcriptional activation is signaled by the Oct-4, Sox2 and Nanog networks that co-occupy gene promoters of regulators of pluripotency and self-renewal, and the c-Myc networks that regulate RNA Pol II and proliferation. The subset of transcription factors (TF) co-occupying promoters varies in response to intracellular signals. Transcriptional repression inhibits the somatic cell program and results from low-level promoter occupancy and the recruitment of histone deacetylase (HDAC) and Polycomb Group (PcG) co-repressor complexes.

STEM CELL ANTIBODIES

Active Motif has a large, ever-expanding collection of antibodies to proteins important in stem cell biology. For the complete, up-to-date list, please visit us at www.activemotif.com/stemcellabs.

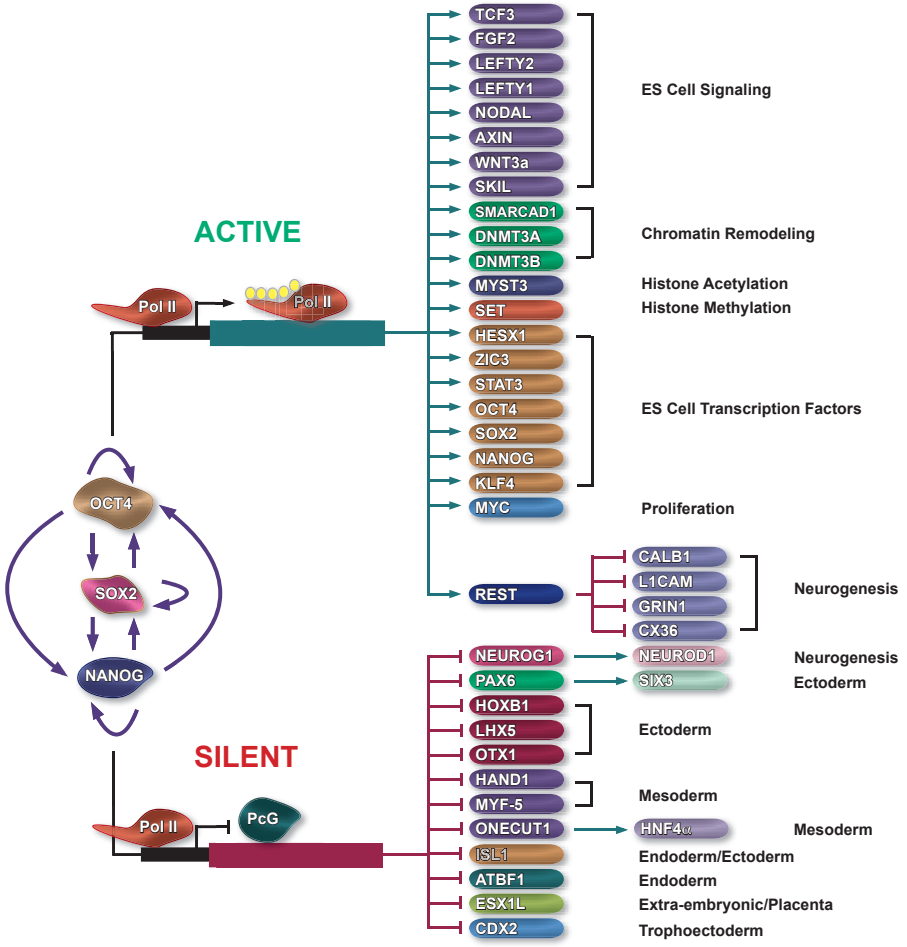
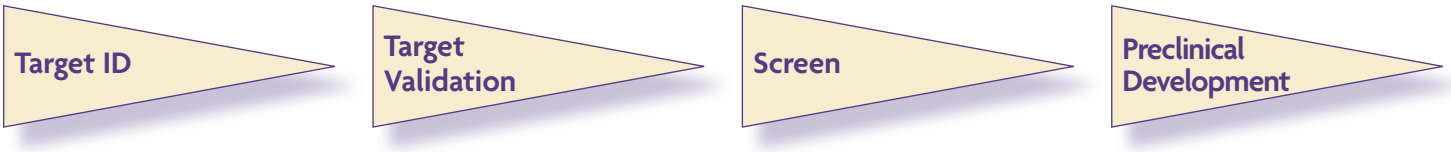


Figure 1: Transcriptional regulatory circuitry in embryonic stem cells.
This "wiring diagram" represents the core transcriptional regulatory circuitry in human embryonic stem cells based on expression data for the OCT4, SOX2 and NANOG target genes. Use of this image was kindly permitted by Dr. Rudolf Jaenisch, Professor of Biology at the Massachusetts Institute of Technology.

Product	Format	Catalog No.
BRG-1 antibody (mAb)	100 µg	39807
DAX-1 / NR0B1 antibody (mAb)	100 µg	39983
GLI1 antibody (pAb)	100 µl	61215
HOXA9 antibody (pAb)	100 µl	39825
KLF4 antibody (pAb)	100 µl	39745
KLF5 antibody (pAb)	100 µl	61099
MLL1/HRX antibody (mAb)	100 µg	39829
Notch1 antibody (mAb)	100 µg	61147
Notch3 antibody (mAb)	100 µg	61149
Oct-4 antibody (pAb)	100 µl	39811
SALL4 antibody (pAb)	100 µl	39957
Sox2 antibody (pAb)	100 µl	39823
TCF7L1 /TCF3 antibody (pAb)	100 µl	61125
YY1 antibody (pAb)	100 µl	39071

Antibodies for Drug Development Assays

Epigenetic targets are the most promising class of drugable targets to emerge in a decade. These targets are not only relevant to oncology research, but they also have potential in metabolic, neurological, inflammatory and cardiovascular disorders. In drug development, antibodies are an important tool used to better understand the epigenetic changes and potential off-target effects. Active Motif's extensive portfolio of antibodies against DNA methylation, histone modifications and histone modifying enzymes will help advance research in all phases of drug discovery.



Minimize risks

Active Motif specializes in manufacturing antibodies against histone modifications and chromatin proteins. Because we manufacture and test our own antibodies, we can deliver the high-quality antibodies that your research requires.

Assay compatible formulations

Antibody formulations containing glycerol, BSA, amine-containing azides, Tris or glycine can wreak havoc on established assays. Active Motif offers antibodies in PBS, so that they can be seamlessly integrated into existing pipelines.

Large lot sizes

We produce large lots to ensure consistency in all phases of the drug discovery process, including antibody-based HTS.

Specificity is rigorously tested

Active Motif histone antibodies are tested for cross-reactivity to multiple histone tail modifications.

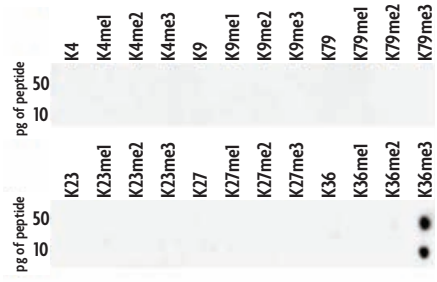


Figure 1: Dot blot analysis of H3K36me3 pAb (Catalog No. 61101) to determine its reactivity to various H3 lysine modifications.

Tools to build assays

Active Motif antibodies are compatible with many applications and can be combined with our recombinant modified histones and recombinant histone modifying enzymes to build assays quickly. Antibodies against modified histone and histone modifying enzyme pairs include: EZH2 & H3K27me3; MMSET & H3K36me2; LSD1 & H3K4me2; DOT1L & H3K79me2.

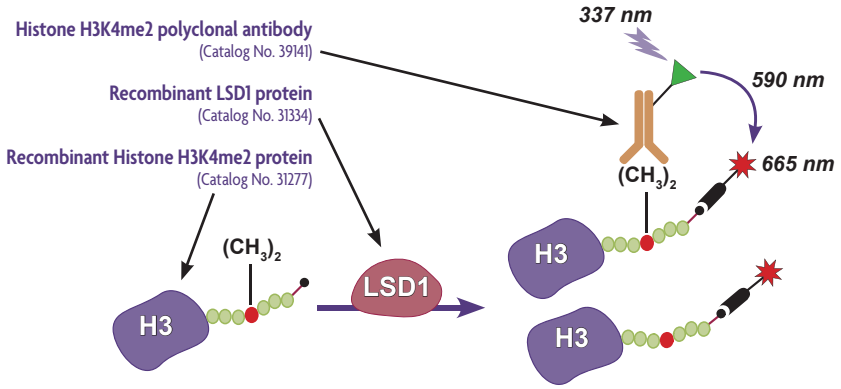


Figure 2: Time-Resolved FRET assay to monitor demethylation of H3K4 by LSD1.

DRUG TARGET ANTIBODIES

Active Motif provides antibodies against proteins in all major epigenetic drug target classes.

TARGET	Antibody	Format	Catalog No.
HMTs	MLL antibody (pAb)	100 µg	61295
	PRMT5 antibody (pAb)	100 µl	61001
	SUV39H1 antibody (mAb)	100 µg	39785
HDMs	JARID1C antibody (pAb)	200 µl	39229
	JMJD2A antibody (mAb)	100 µg	39815
	JMJD2B / KDM4B antibody (pAb)	100 µl	61221
HATs	GCN5 antibody (mAb)	100 µg	39975
	MOF / MYST1 antibody (pAb)	100 µl	61245
HDACs	HDAC1 antibody (pAb)	100 µg	40967
	HDAC2 antibody (mAb)	200 µl	39533
	SIRT1 antibody (mAb)	200 µg	39353
Readers	BRD4 antibody (pAb)	100 µl	39909
	PELP1 antibody (pAb)	100 µl	61263
DNMTs	DNMT1 antibody (mAb)	100 µg	39204
	DNMT2 antibody (pAb)	100 µg	39205
	DNMT3A antibody (mAb)	100 µg	39206

Chromatin State Analysis

Chromatin plays a central role in mediating the regulatory signals that impact DNA accessibility, gene transcription and regulation. Recent studies have utilized mathematical modeling systems to describe a number of "chromatin states", or regulatory regions that indicate the biological function of given genomic regions in a particular cell type.

Modeling Systems

It is well established that different histone modifications are associated with the transcriptional status of genes. However, given the variety of these modifications as well as the presence of histone variants and chromatin interacting proteins, researchers have struggled to understand the combinatorial nature of these modifications and how they impact gene function. Computational modeling systems based on hidden Markov models[1-5] have assessed the combination of histone modifications to discover "chromatin states" that define regulatory regions of biological significance. These models analyze genome-wide ChIP-Seq data sets from published data or from publicly available databases such as ENCODE[6] or the NIH Epigenomics Roadmap[7]. Histone modifications known to be associated with active or silenced genes include H3K27me3, H3K36me3, H4K20me1, H3K4me1, H3K4me2, H3K4me3, H3K27ac, and H3K9ac.

Chromatin States

By analyzing data from up to 12 individual histone modifications in *Arabidopsis*, 4 main chromatin states were identified: active genes, repressed genes, silent repeat elements and intergenic regions[3]. In multiple mammalian cell types, analysis of 8 histone modifications led to the description of 15 different functional chromatin states including weak, strong and poised promoters, weak and strong enhancers, insulators and repetitive DNA[1,2] (Figure 1). In *Drosophila*, 18 histone modifications were used to identify 9 chromatin states, providing a comprehensive picture of the chromatin landscape in this model organism[4].

Biological significance

Chromatin states were shown to have functional significance by correlating them with functional elements and expected patterns of gene expression. In a lymphoblastoid cell line, gene regions identified as "chromatin state 1 – active promoters" were involved in the immune response whereas in Hep G2 liver cells "chromatin state 1" annotated genes were involved in cholesterol transport[2]. Enhancers were shown to display significant cell type-specific signatures, and their classification enabled the prediction of transcription factor interactions[2].

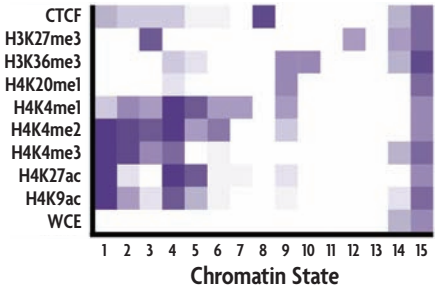


Figure 1: Chromatin states in mammalian cells. Graphical representation of the frequency of 8 histone marks defining 15 chromatin states. Adapted from References 1 & 2.

In *Drosophila*, transcriptionally active genes classified according to their chromatin state correlated with expression magnitude, gene structure and genomic context[4]. Furthermore, chromatin states were used to classify cell differentiation status with almost 100% accuracy[5].

Conclusion

Studying the combination of histone modifications alone or in combination with transcription factors and chromatin binding proteins by ChIP-Seq and mathematical modeling is leading to a greater appreciation of the biological role of chromatin and its function in regulating gene expression. Chromatin states provide a global map of the epigenome, highlight the principles of domain organization and form a basis for further investigation of their central role in the genome.

References

1. Ernst J, Kellis M (2010) *Nat Biotechnol* 28(8):817-825.
2. Ernst J, *et al.* (2011) *Nature* 473(7345):43-49.
3. Roudier F, *et al.* (2011) *EMBO J* 30(10):1928-1938.
4. Kharchenko PV, *et al.* (2011) *Nature* 471(7339):480-485.
5. Larson JL, Yan G-C (2012) *PLoS ONE* 7(2):e31414.
6. Birney E, *et al.* (2007) *Nature* 447(7146):799-816.
7. Bernstein BE *et al.* (2010) *Nat Biotechnol* 28(10):1045-1048.

CHIP, CHIP-SEQ & CHIP-CHIP VALIDATED ANTIBODIES

To see a complete, up-to-date list of our antibodies that have been validated for use in ChIP, ChIP-Seq and/or ChIP-chip, please visit us at www.activemotif.com/chipabs.

Product	Format	Catalog No.
CTCF antibody (pAb)	100 µl	61311
Histone H3K4me1 antibody (pAb)	200 µl	39297
Histone H3K4me2 antibody (pAb)	200 µl	39141
Histone H3K4me3 antibody (pAb)	200 µl	39159
Histone H3K4me3 antibody (pAb)	100 µg	39915
Histone H3K9ac antibody (pAb)	200 µl	39137
Histone H3K9ac antibody (pAb)	100 µg	39917
Histone H3K9me3 antibody (pAb)	200 µl	39161
Histone H3K27ac antibody (pAb)	200 µg	39133
Histone H3K27me3 antibody (pAb)	200 µg	39155
Histone H3K36me3 antibody (pAb)	100 µl	61101
Histone H3K56ac antibody (pAb)	200 µl	39281

Chromatin and Cell Signaling

During the last decade, many researchers have explored the role of cellular signaling cascades on gene expression. As a result of this work, there is now a clear understanding that the systematic activation of signaling cascade enzymes leads to downstream changes in gene expression. However, to date very little work has explored the role that these signaling cascades play in regulating the epigenome. Epigenetic control of gene expression can occur at the chromatin level through protein-mediated changes to DNA accessibility or by enzyme-mediated changes in the DNA modification state. Such modifications serve to alter the histone-DNA contacts, causing the chromatin to either "loosen" or "tighten", and thereby facilitate or limit the availability of the genomic loci to transcriptional or other regulators. The process of changing the chromatin or DNA modification state often occurs in response to external stimuli, thereby creating an inflection point between cell signaling biology and gene expression patterns. Deciphering the connectivity between signaling cascades and the epigenome is undoubtedly an area of scientific opportunity, and Active Motif is committed to providing researchers with validated kits and reagents to make this research accessible.

A new view for cell signaling

The traditional schematics used to show signaling cascades typically end with induction of regulatory transcription factors, such as SMADs (Catalog No. 61249) or TCF/LEF (Catalog No. 61125). However, epigenetic control mechanisms have been shown to tightly regulate transcriptional activation, which therefore implies that signaling cascades also have a mode of action within the epigenome. Indeed, recent work looking at post-translational modifications for key chromatin regulators, such as EZH2 (Catalog No. 39875), has shown that cell cycle-dependent phosphorylation plays an important role in enzyme activation[1]. This early stage research suggests that a new view for cell signaling research should be adopted if biologists are to fully decipher the significance of signal transduction (Figure 1).

Next-Generation sequencing further confirms the connectivity between cell signaling and the epigenome

Highly parallel Next-Generation sequencing (NGS) has enabled researchers to explore important biological questions in new ways. And, because signaling

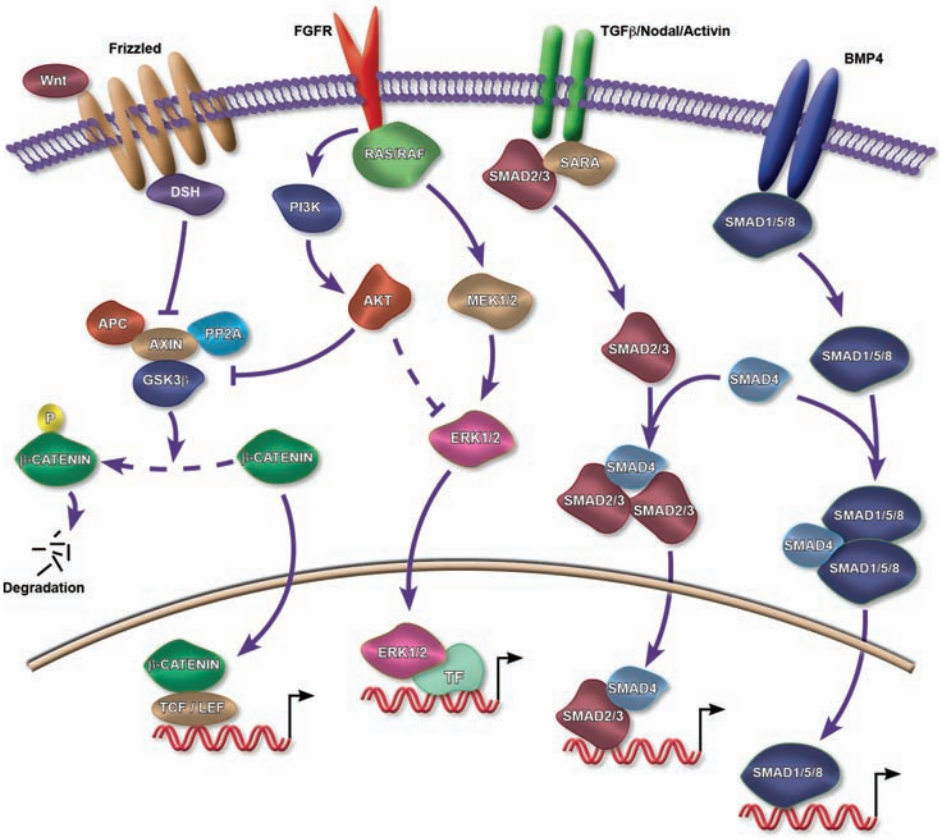


Figure 1: Cell signaling pathways controlling pluripotency and self-renewal.
The primary cell signaling pathways involved in growth and differentiation are depicted. The Wnt, TGF-β/Activin/Nodal, and FGFR pathways are critical for pluripotency and self-renewal of embryonic stem cells. These signaling pathways regulate the activity of the downstream transcription factors, including Oct-4, NANOG and SOX2, that act as master regulators to drive the expression of stem cell-specific genes. During the process of differentiation, other signaling pathways, such as BMP and Notch, function to activate the downstream expression of lineage-specific genes. The culmination of the crosstalk between these various cell signaling pathways ultimately determines the cell's decision of whether to maintain its stem cell identity or to differentiate into a specific cell type.

Reference

1. Kaneko S *et al.* (2010) *Genes & Dev* 24(23):2615-2620.

cascades are known to modulate the activity of regulatory transcription factors, it is possible to look at the global changes in transcriptional activity while exploring underlying epigenetic mechanisms. To emphasize the power of this approach, Active Motif utilized NGS to analyze the relationship between STAT1 activation and trimethylation of histone H3 at lysine 4 (a gene activation mark) following stimulation by interferon gamma (INFγ). As seen in Figure 2, INFγ stimulation results in epigenetic changes within the same gene loci as those being occupied by STAT1α (Catalog No. 39059), emphasizing the relationship between these events. Obtaining a clear view of how such events are coordinated and where they may differ will undoubtedly provide valuable insights into how the environment signals to the epigenome.

Histone phosphorylation

A further example of the interconnectivity between cytoplasmic signaling cascades and the epigenome occurs directly at the level of the histones. Specifically, phosphorylation of serine residues (Ser10 and Ser28) has been shown to occur rapidly following stimulation of commonly studied signaling pathways, such as the heat shock pathway. The presence of phosphorylated serines has been shown to cause important epigenetic and transcriptional changes, and is commonly used as an early stage marker of apoptosis. The rapid response of the epigenome to external stimuli and its importance in the regulation of key cellular processes, such as apoptosis, serve to further emphasize why it is becoming essential to analyze epigenetic changes as part of signaling cascade research.

Where to begin?

Many of the methods employed in the field of epigenetics, such as chromatin immunoprecipitation (ChIP) and ChIP followed by NGS, are technically demanding. Therefore, Active Motif's objective is to expand the reach of epigenetic research by making technological advances available to all researchers, independent of their background discipline. If you are a cell signaling researcher interested in expanding your research by exploring the role of epigenetics within your model system, give us a call or visit us at www.activemotif.com to learn how Active Motif products can aid you in this process.

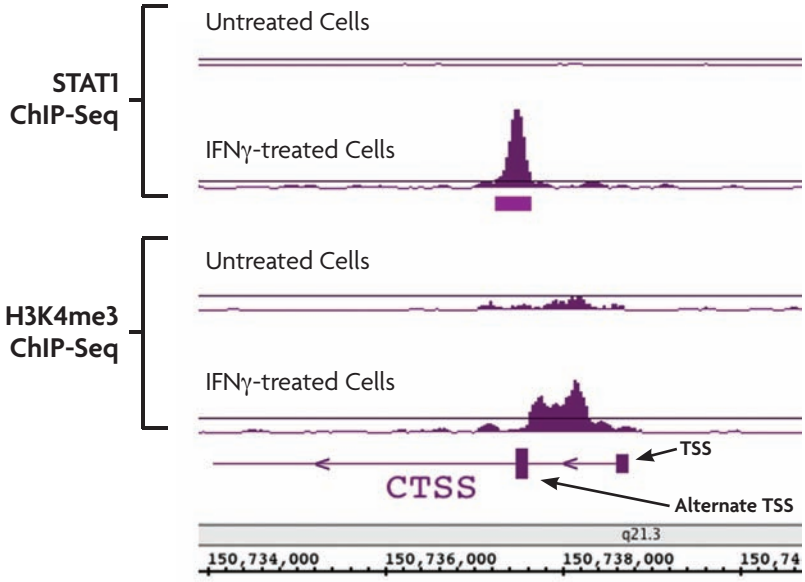


Figure 2: IFNγ induced STAT1 binding correlates with induced epigenetic changes.
Human colon cancer cells were treated with IFNγ to stimulate signaling cascades that lead to STAT1 phosphorylation and activation. ChIP-Seq was performed to map all STAT1 binding sites and H3K4me3 ChIP-Seq was performed to identify epigenetic changes. The image above is zoomed in to show an example of a single gene where induced STAT1 binding correlates with induced H3K4me3 occupancy.

CHROMATIN MODIFICATION AND CELL SIGNALING ANTIBODIES

Active Motif has a large, ever-expanding collection of antibodies to proteins important in chromatin modification, transcriptional regulation and cell signaling. For the complete, up-to-date list, please visit us at www.activemotif.com/abs.

Product	Format	Catalog No.
EZH2 antibody (mAb)	100 µg	39875
EZH2 phospho Thr345 antibody (pAb)	100 µl	61241
Histone H3K4me3 antibody (pAb)	200 µl	39159
NFκB p50 antibody (pAb)	200 µl	39037
NFκB p65 phospho Ser529 antibody (pAb)	100 µl	39691
NFκB p65 phospho Ser536 antibody (pAb)	100 µl	39675
RNA pol II antibody (mAb)	100 µg	61081
RNA pol II CTD phospho Ser2 antibody (mAb)	100 µg	61083
RNA pol II CTD phospho Ser5 antibody (mAb)	100 µg	61085
RNA pol II CTD phospho Ser7 antibody (mAb)	100 µg	61087
RNA Pol II CTD phospho Thr4 antibody (pAb)	100 µg	61307
STAT1α antibody (pAb)	100 µl	39059
STAT1 phospho Ser727 antibody (pAb)	200 µl	39633

ACTIVE MOTIF®

Enabling Epigenetics Research

Now offering **ChIP-Seq** validated antibodies

Active Motif is dedicated to developing quality antibodies and kits to characterize key epigenetic events.

Visit us at www.activemotif.com for a complete list of antibodies for DNA methylation, ChIP and ChIP-Seq.

Superior Antibodies & Kits for EPIGENETICS RESEARCH

Visit us at **www.activemotif.com**

- H3K4me3
- H3K9me3
- H3K27me3
- H3K36me3
- H3S10ph
- H3K9ac
- EZH2
- 5-mC
- 5-hmC
- 5-fC
- 5-caC
- 3-mC
- Oct-4
- MyoD
- RNA p
- DNMT
- HDAC
- H3.cs1
- H2A