# Chromatin and Epigenetic Regulation Pathways from Cell Signaling Technology

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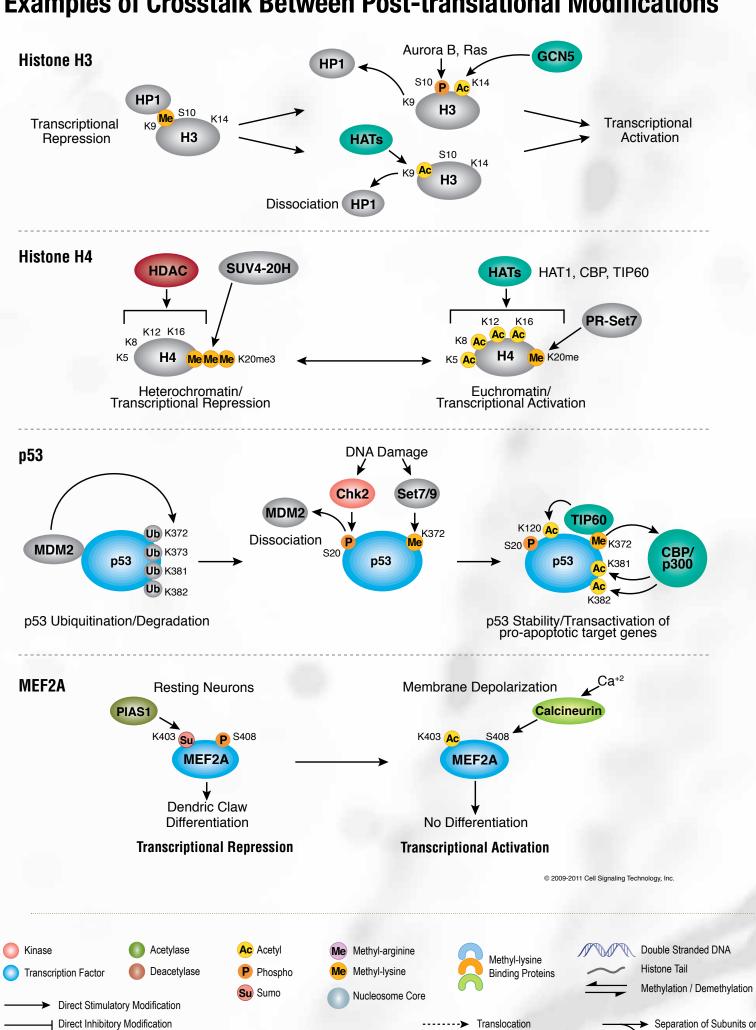
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### **Protein Acetylation** Regulatory Acetylation Signaling Pathways **МСМЗАР** ATF-2 MORF CDY CLOCK PCAF EWI p/CIP Elp3 SRC-1 p53 Rb GCN5L hTAF<sub>250</sub> BRCA1 α-Importin GRIP TFIIB HAT1 Tip60 HBO1 DNA Stability Transcription TFIIH P P Pluripotency TAFS TFIIF TFIIA TBP TFIIB RNA Pol II **Neural Stem Cells DNA Damage** Deacetylation **Known Acetylated Proteins** Acetyl-CoA Acetyl CoA MEF2 Synthetase Synthetase Ku70 p130 AP endonuclease MFF2A PCAF **Sirtuins** MHG17 Smad2 eNOS PGC-1α Mitochondrial FoxO SREBP GCN<sub>5</sub> Rb, p107 E2F1, -2, -3 NcoR Sin3 Ku70 TAF(1)68 NF-κB tubulin TCF TFIIB FoxO1, 3, 4 **HDAC Classes** TFIΙΕβ p73 Class II: HDAC4-7 9 10 TR/RXR Histones Signaling Pathways Class III: SIRT1-7 HMG A1 PGC-1α α-tubulin Class IV: HDACII HSP90 WRN © 2006-2011 Cell Signaling Technology, Inc

# **Histone Methylation** Transcriptionally Inactive Transcriptionally Active Chromatin Chromatin Transcription Initiation and Elongation SUV39H, G9a, ESÉT H3K79me H3K9me LSD1, JMJD1, Pericentric Heterochromatin Inactive X Chromosome Inactive X Chromosome

# **Examples of Crosstalk Between Post-translational Modifications**



## **Histone Modification Table**

Hox Gene Repression

		Histone-modifying Enzymes	Pronosed Function	PMID
stone :A				10082517
.^	, , , ,			10096020, 9880483
				9427644
	Lyor (o. coroviolas)			10082517
В	LvoE		·	
D	Lys5		· · · · · · · · · · · · · · · · · · ·	9880483, 10821277
				11545749 9880483, 10821277
			· · · · · · · · · · · · · · · · · · ·	9880483, 10821277
		<u> </u>		11545749
	Lys20			9880483
			' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '	
}	Lys4 (S. cerevisiae)		·	10082517
	10	<u>'</u>		10600387
	Lys9		·	7862667
	Lund 4			10026213, 9296499
	Lys14			7862667
				9880483, 12353039
		Esal, Tip60		10082517, 10096020
		000 /	· · · · · · · · · · · · · · · · · · ·	12353039, 10966108
				9296499
		FID3		11904415
		Haso	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	1000007
				10600387
				10523658
				8980232
				12626510
		Sas3		11731478
		n300		9880483
	Lys18			
	2,0.0	p300/CBP	DNA replication, transcriptional activation	9880483, 12498683
	Lys23	unknown	histone deposition	7862667
	ĺ	Gcn5		10026213
		Sas3	transcriptional activation (elongation)	11731478
		p300/CBP	transcriptional activation	9880483, 12498683
	Lys27	Gcn5	transcriptional activation	11545749
	Lys56 (S. cerevisiae)	Spt10	transcriptional activation	15882620
			DNA repair	16015338
	Lys5	Hat1	histone deposition	8858151
	3,00			10082517, 10096020
		,		12353039, 10966108
		ATF2		10821277
				10600387
		s4 (S. cerevisiae)         Esa1         transcriptional activation         1           s5 (mammals)         Tip60, p300/CBP         transcriptional activation         1           s7 (S. cerevisiae)         Hat1         unknown         9           s5         p300, AFF2         transcriptional activation         1           s5         p300, CBP, AFF2         transcriptional activation         9           s12 (mammals)         p300/CBP, AFF2         transcriptional activation         9           s16 (mammals)         p300/CBP, AFF2         transcriptional activation         9           s16 (S. cerevisiae)         Gcn5, Esa1         transcriptional activation         9           s20         p300         transcriptional activation         1           s20         p300         transcriptional activation         1           s4 (S. cerevisiae)         Esa1         transcriptional activation         1           s4 (S. cerevisiae)         Esa1         transcriptional activation         1           s4 (S. cerevisiae)         Esa1         transcriptional activation         1           s5 (C. cerevisiae)         Esa1         transcriptional activation         1           s6 (S. cerevisiae)         Esa2, Tip60         transcriptional activation	9880483	
	Lys8		9880483, 8805705	
	3,00		10082517, 10096020	
		2001, 11,000		12353039, 10966108
				10821277
		ATF2		10021211
			transcriptional activation	11904415
		Elp3	transcriptional activation (elongation)	9880483
	Lys12	p300	transcriptional activation (elongation) transcriptional activation	
	Lys12	p300	transcriptional activation (elongation) transcriptional activation histone deposition	9880483
	Lys12	p300 Hat1	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing	9880483 8858151
	Lys12	p300 Hat1	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation	9880483 8858151 10982821 10082517, 10096020
	Lys12	p300 Hat1 Esal, Tip60	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair	9880483 8858151 10982821 10082517, 10096020
	Lys12	p300 Hat1 Esal, Tip60	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108
	Lys12	Elp3  p300  Hat1  Esal, Tip60  Hpa2 p300	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown transcriptional activation	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108 10600387
		Elp3  p300  Hat1  Esal, Tip60  Hpa2 p300 Gcn5	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown transcriptional activation transcriptional activation	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108 10600387 9880483
		p300 Hat1 Esal, Tip60 Hpa2 p300 Gcn5 MOF (D. melanogaster)	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown transcriptional activation transcriptional activation transcriptional activation	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108 10600387 9880483 8805705
		p300 Hat1 Esal, Tip60 Hpa2 p300 Gcn5 MOF (D. melanogaster)	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown transcriptional activation transcriptional activation transcriptional activation transcriptional activation transcriptional activation	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108 10600387 9880483 8805705 9155031
		Elp3 p300 Hat1 Esal, Tip60 Hpa2 p300 Gcn5 MOF (D. melanogaster) Esal, Tip60	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown transcriptional activation transcriptional activation transcriptional activation transcriptional activation transcriptional activation	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108 10600387 9880483 8805705 9155031 10082517, 10096020
		Elp3 p300 Hat1 Esal, Tip60 Hpa2 p300 Gcn5 MOF (D. melanogaster) Esal, Tip60	transcriptional activation (elongation) transcriptional activation histone deposition telomeric silencing transcriptional activation DNA repair unknown transcriptional activation transcriptional activation transcriptional activation transcriptional activation transcriptional activation	9880483 8858151 10982821 10082517, 10096020 12353039, 10966108 10600387 9880483 8805705 9155031 10082517, 10096020 12353039, 10966108

Site	Histone-modifying Enzymes	Proposed Function	PMID
Lys9	biotinidase	unknown	16109483
Lys13	biotinidase	unknown	16109483
Lys4	biotinidase	gene expression	16098205
Lys9	biotinidase	gene expression	16098205
Lys18	biotinidase	gene expression	16098205
Lys12	biotinidase	DNA damage response	15153116, 16177192
	Lys9 Lys13 Lys4 Lys9 Lys18	Lys9 biotinidase Lys13 biotinidase Lys4 biotinidase Lys9 biotinidase Lys18 biotinidase	Lys9     biotinidase     unknown       Lys13     biotinidase     unknown       Lys4     biotinidase     gene expression       Lys9     biotinidase     gene expression       Lys18     biotinidase     gene expression

Methy	/lation			
Histone	Site	Histone-modifying Enzymes	Proposed Function	PMID
H1	Lys26	Ezh2	transcriptional silencing	16127177, 15099518
Н3	Lys4	Set1 (S. cerevisiae)	permissive euchromatin (di-Me)	11751634
		Set7/9 (vertebrates)	transcriptional activation (tri-Me)	11779497
		MLL, ALL-1	transcriptional activation	12453419, 14603321
		Ash1 (D. melanogaster)	transcriptional activation	12397363
	Arg8	PRMT5	transcriptional repression	15485929
	Lys9	Suv39h,Clr4	transcriptional silencing (tri-Me)	10949293, 11283354
		G9a	transcriptional repression, genomic imprinting	11316813
		SETDB1	transcriptional repression (tri-Me)	11959841
		Dim-5 (N.crassa), Kryptonite (A. thaliana)	DNA methylation (tri-Me)	11713521, 12194816
		Ash1 (D. melanogaster)	transcriptional activation	12397363
	Arg17	CARM1	transcriptional activation	12498683
	Lys27	Ezh2	transcriptional silencing	12351676
			X inactivation (tri-Me)	
		G9a	transcriptional silencing	11316813
	Lys36	Set2	transcriptional activation (elongation)	12773564
	Lys79	Dot1	euchromatin	12123582
			transcriptional activation (elongation)	12667454
			checkpoint response	15525939
H4	Arg3	PRMT1	transcriptional activation	11448779
		PRMT5	transcriptional repression	15485929
	Lys20	PR-Set7	transcriptional silencing (mono-Me)	12086618
		Suv4-20h	heterochromatin (tri-Me)	15145825
		Ash1 (D. melanogaster)	transcriptional activation	12397363
		Set9 (S. pombe)	checkpoint response	15550243
	Lys59	unknown	transcriptional silencing	12937907

Nuclear Receptor-mediated Activation

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Histone	Site	Histone-modifying Enzymes	Proposed Function	PMID
H1	Ser27	unknown	transcriptional activation, chromatin decondensation	16127177, 15099518
H2A	Ser1	unknown	mitosis, chromatin assembly	15133681
		MSK1	transcriptional repression	15010469
	Thr119 (D. melanogaster)	NHK1	mitosis	15078818
	Ser122 (S. cerevisiae)	unknown	DNA repair	15781691
	Ser129 (S. cerevisiae)	Mec1, Tel1	DNA repair	11140636, 15458641
	Ser139 (mammalian H2AX)	ATR, ATM, DNA-PK	DNA repair	11673449, 11571274, 14627815
H2B	Ser10 (S. cerevisiae)	Ste20	apoptosis	15652479
	Ser14 (vertebrates)	Mst1	apoptosis	12757711
		unknown	DNA repair	15197225
	Ser33 (D. melanogaster)	TAF1	transcriptional activation	15143281
Н3	Thr3	Haspin/Gsg2	mitosis	15681610
	Ser10	Aurora-B kinase	mitosis, meiosis	9362543, 10975519
		MSK1, MSK2	immediate-early gene activation	12773393
		IKK-α	transcriptional activation	12789343
		Snf1	transcriptional activation	11498592
	Thr11 (mammals)	Dlk/Zip	mitosis	12560483
	Ser28 (mammals)	Aurora-B kinase	mitosis	11856369
		MSK1, MSK2	immediate-early activation	12773393, 11441012
H4	Ser1	unknown	mitosis, chromatin assembly	15133681
		CK2	DNA repair	15823538

Histone	Site	Histone-modifying Enzymes	Proposed Function	PMID
H2A	Lys126 (S. cerevisiae)	Ubc9	transcriptional repression	16598039
H2B	Lys6 or Lys7 (S. cerevisiae)	Ubc9	transcriptional repression	16598039
	N-terminal tail (S. cerevisiae)	Ubc9	transcriptional repression	14578449

Ubiquitination					
Histone	Site	Histone-modifying Enzymes	Proposed Function	PMID	
H2A	Lys119 (mammals)	Ring2	spermatogenesis	15386022	
H2B	Lys120 (mammals)	UbcH6	meiosis	16307923	
	Lys123 (S. cerevisiae)	Rad6	transcriptional activation	10642555	
			euchromatin		

associated with deacetylase activity. Acetylation complexes (such as CBP/p300 and PCAF) or deacetylation complexes (such as Sin3, NuRD, NcoR and SMRT) are recruited to DNA-bound transcription factors (TFs) in response to signaling pathways. Histone hyperacetylation by histone acetyltransferases (HATs) is associated with transcriptional activation, whereas histone deacetylation by histone acetylases (HDACs) is associated with transcriptional activation, whereas histone deacetylation by remodeling higher order chromatin structure, weakening histone-DNA interactions, and providing binding sites for transcriptional activation complexes containing proteins that possess bromodomains, which bind acetylated lysine. Histone deacetylation represses transcription through an inverse mechanism involving the assembly of compact higher order chromatin and the exclusion of bromodomain-containing transcription activation complexes. Histone hypoacetylation is a hallmark of silent heterochromatin. Site-specific acetylation of a growing number of non-histone proteins, including p53 and E2F, has been shown to regulate their activity, localization, specific interactions, and stability/degradation, therefore controlling a variety of cellular processes, such as transcription, proliferation, apoptosis, and differentiation. At an organismal level, acetylation plays an important role in immunity, circadian rhythmicity, and memory formation. Protein acetylation is becoming a favorable target in drug design for numerous disease conditions. becoming a favorable target in drug design for numerous disease conditions.

─ ☐ Tentative Inhibitory Modification

Transcriptional Inhibition

histones have more recently been shown to be dynamic proteins, undergoing multiple types of post-translational modifications. Two such modifications, methylation of arginine and lysine residues are major determinants for formation of active and inactive regions of the genome. Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the Drosophila Su[var]3-9, Enhancer of zeste, and Trithorax proteins. Lysine methylation has been implicated in both transcriptional activation (H3 Lys4, 36, 70) and eilegging (H3 Lys9, 37, H4 Lys9).

Cleavage Products

Unlike acetylation, methylation does not alter the charge of arginine and lysine residues and is unlikely to directly modulate nucleosomal interactions required for chromatin folding. While the mechanisms by which arginine methylation regulates transcription are unknown, lysine methylation coordinates the recruitment of chromatin modifying enzymes. Chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), Tudor domains (53BP1), and WD-40 domains (WDR5) are among a growing list of methyl-lysine binding modules found in

sation and nucleosoffic mobility in order to maintain local regions of active or inactive chromatin. In addition, lysine methylation can block binding of proteins that interact with unmethylated histones or directly inhibit catalysis of other regulatory modifications on neighboring residues. The presence of methyl-lysine binding modules in the DNA repair protein 53BP1 suggests roles for lysine methylation in other cellular

Histone methylation is crucial for proper programming of the genome during development and misregulamaintaining a gene in an active or inactive state. While there is no argument that methylation is a stable shown that methylation is reversible and provides a rational for how genomes might be reprogrammed

## **Examples of Crosstalk Between Post-translational Modifications**

putative role for each of these modifications, for instance, acetylation correlates with activation and methylation with repression. However, more recent studies indicate that some of these modifications could trigger either activation or silencing in a context dependent manner. For instance, methylation of histone H3 Lys9 correlates with repression, while methylation of H3 Lys4 correlates with activation. Furthermore, each of these moieties can be either mono, di- or tri-methylated, and depending on the degree of methylation, the biological output will be completely different. Until recently, PTMs were considered independently, under the assumption that their functions would not be related to one another. It is now clear that PTMs work in concert, and the crosstalk between different modifications deterto one another. It is now clear that PTMs work in concert, and the crosstalk between different modifications deter mines the final biological read-out. In this context, some modifications can influence others, and it appears that specific combinations of these modifications can form a dynamic "code". We provide a few examples of this type of crosstalk above. Although each of the modifications shown here are occurring in cis, there are now clear examples, at least for histones, where modifications in one histone molecule can regulate modifications in other histones in trans. Although there are now many examples of these "functional networks", it is likely that we have just begun to scratch the surface. Better antibodies and novel technologies will help to complete this crosstalk puzzle, for which the specific fine-tuning appears critical to determine life as we know it.

Multistep Stimulatory Modification

Multistep Inhibitory Modification