*Research in Biotechnology, 2(2): 50-66, 2011* **ISSN: 2229-791X**
www.researchinbiotechnology.com

**Review Article**

# Methylation and loss of Imprinting: Unending rivalries unleashed between "kneaded erasers" and "fate writers"

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**Genome is a complex barcode that is interpreted at molecular level. There are various proteins which are modulating the expression or repression of the genes. Miscellaneous proteins work in collaboration to stimulate or repress the gene expression. Chromatin remodeling factors are the artists which chisel, carve and mould the sculpture of genome. In this review we will emphasize on exemptions and extensions which trigger genomic instability in broad range of molecular anomalies. Doubtlessly therapeutic interventions have shown tremendous promise in cancer therapy, but the selectivity profiles of these compounds have largely relied on serendipity or 'off-target' activities rather than rational drug design. Purposefully designed compounds with activity against methyltransferase, demethylase and HDAC will bring us a step closer to personalized medicine.**

Genome is the complex machinery that is executed by various proteins. The expression or repression of the gene is triggered by a network of proteins that work in conjunction to induce or shutdown the expression of a gene. Acetylation and methylation are the two opposite trends which work antagonistically. These convergent and divergent trends are involved in the dynamics of normal and neoplastic cell. In cancer cell, the oncogenes are switched on and tumor suppressor genes are consequently switched off. These contrasts and commonalities modulate the transition of a normal cell to cancerous cell. Consistent with these facts, another master component that cannot be overlooked is the chromatin. There is a constitutive organization of chromatin. Histones are the foundation stones of the chromatin. The modifications of histone mediate DNA accessibility during replication, transcription and DNA repair. According to current understandings, both genetic alteration and epigenetic aberrations are involved in tumorigenesis.

Oncogenesis is contextualized by histone methyaltion that occurs at H3K4 and H3K27. It is worth mentioning that Histone H3K4 methylation keeps centromeres open for business.Methylation of histone might occur at lysine and arginine residues. This phenomenon is maintained by two antiparallel groups of enzymes. These act as disparate players that that shake uncommon principles. The proportional stoichiometry is necessary enough to abolish or bolster the gene expression mechanisms. The "Writers" and "Erasers" are the types of methylating enzymes which determine the fate of genome by barcoding the histone tails. For instance H3K4 methylation is established by SET1 and mixed lineage leukemia family of histone methyltransferases (HMT). The demethylation is executed by lysine specific histone demethylase (LSD1) and Jumanji AT

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rich Domains (JARID1) family of histone demthylases.

**Histones: The Attributes of Phenomenal wrap artists**

In eukaryotes the DNA and proteins scaffolds evolve into a tertiary structure known as chromatin. Nucleosome is the main entity of chromatin. A nucleosome is referred to as 147 bp of DNA wrapped around an octamer of histone proteins (Zhurkin *et al* 2010). The octamer is composed of two copies of four histone molecules H2A, H2B, H3 and H4 (Manoj *et al* 2006). There is a very tight interaction between the DNA and the hisotne molecules because of the attractive forces formed by negatively charged DNA and basic N-terminal histone tails (Hayes *et al* 2003). The two nucleosomes are linked to each other by another histone protein known as the linker histone or H1 (Manoj *et al* 2006). The repeated coiling of DNA forms a compact and a higher order structure. The nucleosome is an obstacle for various nuclear processes such as transcription, replication and DNA reapir. The DNA that is in compact form or inaccessible is referred to as heterochromatin or inactive DNA. DNA that is present in loose form or is accessible is referred to as euchromatin. The hallmark feature of eukaryotes is the switching between heterochromatin and euchromatin using different machineries. Appropriate regulation of gene expression is necessary for normal functioning of cell. This regulation is carried by remodeling of chromatin structure. The remodeling of chromatin is carried by two classes of enzymes, one that covalently modifying histone DNA contacts and other that uses energy from ATP hydrolysis to disrupt nucleosome. The class one enzymes modify histone by acetylation, methylation, phosphorylation and ubiquitylation (Paolo *et al* 2000). The second class of enzymes includes SWI/SNF (Switch/Sucrose Non Fermenter), NuRD (Nucleosome Remodeling and Deacetylation), ISWI (Imitation SWI), CHD (Chromodomain Helicase like Domain) and RSC (Chromatin Structure Remodeling) (Wang *et al* 2000, Gerd *et al* 2005, Yokoyama *et al* 2009).

***Marks, modules and multivalency***

Histones can be cavalently modified by the addition of various chemical appendages that create binding or effector, modules are known. In most cases, residues that line the pocket of a module dictate the modification state of a mark that is preferentially bound, while residues outside the binding pocket contribute to much of the histone sequence specificity. In several instances, similar folds bind different marks: for example, tudor domains can bind Kme3 (JMJD2A) or Kme2 (53BP1), and PHD fingers can bind preferentially Kme2/3 (ING2) or unmodified K (BHC80). In many cases, there is no longer a distinct correlation between a single histone mark and its function. For example, the Kme3 mark, when in context of the N terminus of H3 (K4me3), can be bound by PHD fingers of proteins in complexes that either activate gene expression (for example, the BPTF subunit of the NURF complex in homeotic gene remodeling enhances transcription) or repress it (for example, the ING2 subunit of the Sin3a-HDAC complex is involved in repression following DNA damage). Analysis of native histone modification states combinations rather than in isolation, which may help to account for this paradox. Perhaps multivalent interactions with discrete patterns dictate composite specificity and enhances the affinity the chromatin associated complexes.

**The Incorporation of Histones on DNA**

Prior to deposition, H3–H4 and H2A–H2B exist as dimers that are complexed to specific histone chaperones. On chromatin disruption at replication, parental H3–H4 tetramers with histone marks can either be preserved (unsplit) or broken up into dimers (split), potentially by interacting with the chaperone

anti-silencing function 1 (ASF1). Nucleosomes with only old H3–H4 are formed when unsplit parental tetramers are transferred directly onto daughter strands or when two parental H3–H4 dimers reassociate. Newly synthesized H3–H4 dimers with their typical marks are complexed with the chaperones ASF1 and chromatin assembly factor 1 (CAF1; also known as CHAF1). Nucleosomes might be formed on the daughter strands from one parental and one new H3–H4 dimer (indicated as mixed) or exclusively from two new H3–H4 dimers. There is maturation of newly formed nucleosome containing new or mixed histones. The other type of chaperone proteins involved in histone corporation areFACT, facilitates chromatin transcription; HIRA, Hir-related protein A; NAP1, nucleosome assembly protein 1.

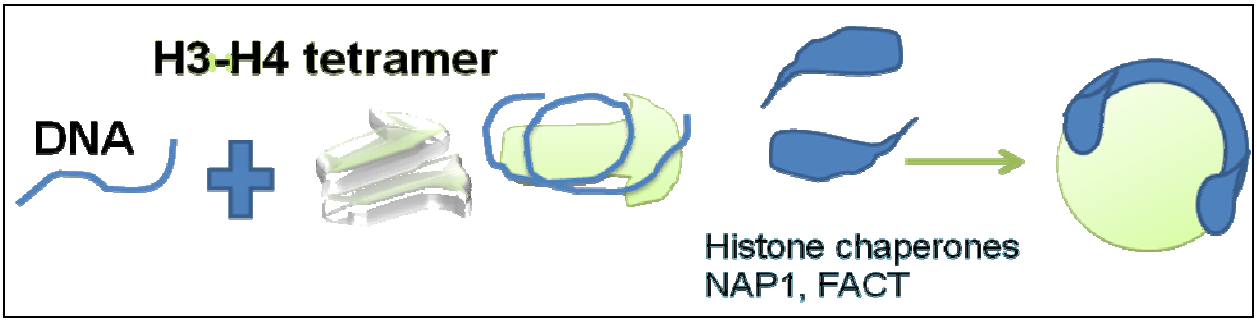


**Figure 1. The incorporation of histone $(H3–H4)_2$ tetramers onto DNA, followed by the addition of two histone H2A–H2B dimers to form a nucleosome core particle. The action of methylases and deacetylases for the maintaining heterochromatin**

In fission yeast the pericenteric heterochromatin contains loads of dimethylated histone H3K9 (H3K9me2) in G2 phase. This H3K9 provides a binding site for heterochromatin protein 1 (HP1) which is the homologue of Swi6. When the yeast cell enters M or mitotic phase the phosphorylation of H3S10 leads to reduced binding of Swi6. This is termed the phospho–methyl switch. Centromeric repeats are transcribed after centromere replication in early S phase and after dilution of histone marks Transcripts are processed by the RNA interference (RNAi) machinery into small interfering RNAs (siRNAs). Hisone methyltransferase Clr4 (the Suv39 homologue) is recruited by RNAi machinery. Clr3 deacetylates H3K9 and Clr4 dimethylates the lysine tails. This leads to the restoration of Swi6 binding and silent heterochromatin. In mice methylated DNA and H3K9me3 is present in pericentric heterochromatin. H3K9me3 is bounded by HP1. The extent to which HP1 is disrupted by the phospho–methyl switch and how HP1 is restored in G1 phase is unclear. Centromeric transcripts accumulate in mitosis and in G1–early S phase however, a direct role for RNA in heterochromatin maintenance in mice is lacking. The DNA and histone modifiers along with chaperone proteins are involved in the maintenance of pericentric heterochromatin. DNA methyltransferase 1 (DNMT1) is involved in DNA methylation, which, together with proliferating cell nuclear antigen (PCNA), performs histone deacetylase activity. HP1 inheritance and H3.1 deposition is ensured by CAF1; also known as CHAF1 by the transfer of parental HP1 to daughter strands, where it is maintained by a self-perpetuating loop that involves SUV39H1 (also known as KMT1A).

The K9 tail of H3.1 can be monomethylated before deposition. This serves as a substrate for further modification in chromatin.

**SWI/SNF: The chromatin Remodeler**

SWI/SNF is an evolutionary conserved complex which is present in yeast and higher organisms such as humans and yeasts (Wang *et al* 2000). The SWI/SNF complex apart from gene regulation is also involved in cell cycle regulation and cell differentiation (Ryan *et al* 2009). In human two types of SWI/SNF complex are present known as complex A or BAF (BRG Associated Factors) and complex B or PBAF (PolyBromo and BRG Associated Factors) (Wang *et al* 2000). SMARCA4 (SWI/SNF Related, Matrix Associated, Actin dependent, Regulator of Chromatin, Subfamily A, Member 4) or BRG (Brahma Related Gene) is a core complex of both BAF and PBAF (Wang *et al* 2000). The gene for *smarca4* is located on chromosome 19p13. SMARCA4 contains a central ATPase subunit that is responsible for hydrolysis of ATP (Wang *et al* 2000). The energy drives the DNA away from the histone molecules. SMARCA4 contains multiple domains that are responsible for its efficient working. The SMARCA4 protein contains a total of 1606 amino acids. The N terminal region of the protein contains three major domains QLQ, HSA and BRK. The QLQ domain contains Gln leu Gln motif which is responsible for mediating protein interactions. The HSA domain is responsible for binding to DNA and is associated with helicase. The BRK function is unknown but is found in association with helicases and transcription factors (Archer *et al* 2003). In the ATPase region DEXDc (Dead-Like Helicases Superfamily) is present, it conatins ATP and $Mg^{++}$ binding sites. Other domain present in the ATPase domain is the HELICc (Helicase superfamily C-terminal domain), this domain is associated with DEXDc and is found in many helicases and helicase like proteins (McKay *et al* 2007). HELICc is responsible for unwinding nuclic acid duplexes by using energy derived from ATP hydrolysis. In the C-terminal region there are acetyl lysin binding site, AT-hook, and bromodomain. The AT-hook motif is an auxillary protein involved in binding of DNA and facilitating changes in DNA structure. The bromodomain recognizes the acetylated histone tails and helps in binding with DNA (Wang *et al* 2000, Mahavir *et al* 2006).

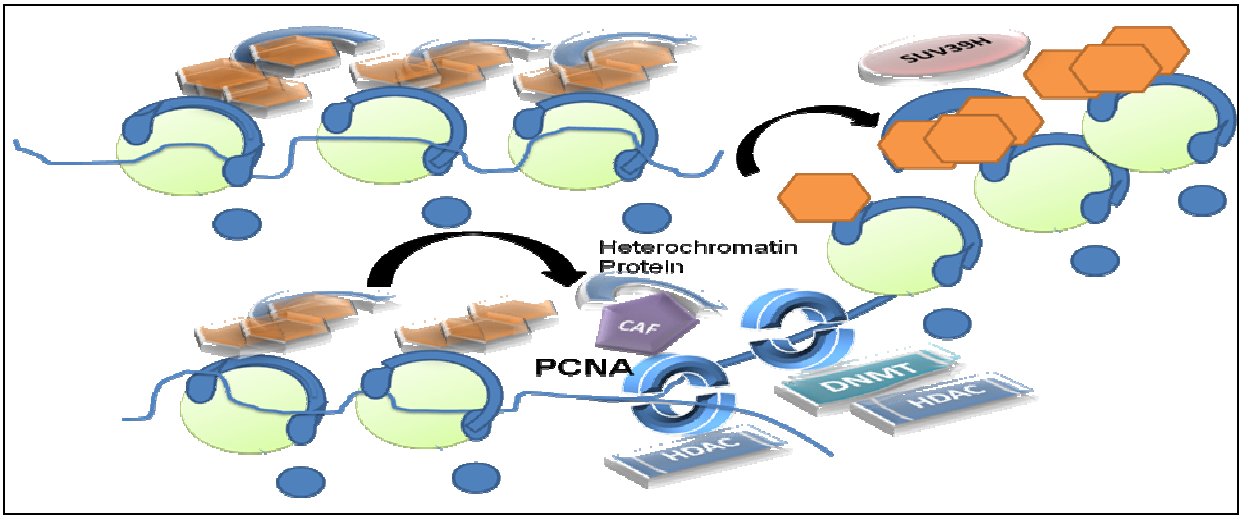


**Figure. 2. The interactive illustration of methyl transferase and deacetylase. Subsequent inactivation of H3K9 is dependent on the methylation of the region by DNMT.**

**Mechanism of Nucleosome Mobilization**

Nucleosome creates a barrier for various DNA binding factors, it is necessary to disrupt histone-DNA contacts to gain excess to the DNA binding sites. Despite altering nuclesome for exposing DNA sites, SWI/SNF complex is also reported to repress gene transcription by mobilizing histone octamer to promoter regions. The recruitment of SWI/SNF complex to DNA is not site specific. SWI/SNF machinery is used by various proteins which direct this to specific DNA sites for gene activation or repression. The characteristic feature of nucleosome mobilization by SWI/SNF is that it creates nucleosome with altered characteristics and topological features. Smarca4 being an important subunit of SWI/SNF complex is essential for binding and catalyzing ATP for nucleosome displacement. Due to technical limitations there are only two ways for assaying nucleosome mobilization; by nuclease activity and by changes in nucleosomal electrophoretic mobility (Blaine *et al* 2008).

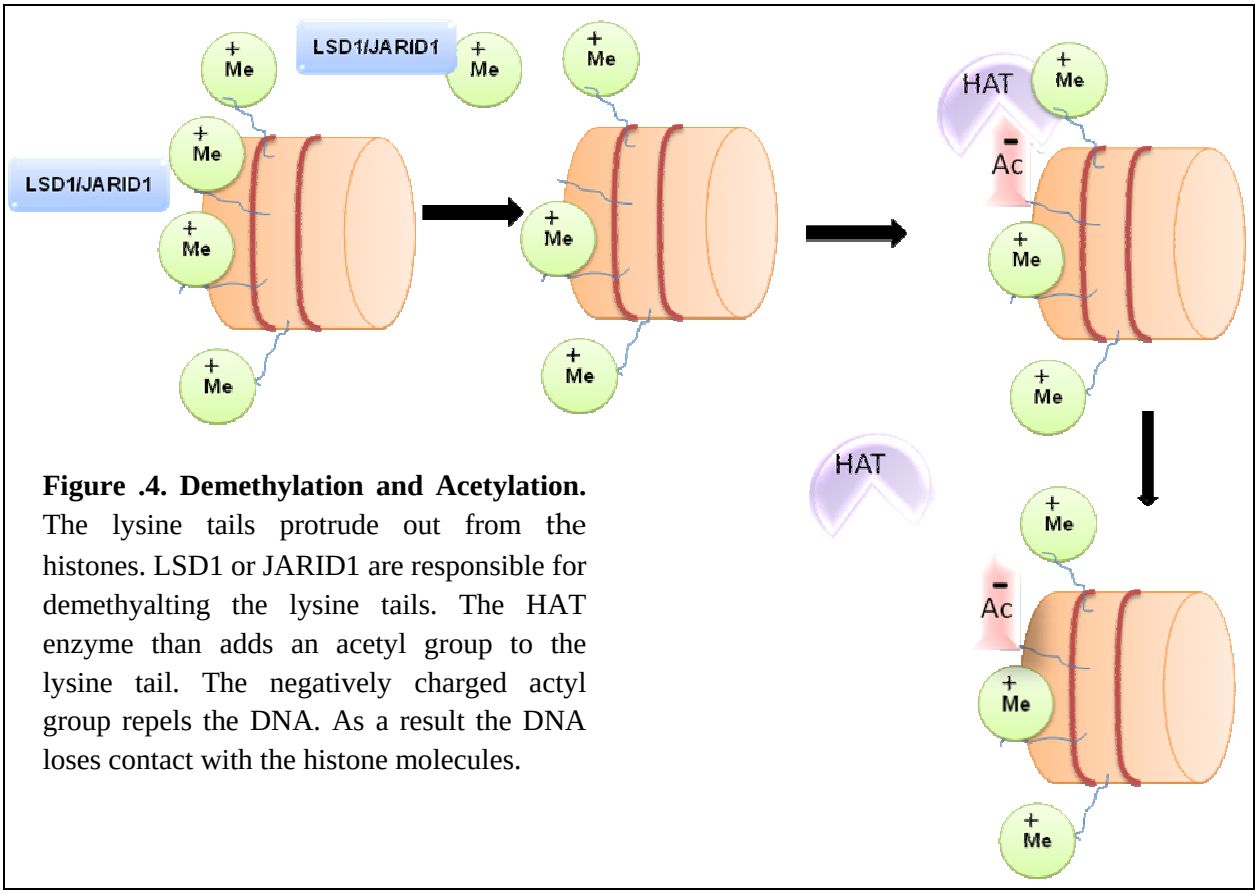


**Figure .4. Demethylation and Acetylation.** The lysine tails protrude out from the histones. LSD1 or JARID1 are responsible for demethyalting the lysine tails. The HAT enzyme than adds an acetyl group to the lysine tail. The negatively charged actyl group repels the DNA. As a result the DNA loses contact with the histone molecules.

**The mechanistic insights of remodeling by SWI/SNF**

The basic mechanism starts with recruitment of SWI/SNF complex to specific DNA sequence by a co activator. The SWI/SNF complex works in collaboration with the HAT enzymes (Hassan *et al* 2007). The acetylation of histones forms a negative charge. This opposite charge relieves the DNA of histone interactions. Electron micrograph and three

dimensional reconstruction of SWI/SNF complex A (PBAF) and RSC complex show that they have a cavity in which a mononucleosome can fit in (Blaine *et al* 2008). The binding of complex is on nuclesome positioning sequences (Martin *et al* 2007). After binding the SWI/SNF complex utilizes the ATP for energy to alter the nucleosome structure.

**Loop formation**

This mechanism involves formation of DNA loop. This starts from detachment of DNA by SWI/SNF complex at the Super Helix Location SHL+2 or SHl-2 near dyaed axis of the nucleosome (Blaine *et al* 2008, Martin *et al* 2007, Kingston *et al* 2009, Narlikar *et al* 2008). The complex detaches the DNA from the edge and pushes it inwards along the histone. In another model the complex attaches to the linker DNA and pushes the DNA in towards the nucleosome creating a force which peels of the DNA (Blaine *et al* 2003). After initial detachment the DNA bulge propagates towards the end of nucleosome. As the bulge propagates it exposes sites on nuclecosome. To these sites the DNA before the bulge gets attached. As more ATP is hydrolyzed the size of the loop increases (Kingston *et al* 2009). The size of loop is determined by how far on the DNA the enzyme can translocate per ATPase cycle therefore with excess of ADP the reaction slows down and SWI/SNF gets detached (Kingston *et al* 2009). Besides ADP accumulation the linker DNA and the nucleosome excluding sequences hinders the formation of loop (Martin *et al* 2007, Kingston *et al* 2009). Firstly as the DNA propagates the linker DNA gets into a collision with the SWI/SNF complex this either result in detachment of the SWI/SNF complex or H1 (Blaine *et al* 2008, Schnitzler *et al* 2003). The nucleosome excluding sequences prevents formation of nucleosme as they contain rigid sequences as a result the DNA propagates in opposite direction (Martin *et al* 2007). In the end a loop may be formed resulting in repositioned histone octamer. The disruption of histone DNA contacts may result in dimer or octamer loss. The loop may get trapped. The loop formed may bind to another exposed histone and result in formation of di-nucleosomes. A single remodeling event occurs in about 1s utilizing atleast 10 ATPs. In remodeling 50bp DNA is exposed (Narlikar *et al* 2008).

**Histone Eviction**

In another model of nucleosomal remodeling the swi/snf complex causes histone exchange or its eviction from its current location. This process occurs after loop formation, but instead of sliding of histone octamer, there is loss of histone molecules. The loss of histone occurs due to instability of the octamer which is maintained by DNA contacts (Tom *et al* 2003). As a result there is either eviction of histone proteins or they are replaced by histone variants (Kingston *et al* 2009). The destabilization of histone octamer results in a complete eviction of the octamer or most probably eviction of H2A/H2B dimer from the nucleosome. This dimer can reassociate it self with the adjacent free DNA or any vacant H3/H4 tetramer. Beside this there is also involvement of histone- chaperone proteins. These proteins are present in nucleus where they act as histone acceptor or donor; they are able to retrieve the whole octamer or only the dimer which dissociates. Members of these proteins such as Asf1 and nucleolin collaborate with the swi/snf complex for the eviction of histones from nucleosomes (Wolfram *et al* 2003, Stefan *et al* 2006, and Philipp *et al* 2006). In another study Swi3 subunits or BAF 155 and BAF 170 has also been reported to have chaperone like ability. Upon recruitment of the remodeling complex to the nucleosome, the N-terminal domain of swi3p subunit forms an interaction with the H2A/H2B dimer. After dissociation of the complex the swi3p detaches the dimer from nucleosome (Peterson *et al* 2007). The result of dimer loss or nucleosome eviction exposes

the DNA for different DNA binding proteins required for replication, transcription or repair mechanisms (Tom *et al* 2009). In recent study by Bartholomew *et al* 2010, a novel mechanism of SWI/SNF remodeling was observed employing dinucleosomes instead of mononucleosomes in experimental procedures. The remodeling occurred in a two steps, in first after binding of SWI/SNF to nucleosome there is eviction of H2A-H2B dimer, in the second step the entire octamer is lost. In the intermediate process the SWI/SNF mobilizes the proximal nucleosome and destabilizes the adjacent nucleosome. It was observed by the researchers that there is no involvement of additional factors or chaperones proteins as acceptors of histones.

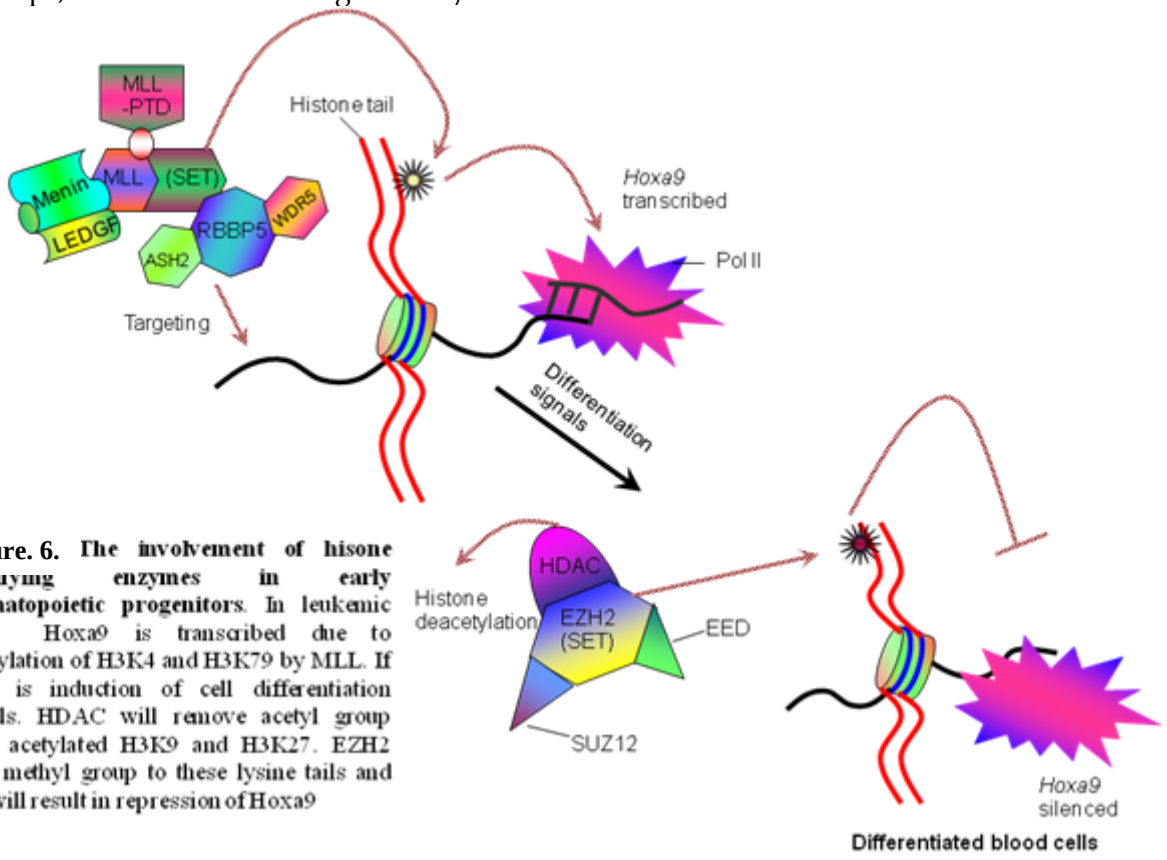


**Figure. 6. The involvement of hisone modifying enzymes in early haematopoietic progenitors.** In leukemic cells Hoxa9 is transcribed due to methylation of H3K4 and H3K79 by MLL. If there is induction of cell differentiation signals. HDAC will remove acetyl group from acetylated H3K9 and H3K27. EZH2 adds methyl group to these lysine tails and this will result in repression of Hoxa9

**Histone modifications in Hematopoietic lineages**

Menin has tumor suppressor ability. Menin loss of function results in the formation of cancer (Yokoyama *et al* 2008). MLL or mixed lineage lymphoma is a proto-oncogene. MLL act as methyl transferase which specifically adds methyl group to H3K4. MLL causes trimethylation of H3K4 (Michael *et al* 2004). The methylation of H3K4 is a mark for active chromatin. MLL is required for the transcriptional regulation of *Hoxa9*. Hoxa9 is involved in establishing embryonic body plan during development. It is also involved in the expansion of promoter progenitor and stem cell renewal in hematopoietic linage cells. Hoxa9 is also an important requisite of the leukemic cells (Jude *et al* 2007). LEDGF or lens epithelium derived growth factor contains PWWP motif that is structurally related to so call 'royal family.' The PWWP motif specifically binds to modified nucleosomes. Menin-MLL-LEDGF forms a complex, LEDGF in this complex act as an

adopter protein (Yokoyama *et al* 2008). This complexs with another protein complex 'SET-ASH2-RBBP5-WDR5' in hematopoietic cells. SET also act as methyl transferase which methylates lysine 4 or H3. ASH2, RBBP5 and WDR5 form a sub-complex with SET enzyme. These proteins are responsible for efficient binding of the histone methyl transferases to the histones (Michael *et al* 2004). This methylation is associated with active chromatin. Due to this activation polymerase II transcribes Hoxa9. In the differentiated progenitor cells the differentiation signals triggers HDAC, this removes acetyl group from H3K9 and H3K27. This deacetylataion of lysine residues leaves an empty site for another moiety such as methyl group. The EZH2 methyltransferase adds methyl group to this vacant lysine tail. This represses the activation of *Hoxa9* and prevents its transcription. This blocks the proliferative ability of hematopoietic cells. In leukemic cells this phenomena is tethered by MLL oncogene and deactivation of Menin which act as tumor suppressor. This leads to continuous transcription of *Hoxa9* and increase in the number of abnormal hematopoietic progenitor cells.

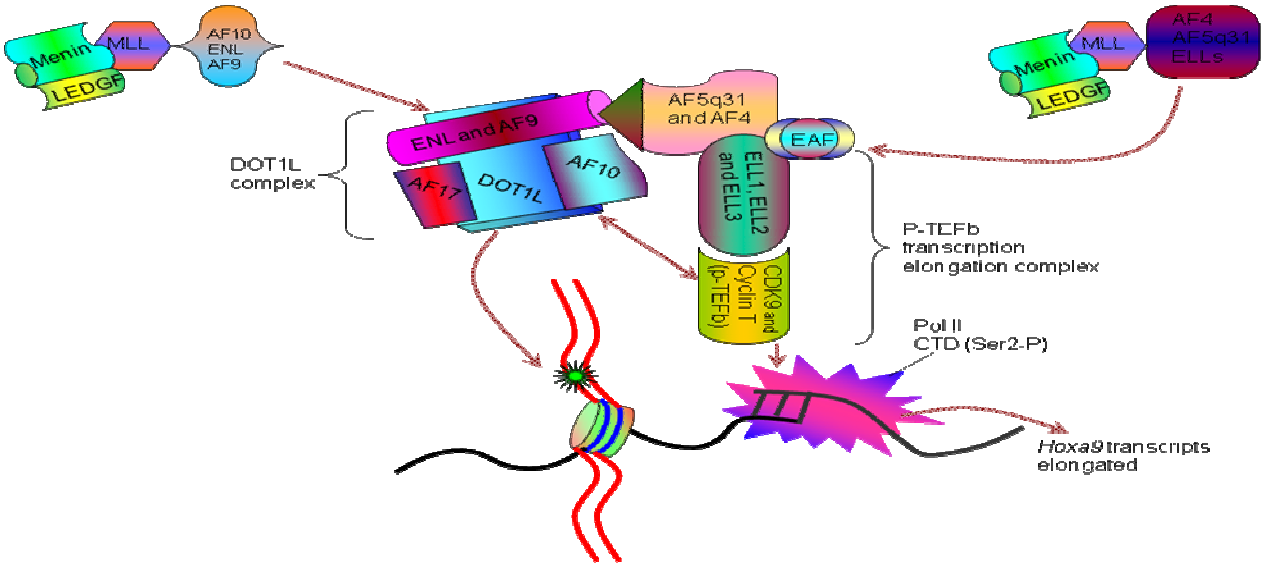


**Figure. 7. The elongation of Hoxa9 transcript.**

**MLL Fusion partners and their role in *Hoxa9* transcript elongation**

In leukemias MLL forms different fusion proteins; the attachment of fusion partners to the MLL demolishes the H3K4 methyl transferase activity of MLL through loss of $MLL^{c}$ subcomplex and $MLL^{n}$-associated HCFs. MLL forms fusion protein with AF10, AF9 and ENL. In the same manner MLL fusion partners may also include AF4, AFq31 and ELLS. Menin-LEDGF-MLL complexes with AF10-ENL-AF9. DOT1L is an associate factor that is recruited by MLL-AF10 fusion protein to MLL target genes. DOT1L is a methyl transferase that specifically methylates H3K79. Once there is hypermethylation of H3K79 there is upregulation of MLL target genes such as *Hoxa9*. This in turn block differentiation and promote proliferation leading to leukemia (Kay *et al* 2007, Zhang *et al* 2004). Menin-LEDGF-MLL also recruits protein complex AF4-AF5q31-ELLS to the transcriptional machinery. AF4 is responsible for stimulating

P-TEFb. P-TEFb is responsible for the phosphorylation of Pol II CTD at Ser-2. This induces the productive elongation of *Hoxa9* (Kay *et al* 2007, Ahn *et al* 2004).

**DNA Damage Repair**

As cell is prone to damage constantly and approximately 10000 lesions occur each day therefore it is necessary to repair the damage. There are different pathways cell has evolved to repair different lesions. These include Nucleotide Excision Repair, Base Excision repair, Single and Double stranded Breaks Repair. The lesions can occur throughout the DNA either in nucleosomal bound or unbound. SMARCA4 being the driving motor of the SWI/SNF complex plays a crucial role in repair mechanisms. The SWI/SNF complex is essential for repair by two ways. Firstly by remodeling nucleosome so the repair machineries have easy access to the damaged DNA and secondly by controlling transcription of genes involved in repair mechanism (Wim *et al* 2010, Imbalzano *et al* 2008).

Different histone marks are associated with different states of chromatin. The methylated histones are usually in repressed state while the acetylated histones with some specific methyl marks are associated active chromatin. Tip60 is an acetyltransferase which is activated in the early stages of DNA Damage Repair. After the induction of DNA damage the Rad50 subunit of MRN complex, complexes with the chromodomain of Tip60. The MRN complex and chromodomain are both necessary for the acetyl transferase activity of Tip60. The chromodomain of Tip60 are the methyl-lysine binding residues and are mainly associated with histone H3. Normally HP1 protein is associated with H3K9. After DNA damage CK2 helps in the removal of HP1 from H3K9. This removal of HP1 is necessary for the induction of DNA repair pathways. H3K9me3 and H3K36me3 are usually associated with repressed chromatin; recent studies have shown that they are necessary for the recruitment and activation of DNA repair machinery. Tip60's chromodomain binds methylated residues of H3K9 and H3K36. This binding of Tip60 to methylated histones is important for the acetyl transferase activity of Tip60. Tip60 in association with the MRN complex is also involved in the acetylation of ATM which is important for the kinase activity of ATM (Zdenko Herceg *et al* 2010).

**Damage Recognition and H2AX Phosphorylation**

The most initial and important response to DNA double stranded breaks is the phosphorylation of H2AX. γH2AX triggers the actetylation of H3 lysine tails in the same and neighboring nucleosomes (Kwon *et al* 2006). This acetylation is carried by HAT enzymes such as Gcn5 (Kwon *et al* 2010). This acetylation of lysine is recognized by the bromodomain of SMARCA4. After recognition the SWI/SNF complex remodels nuclesomal structure and facilitates ATM to further phosphrylate H2AX in the neighborhood nucleosomes (Kwon *et al* 2010). This in turn actylates more H3 and in a cyclic way many nucleosomes are phosphorylated by ATM in association of SWI/SNF complex. As there is formation of single stranded break, Replication Protein A gets phosphorylated and gets bound to ssDNA. RPA act as a platform for the recruitment of different proteins (Junran *et al* 2010). In the case ssDNA breaks resulting from stalled replication forks SMARCAL1 play a major role in controlling the process of homologous recombination. SMARCAL1 was first considered to be a helicase but recent research has proved that it is an annealing enzyme (Yusufzai *et al* 2008). SMARCL1 is member of swi/snf complex. SMARCAL1 becomes phosphrylated by ATM, ATR, DNA-PKc in response to double stranded breaks and stalled replication forks. On phosphory-lation SMARCAL1 makes complex with RPA with its N-terminal (Yusufzai *et al* 2009).

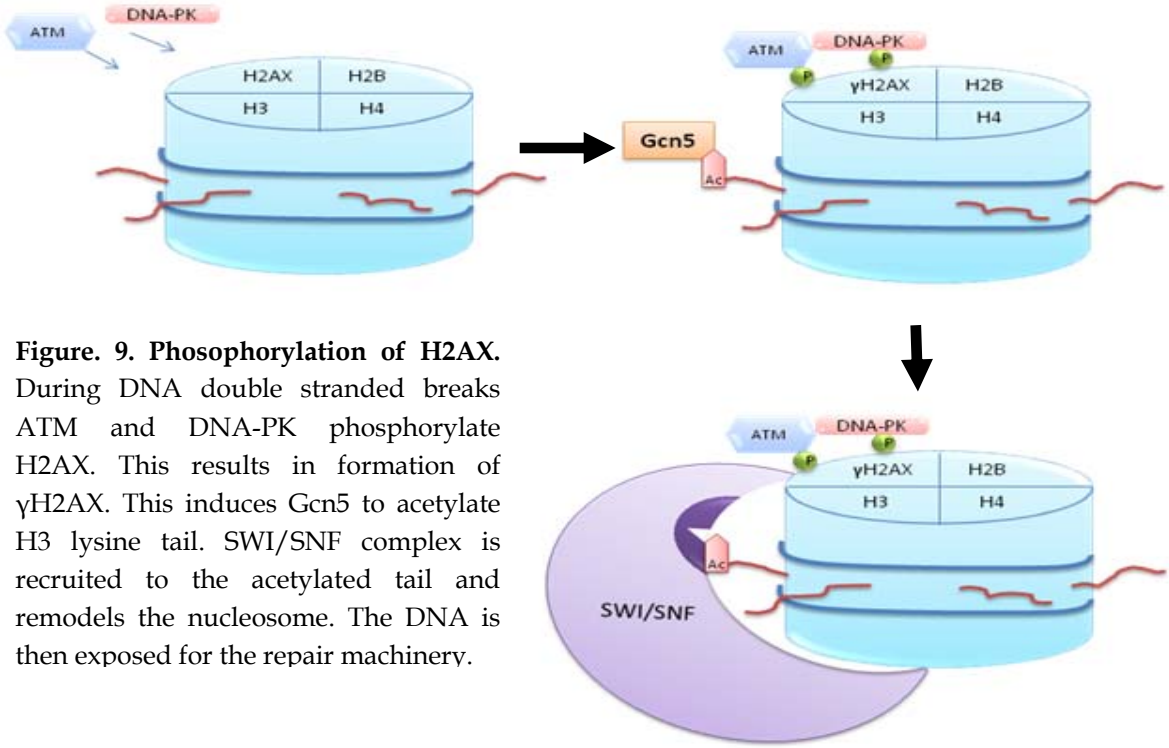


**Figure. 9. Phosophorylation of H2AX.** During DNA double stranded breaks ATM and DNA-PK phosphorylate H2AX. This results in formation of γH2AX. This induces Gcn5 to acetylate H3 lysine tail. SWI/SNF complex is recruited to the acetylated tail and remodels the nucleosome. The DNA is then exposed for the repair machinery.

The roles of SMARCAL1 include first to anneal collapsed replication forks by dissociation of RPA, second it prevents the recruitment of Rad51 to the DSB preventing further breaks and lastly it's associated with single stranded DNA for its stabilization of ssDNA (Postow *et al* 2009). If there is stabilization of RPA on ssDNA it causes the binding of RAD24-RFC (replication factor C-like complex with Rad24) to the site. This complex helps in the loading of checkpoint clamp (Burgers *et al* 2006). The checkpoint clamp consists of three subunits Rad9, Hus1, and Rad1 (9-1-1). Rad9 binds to TopBP1 and activates it which in turn activates ATR (Delacroix *et al* 2007). ATR forms a complex with ATRIP which forms the S-Phase checkpoint complex (Lee *et al* 2006, Junran *et al* 2010). BRCA2 is recruited at the ssDNA-dsDNA junction (Pavletich *et al* 2005), once attached BRC repeats recruits the Rad51 to the junction forming Rad51 filament (Kowalczykowski el al 2009). This forms nuclear foci for the repair. Rad51 replaces the RPA protein from the ssDNA in association with its paralogs (RAD51C/XRCC3) heterodimer and a (RAD51B/ RAD51C/RAD51D/XRCC2) heterotetramer (West *et al* 2001). Other proteins Rad52 and GEMIN2 also collaborate in this process (Shunichi *et al* 2010). Rad51 filament search for the homology of the damaged site with the repair template from a homologous double stranded DNA (Rodney *et al* 2010).SWI/SNF complex upon recognition of the homologous DNA is recruited to donor sequence where it performs remodeling of the nuclesome to expose DNA for strand invasion (Chai *et al* 2005). After pairing the ssDNA invades the double stranded structure which is mediated by Rad54 a homolog of swi/snf complex (Heyer *et al* 2002). Rad 55/57 helps in the stabilization of this structure (Lorraine *et al* 2002). After invasion of strand there is formation of D-loop structure (Shunichi *et al* 2010). There is DNA synthesis along the 3′ invading strand. The D-loop changes and forms a holiday junction.

Rad52 is responsible for the annealing of other strand to site forming a double holiday junction (Tomohiko *et al* 2006). The cleavage of these holiday junctions by resolvase enzymes such as SLX1 and SLX4 results in recombinant products of DNA (Harper *et al* 2009).

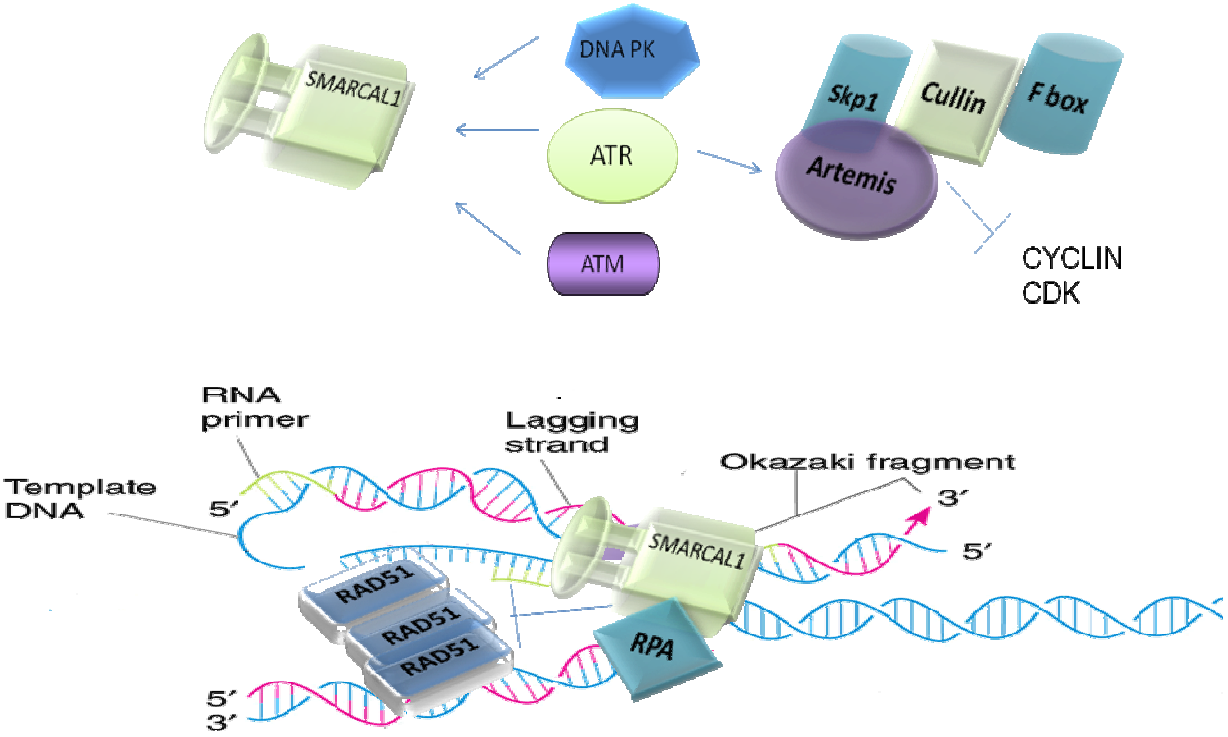


**Figure 10. SMARCAl1 activation and Role.** SMARCAL1 is phosphrylated by ATM, ATR and DNA-PK upon single stranded DNA breaks and stalled replication froks. ATR activates Artemis-skp1-Cullin-Fbox, this complex inhibit Cyclin CDK for cell cycle arrest during repair. RPA is recruited to the DNA during formation of single stranded breaks. Phosphorylated SMARCAL1 forms complex with RPA and prevent collapsing of replication fork. During single stranded DNA breaks it also inhibit Rad51 which is the initiator of Homologous Recombination Repair Pathway.

## Conclusion

Despite the fact that substantial fraction of information has been added into the existing pool of epigenetics, yet there are some outstanding questions in terms of translational medicine and personalized medicine. During the designing of therapeutic interventions it has to be taken into account that activation of Tumor suppressor genes might also activate oncogenes and analogously, silencing of oncogenes might also inactivate tumor suppressor genes. It is obvious that there is a slight demarcation between two diametrically opposed trends which might offer stumbling blocks during the standardization of therapy.

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