Transcription Through Chromatin – Link to Diseases and Therapeutics

Hari Kishore A, Sushma S Iyengar and Tapas K Kundu

The expression of chromosomal genes is regulated by post-translational modification of both histone and nonhistone chromatin proteins and ATP-dependent remodeling of chromatin. Dysfunction of the modification and remodeling machineries can lead to several diseases, which include cancer, cardiac hypertrophy, and asthma. Many genetic diseases can also lead to malfunction of the machinery. The enzymes responsible for chromatin organization are the new targets for therapeutics. Inhibitors and activators of histone acetyltransferases and inhibitors of histone deacetylases may serve as new generation drugs.

Introduction

Chromatin is the highly organized and dynamic nucleoprotein form of the genome that facilitates genes to be turned on and off depending upon the cellular requirements in a very precise manner. In agreement with the number of functional genes, a major part of the genome is transcriptionally inert and is present as heterochromatin[1] whereas, active genes are located in euchromatin[2]. In the active chromatin, core histone tails are dynamically modified by histone acetytransferases, deacetylases, methyltransferases and kinases. Histone acetyltransferases acetylate specific lysine residues of histone H3 (K14 and K10) and histone H4 (K8 and K16) for transcriptional activation. These acetylated residues are deacetylated by specific histone deacetylases when the genes need to be silenced. The active genes are methylated at the K4 site of histone H3 with functional consequences. Interestingly, no demethylase is known so far. However, methylation of histone H3 at K9 position represses transcriptional activity. Presumably, activation and re-

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pression by methylation are regulated by the displacement of methylated histones. Phosphorylation of the histone H3 (at Ser 10) has been shown to be important for transcriptional activation. Furthermore, active gene promoters were found to be devoid of histone H1 and the CpG[3] islands at the promoter are not methylated. ATP-dependent chromatin remodeling machinery facilitates to achieve both active and repressed state of chromatin by altering the pathway of DNA over the nucleosomal core. There are several ATP-dependent chromatin remodeling complexes, which get recruited on the chromatin by different activators and repressors to perform their specific role. However, chromatin is not only the complex of histone and DNA, several nonhistone chromatin-associated proteins are also integral parts of chromatin. Posttranslational modifications (e.g., acetylation/deacetylation/phosphorylation) of nonhistone proteins regulate their dynamic association with chromatin and the consequent function. Dysfunction of any of these machineries leads to one or several diseases in humans. Thus, these are the newly identified therapeutic targets. In this article we will discuss how the mutation or altered function of histone acetyltransferases, histone deacetylases, histone methyltransferases and some of the components of ATP-dependent remodeling cause different diseases. We will also briefly highlight the present status of new therapeutic agents targeted to histone acetyltransferases and histone deacetylases.

Histone Acetyl Transferases (HATs) and Diseases

Acetylation of histones is a reversible process. The balance between acetylation and deacetylation is one of the key factors for the regulation of gene expression. Hyperacetylation of histones associated with normally silenced genes or deacetylation of histones associated with actively transcribed genes may lead to several disorders. Acetylation-associated disorders may occur through i) hyperacetylation and derepression of normally repressed promoters; ii) the hypoacetylation and repression of genes necessary for cell viability, and iii) mistargeting of HAT and HDAC activity.

[1] Heterochromatin comprises of densely staining condensed chromosomal regions, believed to be for the most part, genetically inert. chromatin that remains tightly coiled throughout the cell cycle.

[2] Euchromatin is the chromosomal region that stains poorly or not at all; thought to contain the normally functioning genes. Region of eukaryotic chromosome that is diffuse during interphase. Presumably the actively transcribing DNA of the chromosomes.

[3] CpG stands for cytosine and guanine separated by a phosphate which links the two nucleotides together in DNA. Unlike CpG sites in the coding region of a gene, the CpG sites in the CpG islands are un-methylated. This raises the possibility that methylation of CpG may inhibit the expression of a gene by altering the promoter. CpG islands are often located around the promoters of housekeeping genes (which are essential for general cell functions) or other genes frequently expressed in a cell.

Keywords.
Cancer, chromatin therapy, cardiac hypertrophy, HIV, Rubinstein–Taybi syndrome.

Mutation, mistargeting and translocation[4] (formation of fusion proteins) of the histone acetyltransferases cause aberrant acetylation.

Mutation in HATs

Rubinstein–Taybi Syndrome (RTS) is a developmental haploinsufficiency[5] disorder, which is marked by mental retardation, craniofacial defects, broad big toes and thumb. These patients are highly susceptible to cancer. Mutation in the *cbp* locus results in RTS. It was found that a single mutation at the plant homeodomain (PHD)-type zinc finger in the HAT domain of *cbp* causes this syndrome. The mutation alters a conserved finger amino acid residue E to K at position 1278. Interestingly, this mutation in *cbp* also abolishes its histone acetyltransferase activity.

Mistargeting of HATs – Sequestration and Degradation Manifested in Neurodegenerative Disease.

Spinal and Bulbar Muscular Atrophy (SBMA): Spinal and bulbar muscular atrophy is an inherited neurodegenerative disease caused by CAG repeat expansion in the androgen receptor gene. This results in an expanded polyglutamine tract, which confers a novel, toxic function to the affected protein. The transcriptional activator CBP (Cyclic AMP Response Element Binding Protein) is incorporated into nuclear inclusion bodies formed by polyglutamine-containing proteins. Free CBP levels are reduced in cells expressing expanded polyglutamine, though the CBP mRNA level is not affected. Over expression of CBP can rescue cells from polyglutamine-mediated toxicity.

Huntington's Disease: This disease is a fatal neurodegenerative disease with a late onset and is inherited in a dominant fashion. It is also associated with increase in the length of a CAG triplet repeat present in the huntingtin gene located on chromosome 4p16.3. Mutant huntingtin protein from human brain cells is more resistant to proteolysis than normal huntingtin, which leads to aggregation and toxicity through the sequestration of important targets, including normal huntingtin protein.

[4] Translocations are chromosomal abnormalities which occur when chromosomes break and the fragments rejoin to other chromosomes.

[5] Haploinsufficiency is the situation in which an individual who is heterozygous for a certain gene mutation or hemizygous at a particular locus, often due to a deletion of the corresponding allele, is clinically affected because a single copy of the normal gene is incapable of providing sufficient protein production as to assure normal function.

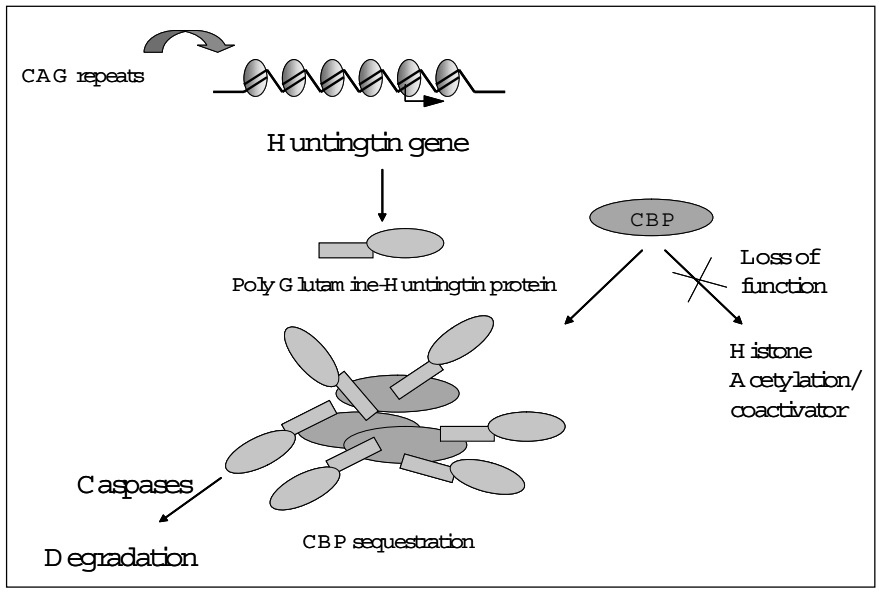


Figure 1. Due to the introduction of CAG repeats in huntingtin gene, a PolyQ-huntingtin protein is expressed which sequesters normal Hunt-ingtin and CBP. This CBP forms inclusion bodies, which are further degraded by caspases[6].

[6] Caspase is a protein that is involved in apoptosis (*Box* 1). Caspases are characterized by their unusual ability to cleave proteins at specific sites. Active caspases can often activate other caspases, leading to a cascade of protein degradation.

Polyglutamine-containing domain of huntingtin directly binds the acetyltransferase domains of CBP and PCAF (p300-CBP Associated Factor) and precipitate them from the cellular environment. The PolyQ-huntingtin also inhibits acetyltransferase activity of p300, CBP and PCAF *in vitro* (*Figure* 1).

Translocations of HATs

MOZ (Monocytic leukemia Zinc finger) gene fusions: MOZ-CBP fusion protein is expressed due to a translocation [t(8;16)(p11;p13)] in Acute Myeloid leukemia (AML). This fusion protein may be mistargeted or misregulated which leads to altered chromatin acetylation leading to either hyperacetylation of nonspecific gene targets or hypoacetylation in case of mistargeting or loss of function (*Figure* 2 A and B).

MLL (Mixed Lineage Leukemia) gene fusions: MLL-CBP fusion protein is expressed in translocation [t(11;16)(q23;p13)] in case of therapy-related *AML*, myelodysplastic syndrome, or chronic myelomonocytic leukemia. MLL is a homolog of the *Drosophila trithorax (trx)* gene involved in proper homeotic gene expression and regulation of chromatin structure. MLL marks

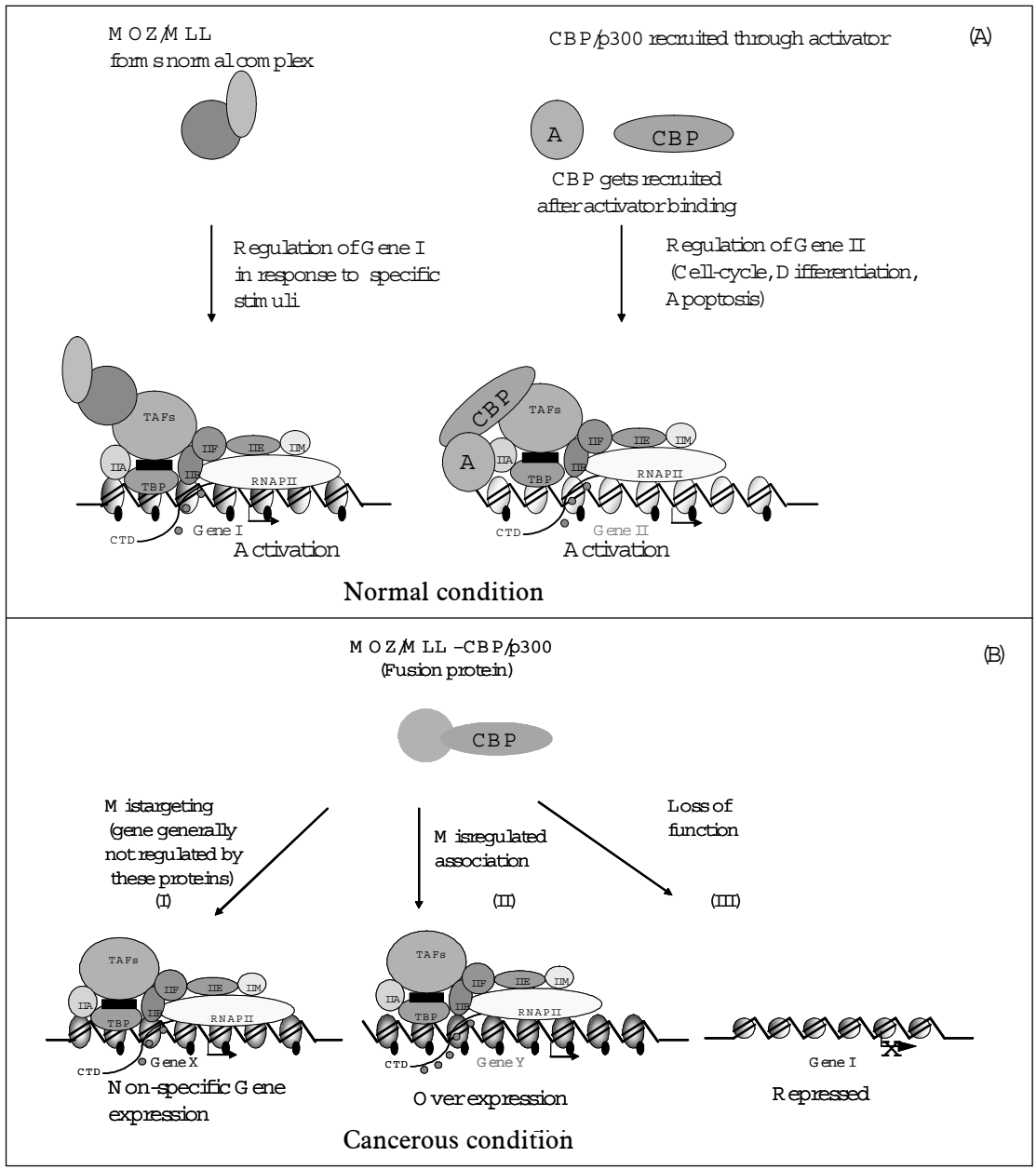


Figure 2. Aberrant gene expression by the HAT containing fusion proteins due to chromosomal translocations. (A) During the normal course of gene expression MOZ/MLL interacts with specific proteins and get recruited to the promoter in response to stimuli. p300/CBP interacts with promoter bound gene specific activator and activates the transcription. (B) In cancerous condition translocation derived fusion proteins are expressed (MOZ-CBP, MOZ-TIF2, MLL-CBP or MLL-p300). These fusion proteins may (I) be mistargeted to a non-specific gene; (II) misregulate a specific target gene (hyper-activation); (III) lose function resulting in inactivation of gene.

Abbreviations

Bad	–	Bcl2-antagonist of cell death protein
BRCA1	–	Breast Cancer 1
CIP1	–	CDK-Interacting Protein 1
CNSL	–	Cashew Nut Shell Liquid
CTPB	–	[N-(4-chloro-3-trifluoromethyl-phenyl)-2- ethoxy-6-pentadecyl-benzamide]
ETO	–	eight-twenty-one (a zinc finger nuclear protein)
FMR1	–	Fragile-X Mental Retardation 1
MORF	–	MOZ-related Factor
MTG16	–	myeloid transforming gene chromosome 16
PLZF	–	promyelocytic leukemia zinc finger
PML	–	promyelocytic leukemia
RSK2	–	p90 ribosomal S6 protein kinase 2
SMYD3	–	SET and MYND containing 3
WAF1	–	Wildtype p53 – Activated Fragment 1

For other abbreviations see [1].

the boundaries of transcriptional activity by discriminating among DNA methylation states. The fusion protein has A/T hooks and cysteine-rich DNA recognition domain of MLL fused to intact CBP, and it lacks C-terminal SET domain, which is highly conserved in several chromatin-associated proteins. The SET domain of MLL is very important for interacting with hSNF5 (component of SWI/SNF, a chromatin-remodeling complex), which has a direct effect on the transcription. Thus the fusion protein MLL-CBP fails to recruit SWI/SNF to its target.

MORF gene fusions: MORF-CBP fusion proteins are expressed in [t(10;16)(q22;p13)] in childhood AML. MORF belongs to MYST family of HATs. The MORF-CBP protein retains the zinc fingers, two nuclear localization signals, the HAT domain, a portion of the acidic domain of MORF and the CBP. CBP retains CREB-binding domain, three Cys/His-rich regions, bromodomain, HAT domain and the Glu-rich domains. The functional implications of this fusion protein are yet to be elucidated.

Histone Deacetylases (HDACs) and Diseases

Three classes (I, II and III) of HDACs, function in a closely coordinated and targeted manner to direct the deacetylation of histones of a gene to be repressed. Transcriptional repressors like YY1, Mad/Max, and NCoR/Smrt form complexes with HDACs. HDACs along with Mad/Max and RB (cell cycle regulators) form a multi-subunit repressor complex, which represses gene expression in a targeted manner. This repressor complex can be disrupted by over expression of *c-Myc* or *v-Ski* which induces cell-cycle progression and transformation. RB associates with transcription factor E2F which represses E2F-dependent genes and regulates S-phase entry in cell cycle. This repression is brought about by interaction of RB with HDACs. Mutations in RB disrupts RB-HDAC complex which triggers uncontrolled cell proliferation. HDACs are associated with NURD complex, which has helicase/ATPase activity and specifically binds to methylated DNA. HDACs also associate with MeCP2[7]. MeCP2 binds to methylated DNA and the associated HDAC deacetylates the chromatin to silence the gene. Thus HDACs have a role in chromatin remodeling and promoter silencing. In various tumors, promoters of RB, $p15^{ink4b}$, and $p16^{ink4a}$ were found to be hypermethylated, as a result these genes are silenced. Presumably, HDACs are also involved to repress these genes.

Protein Fusions Involving HDACs

RARa/PML and RARa/PLZF in Acute Promyelocytic Leukemia (APL): RARa (Retinoic Acid Receptor[8]) represses transcription by HDAC recruitment. Retinoic acid disrupts this complex and activates retinoic acid-inducible genes. In case of fusion proteins like RARa/PML (ProMyelocytic Leukemia) and RARa/PLZF (Promyelocytic Leukaemia Zinc Finger) the repressor complex in association with HDAC becomes insensitive to RA. Thus the RA-responsive genes remain repressed and lead to blockage of hematopoietic differentiation.

AML-fusions: In leukemia, HDAC-mediated repression blocks

[7] Methyl CpG binding Protein (MeCP2): The MeCP2 protein binds to DNA at regions called CpG islands, which frequently occur near the beginning of a gene. The protein then interacts with other proteins to form a complex that turns off the gene.

[8] Retinoic Acid Receptor (RAR): Retinoic Acid receptors (RARs) are nuclear receptors related to the steroid and thyroid hormone receptors, a family of proteins that function as ligand-dependent transcription factors. Several RAR isoforms have been identified in mammals - RAR-alpha, -beta and -gamma as well as novel nuclear receptors known as RXR (Retinoid X receptor), which are distantly related to RARs and respond to high concentrations of RA. Nuclear receptors act as transcription factors by binding to specific DNA recognition sequences generally located upstream of responsive genes. Although RARs can activate gene expression through binding to thyroid hormone response elements, a much more specific and potent Retinoic acid response element (RARE) has been identified recently within the promoter of the RAR-beta gene.

'myeloid differentiation' [9]. It has been found that in these patients AML-1 is present as fusion protein with ETO, MTG16 and TEL. Due to the formation of fusion proteins (DNA binding domain of AML-1 and ETO, MTG16 and TEL) HDAC-association mediated repression becomes irreversible, which blocks myeloid differentiation.

[9] Myeloid differentiation: Differentiation of erythrocytes, megakaryocytes, platelets, basophils, eosinophils, neutrophils, monocytes, macrophages, and osteoclasts from myeloid precursor cells. In acute leukemia differentiation is disturbed by mechanisms typically involving some transcription factors participating in control of differentiation. Genetic aberrations result in a differentiation block and clonal expansion. As a consequence, normal hematopoiesis is suppressed with life-threatening coagulation and immunity disorders.

Methylation of Histones and Diseases

In the past two years, studies on histone methylation have gained immense importance and that has lead to several discoveries. Unlike histone acetylation, the reverse step of methylation is not known yet. Thus, methylation is a sort of permanent mark on the genome. Methylation may activate as well as repress chromatin, based on the position of lysine and arginine residues in different histones (predominantly H3 and H4). Significantly, both methylation-dependent activation and repression of chromatin, function in a highly organized manner along with acetylation and deacetylation. Dysfunction of methyl-transferases thus may lead to several diseases. Many HMTases (Histone methyl transtase) contain the SET domain. The SET domain containing HMTase RIZ1 acts as tumor suppressor, suggesting its function in cancer regulation. The other important example is MLL, which is an HMTase that can be converted into oncoprotein by acquisition of transcription activation domain of EEN. Most recently, a novel HMTase, SMYD3, has been discovered. It has been found that SMYD3 is over expressed in majority of colorectal and hepatocellular carcinomas. It is also a SET domain containing HMTase, which specifically methylates H3-K4. The silencing of the SMYD3 by siRNA in cancer cells resulted in significant growth suppression. Another genetic disease, *Fragile X syndrome* is caused by an expansion of a polymorphic CAG triplet repeat that results in silencing of *FMR1* gene present on the X-chromosome. This is characterized by hypermethylation of CpG island in the *FMR1* gene promoter and deacetylation of histone H3 and H4. In fragile X cells, there is a decrease in methylation of histone H3-K4 with a large increase in methylation at H3-K9. The high level of H3-K9

methylation hampers H3 to be acetylated even after treatment of fragile X cells with HDACi, a treatment that fully restores acetylation of histone H4. This hereditary disorder is marked by mental retardation.

Histone Kinases and Disease

Phosphorylation of histone H1 directs the compaction of chromatin whereas histone H3 phosphorylation leads to gene expression of *c-jun*, *c-fos* and *c-myc*. RSK2 , a histone, kinase is associated with Coffin–Lowry Syndrome (CLS), which is marked by severe mental retardation, short stature, coarse facies, patulous lips. Deletion, nonsense and missense mutations are observed in patients with CLS. Western blot analysis of lymphoblastoid or fibroblast cell lines derived from CLS patients could not detect RSK2, which could be due to truncations. Sometimes though the level of protein does not alter, the RSK2 phosphotransferase activity of the mutant protein is impaired. RSK2 activates CREB through phosphorylation (Ser-133), which is impaired in CLS. RSK2 is also a part of the gene family implicated in cell cycle regulation through MAPK pathway.

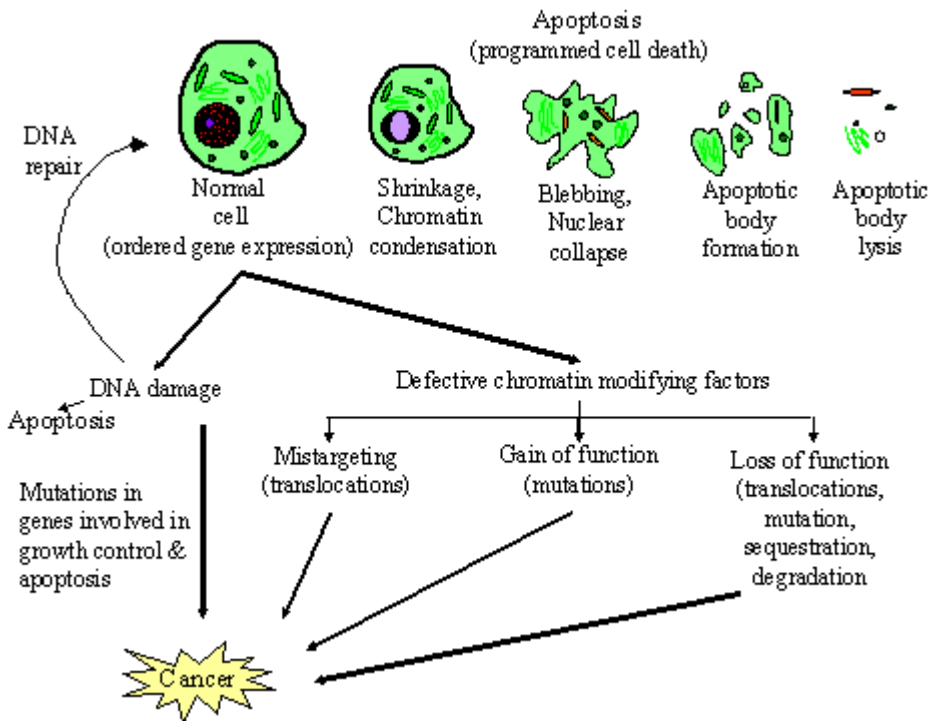
Modulators of Histone Modifying Enzymes as Therapeutics

HAT and HDAC Modulators as Therapeutics of the Future

A) HDAC inhibitors (HDACi): HDACs are important molecular targets for cancer therapy. Hence, HDACi gain importance in the field of chemotherapy. HDAC inhibitors cause cell cycle arrest, differentiation and/or apoptosis (*Box* 1) of many tumours, suggesting their usefulness for chemotherapy and differentiation therapy. Various classes of compounds (short-chain fatty acids, hydroxamic acids, cyclic tetrapeptides, cyclic depsipeptides and phenylene diamines) are known to be potent against HDAC activity. The first HDAC inhibitor was actually used as a ligand attached to a solid support resin (affinity matrix) to purify HDACs. Since then a number of HDAC inhibitors were designed by modifying various naturally occurring molecules

Box 1. Apoptosis

Apoptosis, or programmed cell death, is a regulated physiological process leading to cell death characterized by cell shrinkage, membrane blebbing and DNA fragmentation. Failure of DNA damage repair mechanism may also lead to apoptosis. Caspases, a family of cysteine proteases, are central regulators of apoptosis. Once activated, these caspases cleave and activate downstream effector caspases, which in turn cleave cytoskeletal and nuclear proteins and induce apoptosis. Too little or too much apoptosis plays a role in many diseases. When there is misregulation of programmed cell death, the cells may become immortal which leads to cancer or too many cells may get lysed as in the case of neurodegenerative diseases (Alzheimer, Huntington, Parkinson).



(*Table* 1). HDACi globally affect the repressive activities of HDAC associated transcription factors and various chromatin remodeling complex. HDACi exert growth inhibitory, pre-apoptotic and differentiating activities in various cell lines irrespective of histological origin. Retinoic Acid induced differentiation is enhanced by HDACi. Most of the HDACi upregulate the expression of tumor suppressor proteins like p21WAF1/

HDAC Inhibitors	Properties
SAHA (Suberoylanilide hydroxamic acid)	Potent, cell-permeable inhibitor, induces differentiation in human breast cancer cells, induces apoptosis, displays anti-angiogenesis activity by altering VEGF signaling in HUVECs.
Scriptaid	Reversible medium-potency, hydroxamic acid-type, inhibits tumor growth in vitro and in vivo and, in conjunction with AZA, acts to re-express functional ER.
Suberoyl bis-hydroxamic acid	Low-potency HDAC inhibitor, induces differentiation in murine erythroleukemia cells.
Trichostatin A (TSA)	Potent and reversible HDAC inhibitor, blocks cell cycle progression at G1, it displays immunosuppressive activity in a mouse. Inhibits angiogenesis. modulate CD4+ T-cell responses.
HC Toxin	Potent, cell-permeable, cyclopeptide HDAC inhibitor, it displays antineoplastic activity and up-regulates 15-lipoxygenase in colorectal carcinoma cell lines.
Chlamydocin	Naturally occurring cyclic tetrapeptide, increases the expression of $p21^{cip1/waf1}$, blocks cell cycle progression at G_2/M phase of the cell cycle, induces apoptosis by activating caspase-3, which in turn leads to the cleavage of $p21^{cip1/waf1}$.
Sodium butyrate	Increases the expression levels of p21 (WAF-1) and inhibits G1-S transition of the cell cycle. increases the expression of the Bad protein, decreases Ca^{2+} release from intracellular stores
FK228 (depsipeptide)	Potent inhibitor of HDAC1 and HDAC2.
Psammaplin A (PSMA)	Physiologically stable marine natural product, induces the expression of the cyclin dependent kinase inhibitor $p21^{waf1}$.
Sodium phenyl Butyrate	A chemical chaperone, glutamine trap used as ammonia scavenger (in patients with urea cycle disorder).
Valproic acid	Considered for clinical trials for spinal muscular atrophy
Sirtinol	Inhibits yeast Sir2p transcriptional silencing activity in vivo, yeast Sir2p and human SIRT2 deacetylase activity in vitro.

Table 1. Histone deacetylase inhibitors, potential anticancer therapeutics.

CIP1 (p21WAF1 a cyclin-dependent kinase inhibitor plays a crucial role in G1/S and G2/M transitions of cell cycle). HDACi also induce expression of CD86 possibly stimulating tumor immunity.

There are considerable drawbacks and limitations of various cancer drugs owing to which HDACi are gaining importance. Most of the HDACi have multiple effects in addition to inhibi-

Box 2. Engineered Transcription Factors

Selective manipulation of specific gene expression can be a powerful tool in biomedicine. Typical Transcription Factors (TFs) have DNA binding domain (DBD) and an effector (activation or repressor) domain. By changing DBD and effector domains new TFs can be made which are gene specific. Among DBDs, Zinc Finger Proteins (ZFPs) are widely studied. ZF are small DNA recognition motifs (30 amino acids). A series of ZF can be designed to make a DBD specific for a particular gene. These are called Code-based designed ZFs. ZFP TFs can be designed to upregulate or downregulate a particular target gene of interest. These tailored ZFP TFs mimic natural transcriptional mechanism and thus provide an attractive tool for regulating gene expression. A ZFP TF can be constructed by using DNA sequence of a promoter of the specific target gene which forms a DBD to which a repression domain (e.g., Kruppel – associated box A/B, v-erb or SID) or activation domain (e.g., p65 of NFkB or VP16) is linked.

ZFP TFs in Cancer therapy

Aberrant expression of genes can be detected from microarray analysis and protein profiling. This kind of transcription profile is a diagnostic marker for cancer type and disease progression. By correcting this abnormal expression of genes, disease condition can be corrected using engineered ZFP TFs. This is referred to as 'Transcription therapy'. These ZFP TFs can be delivered at a particular in vivo target using adenovirus or retrovirus, fused to "delivery-short amino acid sequences", directly injected into blood stream. Also these ZFP TFs, can be controlled by linking a ligand-binding domain (e.g., steroid hormone receptors, so that by administration of various doses of steroid hormone analogs gene expression can be controlled).

tion of deacetylase activity and cause toxicity to the cell. So the search continues for stable, small molecular, cell-permeable, non-toxic HDACi. Also an important factor to be considered is the reversibility of action. Compounds like TSA, SAHA, sodium phenyl barbiturate, depsipeptides either alone or in combination are proved to be successful in cancer therapy. Recently compounds like arginine butyrate, cyclo (trp-trp) and cyclo (phe-pro) have been shown to increase histone acetylation in tumor derived cell lines.

HDAC recruitment directed by DNA methylation as in the case of Fragile X syndrome, has given clues to use of HDACi in combination with DMTase (DNA methyltransferases) inhibitors (5-aza-2'-deoxycytidine). This kind of combination therapy restored methylation of DNA and acetylation of histones to nearly wild type. Thus non-toxic, enzyme specific HDACi in combination with other DMTase inhibitors could be very good therapeutics for cancer in future. In the case of tumors harboring fusion proteins like RARa-PML and RARa/PLZF there is formation of an Retionic Acid-unresponsive complex with HDAC. A combination of HDACi and RA has been proved to be successful rather than RA alone as therapeutics. Most importantly HDACi arrest progressive neuronal degeneration induced by polyglutamine repeat expansion. These findings raise the possibility that therapy with HDACi may slow or prevent the progressive neurodegeneration seen in Huntington's disease. Expression of huntingtin leads to hypo-acetylation of H3 and H4, which is reversed by administration of HDACi.

HDAC of the host, inhibits HIV gene expression and virus production and may contribute to latency of HIV within resting CD4 T cells. HDACi are capable of inducing expression of quiescent provirus, without fully activating cells or enhancing *de novo* infection. Thus a combination of antiretroviral therapeutics and HDACi may in future help in treating AIDS.

B) ***HAT modulators:*** Although great progress has been made in the study of HDACi, very little has been done in the area of histone acetyltransferase modulators. Long before the discovery of HATs, polyamine-CoA conjugates were found to inhibit the histone acetyltransferase activity of cell extracts. The discovery of different histone acetyltransferases made it possible to synthesize more specific inhibitors of histone acetyltransferases. Two synthetic inhibitors, Lysyl-CoA specific for p300 and H3-CoA-20 targeted to PCAF were discovered recently. Both of these inhibitors soon became useful tools to understand the mechanism of p300 and PCAF function *in vitro*. However, cells are impermeable to these inhibitors and being CoA conjugates,

these are pharmacogenically poor. More recently, we have been able to isolate two natural inhibitors from plant sources, which permeate the cell and thus could be lead compounds for designing drugs. These are anacardic acid, from CNSL and Garcinol from *Garcinia indica* (Kokam) fruit rind. By using anacardic acid, we have synthesized an amide derivative, CTPB, which enhances the HAT activity of p300 specifically. To date CTPB is the only known small molecular activator of any histone acetyltransferase, which could be highly useful as an alternative for HDACi. Also, CTPB could be used to disrupt the interaction of p300 and PolyQ-Huntingtin and restore the normal levels of histone acetyltransferase activity.

HMTase Inhibitors

Recently, an inhibitor AMI-1, specific to arginine methyltransferase activity, has been reported. It can inhibit the methyltransferase activity *in vitro* and *in vivo*. AMI-1 inhibits *in vivo* arginine methylation of cellular proteins and can modulate nuclear receptor regulated transcription from estrogen and androgen receptor responsible elements. Therefore, it is effective in inhibiting the action of certain hormones.

ATP-Dependent Remodeling Complexes and Diseases

Mutations in hSWI/SNF

SWI/SNF complex is an ATP-dependent chromatin remodeling protein complex, which is composed of at least 11 different proteins. It can either activate or repress transcriptional activity. In *rhabdoid tumours* mutations (frameshift, nonsense and deletion) have been reported in the *hSNF5* gene. It is not clear how these truncations affect SWI/SNF function. But these mutations in malignancies show that SWI/SNF may contribute in regulation of cell cycle. Inhibition of cell cycle could be achieved by both activation and repression function of SWI/SNF. SWI/SNF mediated chromatin-remodeling activity might facilitate transcription factor binding and activate genes that inhibit cell cycle progression (e.g., transcriptional activation of estrogen,

glucocorticoid and RARs). hBRM associates with RB and E2F1 which blocks E2F1 activation function. The blocking of E2F1 activation causes the cell cycle arrest at G1 phase. Since RB recruits HDACs, genes involved in cell cycle progression might be repressed through synergestic action of SWI/SNF and HDACs. Also mutations in *hSNF5* gene may impair its interaction with hBRM. This may alter the function of SWI/SNF.

The SWI2/SNF2 family of DNA-dependent ATPases participates in a number of processes, including DNA repair, chromatin remodeling, recombination and transcription. Individual members of this family of proteins have been demonstrated to interact with and regulate a limited set of transcription factors including steroid receptors (estrogen receptor, androgen receptor, etc.), p53, c-myc, the retinoblastoma[10] protein and BRCA1. Many of these factors have been investigated for their role in the development and/or progression of breast cancer or prostate cancer. Although the steroid receptor has been the primary target for chemotherapy, an alternative target might be ATPase catalytic site functionally coupled to a DNA binding site. Phosphoaminoglycosides, a group of ATPase inhibitors, can inhibit the activity of SWI2/SNF2 complex. Since, SWI2/SNF2 family members are involved in regulation of androgen receptor function, inhibition of the ATP-dependent chromatin remodeling activity selectively inhibits the growth of prostate cancer cells. Regulation of the SWI2/SNF2 family members regulates a limited number of transcription factors that are intimately involved with cell growth and differentiation. SWI2/SNF2 proteins could be novel targets for therapeutic exploitation with specific interest in control of cell growth by phosphoaminoglycosides.

[10] Retinoblastoma is a rare type of eye cancer that develops in the retina. Although this disorder can occur at any age, it usually develops in children. Most cases of retinoblastoma occur in only one eye, but both eyes can be affected. The most common sign of this disorder is a visible whiteness in the normally black pupil (the part of the eye that lets in light). Other signs and symptoms of retinoblastoma include crossed eyes or eyes that do not point in the same direction; persistent eye pain, redness, or irritation; and blindness or poor vision in the affected eye.

Conclusion

Various concerted modifications of the histone tails and chromatin remodeling hold the key to gene regulation. Aberrant gene expression may lead to disease condition. Thus the enzymes involved in chromatin remodeling are the crucial targets

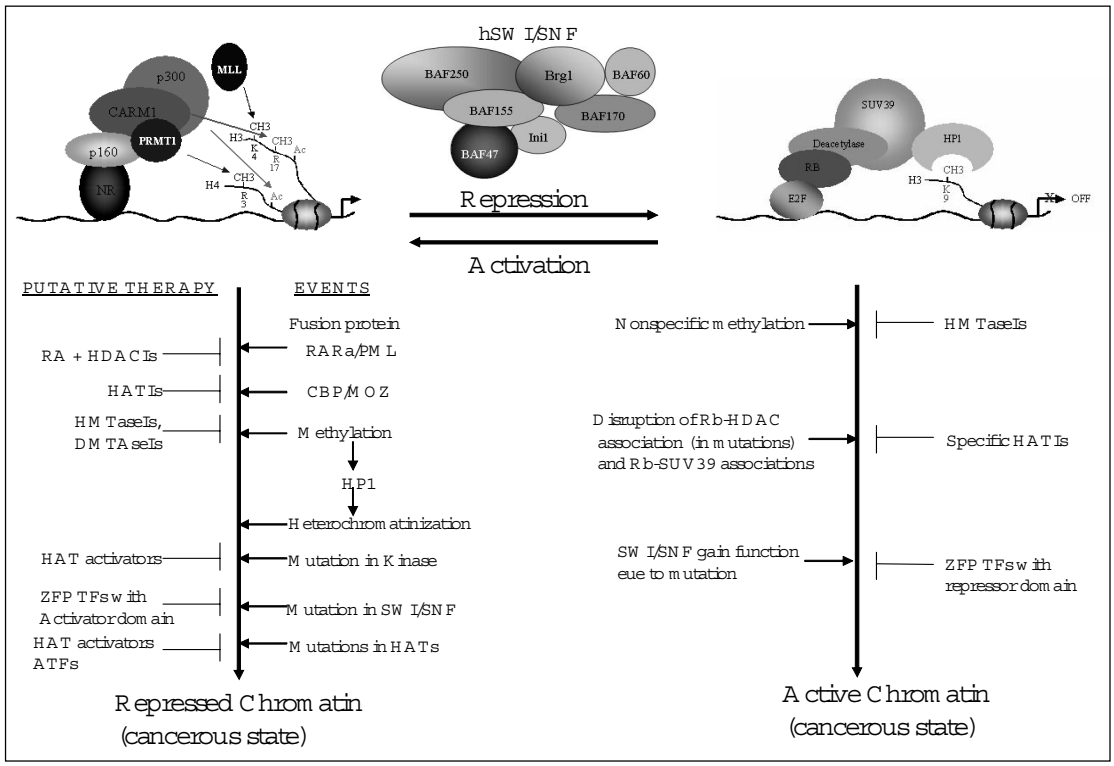


Figure 3. Chromatin therapy. In a disease state, normally active chromatin may be modified to a repressed state and vice versa due to the events indicated. Specific modulators (e.g., inhibitors or activators of HATs, inhibitors of HDACs and inhibitors of HMTase) could be useful to restore the normal activities.

for therapy. By manipulating specific enzymes using modulators (inhibitors and activators), a desired gene expression can be achieved which could be very important in designing the therapeutics for a particular kind of chromatin associated disease. This novel approach is called 'Chromatin Therapy' (*Figure* 3). Much work though remains to be done in this area.

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Suggested Reading

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